Stone Fruit Commodity-based Survey Reference

Cooperative Agricultural Pest Survey (CAPS)

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Draft Log

May 2011 – Draft sent for CPHST review.

June 2011 – Draft sent for CAPS review.

August 2011 – Final draft posted on CAPS Resource and Collaboration website.

March 2012 – Trap and lure information updated to reflect names used in the IPHIS survey supply ordering system. *Lobesia botrana* lure effectiveness changed from three to four weeks. New maps added for pests. *Leucoptera malifoliella* datasheet removed and language added about this pest being unavailable for survey. Minor changes made for a few pest datasheets (host/distribution information primarily). Information about Tort AI- Tortricids of Agricultural Importance, a new diagnostic tool developed by CPHST's Identification Technology Program, was added where appropriate.

June 2013 – Removed '*Candidatus* Phytoplasma australiense' as a secondary pest of stone fruit. The report of this pest on peach from Bolivia was found to be an error and is likely a related species and not '*Ca*. Phytoplasma australiense'. Therefore, it no longer makes sense to include this pest in the stone fruit manual. Updated CAPS approved methods for '*Ca*. Phytoplasma mali' and '*Ca.* Phytoplasma prunorum'.

October 2013 – Added an updated datasheet for *Monilia polystroma* and added a datasheet for *Monilinia fructigena* (a new 2014 AHP pest).

August 2016 – Updated mapping information. Removed outdated maps.

Introduction to Reference

History of Commodity-Based Survey

The CAPS community is made up of a large and varied group of individuals from federal, state, and university organizations who utilize federal (and other) funding sources to survey for, and (in some cases) diagnose exotic and invasive plant pests. By finding pests early, eradication efforts will likely be less expensive and more efficient. For more information on CAPS and other Plant Protection and Quarantine (PPQ) pest detection programs see: http://www.aphis.usda.gov/plant_health/plant_pest_info/pest_detection/index.shtml.

Traditionally, states have been given a list of pests. Each year, states choose (from this list) a number of pests to incorporate in their own specialized surveys. There is certainly value in surveying for plant health threats in terms of discreet pests. However, this may not always be the most efficient means of survey. For example, a single pest may occur on a myriad of different hosts, making a comprehensive survey too time consuming and expensive. An alternative method has been suggested. Grouping important pests under the umbrella of a single commodity could be a more efficient way to look for certain pests. The rationale for choosing a commodity survey in certain instances includes the following:

- Survey area will be smaller and targeted.
- Resources can be better utilized with fewer trips to the field.
- Commodities are easy to prioritize in terms of economic and regional (geographic) importance.

The Center for Plant Health Science and Technology (CPHST) has been charged to develop a commodity-based survey strategy in support of the CAPS program. There are two types of end products being developed for each commodity. Each product serves a valuable yet unique purpose. The result is a set of paired documents developed for each commodity. A description of these documents is provided below:

Commodity-Based Survey Reference (CSR): This document is composed of a series of pest data sheets, mini-pest risk assessments (PRAs), or early detection PRAs. The data sheets are highly graphic and illustrate the biology, survey, and identification of particular pests in appropriate detail for CAPS surveyors. The pests in this document are numerous. The pests were chosen primarily from the CAPS Analytic Hierarchy Process (AHP) prioritized pest list (Appendix C and D) and the Select Agent list (http://www.selectagents.gov/ or http://www.aphis.usda.gov/programs/ag_selectagent/ag_bioterr_toxinlist.shtml). The AHP prioritized pest lists for FY 11' and FY 12' are also given in Appendices C and D. Additional pests may be added if they are cited in scientific literature as being a primary pest of the given commodity and are exotic to the United States, or if specifically

requested by the CAPS National Committee or industry. States are not required to survey for all of the pests in this document, but may choose those that are particularly relevant to include in their survey. In general, this document should serve as a desk reference for survey specialists as they plan their annual surveys.

Commodity-Based Survey Guidelines (CSG): This document is smaller. The list of pests is shorter than those chosen for the CSR. A subgroup of the CAPS National Committee determines which pests from the CSR will be included in the CSG. As such, states that participate in these surveys must survey for all organisms listed in the CSG. The CSG set forth guidelines for survey and identification from a broad scale (site selection, number of acres to survey, number of samples to collect, etc.) and a narrow scale (field methods, survey tools, transporting samples, etc.). States are encouraged to follow the procedure set forth in the CSG. The methods are intended to increase the homogeneity of the national data set and increase the statistical confidence in negative data (*e.g.,* demonstration of "free from" status).

As a pilot project, citrus was undertaken as the first commodity in this initiative. The products were developed for implementation in the 2007 survey season. Citrus was chosen, because it is an economically important commodity that is equally distributed in both PPQ regions but is distributed in few overall states. To date, survey strategies for pests of citrus are also well documented. Shortly after completion of the citrus CSG, several other commodity survey guidelines were initiated, including soybean, small grains, grape, corn, pine forests, and oak forests.

Stone Fruit Commodity Survey Reference

The *Stone Fruit Commodity Survey Reference* (CSR) is a companion document to the *Stone Fruit Commodity Survey Guidelines* (CSG). Both documents are intended to be tools to help survey professionals develop surveys for exotic stone fruit pests. The *Stone Fruit CSR* is a collection of detailed data sheets on exotic pests of stone fruit. Additionally, the authors have tried to identify native pests that these exotic pests may be easily confused with as well as potential vectors of exotic pests. These data sheets contain detailed information on the biology, host range, survey strategy, and identification of these pests. The commonly confused pests and vectors are included in a section of the pest data sheet dealing with the target pest.

In contrast, the *Stone Fruit Commodity Survey Guidelines* companion document is intended to help states focus resources on survey efforts and identification of a smaller group of target pests (usually less than a dozen). The guidelines contain little information about biology. Instead, they focus on survey design, sampling strategies, and methods of identification. There is no survey that would be wholly applicable to each location in the United States. Environment, personnel, budgets, and resources vary from state to state. Thus, the guidelines will provide a template that states can use to increase the uniformity and usability of data across political, geographic, and climatic regions while maintaining flexibility for specificity within individual regions.

Purposes of the Stone Fruit CSR

- To relate scientific information on a group of threatening pests.
- To facilitate collection of pest data at a sub-regional, regional, and national level versus data collection from a single location.
- To aid in the development of yearly surveys.
- To help CAPS cooperators increase their familiarity with exotic pests and commonly confused pests that are currently found in a given commodity.
- To aid in the identification and screening of pests sampled from the field.
- To collate a large amount of applicable information in a single location.

End Users

As previously noted, this document may be used for many purposes. Likewise, it will be of value to numerous end users. As the document was developed, the authors specifically targeted members of the CAPS community who are actively involved in the development and implementation of CAPS surveys.

State Plant Health Director (SPHD): The SPHD is the responsible PPQ official who administers PPQ regulatory and pest detection activities in his or her state. The SPHD is also responsible for ensuring that the expanded role of CAPS is met in his or her state. In many states, the SPHD provides guidance for the state's ongoing management of pest risk and pest detection. However, SPHD responsibilities will vary according to the extent to which each state carries out the various components of the CAPS program.

State Plant Regulatory Official (SPRO): These individuals are employees of their respective states and generally manage the expanded survey program. The SPRO is the responsible state official who administers state agricultural regulatory programs and activities within his or her respective state.

Pest Survey Specialists (PSS): The PSS, a PPQ employee, is generally (but not always) supervised by the SPHD of the state in which he or she is assigned. A PSS may also be responsible for survey activities and may work with the SSC and the survey committee in more than one state.

State Survey Coordinators (SSC): The SSC is a state employee responsible for coordinating each state's CAPS program, participating as a member of the state CAPS committee (SCC), and acting as liaison with the state PPQ office.

Diagnosticians: Diagnostic capabilities vary by state. Some states have advanced networks of diagnosticians, whereas other states access diagnostic support through National Identification Services (NIS) or through contracts with external partners. States are encouraged to utilize qualified diagnosticians in their respective states if expertise is

available. PPQ offers diagnostic support for the CAPS program through National Identification Services (NIS). A major responsibility for NIS's Domestic Identifiers is to provide diagnostic support to CAPS programs. There are plant pathology and entomology domestic identifiers in each of the PPQ regions. A forest entomology domestic identifier oversees both regions. To learn more about diagnostic resources available to you, discuss your diagnostic requirements and options with your State Plant Health Director, one of the regional Domestic Identifiers, and/or NIS. Appendix A has a listing of NIS and Domestic Identifier contact information.

Organisms Included in the Stone Fruit Survey Reference

Organisms included in the stone fruit survey reference are organized first by:

1. Pest type, (*e.g.,* arthropods and plant pathogens).

2. Organisms are then divided by their pest status on stone fruit [*e.g.,* primary pest (major pest) and secondary (minor pest)]. Primary and secondary is determined by reviewing the literature, host association, yield loss, and etc. associated with the pest on a given commodity

   A. Primary Pests: Full pest datasheets will be developed for primary pests. All pests must be exotic to the conterminous United States.

   - Pests found on the AHP Prioritized Pest List (for the fiscal year of interest) and that are major pests on the commodity will be considered primary pests.

   - Additional **exotic** pests that the author finds in the literature that are major pests on the commodity will be included as primary pests and given the designation of "National threat".

   B. Secondary Pests: Truncated pest datasheets will be developed for secondary pests.

   - Pests found on the AHP Prioritized Pest List (for the fiscal year of interest) that are **not** identified as major pests of the commodity in the literature.

   C. PPQ Program and Line Item Pests: Plant Protection and Quarantine Program pests and pests with their own line item funding should be listed by scientific name and common name **only**. These pests will **not** receive pest datasheets, unless specifically requested by the National CAPS committee. If a PPQ website exists for the pest, a link should be provided to that site. CPHST Ft. Collins can assist in determining which program pests and line item pests are relevant to the commodity.

   D. Other Pests Determined by the National CAPS Committee or

requested by the CAPS Community: Full pest datasheets will be developed for specific pests requested by the CAPS community.

3. Finally, organisms are arranged alphabetically by their scientific names. Common names are provided as well. Previous manuals have included pests from the Eastern and Western Region pest lists. The restructuring of the CAPS program and shift from regional guidelines to a single set of national guidelines has made these lists obsolete. Therefore, pests from these lists were not included in this CSR. States now have more flexibility to survey for pests of state concern, and most regional pests were captured in one or more state CAPS pest lists.

To help provide a rationale for the inclusion of each pest in the reference, the authors have included a section titled, “Reason for Inclusion in Manual”. Pests are either considered to be a CAPS target and are listed in the CAPS prioritized pest list or a national threat. The pests considered as national threats are not known to be present in the United States; however, they are not associated with the CAPS prioritized pest lists but are found on another list or identified through the literature. An additional category, requested by the CAPS community, is present in some manuals if a pest is suggested that is a primary pest, exotic to the United States, or is of regulatory significance.

Appendix M1

The survey methodology presented in Appendix M1 in the 2012 CAPS National Survey Guidelines (http://caps.ceris.purdue.edu/webfm_send/1063; http://caps.ceris.purdue.edu/node/223) lists the most up-to-date, CAPS-approved methods for survey and identification/diagnostics of CAPS target pests from the Priority Pest List, consisting of pests from the 1) commodity- and taxonomic-based surveys and 2) AHP Prioritized Pest List. The information in this table supersedes any survey and identification/ diagnostic information found in any other CAPS document (i.e., Commodity-based Survey References and Guidelines, EWB/BB National Survey Manual, etc.). All other CAPS documents will be revised to include the information contained in this table; however, this table should always be the authoritative source for the most up-to-date, CAPS-approved methods.

Introduction to Stone Fruit

The term stone fruit can be a synonym for "drupe" or, more typically, it can mean just the fruit of *Prunus* species. A drupe is a type of fruit in which an outer fleshy part (exocarp, or skin; and mesocarp, or flesh) surrounds a shell (the pit or stone) of hardened endocarp with a seed inside. These fruits develop from a single carpel and mostly from flowers with superior ovaries. The definitive characteristic of a drupe is that the hard, lignified stone (or pit) is derived from the ovary wall of the flower.

Stone fruits (apricots, cherries, nectarines peaches, plums, and prunes) are widely consumed and have become popular in world markets. These fruits are called stone fruits because they have a hard, stony pit. They can be eaten fresh, or saved for future enjoyment by canning, preserving, freezing, or drying. Sour cherries are most often used in pies.

Stone Fruit Production
Stone fruit production in 1998 (from selected countries) was estimated at 13.8 million metric tons. Peaches and nectarines account for the bulk of stone fruit production, followed by plums and prunes. Italy, Spain, and the United States are the leading stone fruit exporters in the Northern Hemisphere. In the Southern Hemisphere, Chile is the major exporter of stone fruit. Of all selected country stone fruit exports in 1998, peach and nectarine shipments comprised more than a 70 percent share, by volume, followed by plums and prunes at 17 percent. France, the United States, Italy, and Brazil are major stone fruit importers, accounting for more than 60 percent of selected country imports in 1998.

Apricots
The apricot, *Prunus armeniaca*, was first cultivated about 4,000 years ago in China. As a result of commerce, apricots were introduced into southwest Asia, then into Italy by 100 B.C., into England by 1620 A.D., and into the United States (Virginia) about 1629. The former Soviet Union produces the most apricots annually, followed by Turkey, Italy, Spain, Greece, France and the United States. California produces about 97% of the U.S. crop, primarily in the San Joaqin Valley. Other states with apricot production include Washington, Utah, Michigan, and Arizona. A commodity acreage map for apricot is provided in Figure 1.

Apricot production in 1998 (from selected countries) was estimated at 429,225 tons, down almost 20 percent from the 1997 output. U.S. apricot production in 1998 was estimated at 107,320 tons, down 15 percent from 1997.

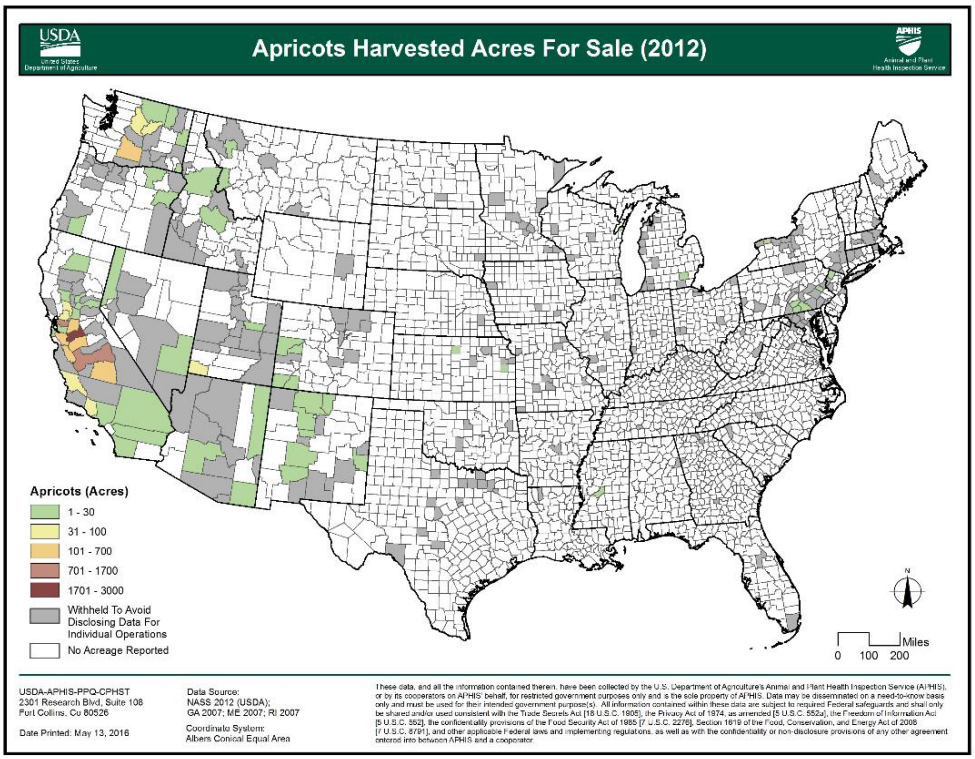

Figure 1. Apricot commodity acreage map. Map courtesy of USDA-APHIS-PPQ-CPHST.

Peaches and Nectarines

The peach, *Prunus persica*, originated in China, where records of cultivation date back 3,000 years. Peaches were probably brought to Persia through mountain trading routes, becoming known there as Persian fruit, hence the name persica or peach. These derived terms led to the misconception that peaches originated in Persia. By 330 B.C., peaches reached Greece. During the Middle Ages, their culture spread throughout Europe. The Portuguese apparently introduced the peach to the east coast of South America. The Spanish brought peaches to Florida and Mexico, the French to Louisiana, and the English settlers to Virginia and Massachusetts. Commodity acreage maps for peaches and nectarines are provided in Figure 2.

The nectarine, *Prunus persica* var. *nucipersica*, is a hairless or fuzzless peach, derived by mutation of the dominant gene for fuzziness to the recessive gene for smooth skin.

China, Italy, and the United States are the major peach and nectarine producing countries, accounting for 65 percent of total output in 1998 from selected countries. Other important producing countries include Spain, Greece, France, and Argentina. In the United States, production of peaches and nectarines in 1998 was estimated at 1.3 million tons, down from 1.4 million tons in 1997. U.S. production normally consists of about 55 percent freestone peaches, 30 percent clingstone peaches, and 15 percent nectarines. California leads the United States in peach and nectarine production with more than 70 percent of the peach crop and more than 90 percent of the nectarine crop. South Carolina and Georgia follow California's 70 percent share of peach

production, accounting for about 6 and 4 percent of the U.S. total, respectively. South Carolina and Georgia typically market their peaches from May through August while California's season runs June through September.

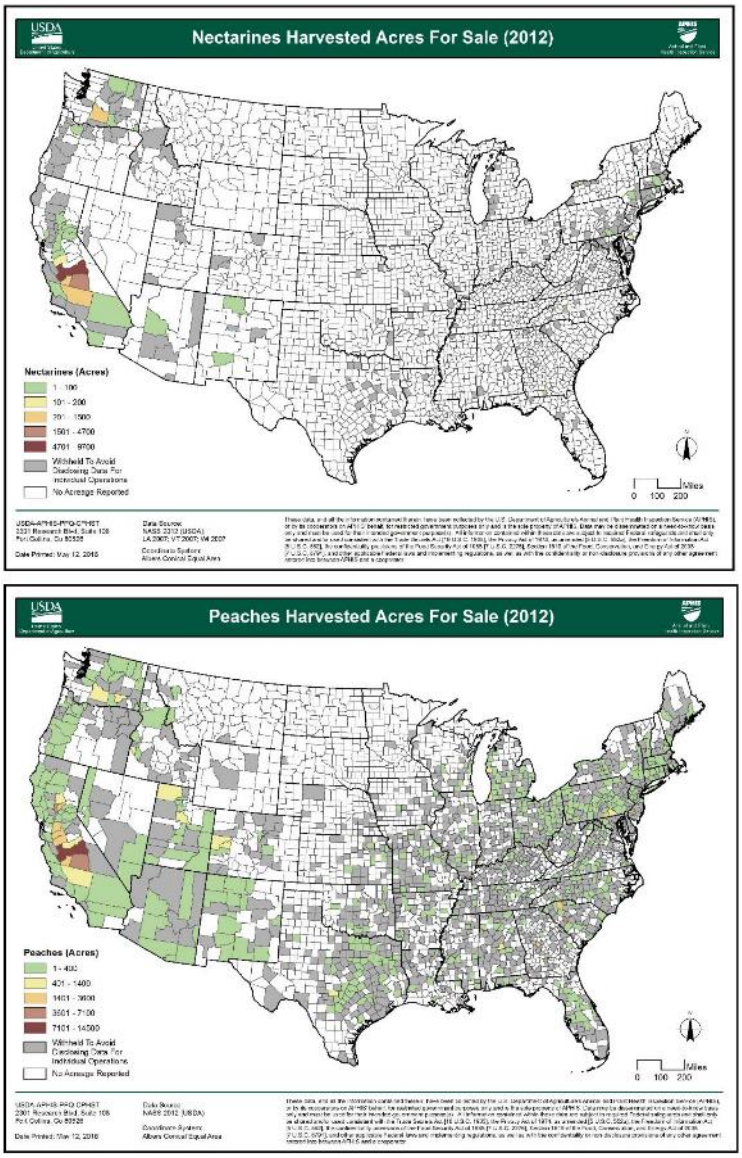

Figure 2. Peach and nectarine commodity acreage maps. Map courtesy of USDA-APHIS-PPQ-CPHST.

Plums and Prunes

Although some plum species are native to North America, commercial cultivars grown in the United States originated in Europe and Japan. Plums are classified into two groups: the European plum, *Prunus domestica*, was introduced from Europe; the Japanese plum, *Prunus salicina*, is native to China and was introduced from Japan. The leading plum-producing regions are China, France, and the United States. Other

important plum and prune production countries are Germany, France, Turkey, Hungary, Italy, Spain, Bulgaria, Chile, South Africa, and Australia. The 17,000 ha grown in California account for over 90% of the production in the United States. A commodity acreage map for plums/prunes is provided in Figure 3.

A prune, by definition, is a dried plum. California's production constitutes 70% of the world's prune supply and 99% of that of the United States.

U.S. plum and prune production in 1998 is estimated at 486,796 tons, down sharply from the 832,071 tons produced in 1997. Adverse spring weather in many producing states, including severe hail in California, reduced overall U.S. output in 1998.

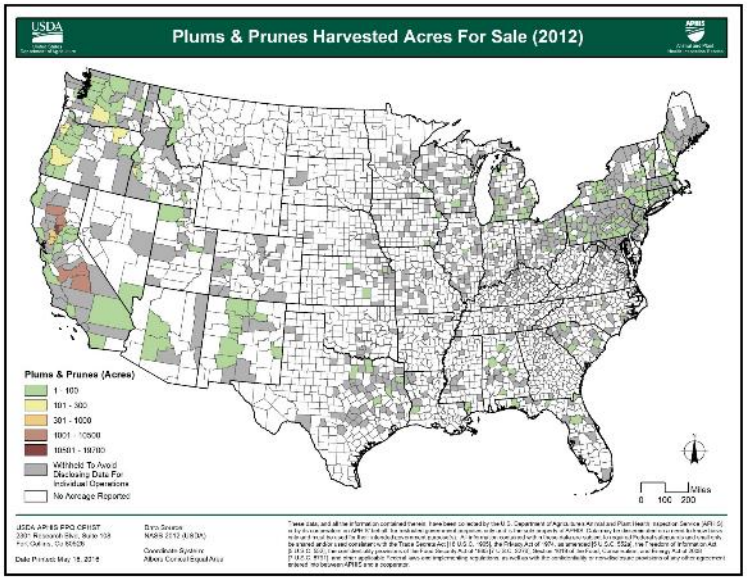

Figure 3. Plum/prune commodity acreage map. Map courtesy of USDA-APHIS-PPQ-CPHST.

Cherries

Cherries were first described in 300 B.C. by Theophrastus and had probably been grown for several centuries earlier for their wood. The sweet cherry, *Prunus avium*, is believed to be native to southwestern Asia in the area around the Caspian and Black seas. Beginning in the 16th century, cherries were widely planted in Europe, particularly in Germany. Europeans first brought sweet cherries to North America. Sour cherry (*Prunus cerasus*), also called tart cherry, is believed to be an interspecific hybrid between sweet cherry and ground cherry (*P. fruticosa*). Sour and ground cherry are tetraploids with a haploid number of 16 chromosomes; while sweet cherry is diploid. There is disagreement about the center of origin for sour cherry. Some believe it arose in the Near East, which includes portions of Asia Minor, Iran, Iraq, and Syria; while others believe Switzerland and the Adriatic Sea are the center of origin.

Cherry production in 1998 is estimated at 585,855 tons for selected countries, about the same as the previous year's output. The United States is the leading cherry producing country, accounting for about 60 percent of selected country output in 1998. U.S. cherry production in 1998 is estimated at 348,405 tons, up slightly from 1997. Sweet cherries comprise about 55 percent of total U.S. cherry output and sour cherries account for the remaining 45 percent. Sweet cherry production in the United States is concentrated in the states of Washington, Oregon, Michigan, and California, in that order. Michigan produces the bulk of the U.S. sour cherry crop. Commodity acreage maps for sweet and sour (tart) cherries are provided in Figure 4.

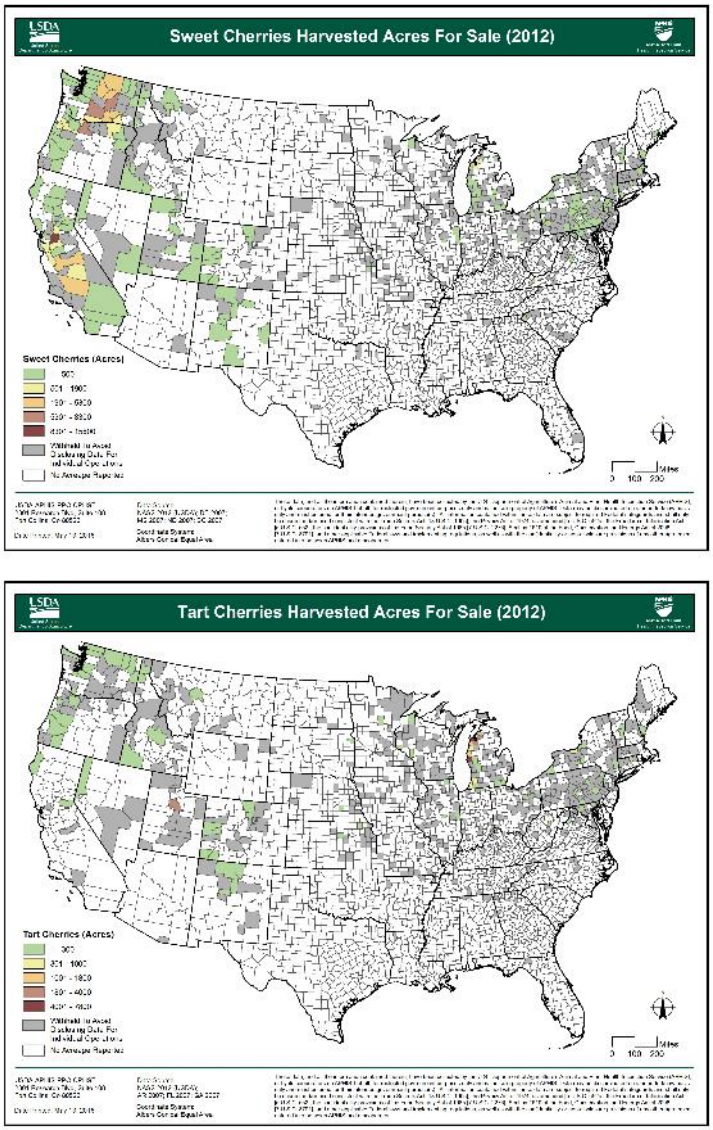

Figure 4. Sweet and sour cherry commodity acreage maps. Map courtesy of USDA-APHIS-PPQ-CPHST.

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Primary Information Services. Stone Fruits. http://www.primaryinfo.com/stone-fruits.htm.

Arthropods

Primary Pests of Stone Fruit (Full Pest Datasheet)

Adoxophyes orana

Figure 1. Eggs and larva of *A. orana.* Photos courtesy of R. Coutin/OPIE and Jae-Cheon Sohn, South Korea. http://www.invasive.org.

Scientific Name
Adoxophyes orana Fischer von Roeslerstamm

Synonyms:
Adoxophyes reticulana, Capua reticulana, Cacoecia reticulana, Capua orana, Tortrix orana, Tortrix reticulana, Capua congruana, Adoxophyes tripsiana, Adoxophyes fasciata, Adoxophyes congruana, and *Acleris reticulana.*

Common Name
Summer fruit tortrix, reticulated tortrix, apple peel tortricid

Type of Pest
Moth

Taxonomic Position
Class: Insecta, **Order:** Lepidoptera, **Family:** Tortricidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2006 through 2012

Pest Description
Eggs: Females lay yellow egg masses of 30 to 50 eggs [3 to 10 mm (0.118 to 0.39 in.) in diameter] on the surfaces of the leaves in early spring (Fig. 1). The eggs are easily observed in the inner parts of the tree top (Dickler, 1991). The greenish larvae hatch and leave behind the transparent shell of the eggs (CABI, 2009).

Larvae: Mature larvae are 18 to 22 mm (0.71 to 0.87 in.) long and greenish in color with light hairs and warts. The head is light brown to brownish yellow (sometimes somewhat spotted due to white blotches on the first, second, and sixth stemmata in fresh specimens) as is the thoracic shield and the anal shield. The anal comb is very fine and long with light colored teeth. The thoracal legs are brown to black. The head is long and wide. Abdominal and anal prolegs are greenish (Sakamaki and Hayakawa, 2004; CABI,

2009). When disturbed, the larvae spin a silken thread and descend to escape. This thread is also a possible method for movement via wind.

Pupae: The pupae of *A. orana* are initially greenish brown to light brown, but become dark brown towards the time of emergence of the adult moth. The length is between 8 and 11 mm (0.31 to 0.43 in.). Sakamaki and Hayakawa (2004) found lengths between 8.5 and 12 mm (0.33 to 0.47 in.). The posterior margin of abdominal segments 2 to 8 of the pupae contains very small bristles. These bristles cannot be distinguished with a regular magnifying glass and are hence visible as a line. The specific fork-shape of wing veins 7 and 8 is already visible in the pupal stage.

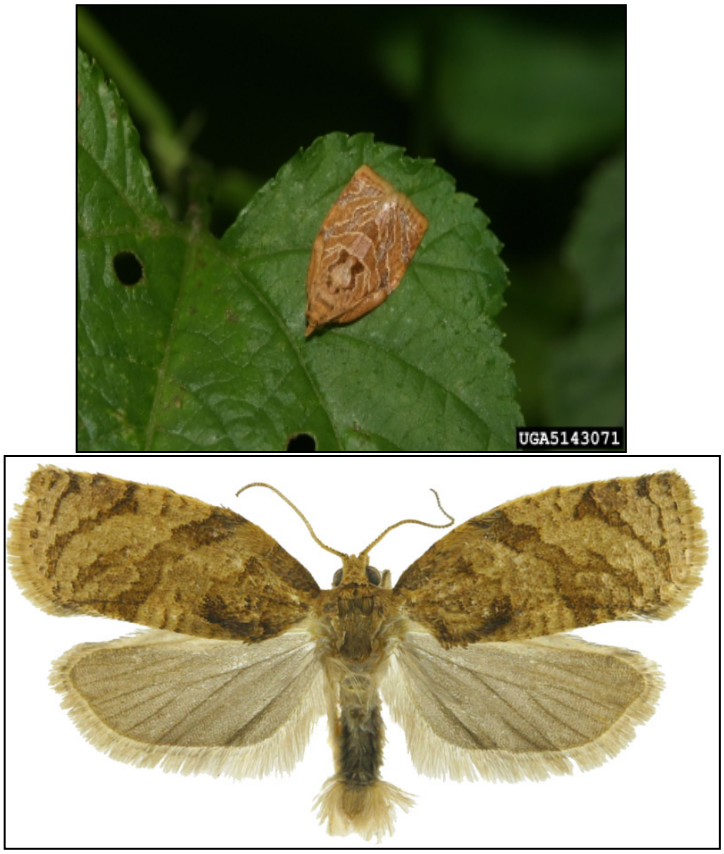

Figure 2: Adult *Adoxophyes orana*. Photos courtesy of Jae-Cheon Sohn, South Korea (top). http://www.invasive.org and Todd Gilligan, Colorado State University (bottom).

Adults: Adults (Fig. 2) range from a dull grayish brown (female) to yellowish brown (male) with a variable dark-brown marking pattern and a 15 to 19 mm (0.59 to 0.75 in.) (male) or 18 to 22 mm (0.71 to 0.87 in.) (female) wingspan (Bradley et al., 1973). Dickler (1991) reports the wing span of the adult moth measuring from 16 to 22 mm (0.63 to 0.87 in.). Adult males are smaller and more brightly colored than adult females.

A very specific characteristic of *A. orana* is the fork-shaped structure of the wing veins 7 and 8. The forewing of the female is rather dull grayish brown, while in the male the coloration is brighter and is a yellowish brown. Sexual dimorphism pronounced; antenna of male shortly ciliate, forewing with broad costal fold from base to about one-third, markings usually conspicuous, contrasting with paler ground color; female usually larger, antenna minutely ciliate, forewing without costal fold, with darker general coloration and less contrasting markings (Bradley et al., 1973).

Biology and Ecology

Two generations occur per year in central Europe in apple orchards. The generations may overlap. A partial third generation is possible if warm temperatures persist in the fall. The generations usually occur from the end of May till the beginning of August and from August till mid-September in Belgium. Cross (1994) describes the life cycle of the

summer fruit tortrix in the United Kingdom. The first generation of larvae hatch from batches of eggs laid on the undersides of leaves in mid-June. The larvae graze on leaves and characteristically feed within a protective silk mesh. After undergoing five larval instars, the larvae pupate. The adult moths fly on warm August evenings and lay their eggs directly on the surface of fruit and leaves. The second generation of larvae hatches in August and feeds mainly on the fruit. They overwinter as second or third instars in silk cocoons in crevices in the bark. They become active again in the warmer spring months and pupate to emerge as moths in May and June (Cross, 1994).

Three to four generations occur in northern Greece in peach orchards. *A. orana* overwinters in bark crevices as a 3rd instar and emerges in the spring to feed on flower buds (Milonas and Savopoulou-Soultani, 2000). Larval activity begins in early spring (March) and is completed by the end of April. *Adoxophyes orana* has a facultative diapause that occurs in response to decreasing photoperiods at the end of October (Milonas and Savopoulou-Soultani, 2004). Each year adult activity occurs in early May or after 418 degree days have accumulated from February 1st, when diapause development is completed (Milonas and Savopoulou-Soultani, 2006). Two additional adult flights occur during July and from late August until mid-September.

Flight periods last for approximately four weeks (Barel, 1973). Moths fly at temperatures above 12°C (54°F) and are typical night fliers with maximum activity around midnight (Minks and Noordink, 1971). Males precede females in flight by a few days and may disperse up to 400 meters (1,300 feet). Female dispersal is limited (Barel, 1973). Mating occurs at night or in the early morning hours about a day after emergence (De Jong et al., 1971; Whittle, 1985; Van der Kraan and van der Straten, 1988; He et al., 1996). *Adoxophyes orana* typically mates once (50 to 60% of the time) but can mate two or more times (Minks and Noordink, 1971).

The number of eggs per female is quite variable and temperature dependent (Charmillot et al., 1984). Charmillot et al. (1984) found that females produced on average 271.1 eggs in a 25°C (77°F) temperature cabinet and 328.6 eggs in an insectary. Janssen (1958) observed similar numbers; while De Jong and Van Dieren (1974) recorded 160 to 329 eggs per female. Van der Kraan and ver der Straten (1998) conducted a study where egg production ranged from 20 to 560, with an average of 292. Although the temperature development threshold is 10.6°C (51°F) for eggs (De Jong et. al., 1965), most eggs are laid at temperatures above 13°C (55°F) or higher (Ankersmit et al., 1976). Egg mortality is common at temperature at or below 13 to 14°C (55-57°F) (Ankersmit et al., 1976). On average the lifespan of the females ranged from 3 to 22 days (14.6 days on average) (van der Kraan and van der Straten, 1988).

According to Carmillot and Megevand (1983), laboratory and field test have shown that the threshold temperature for eggs is 10°C (50°F), while those for summer and overwintering larvae are 7 to 8°C (45 to 46 °F) and 10°C, respectively. The threshold for development of pupae is slightly over 10°C. The duration of embryonic and pupal phases is 90 degree days above 10°C. The summer larval phases last an average of 430 degree days above 7°C (Carmillot and Megevand, 1983).

Milonas and Savopoulou-Soultani (2000) examined the effect of temperature (14, 17, 21, 25, and 30°C; 57, 63, 70, 77, and 86°F) on development, survival, fecundity, and longevity on *A. orana.* The optimal temperature for development was 25°C (77°F). Total developmental time ranged from 50.2 days at 14°C (57°F) to 20.7 days at 25°C (77°F). On average, 333.3 degree days were required for total larval development. The mean longevity for females was 13.5 days at 14°C and 7.6 days at 30°C (86°F); whereas for males mean longevity ranged from 14.9 days at 21°C (70°F) and 7.9 at 30°C. Females laid the fewest eggs (70.6) at 14°C. Extreme temperatures had a negative effect on all life table parameters.

Symptoms and Signs

External feeding will be visible on leaves and fresh growth of twigs. Feeding will deform leaves and create areas with necrosis (dead tissue) (Fig. 3). Damaged flesh heals leaving 'corky' scars. Leaves may appear wilted, yellow, shredded, or dead. Leaves are likely to be rolled or folded and held together with silk webbing. Feeding on new growth of twigs will leave lesions. If the insect is feeding on flowers, external feeding damage and silk webbing will be evident. In all areas where the insect has fed, frass (excrement) should also be visible.

Summer generation larvae feed extensively and severely damage fruit (Fig. 3). Feeding on fruits or pods causes scabs or pitting, and frass may be present. On fruit crops, larvae prefer to feed sheltered under a leaf bound to fruit and silk.

Figure 3. *A. orana* damage to fruit and leaves. Photos courtesy of Magnus Gammelgaard Nielson. http://www.plante-doktor.dk/frugtskraelvikler.htm.

Pest Importance

The summer fruit tortrix moth has become a serious pest in peach and cherry orchards in northern Greece in the last 20 years (Milonas and Savopoulou-Soultani, 2006). In central and northern Europe, it is considered to be an important pest of apple orchards. *Adoxophyes orana* is not host specific as it reportedly feeds and develops on more than 50 plant species in multiple families. Potential host plants, both cultivated and wild, are

common in the United States and often occur at high densities (Davis et al., 2005).

The larvae feed on both foliage and fruit. Damage to foliage is insignificant, but damage to fruit can be serious. Secondary fungal infection is common where insect damage has occurred. On apples, it can be expected that damage from the first generation will result in large deep holes, whereas the second generation produces small holes of less than 5 mm (0.20 in.) in diameter (CABI, 2009).

Known Hosts

Although the host range includes more than 50 plant species in multiple families, *A. orana* feeds preferentially on apples, pears, stone fruit, and other Rosaceous hosts.

Major hosts include: *Cydonia oblonga* (quince), *Malus* spp. (apple), *Prunus armeniaca* (apricot), *Prunus avium* (sweet cherry), *Prunus domestica* (plum), *Prunus persica* (peach), *Pyrus* spp. (pear), and *Rubus* spp. (raspberry).

Minor hosts include: *Acer* spp. (maple), *Alnus* spp. (alder), *Arachis hypogaea* (peanut)**,** *Beta* spp. (beet), *Betula* spp. (birch), *Carpinus* spp. (hornbeam), *Castanea crenata* (Japanese chestnut), *Castanopsis fissa* (evergreen chinkapin), *Chenopodium album* (lambsquarters), *Citrus* spp. (citrus), *Convolvulus arvensis* (field bindweed), *Corylus* spp. (hazelnut), *Cotoneaster dielsianus* (cotoneaster), *Crataegus* spp. (hawthorne), *Dimocarpus longan* (longan)**,** *Diospyros* spp. (persimmon), *Eriobotrya* spp. (loquat), *Fagus sylvatica* (beech), *Ficus* spp. (fig), *Forsythia suspensa* (forsythia), *Fragaria* spp. (strawberry), *Fraxinus* spp. (ash), *Glycine max* (soybean), *Gossypium* spp. (cotton), *Humulus* spp. (hops), *Laburnum* spp. (laburnum), *Ligustrum spp.* (privet), *Litchi chinensis* (litchi), *Lithocarpus glaber* (Japanese oak), *Lonicera* spp. (honeysuckle), *Malus* spp. (crabapple), *Medicago* spp. (alfalfa), *Menyanthes trifoliata* (buckbean), *Morus* spp. (mulberry), *Olea* spp. (olive), *Parrotia* spp. (ironwood), *Physalis peruviana* (Peruvian groundcherry), *Pistacia* spp. (pistachio), *Populus* spp. (poplar), *Potentilla* spp. (cinquefoil), *Prunus cerasus* (sour cherry), *Prunus padus* (European bird cherry), *Prunus salicina* (Japanese plum), *Prunus triloba* (almond tree), *Punica* spp. (pomegranate), *Quercus* spp. (oak), *Rhododendron catawbiense* (Catawba rosebay), *Ribes* spp. (currant), *Rosa* spp. (rose), *Rubus* spp. (blackberry), *Rumex* spp. (dock), *Salix* spp. (willow), *Solanum* spp. (nightshade), *Sorindeia juglandifolia* (damson), *Symphoricarpos* spp. (snowberry), *Syringa* spp. (lilac), *Tilia* spp. (basswood), *Ulmus* spp. (elm), *Urtica* spp. (nettle), *Vaccinium* spp. (blueberry), *Vicia faba* (faba bean)*,* and *Vitis vinifera* (grapevine) (Davis et al., 2005; CABI, 2009).

Known vectors (or associated organisms)

Adoxophyes orana is not known to be a vector and is not known to be vectored by another organism. Although the damage to the fruit is usually superficial, fungal pathogens can infect the damaged fruit through these wounds and significantly reduce fruit quality.

Known Distribution

This pest is present in: **Asia:** Armenia, Azerbaijan, China, Republic of Georgia, India, Japan, and Korea. **Europe:** Albania, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Norway, Poland, Romania, Russia, Serbia and Montenegro, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, and the United Kingdom (Davis et al., 2005; CABI, 2009).

Potential Distribution within the United States

Surveys should be focused where the greatest risk for pest establishment occurs. A recent risk analysis by USDA-APHIS-PPQ-CPHST indicates that most states in the United States have a low to moderate risk rating for *A. orana* establishment based on host availability and climate within the continental United States. Areas of the southeastern United States, California, Illinois, Indiana, Maryland, Missouri, Oklahoma, and Texas have the highest risk of *A. orana* establishment.

Survey

CAPS-Approved Method*: The CAPS-approved method is a trap and lure combination.

Any of the following Trap Product Names in the IPHIS Survey Supply Ordering System may be used for this target:

1) Paper Delta Trap, 2 sticky sides, Brown
2) Paper Delta Trap, 2 sticky sides, Green
3) Paper Delta Trap, 2 sticky sides, Orange

The Lure Product Name is "*Adoxophyes orana* Lure." The lure is effective for 84 days (12 weeks).

Trap Spacing: When trapping for more than one species of moth, separate traps for different moth species by at least 20 meters (65 feet).

Method Notes: Trap should be used with ends open. Trap color is up to the State and does not affect trap efficacy.

Lure Placement: Placing lures for two or more target species in a trap should never be done unless otherwise noted here.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Trapping: Several monitoring techniques have been developed and applied to *A. orana*. The most effective approach involves sex pheromone-baited traps. The sex pheromone is a blend of (*Z*)-9-tetradecenyl acetate and (*Z*)-11-tetradecenyl acetate (Tamaki et al., 1971; Meijer et al., 1972). These two compounds are most attractive to males in a 9:1

blend of (*Z*)-9:(*Z*)-11 isomers; *E*-isomers of either compound had a strong inhibitory effect (Minks and Voerman, 1973; Davis et al., 2005). The 9:1 pheromone blend is available commercially as Adoxomone (Murphy Pherocon™ Summer Fruit Tortrix Moth Attractant) for use with Pherocon 1C traps. Den Otter and Klijnstra (1980) showed that male *A. orana* not only respond to the mixture of these components but also to its separate components. The authors speculated that that the sex pheromone released by females contains more than two components, because copulation behavior is not seen with the synthetic pheromone but readily occurs in the presence of virgin females. Guerin et al. (1986) identified twelve products related to the sex pheromone main components (*Z*)-9- and (*Z*)-11-tetradecenyl acetate (*Z*9-14:Ac and *Z*11-14:AC, respectively). These were the geometric isomers and the alcohols of the main components, (*Z*)-9-dodecnyl acetate, (*Z*)-11-hexadecenyl acetate, and saturated acetates of 12 to 22 carbons. The addition of either of the two alcohols to a blend of the two acetates augmented trap catch in the field (Guerin et al., 1986; Yang et al., 2009). Temperatures below 12°C (54°F) lower flight activity and also trap catches (Minks and Noordink, 1971).

Milonas and Savopoulou-Soultani (2006) installed three Pherocon pheromone traps during the vegetative period in peach, apple, cherry, and pear orchards in Greece. The traps were installed 2 m (6.5 ft) above the ground in a shaded part of the canopy at a distance of 60 m (197 feet) from each other to prevent interaction among traps. Traps were baited with sticky inserts loaded with synthetic sex pheromone. Traps were checked at weekly intervals, and pheromone lures and trap bottoms were changed every 4 weeks. Polyethylene caps treated with 100 µg of the pheromone blend remained effective for over 7 weeks (Minks and Voerman, 1973).

Visual survey: Visual sampling and beat sampling may also be used to inspect plants for eggs and larvae. Eggs may be observed on the stems and leaves; late instars may be found in the crown on new shoot growth; and pupal cocoons may be found in leaves, on stems, or in mummified pods/seeds. Both methods are time consuming. Visual sampling or beat sampling are not commonly recommended (Davis et al., 2005).

In the spring (end of April), *A. orana* can be surveyed by sampling flower cluster just before the bloom of apple. This sampling is labor intensive, because it is often necessary to examine a large number of clusters and because identification of the larvae is often difficult (Charmillot and Brunner, 1989). Sampling for the summer generation larvae is easier because the damage is easily visible when third instar larvae and older begin to roll the leaves. In Switzerland, the first damage on shoots is apparent from the end of June to the end of July about 200 degree days after the start of the first flight (Charmillot and Brunner, 1989). Fruit can also be sampled in August and September by looking for small holes in fruit from the feeding of the third instar larvae. A visual examination of about 2,000 fruit at harvest can be used to show damage from the first and second generation of *A. orana* (Charmillot and Brunner, 1989).

Milonas and Savopoulou-Soultani (2006) cut two shoots at weekly intervals randomly from four sides (N, S, E, W) of trees during the vegetative period from April until

October. Thirty-two trees were randomly chosen and eight 30-cm (11.81 in.) long shoots were cut from each tree from the beginning of plant development. Shoots were examined in the laboratory using a stereoscope. Ripe fruits were also sampled randomly from the orchard and examined in the laboratory for superficial damage caused by *A. orana* larvae.

Not recommended: As an alternative to pheromone traps, Robinson light traps with 125W mercury vapor bulbs, 125 W black light bulbs, or 100W flood lights can be used. While sex pheromone traps attract males of a targeted species, light traps non-selectively draw in many flying insects.

Key Diagnostics/Identification

CAPS-Approved Method*: Confirmation requires a morphological identification. *Adoxophyes orana* may occur in mixed populations with other morphologically similar species, including other *Adoxophyes* species. Final identification is by dissection of male genitalic structures.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Because of their very secretive nature, leafrollers are difficult to detect. Distinguishing between males and females of adult *Adoxophyes* is difficult in general (Balachowsky, 1966). According to Yasuda (1998), the extensive color and pattern variation of the forewing and morphological resemblance among *Adoxophyes* species have created difficulties in the identification of the species.

A new identification tool, *Tort AI – Tortricids of Agricultural Importance*, is available at http://idtools.org/id/leps/tortai/ from CPHST's Identification Technology Program. This tool contains larval and adult keys, fact sheets, an image gallery, molecular search capacity, and more. *Adoxophyes orana* is included in this tool.

Easily Confused Pests

Adoxophyes orana very closely resembles two U.S. species, *Adoxophyes furcatana* and *A. negundana*, but there are slight differences in male genitalia. Any identification should be confirmed by an appropriately trained entomologist.

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Bactrocera zonata

Scientific Name
Bactrocera zonata (Saunders)

Synonyms:
Chaetodacus zonatus, Dacus (Strumeta) zonatus, Dacus ferrugineus var. *mangiferae* Cotes*, Dacus persicae, Dacus zonatus, Dasyneura zonatus, Rivellia persicae* Bigot*,* and *Strumeta zonata*

Common Names
Peach fruit fly, guava fruit fly (also refers to *Bactrocera correcta)*, Oriental fruit fly

Type of Pest
Fruit fly

Taxonomic Position
Class: Insecta, **Order:** Diptera, **Family:** Tephritidae

Reason for Inclusion in Manual
Requested by the CAPS community – not being surveyed for regularly with fruit fly funding.

Pest Description (From Fletcher (1987) and Rahman et al. (1993) unless otherwise noted)

Eggs: Elongated, elliptical, whitish and 1.0 to 1.2 mm (0.04 to 0.05 in.) long, somewhat rounded at the posterior end, slightly pointed anteriorly with a distinct micropyle.

Larvae: In general, the larvae have typical maggot characteristics with an involuted (rolled inward, spirally) head, three thoracic segments and eight abdominal segments. The three most important features of *Bactrocera* spp. (and other dacine species) are: (1) the mouth hooks, (2) the anterior spiracles, and (3) the posterior spiracles. All three change during larval development. Specific to *B. zonata*, this species has three larval instars and its spiracular openings of the respiratory system are restricted to a pair each on the prothorax and the posterior of the abdomen.

First Instar: The first instar larvae are elongated, white, and 1.7 to 2.3 mm (0.07 to 0.09 in.) long. The anterior end of the larva is narrow and pointed; while the posterior end is broad and somewhat rounded. The head region has minute yellowish-brown mouth hooks. The cephalopharyngeal (head region) skeleton is readily visible through the semi-transparent body of newly hatched larvae. The anterior portion of pharangeal sclerites is visible as small brownish dots.

Second Instar: The second instar larvae are elongated, white, and 4.0 to 6.5 mm (0.16 to 0.26 in.) long. This larval instar is characterized by the presence of anterior spiracles, each having 13 to 15 apical lobes. Each lobe has an opening. In general, most spiracles are further developed than in the first instar with a greater scleritization apparent here and in the cephalopharyngeal region.

Third Instar: The third instar larvae are yellowish-white and 9 to 10 mm (0.35 to 0.39 in.) long. The head segment now has two small jointed antennae and a single jointed maxillary palpus. The anterior respiratory spiracles with 13 to 15 lobes are still present. The anal lobes are well developed and the posterior spiracles now have fully developed transverse bars.

Pupae: The pupae are barrel-shaped, yellowish to yellowish-brown, 11-segmented, and 4.2 to 5.8 mm (0.16 to 0.23 in.) long x 2.3 to 2.5 mm (0.90 to 0.98 in.) wide. The anterior end has two anterior spiracles; while the posterior end is rounded. The posterior spiracles occupy the same position as in the larvae (CABI, 2009).

Figure 1. *Bactrocera zonata* adult. Photo courtesy of Natasha Wright (Florida Dept. Ag.). www.bugwood.org.

Adults: Adults (Fig. 1) are about 6 mm (0.24 in.) long and reddish brown with yellowish thoracic markings. They have transparent wings with a small brown spot on the tip. The face has a spot in each antennal furrow. The scutum has lateral yellow or orange vittae. The scutellum is entirely pale colored, except sometimes they possess a narrow black line across the base. The costal margin of wing without a colored band along whole length of cell r1; cell sc is usually yellow, and apex of vein R4 + 5 often with a brown spot; crossveins R-M and Dm-Cu not covered by any markings (EPPO, 2005).

Head: The head has reduced chaetotaxy, lacking ocellar and post-ocellar setae. The first flagellomere is at least three times as long as broad. It also should have a dark spot in each antennal groove, rather than a broken transverse line as in *B. correcta* (Fig. 4).

Thorax: The thorax has reduced chaetotaxy, lacking dorsocentral and katepisternal setae. Post-pronotal lobes without any setae (sometimes with some small setulae or hairs); scutum with anterior supra-alar setae and prescutellar acrostichal setae; scutellum not bilobed, with only two marginal setae (the apical pair).

Wing: Wing vein sc is abruptly bent forward at nearly 90°, weakened beyond this bend and ending at subcostal break; vein R1 with dorsal setulae; cell bcu (=cup) extension

very long, equal or longer than length of vein A1 + CuA2; 4 to 6 mm (0.16 to 0.24 in.) long. Raised narrow subbasal section of cell br lacking microtrichiae.

Abdomen: All tergites separate (view from side to see overlapping sclerites); tergite five with a pair of slightly depressed areas (ceromata); male with a row of setae (the pectin) on each side of tergite three.

Biology and Ecology

Bactrocera zonata is a pest mainly on peach (and other stone fruit), mango, guava, and papaya, but it can be found on many other wild and cultivated fruits, including *Citrus* spp. It readily disperses as far as 15 miles (79200 ft.; 24140 m) both early and late in the growing season, even when hosts are abundant (Fletcher, 1989). It can outcompete other fruit fly pests (due to its shorter larval development time, larger egg size, longer lifespan and/or reproductive output) including *Ceratitus capitata, C. rosa* and *B. dorsalis* (Duyck et al., 2006a, 2007; CABI, 2009). *Bactrocera zonata* lives and reproduces over a longer time frame, tolerates a lower relative humidity, and its pupae can survive submersed in soil much longer than these competitor species (Marwat et al., 1992; Saafan et al., 2005; Duyck et al., 2006b).

This fruit fly is active throughout the year at temperatures at or above 10°C (50°F), and development of all life stages stops at temperatures below 10°C. The optimum temperatures for activity (feeding, egg laying, etc.) and development is 25 to 29°C (77 to 85°F). Flies are not active at temperatures over 35°C (95°F) or at night. Adults have been seen as early as the end of March and as late as mid-November (Qureshi et al., 1993; Hussain, 1995; Duyck et al., 2004). When reared in the laboratory, the average adult lifespan is 56 days for males, 62 for females (Hussain, 1995), with three to nine overlapping generations per year. Adults need to feed on nectar, plant sap, and decaying fruit to mature sexually and for general survival. Feeding normally takes place in the morning, but is also done during full daylight and this activity is probably temperature dependent. Night is spent under foliage or any other protective crevices of hosts and non-hosts.

From pupae, adults emerge in the morning and then need a 10- to 16-day maturation process before they become reproductively mature. After this, they begin mating, which normally takes place at dusk. Oviposition begins when immature fruit appear, and seems to be greatest (and most successful) when adult females are 35 days old and immature fruits are about 38.1 mm (1.5 in.) in diameter (Hussain, 1995; CABI, 2009). Females puncture the skin of the fruit (yellow-colored hosts seem to be preferred over green and colorless hosts (Hussain, 1995), create a small cavity and lay three to nine eggs. An average female lays 137 eggs in her lifetime (CABI, 2009). Oviposition can occur at any time during the day, but most often happens in the late afternoon and early evening (Rahman et al., 1993). Larvae hatch as early as one day after the eggs are laid and feed within the fruit for four to 21 days. After maturing, they drop to the ground and burrow into the soil to pupate. The pupal stage can last from four weeks in the summer to six weeks in the winter. This species overwinters as pupae in areas where true diapause is necessary (CDFA, 2011).

Two temperatures, 16°C (60°F) and 25°C (77°F), seem to be important thresholds for *B. zonata*. While development can continue at temperatures lower than 16°C, adult emergence dropped to 1%, and egg (10%), larval (46%), and pupal (13%) survival are much lower than the corresponding results seen at 20°C (68°F), 25°C, and 30°C (86°F). The highest emergence and survival rates were recorded at 25°C; adult emergence (70%), egg (71%), larval (98%) and pupal (100%) survival were higher at 25°C than at any other temperature (Hussain, 1995; Duyck et al., 2004). Egg development was the fastest at 25°C and 30°C, and larval, pupal and ovarian developments were the fastest at 30°C.

In Egypt, studies performed on different hosts (citrus, stone fruit, mango, guava, etc.) resulted in an average of 493 thermal units (day degrees) required to complete one generation (Khalil et al., 2010).

In Europe, *B. zonata* is most common in private gardens where different host species fruit throughout the year and are available; commercially it is most common in orchards of peach, fig, and guava. Adults can survive winters with temperatures at or close to freezing in North Sinai, Egypt.

Control/eradication measures for *B. zonata* currently use a combination of two techniques; the Male Annihilation Technique (MAT) and the Bait Application Technique (BAT). In the United States, the MAT uses the Jackson trap, lure and insecticide combination, but other types of traps/lures are used as well. The BAT involves placing small amounts (40 to 100 mL (1.35 to 3.38 oz.) of a protein bait mixed with an

Figure 2. Damage from egg laying of *B. zonata* on peaches. Left: Notice the clear resin-like fluid coming from the lower right of the peach. Right: Oviposition scars on peach. Photos courtesy of Ian White and Rui Cardoso.

insecticide on various parts of an infested area (*e.g.,* tree trunks, leaves, inside traps). Adult flies consume this protein/insecticide mix and die. In Pakistan, a BAT formulation of 3 mL (0.1 oz.) malathion 57% *a.i.* EC and 30 mL commercial protein hydrolysate in 1 L (0.26 gal.; 33.8 oz.) of water significantly reduced infestation rates in guava, melon, and jujube at a rate of 7.5 L/hectare/week (Stonehouse et al., 2002). Care should be taken to avoid placing this bait where it would come into contact with children, livestock and/or pets. In addition to malathion, spinosad, diazinon and naled (Dibrom) are also recommended for BAT and MAT.

Symptoms/Signs

The first sign of *B. zonata* attack is a small puncture wound (scar) where a female has oviposited within an immature fruit (Fig. 2). Oftentimes, a droplet of fluid exudes from the fruit and dries as a clear or brown resinous spot. As larvae develop inside the fruits, the second and third instar larvae feed deeper in the fruit. They seem to be the main reason for the complete deterioration of the individual fruit and cause premature fruit drop.

Pest Importance

Economic impacts are twofold; the direct loss of the crop from larval damage and the loss of export markets or costly quarantine/importation/eradication treatments required by importing countries once *B. zonata* has been detected. Across Asia, crop losses due to *B. zonata* can be significant (25 to 100% loss in some areas and fruits). Egypt has seen infestation rates of 20% in apricot (Saafan et al., 2005). In Pakistan, infestation rates are so high that certain crops (*e.g.,* papaya) are not grown or harvested.

On November 10, 2010, an adult *B. zonata* male was captured in a trap in a *Psidium guajava* (guava) tree in Miami-Dade County, Florida. This is the first report of *B. zonata* in Florida. Trapping has been intensified in the 81-square-mile area surrounding the detection site.

Known Hosts

Major hosts include: *Carica papaya* (papaya), *Mangifera indica* (mango), *Prunus persica* (peach), and *Psidium* spp. (guava).

Minor hosts include: *Abelmoschus esculentus* (okra), *Aegle marmelos* (bael fruit), *Annona* spp. (custard/sugar apple), *Afzelia xylocarpa* (makamong), *Careya arborea* (kumbhi), *Citrofortunella* spp. (calamondin), *Citrullus lanatus* (watermelon), *Citrus* spp. (citrus), *Coccinia grandis* (ivy gourd), *Cucurbita* spp. (gourd), *Cucumis* spp. (cantaloupe, cucumber), *Cydonia oblonga* (quince), *Elaeocarpus* spp. (Japanese blueberry), *Eriobotrya japonica* (loquat), *Eugenia* spp. (kelat), *Feijoa sellowiana* (feijoa), *Ficus* spp. (fig), *Fortunella japonica* (round/Marumi kumquat), *Grewia asiatica* (phalsa), *Lagenaria siceraria* (bottle gourd), *Luffa* spp. (loofah), *Madhuca indica* (butter tree), *Malpighia glabra* (acerola), *Malus* spp. (apple), *Manilkara* spp. (Brazilian redwood/sapodilla), *Momordica charantia* (balsam apple/bitter gourd), *Ochrosia elliptica* (elliptic yellowwood), *Olea europaea* (olive), *Persea americana* (avocado), *Phoenix spp.* (date palm), *Prunus armeniaca* (apricot), *Punica* spp. (pomegranate), *Putranjiva roxburghii*

(putranjiva), *Pyrus* spp. (pear), *Sapota* spp. (sapota), *Solanum lycopersicon* (tomato), *Solanum melongena* (eggplant), *Syzygium jambos* (rose apple), *Terminalia catappa* (beach/tropical almond/myrobalan), and *Ziziphus mauritiana* (jujube) (Kapoor, 1993; Allwood et al., 1999; CABI, 2009; CDFA, 2011).

According to CDFA (2011), *B. zonata* attacks early fruit such as jujube, loquat, and peach, then moves to cucurbits, mango, citrus, guava, pomegranate, and sapodilla for the rest of the year.

Known vectors (or associated organisms)

Bactrocera zonata is not a known vector and does not have any associated organisms.

Known Distribution

B. zonata is native to south and southeast Asia.

Asia: Bangladesh, Bhutan, India, Iran, Laos, Moluccas Islands, Myanmar, Nepal, Oman, Pakistan, Saudi Arabia, Sri Lanka, Thailand, United Arab Emirates, Vietnam and Yemen. **Africa:** Egypt, Libya, Mauritius, and Réunion.

Bactrocera zonata has been previously eradicated in Israel and the United States (California (2006) and Florida (2011)). *B. zonata* has also been erroneously listed as present in Indonesia and Nepal.

Potential Distribution within the United States

Bactrocera zonata and *B. dorsalis* occupy the same ecological niche. In other words, places where *B. dorsalis* can invade are nearly identical to the area where *B. zonata* can exist as well.

Bactrocera zonata is a tropical species, and any areas with an upper temperature threshold of 35°C (95°F) are potential establishment sites (Duyck et al., 2004). Also, in field observations, adults of *B. zonata* were seen to be most abundant when the temperatures were 26 to 30°C (79 to 86°C) and the relative humidity was 70 to 75% (Saafan et al., 2005 and references therein). Areas with these climatic characteristics can be considered acceptable habitats for *B. zonata*.

B. zonata is only considered a threat to the following states and territories: Alabama, Arizona, California, Florida, Georgia, Guam, Hawaii, Louisiana, the Mariana Islands, Mississippi, New Mexico, Puerto Rico, South Carolina, Texas, and the U.S. Virgin Islands.

Survey

CAPS-Approved Method*: The CAPS approved method is a trap and lure combination. The trap type is a Jackson trap. The lure is methyl eugenol with an insecticide. The Jackson trap is a delta-shaped, disposable trap with an adhesive-coated, removable insert that is used to capture male *B. zonata*, as well as other *Bactrocera* spp.

IPHIS Survey Supply Ordering System Product Names:
1) Fruit Fly, Methyl Eugenol Lure
2) Jackson Trap Body

Before planning a *B. zonata* survey, it is IMPERATIVE that you work with your CAPS Regional Program Manager and your regional PPQ Fruit Fly Program Manager for guidance in planning your survey (see contact information below).

Joe Beckwith-- PPQ Eastern Region Fruit Fly Program Manager
919-855-7345
joseph.s.beckwith@aphis.usda.gov

Shaharra J Usnick-- PPQ Western Region Fruit Fly Program Manager
970-494-7571
Shaharra.j.usnick@aphis.usda.gov

Lure Placement: Placing lures for two or more target species in a trap should never be done unless otherwise noted here.

Lure Notes: The lures used for *B. zonata* surveys require specific equipment and certification of personnel, due to the necessary addition of an insecticide. For this reason, please consult with your regional Plant Protection and Quarantine (PPQ) Fruit Fly Program Manager before ordering lures. The insecticide component is not available through the PPQ Survey Supply Ordering System. Please work with your Fruit Fly Program Manager for assistance in procuring the insecticide.

Due to the climatic requirements of *B. zonata*, surveys are only relevant for the following states: Alabama, Arizona, California, Florida, Georgia, Louisiana, Mississippi, New Mexico, South Carolina, and Texas.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Adults are the main survey life stage; reproductively mature males of *B. zonata* (and other *Bactrocera* species) are easily attracted to methyl eugenol (4-allyl-1,2-dimethoxybenzene or 3,3,dimethoxy(1)2 propenyl benzene), and this chemical attractant is currently used in the United States to monitor for *Bactrocera* spp. A cotton wick or polymeric plug of methyl eugenol and an insecticide (malathion, naled or Dichlorovos) in a 3:1 ratio is suspended inside a delta-shaped Jackson trap with sticky material. When male fruit flies enter the trap and feed on the lure, they die and become stuck. Thickeners (*e.g.,* Min-U-Gel 400) have also been used to increase the efficacy and longevity of methyl eugenol (Kapoor, 1993). In Pakistani guava/ mango orchards, pheromone traps performed best at capturing males when they were colored yellow or green (Hussain et al., 1995). Male attraction toward methyl eugenol increases as they sexually mature and wanes when they have previously been exposed to a significant amount of this chemical; methyl eugenol is found naturally in some of their

foods (Shelly, 1994). Steiner traps can be used as a substitute for Jackson traps in male survey programs.

Key Diagnostics/Identification

CAPS-Approved Method*: This species can be identified by examining its form and structure (morphological characteristics). It can be distinguished from many of the *Bactrocera* species by wing patterns, spots on its head, lines on its thorax, and abdominal markings.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Larvae of *B. zonata* can be differentiated from *B. cucurbitae* and *B. dorsalis* by differences in the anterior spiracles, cephalopharyngeal skeleton and the posterior spiracular plate (Khan and Khan, 1987). Rearing larvae through to adults, however, is the most accurate way to differentiate between different larvae found in stone fruit. Important references for larvae include White and Elson-Harris (1992) and Pest fruit flies of the world – larvae (http://delta-intkey.com/ffl/www/_wintro.htm).

An excellent online resource by White (2000) providing distinguishing characteristics between *B. zonata* adults and all other known species in the tribe Dacini (includes the genera *Bactrocera, Dacus,* and *Monacrostichus*) can be found at: http://www.iaea.org/programmes/.nafa/d4/public/zonata.html. More extensive keys to *Bactrocera* species can be found in White (2006).

This website walks the reader through marks on the wing (costal band reduced to a small apical spot, lack of microtrichia and anal streak, Fig. 3), head (two spots, one in each antennal furrow, Fig. 4), thorax (thin lateral yellow stripe running down each side, Fig. 5) and abdomen (usually two dark marks on tergite 3, Fig. 5) to identify *B. zonata*.

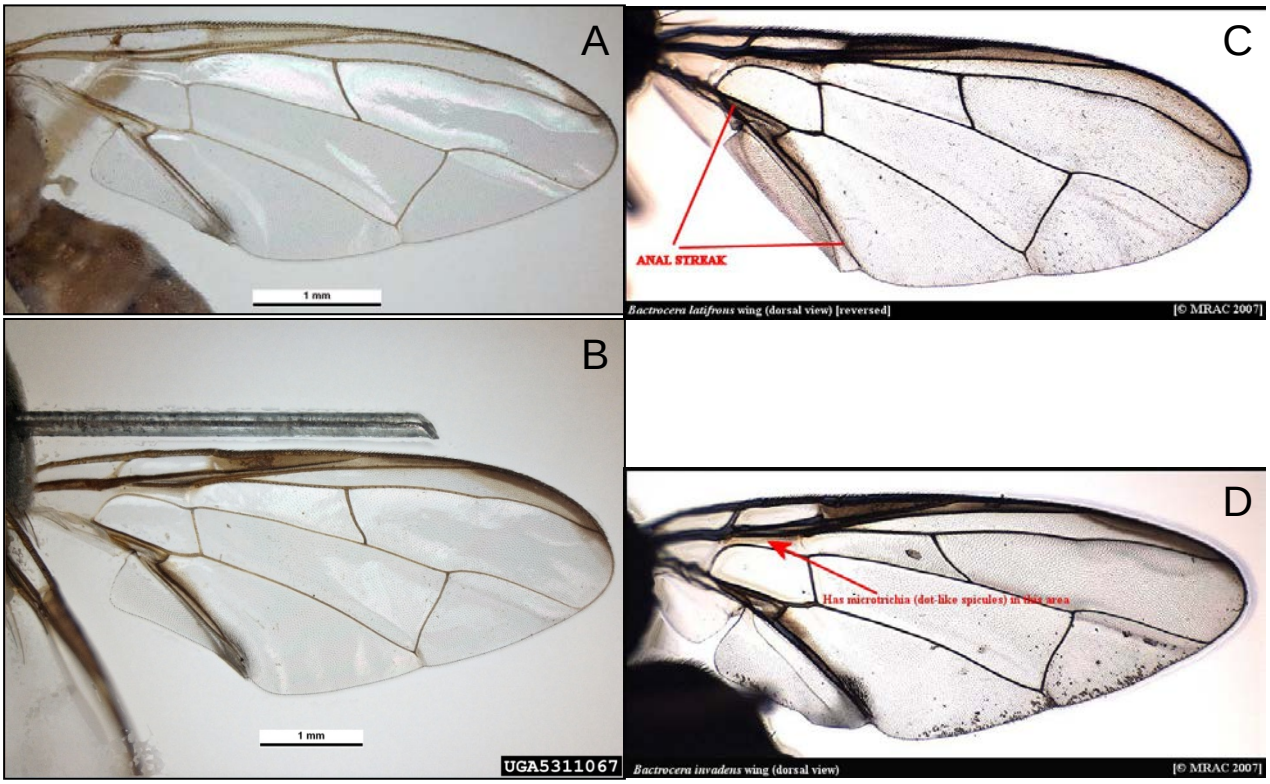

Figure 3. A. Wing of *B. zonata*: note the small brown apical spot on the tip of the wing (Ken Walker, http://www.padil.gov.au/). **B.** Wing of *B. dorsalis*: the costal band at the top of the wing is now not just a spot, but a full band (Pest and Diseases Image Library, http://www.bugwood.org/). **C and D.** Wing of *B. latifrons* with an apparent anal streak and microtrichia, something the wing of *B. zonata* lacks (Royal Museum for Central Africa).

Finally, molecular Identification has been studied to identify *B. zonata*, and a protocol using mitochondrial cytochrome oxidase I has been developed (Asokan et al., 2007).

Easily Confused Pests

Bactrocera zonata can be confused with other *Bactrocera* spp.

Figure 4. Left: Head of *B. dorsalis* with brown spot in the antennal furrow (red arrow) (Ken Walker, http://www.padil.gov.au/). Right: Head of *B. correcta* with a broken transverse line in the antennal grove instead of a spot (red arrow) (Pest and Diseases Image Library, http://www.bugwood.org/).

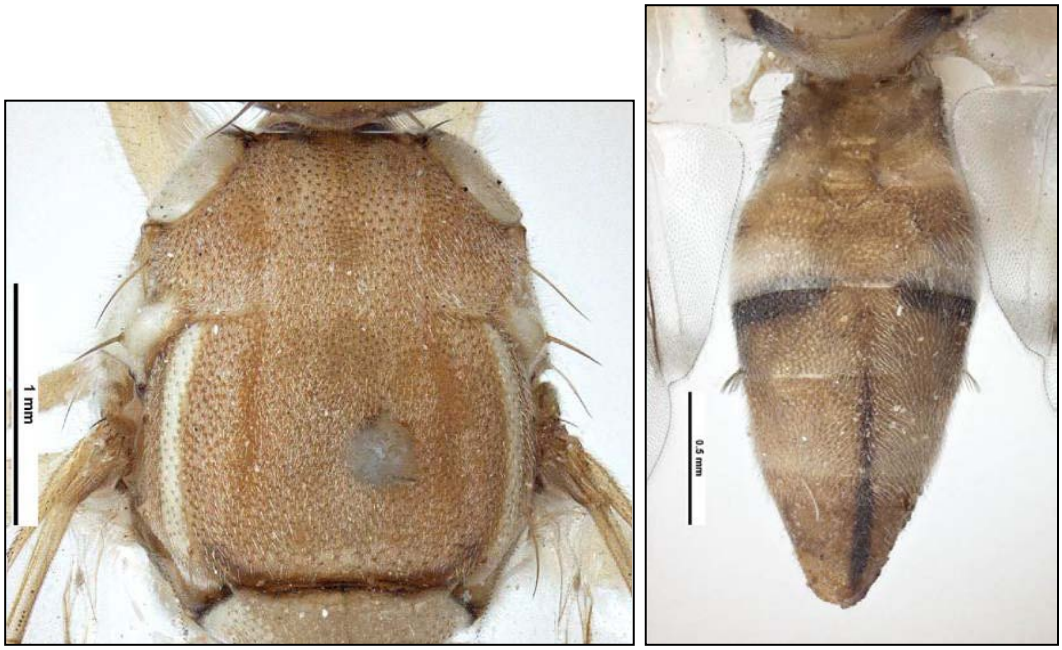

Figure 5. Left: *B. zonata* thorax with the two lateral yellow stripes. Right: *B. zonata* abdomen, with the stripes on tergite 3. Photos courtesy of Ken Walker, http://www.padil.gov.au/.

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Enarmonia formosana

Scientific Name
Enarmonia formosana (Scopoli)

Synonyms:
Tortrix ornatana, Tortrix scriptana, Pyralis woeberana, Tortrix woeberiana

Common Names
Cherry bark tortrix

Type of Pest
Moth

Figure 1. Cherry bark tortrix adult female. Photo courtesy of Todd Gilligan, Colorado State University.

Taxonomic Position
Class: Insecta, **Order**: Lepidoptera, **Family**: Tortricidae

Reason for Inclusion in Manual
Requested by the CAPS community – Limited distribution in the United States

Pest Description
Eggs: Eggs are about 0.7 x 0.6 mm (0.28 x 0.24 in.) in size and creamy white when laid. Eggs later become reddish (Alford, 2007).

Larvae: Larvae are up to 11 mm (0.43 in.) long in size. The body is translucent grayish white, with brownish-gray pinacula; head light brown; prothoracic and anal plates light grayish brown (Alford, 2007).

Pupae: Pupae are 7 to 9 mm (0.28 to 0.35 in.) long; light brown; cremaster broad and blunt (Alford, 2007).

Adults: Adults (Fig. 1) have a 15 to 18 mm (0.59 to 0.71 in.) wingspan. The forewings are more or less brown to black, with a purplish sheen, and with irregular, yellowish-orange markings and silvery-white costal strigulae; hindwings dark brown (Alford, 2007).

Male genitalia: Tegumen long; uncus simple, rather weakly sclerotized, haired dorso-laterally; socii broad, lateral, hairy; tuba analis membranous. Valva small, with elongated basal opening, neck indistinct; cucullus small, bristled, expanding terminally, provided with short modified setae before apex. Aedeagus simple; cornuti missing (Meijerman and Ulenberg, 2000).

Biology and Ecology

In Washington, *E. formosana* has one generation per year and is active from April to September (Beers et al., 1993). *E. formosana* is active during the day, mainly in the early morning (Beers et al., 1993). Gravid females can attack pruning scars and winter damaged areas when choosing oviposition sites, preferring previously infested or damaged host plants (Beers et al., 1993). Females lay eggs either singly or in batches of two to three (Alford, 2007) depositing from 14 to 84 eggs (McNair, 1997).

Hatching occurs in two to three weeks. Larvae then attack bark to feed beneath the surface (Alford, 2007), mining between the bark and cambium of host plants creating frass tubes (Breedveld and Tanigoshi, 2007). Larvae show a preference for scar tissue (Orr, 1991) and make irregular tunnels while feeding. Larvae pass through five instars (Beers et al., 1993). Successive generations can be found using the same tunnels as previous generations (Meijerman and Ulenberg, 2000). Overwintering occurs in the larval stage. Larvae will continue to feed when temperatures reach above freezing during the winter months (Tanigoshi and Murray, 2002). Usually by the following spring or summer, pupation will occur in a silken cocoon (Alford, 2007) close to the surface of the bark (Meijerman and Ulenberg, 2000).

Symptoms/Signs

This pest prefers old wounds in mature trees (Orr, 1991) although it has been found to infest younger plants in the United States (Murray et al., 1998). Larvae can attack areas on damaged trunks, limbs, or pruning scars on branches (Beers et al., 1993). Larvae of successive generations can infest the same areas of the host trees, enlarging the tunnels (Alford, 2007).

Figure 2. Damage caused by cherry bark tortrix. Photo courtesy of Todd Murray, Washington State University, Skamania County Extension.

Feeding tunnels caused by larvae can crack the bark (Fig. 2), and a gum-like resin can be found oozing from the cracks (Beers et al., 1993; Carter, 1984). Infested trees often have brown, silk-lined tubes of frass protruding from the bark (Alford, 2007).

Pupae can be found protruding from the bark even after adults have emerged (Carter, 1984; Alford, 2007). Although damage is usually limited to bark tissue, *E. formosana* may also damage the cambium layer of the tree (Beers et al., 1993). Damage to bark can cause swellings and cankers and can eventually lead to the death of limbs or trees when infestations are high (Beers et al., 1993). The most damage comes from “boring

into the trunks and causing large masses of gum to exude from the bark (Massee, 1946).

Infestations of cherry trees usually occur near the base of the trunk while infestations in apple trees commonly occur on the undersides of the main branches near the trunk and on the trunk near these areas (Alford, 2007). Tunnels may become two to three inches broad and up to three inches deep (Massee, 1946).

Figure 3. Larvae of *E. formosana* along with damage. Photo courtesy of Todd Murray, Washington State University, Skamania County Extension.

Pest Importance

E. formosana is considered a pest of minor importance in Eurasia (Jenner *et al*., 2004; Orr, 1991). This species can be a pest of almond, apricot, apple, cherry, pear, peach, and plum (Alford, 2007).

Massee (1946) states that this species 'occasionally occurs in fruit plantations, but is by no means an important pest,' while Carter (1984) states that this species serves as a pest of a wide range of fruit trees in continental Europe.

E. formosana can cause death of limbs or trees when infestations are high (Beers *et al*., 1993). This pest can cause indirect damage to host plants by making the weakened and wounded trees more attractive to secondary pests (Orr, 1991). Damage by *E. formosana* can also increase the susceptibility of the tree to unfavorable weather conditions (Orr, 1991).

Known Hosts

Known hosts*: *Crataegus douglasii* (black hawthorn)**, *C. monogyna* (single seed hawthorn)**, *Cydonia* (quince), *Malus oregonensis* (native crab apple), *M. pumila* (apple), *M. sylvestris* (European crap apple), *Malus* spp. (ornamental crab apple), *Photinia* spp. (photinia), *Prunus armeniaca* (apricot), *P. avium* (sweet cherry), *P. cerasifera* (flowering plum), *P. cerasus* (sour cherry), *P. domestica* (fruiting plum), *P. dulcis* (almond), *P. emarginata* (bitter/wild cherry), *P. laurocerasus* (cherry laurel), *P. lusitanica* (Portuguese laurel)**, *P. persica* (peach), *P. serrulata* (oriental flowering cherry), *P. subhirtella* (weeping cherry), *P. triloba* (flowering plum), *P. yadoensis* (Japanese flowering cherry), *Pyracantha* spp. (firethorn), *Pyrus communis* (pear), and *Sorbus* spp. (mountain ash) (Brewer et al., 1985; Dang and Parker, 1990; van der Geest and Evenhuis, 1991; Beers et al., 1993; Murray et al., 1998; Tanigoshi and Starý, 2003).

* The literature does not distinguish between major and minor hosts.
** New host records for *E. formosana* in Washington State, from Murray et al. (1998).

Known vectors (or associated organisms)

This pest is not currently known to vector any pathogens or other associated organisms. Damage caused by this pest, however, may make host plants more attractive to secondary pests (Orr, 1991).

Known Distribution

This pest is widely distributed throughout Europe, extending into Siberia (Alford, 2007; Carter, 1984). It is also found in northern Africa (Meijerman and Ulenberg, 2000) and has recently been introduced into both Canada and the United States (Beers et al., 1993; Tanigoshi and Starý, 2003).

Africa: Northern Africa. **Europe:** Albania, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Channel Islands, Corsica, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Hungary, Ireland, Italy, Kazakhstan, Latvia, Lithuania, Luxembourg, Macedonia, Malta, the Netherlands, Norway, Poland, Romania, Russia, Sardinia, Sicily, Slovakia, Slovenia, Spain, Sweden, and Switzerland. **North America:** Canada and the United States (Fauna Europaea, 2010; Jenner et al., 2004; Meijerman and Ulenberg, 2000).

Potential Distribution within the United States

This pest was discovered in the state of Washington in 1991 (Beers et al., 1993). Since its introduction into the United States, it has spread to Oregon (Tanigoshi and Starý, 2003) and is now considered a pest of ornamental cherries along the Pacific Coast (Jenner et al., 2004). A recent risk analysis by USDA-APHIS-PPQ-CPHST indicates that most states in the United States have a low to moderate risk rating for *A. orana* establishment based on host availability and climate within the continental United States. Areas of the Connecticut, Maine, Massachusetts, Michigan, New Hampshire, New York, Ohio, Vermont, West Virginia, and Wisconsin have the highest risk of *E. formosana* establishment.

Spread may be inhibited by warmer, drier areas as eggs apparently cannot develop when temperatures are above 32.2°C (90°F) (Westcott, 1993).

Survey

CAPS-Approved Method*: Trap with lure. The approved trap type is a paper delta trap. The lure information is provided below:

Any of the following Trap Product Names in the IPHIS Survey Supply Ordering System may be used for this target:

1) Paper Delta Trap, 2 sticky sides, Brown
2) Paper Delta Trap, 2 sticky sides, Green
3) Paper Delta Trap, 2 sticky sides, Orange

The Lure Product Name is "*Enarmonia formosana* Lure." The lure is effective for 28 days (4 weeks).

Trap Spacing: When trapping for more than one species of moth, separate traps for different moth species by at least 20 meters (65 feet).

Method Notes: Trap should be used with ends open. Trap color is up to the State and does not affect trap efficacy.

Lure Placement: Placing lures for two or more target species in a trap should never be done unless otherwise noted here.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:
Visual: In areas where the pest is found in the United States, populations can be monitored by examining injured bark for signs of *E. formosana*, including gummosis (a gummy substance caused by sap exuding from wounds) and frass (Beers et al., 1993). Frass can be seen at tunnel openings in late winter and early spring, however, this method is time consuming and damage can be similar to other native moths in the Sesiidae family (Beers et al., 1993).

Trapping: Two lure compounds: (*E*)-9-dodecenyl acetate (0.5mg/lure) and (*Z*)-9-dodecenyl acetate (0.5mg/lure) have been used to trap *E. formosana*. In Breedveld and Tanigoshi (2007) surveyors used diamond sticky traps with pheromone lures to monitor flight activity in Seattle, Washington. Traps were placed in host trees at a height of 1 to 2.5 m (3.2 to 8.2 ft.) and were checked weekly (Breedveld and Tanigoshi, 2007). Lures were replaced every four weeks (Breedveld and Tanigoshi, 2007). Results showed that flight began in early to mid-May, extending to mid-September (Breedveld and Tanigoshi, 2007). The pheromone for *Eucosma sonomana* has also been found to attract *E. formosana* males (Brewer et al., 1985).

Not Recommended: In the past, light traps were used to catch this species (Winfield, 1964). However, this method is not specific for *E. formosana*.

Key Diagnostics/Identification

CAPS-Approved Method*: The CAPS approved method is a morphological identification. Adults can be recognized by the black forewings with distinctive yellow, orange, and silvery markings. Male and female genitalia are also diagnostic. Descriptions of adult morphology can be found in Dang and Parker (1990).

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

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Two sites with reference images are:
http://mothphotographersgroup.msstate.edu/species.php?hodges=3399.3 and
http://bugguide.net/node/view/171315.

A new identification tool, *Tort AI – Tortricids of Agricultural Importance*, is available at http://idtools.org/id/leps/tortai/ from CPHST's Identification Technology Program. This tool contains larval and adult keys, fact sheets, an image gallery, molecular search capacity, and more. *Enarmonia formosana* is included in this tool.

Easily Confused Pests

E. formosana can be recognized by the color and pattern of the forewing as well as the distinctive genitalia (Dang and Parker, 1990). In North America, *Eucosmomorpha nearctica* is similar in size and has a similar forewing pattern, but the two can be easily separated by male or female genitalia. Gilligan et al. (2008) provide illustrations of *E. nearctica*.

Commonly Encountered Non-targets

The following are non-target insects that have been previously found in *E. formosana* traps in the state of Washington (E. LaGasa, personal communication):

Family	Genus	Species
Gelechiidae	*Recurvaria*	*nanella*
Geometridae	*Chloroclystis*	*rectangulata*
Lymantriidae	*Orgyia*	*antiqua badia*
Oecophoridae	*Batia*	*lunaris*
Tortricidae	*Acleris*	*variegana*
Tortricidae	*Archips*	*fuscocupreanus*
Tortricidae	*Archips*	*rosanus*
Tortricidae	*Argyrotaenia*	*franciscana*
Tortricidae	*Cacoecimorpha*	*pronubana*
Tortricidae	*Choristoneura*	*carnana californica*
Tortricidae	*Choristoneura*	*rosaceana*
Tortricidae	*Croesia*	*holmiana*
Tortricidae	*Cydia*	*cupressana*
Tortricidae	*Cydia*	*sedatana*
Tortricidae	*Ditula*	*angustiorana*
Tortricidae	*Episimus*	*argutanus*
Tortricidae	*Eulia*	*ministrana*
Tortricidae	*Grapholita*	*lunatana*
Tortricidae	*Grapholita*	*prunivora*
Tortricidae	*Notocelia*	*rosaecolana*
Tortricidae	*Pandemis*	*limitata*

Tortricidae	*Pandemis*	*pyrusana*
Tortricidae	*Retinia*	*picicolana*
Tortricidae	*Rhopobota*	*naevana*
Tortricidae	*Rhyacionia*	*buoliana*
Tortricidae	*Spilonota*	*ocellana*
Yponomeutidae	*Swammerdamia*	*pellicaria*

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Epiphyas postvittana

Scientific Name
Epiphyas postvittana Walker

Synonyms:
Austrotortrix postvittana, *Dichelia foedana*, *D. retractana*, *D. reversana*, *D. vicariana*, *D. vicaureana*, *Pandemis consociana*, *Teras basialbana*, *T. scitulana*, *T. secretana*, *Tortrix dissipata*, *T. oenopa*, *T. phaeosticha*, *T. pyrrhula*, and *T. stipularis.*

Common Names
Light brown apple moth (LBAM), apple leafroller, Australian leafroller

Type of Pest
Moth

Taxonomic Position
Class: Insecta **Order**: Lepidoptera **Family**: Tortricidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List -2003 through 2008; PPQ Program Pest

Pest Description
Eggs: *Epiphyas postvittana* egg masses (Fig. 1) are flat, broadly oval, translucent, and appear pale yellow to white in color (Brown et al., 2010). The chorion is reticulated, which separates eggs of this species from some, but not all, tortricids in North America (Peterson, 1965). There are approximately 35 eggs in a mass, overlapping like “roof tiles or shingles”. Females lay on average 100 to 300 eggs beginning at two to three days of age.

Figure 1: Eggs of LBAM on a leaf surface. Photo courtesy of T. M. Gilligan & M. E. Epstein, *LBAM ID* (CSU, CDFA, and USDA/APHIS/ PPQ/CPHST).

Larvae: First instar larvae (Fig. 2) are approximately 1.5 to 1.6 mm (~0.06 in.) long with a dark head and light-colored body, and final instar larvae range from 10 to 20 mm (0.39 to 0.79 in.) in length. The body of a mature larva is yellowish green with paler subdorsal, subventral, and ventral lines. The first larval instar has a dark-brown head; all other instars have a light-fawn head and prothoracic plate. The succeeding instars have a darker body than fully grown instars. The head, prothoracic shield, legs, and anal plate are pale brown, the genal dash is present or absent, and the prothoracic shield is only slightly darker than the rest of the integument. All instars are dark dorsally, and the pinacula of later instars are paler than the surrounding integument (Gilligan and Epstein, 2009; Brown et al., 2010).

Pupae: Pupae (Fig. 3) are green after pupation, but become brown within one day. Male pupae average 2.5 by 7.6 mm (0.098 to 0.30 in.); females average 2.9 by 9.8 mm (0.11 to 0.39 in.). The pupal stage is completed within the “nests” made up of rolled up leaves (Gilligan and Epstein, 2009; Brown et al., 2010)

Adult: *Epiphyas postvittana* is sexually dimorphic. Forewings of both sexes are light brown to pale yellow with brown to dark brown markings. Male light brown apple moth adults are usually smaller than females. Male forewing length ranges from 5.3 to 11.1 mm (0.21 to 0.44 in.), compared with 5.4 to 12.5 mm (0.21 to 0.49 in.) in females (Gilligan and Epstein, 2009). Males are more variable than females, although in most males the basal half of the forewing is lightly marked, the median fascia is well defined, and there is a dark mark on the costa distal to the median fascia. In California, males tend to be of three phenotypes (Fig. 4); the form with solid dark markings on the distal half of the forewing is the most uncommon. All males have a forewing costal fold. The female (Fig. 4) forewing color is more uniform, with a poorly defined median fascia and overall mottled or speckled appearance. Most females have a dark mark on the dorsum of each forewing and two dark spots on the posterior of the thorax. All females lack a forewing costal fold. The hindwing in both males and females is mottled with dark scales, although this pattern is usually more evident in females. Adults in other areas of the world can have incredibly variable forewing patterns (Gilligan and Epstein, 2009).

Figure 2: Early- (top), mid- (middle), and late- (bottom) instar larvae of LBAM. Photos courtesy of T. M. Gilligan & M. E. Epstein, *LBAM ID* (CSU, CDFA, and USDA/APHIS/PPQ/ CPHST).

Male genitalia (Fig. 5) are distinctive and a dissection can be used to verify male specimen identity. Males possess a combination of the following characters: spatulate (spoon-shaped) uncus; reduced socii; short valva with a broad sacculus; membranous lobe on the apex of the valva; and an aedeagus with 2 to 4 deciduous cornuti (Gilligan and Epstein, 2009).

Female genitalia (Fig. 5) are typical of many Archipini and females may be difficult to verify based on dissection alone. LBAM females possess a combination of the following characters: simple sterigma; long, straight ductus bursae which is 2/3 or more the length of the abdomen; and corpus bursae with a single, hook-shaped signum (Gilligan and Epstein, 2009).

Biology and Ecology

Figure 3: LBAM Pupa. Photo courtesy of T. M. Gilligan & M. E. Epstein, *LBAM ID* (CSU, CDFA, and USDA/APHIS / PPQ/ CPHST).

Epiphyas postvittana has two to four annual generations over much of its range; the exact number of generations varies by latitude. There is considerable overlap between generations, with development driven by temperature and larval host plant (Danthanarayana, 1975; Geier and Briese, 1980; Thomas, 1989). The highest rate of population increase was on *Plantago lanceolata* (ribwort plantain), followed by *Rumex crispus* (curly dock), apples (*Malus domestica* cv. Granny Smith) and *Trifolium repens* (white clover) (Danthanarayana et al., 1995). In northern New Zealand, four overlapping generations occur, with adults flying during September to October, December to January, February to March, and April to May. In southern Australia, three overlapping generations are completed, with adults flying during December to January, April to May, and September to October. Populations in California appear to complete at least four overlapping generations, with adults present almost continuously from March to November. The upper and lower temperature thresholds for *E. postvittana* development have been determined to be 7.5°C (46°F) and 31°C (88°F) in laboratory studies, with an ideal development temperature of 20°C (68°F) (Danthanarayana, 1975; Brown et al., 2010).

Females lay eggs in a mass that contains from 4 to 96 eggs (mean 35) overlapping individual eggs (Wearing et al., 1991). Females deposit eggs at night (USDA, 1984). Eggs are laid on the upper surface of host plants with smooth leaf surfaces; females will refrain from depositing eggs on hairy leaves (Danthanarayana, 1975; Geier and Briese, 1981; Foster and Howard, 1998). Females often select the depression along the upper side midrib of leaves (Powell and Common, 1985). Egg development time varies with temperature and eggs will hatch in approximately 8 to 9 days at 20°C (68°F) (Gilligan and Epstein, 2009).

Larvae pass through five to six instars during their development. Larvae do not overwinter, although development during colder months is slower. The rate of development varies with temperature and host plant utilized; larval development takes approximately 25 days at a temperature of 20°C (68°F). Early instar larvae feed on the underside of leaves within a silk chamber. Later instar larvae may fold single leaves, create a nest of several leaves webbed together, or web leaves to fruit and feed on the surface.

Pupation occurs within the larval nest. Complete pupal development takes approximately 10 days at a temperature of 20°C (Danthanarayana, 1975). Adult moths emerge after one to several weeks of pupation. Female moths emerge from protective pupal nests and mate soon after emergence (Geier and Briese, 1981). Danthanarayana (1975) suggests the preoviposition period is 2 to 7 days. Females copulate for slightly less than one hr. (Foster et al., 1995). Oviposition does not begin until females are two- to three-days old (Geier and Briese, 1981). The oviposition period lasts from one to 21 days (Danthanarayana, 1975). Adult longevity is influenced by host plant and temperature. In the laboratory, female longevity can vary between 10 days (Geier and Briese, 1981) and 32.7 days (Danthanarayana, 1975); males can live up to approximately 33 days (Danthanarayana, 1975). Under field conditions in Australia, the life span of adult *E. postvittana* is 2 to 3 weeks (Magarey et al., 1994).

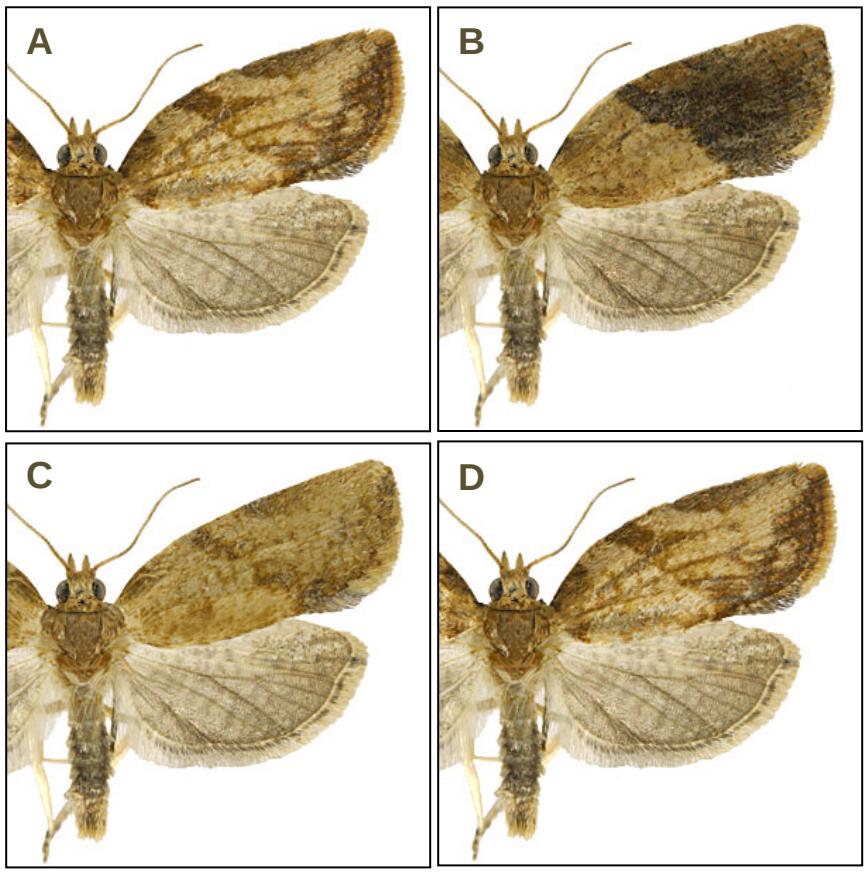

Figure 4. A. Typically marked male. B. Male with dark wings. C. Make with light wings. D. Typically marked female. Photos courtesy of T. M. Gilligan & M. E. Epstein, *LBAM ID* (CSU, CDFA, and USDA/APHIS / PPQ/ CPHST).

Moths are quiescent during the day and may be found on foliage of hosts (Geier and Briese, 1981). Long distance dispersal is typically achieved by adults (Geier and Briese, 1980; Suckling et al., 1994), although larval dispersal occurs over a short range. Flight occurs at dusk in calm conditions (Geier and Briese, 1981; USDA, 1984; Magarey et al., 1994). Adults are unlikely to disperse from areas with abundant, high-quality hosts (Geier and Briese, 1981). Males will disperse farther than females. In a mark-release-recapture study, 80% of recaptured males and 99% of recaptured females occurred within 100 m (328 ft.) of the release point (Suckling et al., 1994). Females do not appear to rely on plant volatiles to locate a host, but tactile cues are important (Foster and Howard, 1998). Humidity influences the dispersal ability of the pest (Danthanarayana et al., 1995).

Although they are sheltered in silk, first instar larvae are more exposed to weather and insecticide treatments than are second and third instar larvae (Madge and Stirrat, 1991; Lo et al., 2000). After approximately three weeks, larvae leave the silken tunnels for a new leaf (USDA, 1984). Second and later instars have the ability to create their own protective feeding shelter by rolling a leaf or webbing multiple leaves together (Danthanarayana, 1975; Lo et al., 2000); behaviors characteristic of the Tortricidae.

Larvae move vigorously when disturbed, but are always connected to the leaf by a silken thread to avoid being removed from the leaf (Nuttal, 1983; USDA, 1984). When larvae happen to fall to the ground, they feed on ground-cover hosts or can survive without feeding for several months (Evans, 1937; Thomas, 1975; USDA, 1984).

E. postvittana is more abundant during the second generation than during other generations (MacLellan, 1973; Madge and Stirrat, 1991). Thus, the second generation causes the most economic damage (Evans, 1937; Thomas, 1975; Madge and Stirrat, 1991; Lo et al., 2000) as larvae move from foliage to fruit (MacLellan, 1973; Magarey et al., 1994).

Figure 5. Top: Male genitalia. Bottom: Female genitalia. Photos courtesy of T. M. Gilligan & M. E. Epstein, *LBAM ID* (CSU, CDFA, and USDA/APHIS / PPQ/ CPHST).

Symptoms/Signs

The insect will feed on foliage (Fig. 6), flowers, and fruit. In spring, the pest feeds on new buds while later generations feed on ripened fruits (Buchanan et al., 1991). After the first molt, they construct typical leaf rolls (nests) by webbing together leaves, a bud and one or more leaves, leaves to a fruit, or by folding and webbing individual mature leaves. During the fruiting season, they also make nests among clusters of fruits, damaging the surface and sometimes tunneling into the fruits (Danthanarayana, 1975).

Fruit surface feeding is common within larval nest sites and is typically caused by later instars (Lo et al., 2000). Clusters of fruit are particularly susceptible. *E. postvittana* has been shown to introduce *Botrytis cinerea*, a fungal that causes gray mold, spores into

wounds via contaminated larvae, with up to 13% of berry damage (by weight) as a result (Bailey, 1997). On a fruit, the calyx offers protection from parasitoids and is probably the best feeding location for young larvae (Lo et al., 2000). Larvae entering the fruit through the calyx may cause internal damage. Wet conditions may allow the entry of rot organisms. Feeding on the foliage by larvae causes ragging and curling of the foliage.

Figure 6: LBAM typical damage to host plant foliage. Photo courtesy of T. M. Gilligan & M. E. Epstein, *LBAM ID* (CSU, CDFA, and USDA/APHIS / PPQ/ CPHST).

Damage to apples is in the form of either pinpricks, which are flask-shaped holes about 3 mm (0.12 in.) deep into the fruit, or entries, which are holes extending deeper than 3 mm into the fruit that leaves some frass and webbing at the surface. On apples, skin damage or blemishes have an irregular cork-like appearance. Larvae may excavate small round pits and produce scars similar to the 'stings' of the larvae of *Cydia pomonella*, the codling moth. The first generation (in spring) causes the most damage to apples; while the second generation damages fruit harvested later in the season (Terauds, 1977). Peaches are damaged by feeding that occurs on the shoots and fruit.

Pest Importance

The larva of *E. postvittana* is a serious pest of fruit and ornamentals in Australia and New Zealand. As a pest of pome fruits, particularly apples, it probably ranks second to *Cydia pomonella*, the codling moth. During a severe outbreak, damage by *E. postvittana* to fruit may be as much as 75%. In Tasmania, this species is the most injurious pest of apples. In years of abundance, populations of the light brown apple moth may cause as much as 25% loss of the apple crop. This pest damages fruit in storage; a few larvae may ruin a whole case of fruit. The markings on the fruit render it unfit for export (USDA, 1984).

E. postvittana is a highly polyphagous pest that attacks a wide number of fruits, ornamentals, and other plants. According to Geier and Briese (1981), "Economic damage results from feeding by caterpillars, which may destroy, stunt or deform young seedlings, spoil the appearance of ornamental plants, and/or injure deciduous fruit-tree crops, citrus, and grapes." Losses in Australia were estimated to be AU$21million (~US22.15 million) per year, but there has been no similar estimation in other countries.

The larvae can be very damaging to grape, apple, and peach. In grape, 70,000 larvae/ha were documented to cause a loss of 4.7 tons of chardonnay fruit in 1992 with

an estimated cost of $2000/ha (Bailey et al., 1995). A single larva can destroy about 30 grams of mature grapes.

Mature larvae are the most difficult stage to control. *E. postvittana* is also difficult to control with sprays because of its leaf-rolling ability, and because there is evidence of resistance due to overuse of sprays (Geier and Briese, 1981).

Known Hosts

Epiphyas postvittana is a polyphagous pest and can damage nursery stock, stone fruit (peaches and apricots), pome fruits (apples and pears), grapes, and citrus. This pest can feed on >500 plant species in 121 families and 363 genera giving it the potential to become extremely destructive (Brown et al., 2010; Suckling and Brockerhoff, 2010). Larvae prefer herbaceous plants over woody ones (Brown et al., 2010).

Major Hosts: *Acacia* spp. (wattles*), Actinidia* spp. (kiwi/Chinese gooseberry), *Chrysanthemum* spp. (chrysanthemum), *Citrus* spp. (citrus), *Cotoneaster* spp., *Crataegus* spp. (hawthorns), *Diospyros* spp. (malabar ebony), *Eucalyptus* spp. (eucalyptus), *Humulus lupulus* (hops), *Jasminum* spp. (jasmine), *Ligustrum vulgare* (privet), *Litchi chinensis* (lychee), *Malus* spp. (apple), *Medicago sativa* (alfalfa), *Persea americana* (avocado), *Pinus* spp. (pines), *Pinus radiata* (radiata pine), *Populus* spp. (poplars), *Prunus armeniaca* (apricot), *Prunus persica* (peach), *Pyrus* spp. (pears), *Ribes* spp. (currants), *Rosa* spp. (roses), *Rubus* spp. (blackberry, raspberry), *Solanum* spp. (potato/tomato), *Trifolium* spp. (clovers), *Vaccinium* spp. (blueberries), *Vicia faba* (broad bean), and *Vitis vinifera* (grapevine) (CABI, 2009).

Other Documented Hosts: *Acca* sellowiana (horn of plenty), *Adiantum* spp. (maidenhead fern), *Alnus glutinosa* (black alder), *Amaranthus* spp. (amaranth), *Aquilegia* spp. (columbine), *Arbutus* spp. (madrone), *Arctotheca calendula* (capeweed), *Artemisia* spp. (sagebrush), *Astartea* spp. (astartea), *Aster* spp. (aster), *Baccharis* spp. (baccharis), *Billardiera* spp. (billadriera), *Boronia* spp. (baronia), *Brassica* spp. (mustards), *Breynia* spp. (breynia), *Bursaria* spp. (bursaria), *Buddleja* spp. (butterfly bush), *Calendula* spp. (marigold), *Callistemon* spp. (bottlebrush), *Camellia japonica* (camellia), *Campsis* spp. (trumpet-vine), *Cassia* spp. (senna), *Ceanothus* spp. (red-root/lilac), *Centhranthus* spp. (fox-brush), *Chenopodium album* (lambsquarters/fat-hen), *Choisya* spp. (choisya), *Clematis* spp. (virgin's-bower), *Clerodendron* spp. (glory-bower), *Correa* spp. (correa), *Crocosmia* spp. (montbretia), *Cupressus* spp. (cypress), *Cydonia* spp. (quince), *Cytisus scoparius* (Scotch broom), *Dahlia* spp. (dahlia), *Datura* spp. (thorn-apple), *Daucus* spp. (carrot), *Dodonaea* spp. (dodonea), *Eriobotrya* spp. (loquat), *Eriostemon* spp. (eristemon), *Escallonia* spp. (escallonia), *Euonymus* spp. (euonymus), *Forsythia* spp. (forsythia), *Fortunella* spp. (kumquat), *Fragaria* spp. (strawberry), *Gelsemium* spp. (jasmine), *Genista* spp. (broom), *Gerbera* spp. (daisy), *Grevillea* spp. (spider-flower), *Hardenbergia* spp. (hardenbergia), *Hebe* spp. (hebe/speedwell), *Hedera* spp. (ivy), *Helichrysum* spp. (everlasting), *Hypericum perforatum* (St. John's wort), *Juglans* spp. (walnut), *Lathyrus* spp. (sweet pea), *Lavandula* spp. (lavender), *Leucadendron* spp. (leucodendron), *Leptospermum* spp. (manuka), *Lonicera* spp. (honeysuckle), *Lupinus* spp. (lupine), *Macadamia* spp.

(macadamia), *Mangifera* spp. (mango), *Melaleuca* spp. (bootlebrush), *Mentha* spp. (mint), *Mesembryanthemum* spp. (ice-plant), *Michelia* spp. (banana-shrub), *Monotoca* spp. (monotoca), *Myoporum* spp. (sandle-wood), Oxalis spp. (wood-sorrel), *Parthenocissus* spp. (ivy), *Pelargonium* spp. (geranium), *Persoonia* spp. (persoonia), *Petroselinum* spp. (parsley), *Philadelphus* spp. (mock-orange), *Photinia* spp. (photinia), *Phyllanthus* spp. (phyllanthus), *Pittosporum* spp. (pittosporum), *Plantago lanceolata* (plaintain/ribwort), *Platysace* spp. (platysace), *Polygala* spp. (milkwort), *Polygonum* spp. (knotweed), *Pteris* spp. (brake-fern), *Pulcaria* spp. (fleabane), *Pyracantha* spp. (fire-thorn), *Quercus* spp. (oak), *Ranunculus* spp. (buttercup), *Raphanus* spp. (radish), *Reseda* spp. (coneflower), *Rumex* spp. (dock), *Salix* spp. (willow), *Salvia* spp. (sage), *Senecio* spp. (ragwort), *Sida* spp. (side), *Sisymbrium* spp. (mustard), *Smilax* spp. (cat-brier), *Tithonia* spp. (sunflower), *Trema* spp. (trema), *Triglochin* spp. (arrow grass), *Ulex europaeus* (gorse), *Urtica* spp. (nettle), *Viburnum* spp. (arrow-wood), and *Vinca* spp. (periwinkle) (Danthanarayana, 1975; Wearing et al., 1991; Venette et al., 2003; Brown et al., 2010).

Known vectors (or associated organisms)

An association between larvae of *E. postvitanna* and *Botrytis cinerea* (Fig. 7), gray mold, has been shown in grapes.

Figure 7. Discolored, shriveled berries caused by *Botrytis* bunch rot (left) and *Botrytis cinerea* sporulating on grape berries. Photos courtesy P. Sholberg, Agriculture & AgriFood Canada.

Known Distribution

Epiphyas postvittana is indigenous to Australia. *E. postvittana* is widespread throughout Australia and New Zealand on many weedy hosts including gorse (*Ulex europaeus*) and broom (*Cytisus scoparius*). It is commonly present in gardens and unsprayed horticultural crops.

Europe: Netherlands, Sweden, United Kingdom. **North America:** United States. **Oceania:** Australia and New Zealand (Meyrick, 1937; Bradley, 1973; Wolschrijn and Kuclein, 2006; Svensson, 2009).

Although it was reported from New Caledonia, its presence in that country could not be verified by Suckling and Brockerhoff (2010).

Potential Distribution within the United States

E. postvittana has been reported to occur in Hawaii since 1896 (Zimmerman, 1978). On March 16, 2007, *E. postvittana* was confirmed in Alameda County, California. As of March 2012, further detections have occurred in Alameda, Contra Costa, Los Angeles, Fresno, Madera, Marin, Monterey, Napa, Sacramento, San Benito, San Diego, San Francisco, San Joaquin, San Luis Obispo, San Manteo, Santa Barbara, Santa Clara, Santa Cruz, Solano, Sonoma, Ventura, and Yolo Counties. A single moth of *E. postvittana* was detected in the summer of 2010 in Oregon. To date, despite extensive trapping, no additional moths have been trapped indicating that the moth is not established in Oregon.

A recent risk analysis by USDA-APHIS-PPQ-CPHST indicates areas of California and the southern and southeastern United States have a moderate to high risk rating for *E. postvittana* establishment based on host availability and climate within the continental United States. Establishment is precluded in areas of the northern and northeastern United States.

Survey

CAPS-Approved Method*: The CAPS approved method is a trap and lure combination. The preferred trap type is a Jackson trap. The lure is effective for 42 days (6 weeks).

In order to standardize data reporting and trap procurement for the LBAM Program, it is preferable that states use the Jackson trap. However, if states prefer to use the large plastic delta traps, the traps must be purchased with their own funding. Negative data may then be reported from the large plastic delta traps. Trap color is up to the state and does not affect trap efficacy.

Large plastic delta traps for *Epiphyas postvittana* should not be ordered through the IPHIS Survey Supply Ordering System.

IPHIS Survey Supply Ordering System Product Names:

1) Jackson Trap Body
2) *Epiphyas postvittana* Lure

Trap spacing: When trapping for more than one species of moth, separate traps for different moth species by at least 20 meters.

Lure Placement: Do not place lures for two or more target species in a trap unless otherwise recommended.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: (Taken from Venette et al., 2003 and CABI, 2009)
Trapping: Pheromone traps have been widely used for detection and monitoring of populations of this species (Bellas et al., 1983). Two key components of the pheromone are (*E*)-11-tetradecenyl acetate and (*E*,*E*)-(9,11) tetradecadienyl acetate (Bellas et al., 1983). These compounds in a ratio of 20:1 are highly attractive to males. This lure is typically formulated on a rubber septum (1 to 3 mg). Due to the recent detections of *E. postvittana* in California, new formulations (*e.g.,* plastic laminate) are under development and testing is planned at Otis. Delta traps have been used and placed from 5 to 6.5 ft (1.5 to 2 m) above ground level.

Foster and Muggleston (1993) provide a detailed analysis of different designs of delta traps. In general, they found that traps with a greater length (*i.e.*, the distance between the two openings of the trap) capture significantly more *E. postvittana* than shorter traps. This effect is not related to saturation of smaller sticky surfaces with insects or other debris. The addition of barriers to slow the exit of an insect from a trap also improves catch. In a separate analysis, Foster et al. (1991) found that placing the pheromone lure on the side of the trap helped to improve trap efficiency. The orientation of the trap relative to wind direction did not affect the number of *E. postvittana* that were attracted to the pheromone or were subsequently caught by the trap (Foster et al., 1991).

Visual survey: Visual inspections have been used to monitor population dynamics of *E. postvittana* eggs and larvae. In grape, 40 vines were inspected per sampling date (Buchanan, 1977). In apple and other tree fruits, 200 shoots and 200 fruit clusters (10 of each on 20 different trees) are often inspected (Bradley et al., 1998). Egg masses are most likely to be found on leaves (USDA ,1984). The egg masses may be jet black if parasitized by *Trichogramma* spp. (a trichogrammatid wasp) (Glenn and Hoffman, 1997). Larvae are most likely to be found near the calyx or in the endocarp; larvae may also create “irregular brown areas, round pits, or scars” on the surface of a fruit (USDA, 1984). Larvae may also be found inside furled leaves, and adults may occasionally be found on the lower leaf surface (USDA, 1984).

Not recommended: Adults are also attracted to fruit fermentation products as a 10% wine solution has been used as an attractant and killing agent for adults (Buchanan, 1977; Glenn and Hoffmann, 1997). The dilute wine (670 ml) in 1 liter jars was hung from grapevines on the edge of a block of grapes (Buchanan, 1977). Black light traps have been used to monitor adults of *E. postvittana* (Thwaite, 1976).

Key Diagnostics/Identification

CAPS-Approved Method*: Confirmation of *E. postvittana* is by morphological identification. Many native tortricids could be confused with *E. postvittana*. Identification requires dissection of male genitalia. Female specimens should be sent to a Lepidopteran specialist for identification. Sorting and Level 1 Screening may be performed without dissection by using Passoa et al. (n.d.).

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: *E. postvittana* is similar to *E. pulla* and *E. liadelpha*, both not known to be present in the United States. Geier and Springett (1976) reported possible hybridization based on demographic characteristics. Larvae are similar to larvae of other leafrollers, which may be present (for example, in New Zealand, *Planotortrix octo*, *P. excessana, Ctenopseustis obliquana,* and *C. herana* may be present). Identity of the species must often be confirmed by examination of adult genitalia. Molecular diagnostics based on PCR amplification of ribosomal DNA have been developed and are especially useful for the identification of immature specimens (Armstrong et al., 1997).

TortAI: Tortricids of Agricultural Importance is designed for use by persons in the continental United States performing domestic surveys for exotic species. *TortAI,* which includes all of the tortricid species found in the digital identification tool *LBAM ID*, includes two image-rich interactive identification keys (adult and larvae), diagnostic fact sheets, a visual dictionary, support pages for the dissecting and preparing specimens, an image gallery, and a molecular search page. Because the world tortricid fauna is too large to treat as a whole, this digital identification tool is not designed to identify every tortricid encountered, but rather to reliably eliminate or confirm target taxa if or when they are encountered. This tool is available via the internet [http://idtools.org/id/leps/tortai/] and on CD.

Easily Confused Pests

*E. postvittan*a may be confused with many native tortricids. It can also be confused with *Amorbia emigratella* (Mexican leafroller), which has been reported from the United States, however, *E. postvittana* has ocelli which are lacking in *A. emigratella.* The undersides of *E. postvittana* hindwings are conspicuously immaculate as in *A. emigratella*, and the second abdominal tergite lacks the conspicuous median pit near the base which is present in *A. emigratella* (USDA, 1984).

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Grapholita funebrana

Scientific Name
Grapholita funebrana (Treitschke)

Synonyms:
Carpocapsa funebrana, Cydia funebrana, *Enarmonia funebrana, Endopisa funebrana, Grapholita funebrana, Grapholitha funebrana, Laspeyresia cerasana, Laspeyresia funebrana, Opadia funebrana,* and *Tortrix funebrana.*

Note: *Grapholita funebrana* is often incorrectly referred to as *Cydia funebrana*. The correct generic placement is in *Grapholita* (see Komai (1999) for more details).

Common Names
Plum fruit moth, prune moth, red plum maggot

Type of Pest
Moth

Taxonomic Position
Class: Insecta, **Order:** Lepidoptera, **Family:** Tortricidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2003 through 2009

Pest Description
Grapholita funebrana is able to develop on many wild and cultivated stone fruits and other plants in the family Rosaceae. This pest occurs in Europe, the Middle East, and northern Asia with losses of 25 to 100% reported.

The information provided below is from Alford (1978), Bradley et al. (1979), and Whittle (1984).

Eggs: Eggs are deposited singly and measure about 0.7 mm (0.28 in.) across by 0.6 mm (0.24 in.) wide, are lenticular to ovate (flattened and slightly elliptical), and are translucent white, becoming yellow as they mature. When they turn yellow, the egg has a central dome-shape area, circled by a flat ring. Eggs are generally laid during June and July at the base of a fruit stalk, hatching in about 10 days.

Larvae: At their longest, larvae are about 10 to 12 mm (0.39 to 0.47 in.) long. The head is dark brown to black. The prothorax is pale yellow; while the prothoracic plate is pale brown with the posterior margin mottled darker brown. The thoracic legs are pale yellow. The abdomen is translucent white but turns pink dorsally and yellowish ventrally

as the larvae develop through the instars. The pinacula is light brown and inconspicuous. The peritreme is brown and inconspicuous. The anal plate is pale brown with small blackish spots. The anal comb has four to seven prongs with one to three small additional prongs laterally.

Pupae: Pupae are light brown, 6 to 7 mm (0.24 to 0.28 in.) long, and contained in a silken cocoon.

Figure 1. *Grapholita funebrana* adult male. Image courtesy of Todd Gilligan, Colorado State University.

Adults: The average wingspan of an adult (Fig. 1) is 12 to 15 mm (0.47 to 0.59 in.). The forewings are triangular, narrow at the base, dark gray brown becoming clearer towards the apex, turning to an ashy gray spot. At the center of this spot, four small horizontal black dashes are present. Adults have brownish gray hind wings, and the underside of the body and legs is grayish.

Labial palpus, frons fuscous (brownish-gray); also (along with the head) described as ocherous (yellow-orange). Forewing mainly overlaid with fuscous brown except obscure pairs of white interspaces between poorly defined blackish brown costal strigulae; fasciate marking blackish brown, indeterminate except outer edge of sub-basal fascia weak dorsally; discocellular spot minute, indistinct, white; distal area, especially ocellus, irroration (tips of scales) with white or grayish white, similar irroration mediodorsally forms indistinct blotch; ocellus comprising usually four black dots, edged laterally by thick plumbeous stria on inner margin, thinner stria on outer margin; cilia concolorous with wing basally, otherwise gray, with black sub-basal line indented subapically. Hindwing fuscous, lighter basally and along termen, cilia grayish white, fuscous sub-basal line. Simple blackish-gray antennae. Abdomen dark brown. Genitalia with characteristic symmetrical projection on sacculus, and a peg-like projection at the orifice of the aedeagus.

The individual variation in adults of this species is mostly seen in the clarity of the white interspaces on the costa and in the strength of the whitish irroration in the distal and medio-dorsal areas of the forewing.

Biology and Ecology

This pest feeds primarily on stone fruits and many potential wild hosts exist in the United States in the family Rosaceae and has been captured many times at U.S. ports of entry, mostly from fruit in baggage. Adults begin to appear in April or May and can be seen through October. Depending upon the climate, this moth has one to three

overlapping generations per year (Sáringer, 1967). In general, the first generation injures fruit at the end of May through June, and the second generation injures fruit in July and August. In areas where multiple generations per year develop, early season varieties are less susceptible to economic damage than later-maturing fruit (CABI, 2009). Females have a higher reproductive potential in the second and third generations (Bobîrnac, 1958). The moth thrives in climates that have warm January and February temperatures (6°C, 42°F), high precipitation (60 inches/year), and high relative humidity (70 to 78%).

Adult moths are most active at night (resting during the day high in the tree canopy) when temperatures reach (18 to 22°C) (64 to 72°F). Females live longer than males (11 days compared to 8 days, on average). Females are also much more abundant (proportionally) than males as the year progresses (Popova, 1971; Rauleder, 2002). Most mating occurs about two hours before dawn, and females prefer to mate about 10 feet above the ground (Charmillot and Blaser, 1982).

Figure 2. Fruit showing the sticky exudate formed when the larvae of the plum fruit moth enters a fruit. Photos courtesy of Magnus Gammelgaard Nielson (http://www.plante-doktor.dk/blommevikler.htm) and R. Coutin (OPIE).

Beginning in May (when the temperature has reached at least (14°C, 57°F), eggs from the first generation are laid singly or in small groups (three to nine) on the sunny side and at the base of fruit stalks, on fruit surfaces, or on the underside of leaves in the afternoon and evening hours (Touzeau, 1972; Whittle, 1984). Eggs hatch in five to 10 days (mostly five to seven days) and the larvae chew into fruit, usually near the stem. Before feeding, the larvae seal up the entrance hole with deposits of chewed fruit skin bound with silk. In general, larval mortality is high in each generation, either through parasitism, competition, and/or failure to establish within the fruit. Larval feeding causes gummosis (fluid exuding from the entrance hole) (Fig. 2), a premature color change, and/or fruit drop. Larvae feed throughout the fruit, traveling from the outer part to the pit region (Fig. 3), and have been seen feeding on multiple fruit, but usually do not. After 15 to 25 days, larvae complete their development, leaving a large exit hole and find a

place to pupate under bark or other crevices, including on the ground and in the soil. In regions where two or three generations per year develop, these moths overwinter as larvae; where only one generation completes development, this moth overwinters as pupae.

Photoperiod is the main cause for the onset of diapause (temperature and host ripeness do not influence diapause). The light conditions crucial for diapause are perceived during the first half of larval development (second and third instar), and the threshold is likely between 15 to 17 hours of daylight, unless the length of the days are still getting longer (Sáringer, 1967, 1970).

Some orchard-wide pheromone releases for mating disruption have seen success, but not all. It seems that some isolation from other wooded areas is necessary to control *G. funebrana* with pheromones (Charmillot et al., 1982). Male trapping over a period of years also seems to reduce fruit damage by up to 84% (Koltun and Yarchakovskaya, 2006).

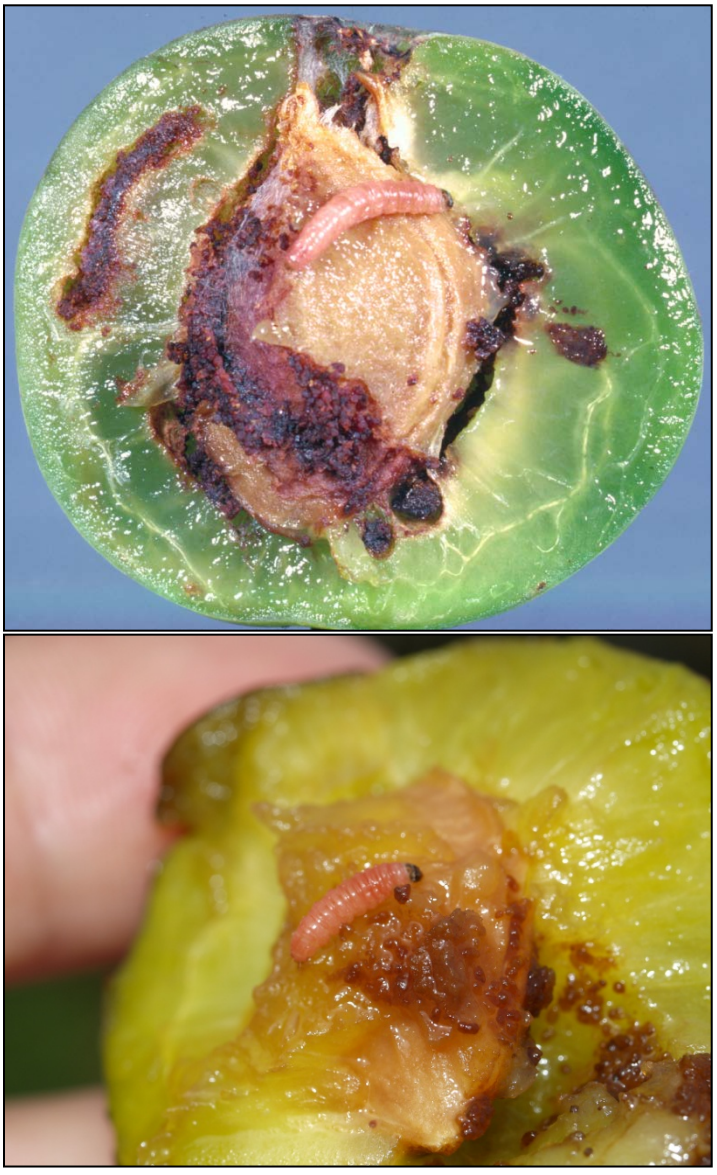

Figure 3. Larva of the plum fruit moth feeding within an unripened and ripened plum. Notice most of the damage occurring near the pit. Photos courtesy of R. Coutin (OPIE) and Magnus Gammelgaard Nielson, respectively. http://www.plante-doktor.dk/blommevikler.htm.

Fenoxycarb (a juvenile hormone mimic) and diflubenzuron (a chitin formation inhibitor) have been used as a control for this moth. These chemicals are used most often at the beginning of the egg laying period. In the Czech Republic, once a degree day value of 290°C is reached, pheromone traps should be monitored. Once a marked flight wave is noticed, these ovicides should be sprayed. The chemicals have shown success controlling the summer (second) generation of *G. funebrana* with only one treatment (Kocourek et al., 1995). It is also been recommended that fenoxycarb should not be

used without a chemical rotation. Organophosphorous insecticides and diflubenzuron (2 to 3 treatments per generation) have also been used to control *G. funebrana (*Andreev and Kutinkova, 2010). Azinphos-methyl at 6.3 g *a.i.* per acre applied at least twice at 14-day intervals was effective at killing larvae in field studies in England. Carbaryl, dimethoate, fenthion and methyl parathion have also seen success in Europe on these larvae (Vernon, 1971).The pyrethrins cypermethrin, bensultap and λ-cyhalothrin were successful against this pest (Tălmaciu et al., 2006).

Symptoms/Signs

Larvae bore into fruits after hatching. Entrance holes, however, are barely visible. Holes at the base of fruit near the stalks and fruit exudates (Fig. 2) that include frass are good diagnostic observations. The feeding activity of the larvae in young fruits usually damages sap vessels near the peduncle, causing a color change in the fruit from green to violet and fruit drop. In the latter part of the season, when fruits are fully-grown, infested ones can be easily detected as they tend to ripen earlier. If you suspect a *G. funebrana* infection, cut the fruit to expose the larvae tunneling in the pulp near the seed (Fig. 3). Finally, inspect and look for cocoons in crevices in the bark of trees, on main branches, on root collars, or even in fruit containers (Whittle, 1984).

Pest Importance

The plum fruit moth is an important pest of plums throughout northern Europe. Yield losses of 40 to 95% have been reported. Total loss has been recorded on the Black Sea coast. Severe losses are more commonly related to the 2nd and 3rd generations, and in regions with warmer summers. In Denmark, this moth prefers cherry to plum (Whittle, 1984, and references therein).

Known Hosts

This pest feeds primarily on stone fruits and wild hosts that exist in the family Rosaceae.

Major hosts: *Prunus* spp. (stone fruit), *P. armeniaca* (apricot), *P. avium* (sweet cherry, gean), *P. cerasifera* (myrobalan plum), *P. cerasus (*sour cherry), *P. domestica* (plum), *P. instititia* (damson plum), *P. japonica* (Japanese plum), *P. persica* (peach), and *P. spinosa* (blackthorn/sloe).

Minor hosts: *Castanea sativa* (chestnut)**,** *Juglans regia* (English walnut), *Malus domestica* (apple), *M. sylvestris* (crabapple), *Prunus dulcis* (almond), and *Pyrus communis* (pear).

Known vectors (or associated organisms)

This insect has been associated with *Monilinia fructigena* (brown rot) and *Botrytis cinerea* (gray mold) (listed as *Molina fructigens* and *M. cinerea*) (Kostarev, 1914).

Known Distribution

Asia: Armenia, Azerbaijan, China, Republic of Georgia, Iran, Japan, Kazakstan, Kyrgyzstan, Syria, Tajikistan, Turkey, Turkmenistan, and Uzbekistan. **Africa:** Algeria.

South America: Argentina. **Europe:** Albania, Austria, Belgium, Bosnia, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Ukraine, and the United Kingdom (Whittle, 1984; CABI, 2009).

Potential Distribution within the United States

Surveys should be focused where the greatest risk for establishment occurs. A recent risk analysis by USDA-APHIS-PPQ-CPHST indicates that most states in the United States have a low to moderate risk rating for *G. funebrana* establishment based on host availability and climate within the continental United States.

Survey

CAPS-Approved Method*: The CAPS approved method is a trap and lure combination. The trap type is a wing trap.

Any of the following Trap Product Names in the IPHIS Survey Supply Ordering System may be used for this target:

1) Wing Trap Kit, Paper
2) Wing Trap Kit, Plastic

The Lure Product Name is "*Grapholita funebrana* Lure." The lure is effective for 28 days (4 weeks).

Trap Spacing: When trapping for more than one species of moth, separate traps for different moth species by at least 20 meters (65 feet).

Lure Placement: Placing lures for two or more target species in a trap should never be done unless otherwise noted here.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Delta trap, Pherocon 1C, or Traptest traps with a rubber septa lure have been used to trap *Grapholita funebrana*. The lure is composed of five compounds, 1) Z,8-12:AC, 2) E,8-12:AC, 3) Z,8-14:AC, 4) Z,10-14:AC, and 5) 14:AC (Venette et al., 2003). These five compounds were identified in the proportions 100:1:30:5:2 in female sex gland extracts of *Grapholita funebrana*, accompanied by saturated acetates from 12 to 20 carbons with tetradecyl acetate predominating (Guerin et al., 1986). The principal components were reported to be Z8-12Ac ("Funemone") and E8-12Ac.

Traps with "Funemone" (*cis*-8-dodecenyl acetate) lures can be placed about 19.68 m (6 ft.) off the ground. These need to be replaced every six weeks and monitored every week. Three to 5% of the *trans* isomer helps in attracting more male moths.

Monitoring with sex pheromones along the edges of fields, rather than in the center, is recommended. Pheromones to detect *G. funebrana* can be placed in the same traps with pheromones of *Cydia pomonella* or *Lymantria dispar* without adverse side effects (Schwalbe and Mastro, 1988). Spatial modeling in Italy has shown some behavioral changes throughout the growing season. During the first flight period, adults aggregate, building up high local densities. During the subsequent one to two flight periods, high rates of dispersal occur along prominent landscape features, such as ravines (Sciarretta et al., 2001). Using pheromone traps to determine population density has given mixed results, and is not seen as reliable as other methods. Pheromone traps also are not species specific, catching many other tortricid species, including males of *G. molesta*.

Key Diagnostics/Identification

CAPS-Approved Method*: Morphological. This species can be identified by examining the male and female genitalia.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Adults of *Grapholita funebrana* are most similar to those of *G. molesta* and *G. tenebrosana*. Genitalia illustrations for all species described here can be found in Razowski (2003).

Grapholita molesta is commonly distributed throughout the United States. It is morphologically very similar to *G. funebrana* and the two species share the same host plants and female pheromones. *Grapholita molesta* can be separated from *G. funebrana* by the absence of a thorn-like projection off the valva in the male and the laterally elongate sterigma with small posterolateral projections in the female.

Grapholita tenebrosana is distributed across Europe to Asia Minor and Siberia. It is not known to occur in North America. Adults can be separated from *G. funebrana* by the elongate valva with a sharply developed anal angle in the male and the large sterigma with triangular lateral lobes in the female.

Larvae may appear similar to those of many other species of *Grapholita* and *Cydia. Cydia pomonella* larvae can be separated from *G. funebrana* by the absence of an anal fork. Other species of *Grapholita* cannot be reliably separated from *G. funebrana* based solely on larval morphology. Chen and Dorn (2009) provide a molecular assay to distinguish *G. funebrana* larvae from similar species using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

A new identification tool, *Tort AI – Tortricids of Agricultural Importance*, is available at http://idtools.org/id/leps/tortai/ from CPHST's Identification Technology Program. This tool contains larval and adult keys, fact sheets, an image gallery, molecular search capacity, and more. *Grapholita funebrana* is included in this tool.

Easily Confused Pests

This pest may be easily confused with *G. molesta*, which is common and widespread in North America. The two species are similar morphologically, share the same host plants, and are attracted to the same female pheromone.

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Leucoptera malifoliella

Scientific Name
Leucoptera malifoliella (Costa)

Common Names
Pear leaf blister moth

Leucoptera malifoliella will not be available as a survey target for the 2012 or 2013 survey season.

Due to the large number of non-targets caught in *Leucoptera malifoliella* traps and the small size of the moth, processing of *Leucoptera malifoliella* traps is too difficult and time-consuming at this time.

Leucoptera malifoliella will be made available for survey when trap improvements and identification support tools can be developed.

Lobesia botrana

Scientific Name
Lobesia botrana [Denis & Schiffermüller]

Synonyms:
Cochylis vitisana, Cochylis botrana, Coccyx botrana, Eudemis botrana, Eudemis rosmarinana, Grapholita botrana, Lobesia rosmariana, Noctua romani, Paralobesia botrana, Penthina vitivorana, Polychrosis botrana, Tortrix botrana, Tortrix vitisana, Tinea premixtana, Tinea reliquana, *Tortrix reliquana,* and *Tortrix romaniana*.

Common Names
European grapevine moth, grape fruit moth, grape leaf-roller, grape vine moth, grape moth, vine moth

Type of Pest
Moth

Taxonomic Position
Class: Insecta, **Order**: Lepidoptera, **Family**: Tortricidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2003 through 2009, Program pest

Pest Description
European grapevine moth (EGVM) is primarily a pest on the flowers and fruits of grape vines, but the moth has been known to infest stone fruit trees, privet and olives as well. Survey for this pest in stone fruit, privet and olives is important because, in general, these secondary hosts flower before grapes and *L. botrana* can be found on these earlier hosts before moving over to grapes, its preferred host.

Eggs: The egg of *L. botrana* is of the so-called ‘flat type’ with the long axis horizontal and the micropyle at one end. Eggs are elliptical, flattened, and slightly convex with a mean eccentricity of 0.65. The egg measures about 0.65 to 0.90 mm (0.03 to 0.04 in.) x 0.45 to 0.75 mm (0.02 to 0.03 in.). Freshly laid eggs are pale yellow, later becoming light gray and translucent with iridescent glints (opalescent). The chorion is macroscopically smooth but presents a slight polygonal reticulation in the border and around the micropyle. The time elapsed since the eggs were laid may be estimated by observing the eggs: there are five phases of embryonic development - visible embryo, visible eyes, visible mandibles, brown head, and black head. As typically occurs in the subfamily Olethreutinae, eggs are laid singly, and more rarely in small clusters of two or three (CABI, 2009; Gilligan et al., 2011).

Larvae: There are usually five larval (Fig. 1A) instars. Neonate larvae are about 0.95 to 1 mm (~0.04 in.) long, with head and prothoracic shield deep brown, nearly black, and

body light yellow to yellowish green. Mature larvae reach a length between 10 and 15 mm (0.39 to 0.59 in.), with the head and prothoracic shield lighter than neonate larvae and the body color varying from light yellowish green to pale brown, depending principally on larval nourishment (CABI, 2009; Gilligan et al., 2011). The head is brown to light yellowish brown to honey colored, the antennae and thoracic legs are brown to black, and the prothoracic shield is variably shaded with dark brown to black on the posterior and lateral margins. All instars have a dark stemmatal area and genal dash (Gilligan et al., 2011).

Important structural features of *L. botrana* larvae include: mandibles without inner teeth or a retinaculum; distance between P1 and AF2 on head equal to distance between P1 and P2; a horizontal line connecting the P2 setae on head passes through AF2; L pinaculum on T1 horizontal, not extending beneath spiracle; SV groups on A1, 2, 7, 8, 9 with 3:3:3:2:2 setae; distance between V setae on A9 approximately 1.5 to 2.0 times the distance between V setae on A8; distance between D1 setae on anal shield equal to distance between D1 and SD1; anal comb with 5 to 6 teeth in California individuals, other authors report 6 to 8 teeth; and body spicules relatively dense (Gilligan et al., 2011).

<u>Pupae:</u> Female pupae are larger (5 to 9 mm; 0.20 to 0.35 in.) than males (4 to 7 mm; 0.16 to 0.28 in.). Freshly formed pupae are usually cream or light brown but

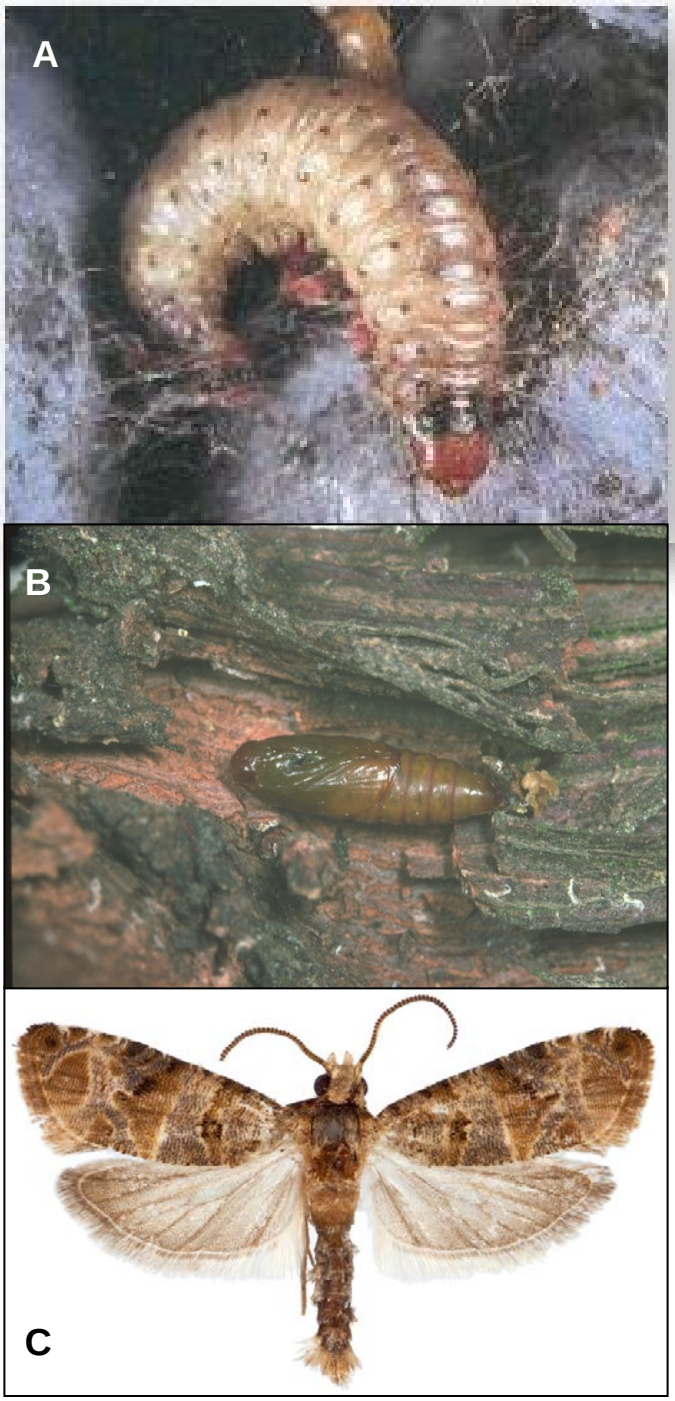

Figure 1. Larva (A), pupa (B), and adult male (C) *L. botrana.* Photos courtesy of Instituto Agrario S. Michele All' Adigen, HYPPZ Zoology, and Todd Gilligan, Colorado State University, respectively.

also light green or blue, but a few hours later become brown or deep brown (Fig. 1B). Cast pupal skins, are somewhat unusual in retaining a greenish tint on the anterior abdominal segments. Pupal age may be estimated as a function of tegument transparency and coloring (CABI, 2009). The sexes may be distinguished by the position of genital sketches that are placed in the IX and VIII abdominal sternites in males and females, respectively. Moreover, the male genital orifice is placed between two small lateral prominences. When adult emergence is imminent, pupae

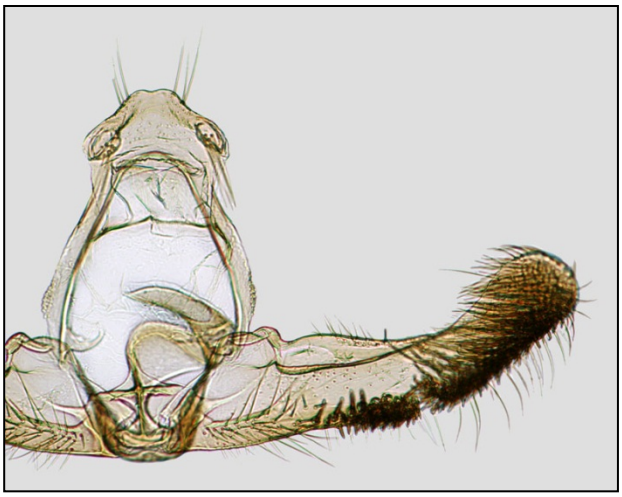

Figure 2. Male genetalia of *L. botrana.* Photo courtesy of Todd Gilligan, Colorado State University.

perforate the cocoon, resting the exuvia fixed outwardly in a characteristic position by cremaster spines.

Important structural features of *L. botrana* pupae include: head unmodified, without projections; clypeus with two pairs of setae; A4 and A5 with 22 to 24 spines between the D2 setae; dorsum of A10 with a patch of spine and no setae present on the anal rice. The cremaster is fan-shaped with a weakly emarginated caudal margin (Gilligan et al., 2011).

Adult: Forewing length ranges from 4.5 to 8.5 mm (0.18 to 0.33 in.) (Gilligan et al., 2011). Adult size is greatly affected by larval food quality (Torres-Vila, 1995). Forewing pattern exhibits little variation and no sexual dimorphism. Forewing pattern is as follows; ground color cream; interfascial areas overlaid with leaden gray; costal stringulae cream, well defined; fasciae brown to dark brown; subbasal fasica well defined, with black scaling medially; median fasica well defined, with triangular medial projection often suffused with black scaling; postmedian fasica broken, forming pretormal patch along dorsum with cluster of black scales; postmedian band forming large brown patch along termen; apex often with conspicuous black dot; termin outlined in cream; fringe brown. The males (Fig. 1C) lack a forewing costal fold. The male hind wing is whitish with a brown periphery; while the female hind wing is completely brown (Gilligan et al., 2011).

Male genetalia (Fig. 2) can be distinguished by a combination of the following characteristics: socii short, lateral, apex with numerous setae; uncus reduced to small bilobed hump on tegument; gnathos weakly sclerotized; valvae long and narrow with dense row of strong spines on ventral margin; cucullus densely setose, separated from sacculus by distinct gap in row of ventral spines; sacculus weakly concave post-medially; phallus small; cornuti absent. Female genetalia (Fig. 3) are characterized by a long, slender ductus bursa that is undifferentiated from the corpus bursae, gradually expanded anteriorly, and an unusual, elongate, somewhat feather-shaped signum (Gilligan et al., 2011).

Figure 3. Female genetalia of *L. botrana.* Photo courtesy of Todd Gilligan, Colorado State University.

Biology and Ecology:

The first flight of adults occurs in spring when daily average air temperature is above the minimal threshold temperature of 10°C (50°F) for 10 to 13 days. The second flight period begins in summer (USDA, 1985). In Israel, adults appear in the vineyard when grapevines flower. Adults are hard to discover during the day and may be noticed only when they take flight after being disturbed. They fly at dusk whenever the temperature is above 12°C (54°F), but rainfall and wind will reduce flight. Adults usually prefer hot, dry places protected from wind so they fly mainly between the first rows of grapevines close to windbreaks and on slopes facing the sun (Avidov and Harper, 1969).

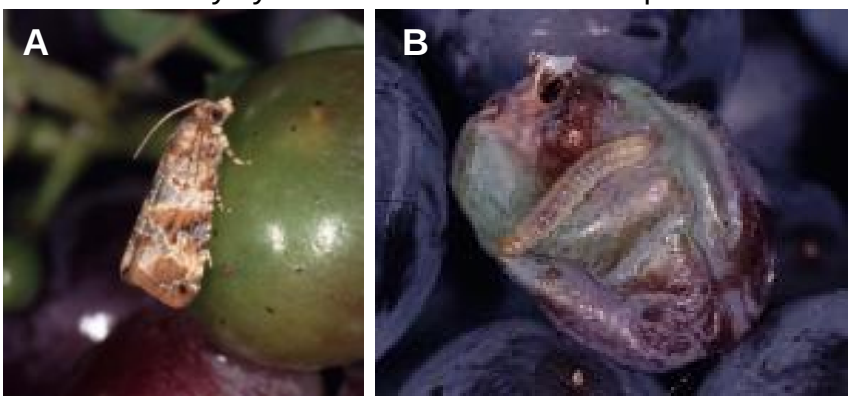

Figure 4. Adult on grape fruit (A) and larvae feeding inside a grape (B). Photos courtesy of Michael Breuer. http://www.bio-pro.de/de/region/freiburg/magazin/01476/index.html.

Within a day or two of mating, females begin to oviposit on the blossoms, leaves, and tender twigs of the grapevine. The female lays 300 or more eggs singly or in groups of two or three at a rate of more than 35 per day. During rearing experiments under laboratory conditions in Czechoslovakia, the optimum temperatures for oviposition were from 20 to 27°C (68 to 81°F) (Gabel, 1981). First generation eggs are laid on the flower buds or pedicels of the vine while second generation eggs are laid on individual grapes (USDA, 1985) (Fig. 4A). Eggs hatch in 5 to 10 days or 75 degree-days above a 10°C (50°F) threshold (Gilligan et al., 2011).

The European grapevine moth is a polyvoltine species (CABI, 2009). The number of generations in a given area is fixed by photoperiod together with temperature, acting on diapause induction and development rate, respectively. Short-day photophases (between 8 and 12 h) during the larval stage induce diapause in larvae that will be later expressed in pupae. The moth achieves two generations in northern cold areas and usually three generations in southern temperate areas, although this general latitudinal pattern is often modified by the altitude-derived gradient and/or microclimatic conditions in a given area. Thus the number of generations has a broader range, reported as one generation in Romania (Filip, 1986) to four generations (often partial) in Spain, Greece, Crete, Italy, and former Yugoslavia (Coscollá, 1997 and references therein). Five generations have been reported in Turkmenistan (Rodionov, 1945).

Figure 5. Glomerules of *L. botrana.* Photo courtesy of EFAPO-ES.

First generation larvae feed on bud clusters or flowers and spin webbing around them (glomerules) before pupating inside the web or under the rolled leaf. Second generation larvae enter an unripened grape (Fig. 4B) and feed before pupating inside the grape. Larvae of the third generation, the most damaging, feed on ripening grapes, migrating from one to another and spinning webs. The third generation larvae leave the fruit and shelter under the bark, among dead leaves, or between clods of earth, where they pupate before overwintering. Few of these larvae pupate before harvest, and many are gathered with the grapes. Larval development is completed in approximately 20 to 28 days or 170 degree days for larvae feeding on flowers and 225 degree-days for larvae feeding on berries (Gilligan et al., 2011). Pupae complete development in approximately 12 to 14 days, or 130 degree-days, for non-diapausing individuals (Gilligan et al., 2011).

Figure 6. Damage by *L. botrana*. Photo courtesy of HYPPZ Zoology.

Moth activity (*i.e.*, flight, feeding, calling, mating, and egg-laying) is principally displayed at dusk, although some activity can also occur at daybreak or at any time on cloudy days. Water availability is necessary for adults to reach their potential reproductive output (Torres-Vila et al., 1996). Females are usually monandrous, but several physiological factors may enhance multiple mating (Torres-Vila et al., 1997). On the other hand, males are largely polygynic (Torres-Vila et al., 1995).

Symptoms/Signs

Documentation on damage to stone fruits is limited, as most information is available on grape. This moth can cause damage to the flowers and the fruit of stone fruit hosts.

On grape inflorescences, neonate (first generation) larvae firstly penetrate single flower buds. Symptoms are not evident initially because larvae remain protected by the top bud. Later, when larval size increases, each larva agglomerates several flower buds with silk threads forming **glomerules** visible to the naked eye (Fig. 5), and the larvae continue feeding while protected inside. Larvae usually make one to three glomerules during their development. Despite hygienic behavior of larvae, frass may remain adhering to the glomerules. On grapes (summer generations), larvae feed externally and when berries are a little desiccated (Fig. 6), they penetrate them, bore into the pulp, and remain protected by the berry peel (Fig. 4B, 7). Larvae secure the pierced berries to surrounding ones by silk threads in order to avoid falling. Each larva directly damages several berries (one to six), but if the conditions are suitable for fungal or acid rot development, a large number of berries placed around may be also affected. Damage is variety-dependent; generally it is more severe on grapevine varieties with dense grapes because this increases both larval installation and rot development. On both inflorescences and grapes, several larvae may co-exist in a single reproductive organ. Larval damage on growing points, shoots, or leaves is unusual.

Figure 7. Larva inside grape fruit. Photo courtesy P. del Estal (CABI, 2009).

First-generation larval feeding on the buds or flowers webs them and prevents further growth. If heavy flower damage occurs during the first moth generation, the affected flowers will fail to develop and yield will be low. Damage by summer larvae of the second and third generation results in many nibbled berries, which later shrivel. The berries may be eaten either partly (leading to rot) or completely (leaving only empty skins at the tip of the bunch). Sometimes berries drop, and only the stalks remain (USDA, 1985).

Pest Importance

The European grapevine moth is a serious pest in the warm vine-growing countries where it is normally found. Larvae feed on flower buds, developing berries, and most destructively, on the ripening fruit of grape. The primary damage to grape berries attracts other insects and predisposes the fruit to fungal infection. Larval boring in grapes may promote a number of fungal rots (CABI, 2009). Loss of up to one-third of the vintage has been reported in areas of the Soviet Union, Syria, and Yugoslavia. Losses in Israel sometimes reach 40 to 50 percent among table grapes and up to 80 percent or more for wine grapes. Further loss is due to the time and labor spent in cleaning the grape bunches. When infestations are heavy, the work days spent in cleaning the fruit account for 30 to 40 percent of the time of those involved in harvesting (USDA, 1985).

On grapes (summer generations), indirect damage is usually more important than direct, at least in the event of less severe attacks. Thus global damage may appear of little importance if it is evaluated exclusively as weight loss (direct damage) because greater damage is due to rot-derived reduction in quality (indirect damage). Larval boring in grapes may promote a number of fungal rots including *Aspergillus*, *Alternaria*, *Rhizopus*, *Cladosporium*, *Penicillium,* and especially *Botrytis cinerea* (Fig. 8) (Fermaud and Le Menn, 1989; CABI, 2009).

Figure 8. Discolored, shriveled berries caused by Botrytis bunch rot (left) and *Botrytis cinerea* sporulating on grape berries (right). Photos courtesy P.Sholberg, Agriculture & AgriFood Canada.

Known Hosts

This pest feeds primarily on the flowers and fruits of grapes. However, *L. botrana* demonstrates a curious behavior of feeding on many different plant families (approximately 27), but only a few species within each family are suitable. Grape cultivars with prolonged blossoming or late-ripening berries are usually more heavily infested than short-flowering or early ripening varieties (Avidov and Harper, 1969). *L. botrana* exhibits an oviposition preference for privet and certain grape cultivars, such as ‘Cabernet Sauvignon’ (Maher et al., 2000, 2001).

Both plums and peaches/nectarines are as suitable as grapes for the development of *L. botrana*. These fruit trees bloom earlier than grapes, and since the female moths are active during this time, oviposition onto *Prunus* flowers and fruits has been observed and studied. The development of larvae and the reproductive capacity of females that are raised on the flowers and fruits of plums and peaches/nectarines are equal to the moths reared on the flowers and fruits of grapevines (Stavridis and Savopoulou-Soultani, 1998). Sour cherries, apricots and other stone fruits do not seem to support the same level of successful development of *L. botrana*, and can be seen as only minor hosts of this moth.

Major hosts
Prunus domestica (plum), *Prunus persica* (peach/nectarine), *Vitis vinifera* (grape), and *Vitis* spp.

Minor hosts
Actinidia chinensis (kiwi), *Clematis vitalba* (traveler's joy), *Coffea* spp. (coffee), *Dianthus* spp. (carnation), *Diospyros kaki* (Japanese persimmon), *Diospyros virginiana* (common persimmon), *Hordeum vulgare* (barley), *Malus pumila* (apple), *Medicago sativa* (alfalfa), *Olea europaea* subsp. *europaea* (olive), *Prunus amygdalus* (sweet almond), *Prunus avium* (sweet cherry), *Prunus dulcis* (sweet almond), *Prunus salicina* (Japanese plum), *Prunus spinosa* (blackthorn), *Punica granatum* (pomegranate), *Pyrus communis* (pear), *Ribes nigrum* (blackcurrant), *Ribes rubrum* (red currant), *Ribes uva-crispa* (gooseberry), *Rosa* spp. (rose), *Rosmarinus officinalis* (rosemary), *Rubes fruticosus* (European blackberry), *Rubus* spp. (raspberry), *Solanum tuberosum* (potato), and *Thymelaea hirsuta* (thymelaea).

Wild hosts
Arbutus unedo (arbutus/strawberry tree), *Berberis vulgaris* (European barberry), *Clematis vitalba* (old man's beard/evergreen clematis), *Cornus mas* (Cornelian cherry), *Cornus sanguinea* (dogwood), *Cornus* spp. (dogwood), *Daphne gnidium* (flax-leaved daphne), *Galium mollugo* (smooth bedstraw), *Hedera helix* (ivy), *Lamium amplexicaule* (henbit), *Ligustrum japonicum* (Japanese privet), *Ligustrum vulgare* (privet), *Lonicera tatarica* (Tatarian honeysuckle), *Menispermum canadense* (common moonseed), *Parthenocissus quinquefolia* (Virginia creeper), *Rhus glabra* (smooth sumac), *Rosmarinus officinalis* (rosemary), *Rubus caesius* (dewberry), *Syringa vulgaris* (lilac), *Tanacetum vulgare* (common tansy), *Trifolium pretense* (red clover), *Urginea maritima*(red squill), *Viburnum lantana* (wayfaring tree), and *Ziziphus jujuba* (common jujube).

Known Vectors (or associated organisms)
It has been shown that the nutritional alteration of berries caused by *Botrytis cinerea* may enhance female fecundity of *L. botrana* (Savopoulou-Soultani and Tzanakakis, 1988). Larval boring in grapes may promote a number of fungal rots including *Aspergillus*, *Alternaria*, *Rhizopus*, *Cladosporium*, *Penicillium,* and especially *Botrytis cinerea.*

Known Distribution
Africa: Algeria, Egypt, Eritrea, Kenya, Libya, and Morocco; **Asia:** Armenia, Azerbaijan, Georgia, Iran, Israel, Japan, Jordan, Kazakhstan, Lebanon, Syria, Tajikistan, Turkey, Turkmenistan, and Uzbekistan; **Europe:** Austria, Bulgaria, Cyprus, Czech Republic, Czechoslovakia, France, Germany, Greece, Hungary, Italy, Luxembourg, Macedonia, Malta, Moldova, Portugal, Romania, Russia, Serbia and Montenegro, Slovakia, Slovenia, Switzerland, Ukraine, and the United Kingdom (CABI, 2009). **North America:** United States. **South America**: Argentina and Chile.

In 2008, the first report of *L. botrana* in Western Hemisphere occurred in Chile (Gonzalez, 2008). In March 2010, the Argentinean National Service for Agrifood Health and Quality reported *L. botrana* in Argentina at locations in the Maipu Department, Mendoza Province, close to the Chilean border (SENASCA, 2010).

North American records from the mid- to late- 1800s are misidentifications of *Paralobesia viteana* (Kearfott, 1904), a native North American grape-feeding tortricid that is extremely similar in morphology to *L. botrana* (Gilligan et al., 2011).

Potential Distribution within the United States

Climatic conditions in the major grape growing areas of the United States favor the establishment of *L. botrana* (USDA, 1985; Venette et al., 2003). Venette et al. (2003) estimated that approximately 29% of the continental United States may be suitable for *L. botrana* establishment. This projection includes the major California wine-producing counties of Napa, Sonoma, Amador, Monterey, and San Luis Obispo.

A recent risk analysis by USDA-APHIS-PPQ-CPHST indicates, however, that areas of the southeastern United States have the greatest risk for *L. botrana* establishment based on host availability and climate within the continental United States. *L. botrana* is excluded from establishment in areas of the western and northeastern United States.

On September 15, 2009, *Lobesia botrana* was detected in commercial vineyard in Napa County, California. Since 2010, , the moth has been detected and a quarantine is in place for portions of 10 counties (Fresno, Mendocino, Merced, Napa, Nevada, San Joaquin, Santa Clara, Santa Cruz, Solano, and Sonoma)in California. In March 2012, *L. botrana* was declared eradicated from Fresno, Mendocino, Merced, and San Joaquin counties leaving only, Napa, Nevada, Santa Clara, Santa Cruz, Solano, and Sonomo under quarantine.

Survey

CAPS-Approved Method*: The CAPS-approved method is a trap and lure combination. The approved trap is a delta trap.

Any of the following Trap Product Names in the IPHIS Survey Supply Ordering System may be used for this target,

1) Paper Delta Trap, 2 sticky sides, Brown
2) Paper Delta Trap, 2 sticky sides, Green
3) Paper Delta Trap, 2 sticky sides, Orange
4) Paper Delta Trap, 3 sticky sides, Orange
5) Large Plastic Delta Trap Kits, Red

The Lure Product Name is "*Lobesia botrana* Lure." The lure is effective for 28 days (4 weeks).

Trap Spacing: When trapping for more than one species of moth, separate traps for different moth species by at least 20 meters (65 feet).

Method Notes: The paper delta trap (with 2 sticky sides) has been added as an approved method. Both the large plastic delta trap (red) and the orange paper delta trap (with 3 sticky sides) are acceptable for use and for data reporting. For 2011 and the foreseeable future, the PPQ Lobesia Program has chosen the 2-sided paper delta trap as the preferred trap for the program. When using the 2-sided trap, the lure should be placed in a lure hanger inside the trap.

The trap color may be decided by the State and does not affect trap efficacy. For the paper delta traps, all of the standard colors used for gypsy moth (brown, green, or orange) are acceptable. Red was the recommended color for the large plastic delta trap as it has been shown to reduce trap catches of non-target (beneficial) insects. Trap color has not been shown to increase or decrease catches of *L. botrana*.

Lure Placement: Placing lures for two or more target species in a trap should never be done unless otherwise noted here.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: From Venette et al. (2003)
Trapping: A sex pheromone has been identified that is highly attractive to males. Males are most attracted to a five component blend of (*E*,*Z*)-(7,9)-dodecadienyl acetate, (*E*,*Z*)-(7,9)-dodecadien-1-ol, (*Z*)-9-dodecenyl acetate, (*E*)-9-dodecenyl acetate and 11-dodecenyl acetate in a ratio of 10: 0.5: 0.1: 0.1: 1. Males are slightly less attracted to a three component blend of (*E*,*Z*)-(7,9)-dodecadienyl acetate, (*E*,*Z*)-(7,9)-dodecadien-1-ol, (*Z*)-9-dodecenyl acetate (ratio of 10:0.5:0.1). Males were still attracted, but much less so, to the main pheromone component (*E*,*Z*)-(7,9)-dodecadienyl acetate. The main pheromone component has been used to disrupt mating as a method of pest control and to monitor the flight period of males. However, this compound is sensitive to sunlight and degrades, becoming non-attractive to *L. botrana* after 60 minutes of exposure to UV radiation.

Pheromone-baited traps (*e.g.*, Pherocon 1C, Zoecon) have been used to monitor male flight activity (Anshelevich et al., 1994) and to make informed treatment decisions in grape production areas. Traps placed 4 ft high (1.3 m) are generally more effective than traps placed at only 1 ft (0.3 m). Delta traps catch relatively fewer moths than traps with a more open design, *e.g.,* traptest traps described as “commercial type (Montedison, Milan, Italy), consisting of two triangular plastic roofs in Havana brown; with a sticky area of 9.89 dm^2 [152 in^2]”. When pheromone traps are used, care should be taken to keep foliage away from the entry to the trap (PPQ, 1993). Rubber septa used to dispense the pheromone should be replaced every 3 weeks (PPQ, 1993). Traps should be placed approximately 100 ft (30.5 m) apart to avoid inter-trap interference.

Visual survey: USDA (1985) suggests visually inspecting for eggs on flower buds or pedicels of vines and grapes. It is preferable to look for larval damage rather than for

eggs, because detection of eggs is very tedious and time-consuming, especially under field conditions. Look for webbed bud clusters (glomerules) or flowers where the spring generation larvae feed. Inspect for pupae under rolled leaves in spring. Inspect grapes and look for eggs or damaged berries. Cut open grapes and search for summer generation larvae (Fig. 5) and pupae. Suspect adult specimens should be pinned and labeled for subsequent identification. Submit suspect larvae or pupae in alcohol. For field surveys, Badenhauser et al. (1999) recommended a sample unit of a grapevine. Sample units should be selected at random.

Not recommended: Light traps have been used, but their lack of specificity and the fact that this moth flies at dusk competing for light makes their use inadvisable when the appropriate pheromones are available. Feeding traps were largely used in the past before pheromone traps were developed, but may still be useful in particular situations. An earthen or glass pot is baited with a fermenting liquid (fruit juice, molasses, etc.), and the scents produced attract adults, which are then drowned. Practical problems include irregularity in trapping because fermentation strongly depends on seasonal temperature, trap maintenance (lure replenishment and foam elimination), and low selectivity.

A corrugated paper band technique has sometimes been employed to trap and quantify overwintering pupae. Bands are placed around grapevine trunks or primary branches, and diapausing larvae pupate inside. However, this method is only useful in the latter generations, and its reliability is uncertain.

Key Diagnostics/Identification

CAPS-Approved Method*: Confirmation of *L. botrana* is by morphological identification. Larvae can be keyed out using Gilligan et al. (2008). Identification of adults requires dissection of the male genitalia; use Brown, (2009) and Passoa, (2009).

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Morphological identification is required for *L. botrana*. Hindwing coloration and the male clasper lacks spine at base (Venette et al. 2003). The key in MacKay (1959), or the simplified version in Passoa (2008), can be used to separate *L. botrana* larvae from many other Olethreutinae in the United States.

A new identification tool, *Tort AI – Tortricids of Agricultural Importance*, is available at http://idtools.org/id/leps/tortai/ from CPHST's Identification Technology Program. This tool contains larval and adult keys, fact sheets, an image gallery, molecular search capacity, and more. *Lobesia botrana* is included in this tool.

Easily Confused Pests

Paralobesia viteana is a native North American pest of grapes with an almost identical larval morphology to *L. botrana* (Gilligan et al., 2011). Pupae of the two species are distinct. The broad cremaster lacking thick curved hooks at the lateral margin, the

presence of spines on A9, the lack of setae on the anal rise and presence of a spine patch on A10 define *Lobesia*. Adults of the two species are similar in size and wing pattern but can be separated by genitalic structures. *P. viteana* has a sclerotized lobe projecting from the ventral base of the male cucullus that is absent in all other Nearctic olethreutines, and the female corpus bursae lacks a signum and has two small lobelike anterior accessory bursae (Gilligan et al., 2011). The two species presently have different distributions: *P. viteana* occurs in the eastern United States, ranging as far west as Colorado; while *L. botrana* is currently restricted to California.

L. botrana can also be confused with *Endopiza viteana* (present in the United States) and *Eupoecilia ambiguella* (not present in the United States). The American grape berry moth, *Endopiza viteana* [*Polychrosis viteana*], occurs in the eastern United States and presents similar bionomics to *L. botrana* (Venette et al., 2003).

In the Palaearctic vine-growing areas, other lepidopteran species have an ecological niche similar to that of *L. botrana*, including *Eupoecilia ambiguella*, *Argyrotaenia pulchellana* [*Argyrotaenia ljungiana*], *Clepsis spectrana*, *Cryptoblabes gnidiella*, *Euzophera bigella,* and *Ephestia parasitella*. Even the primarily phytophagous *Sparganothis pilleriana* may sometimes damage grapes. However, only the first of these, *E. ambiguella*, may cause comparable damage to *L. botrana*, at least in northern European vineyards. Adults of these species may be easily differentiated macroscopically using a photographic key. *E. ambiguella* forewings are cream with a median fascia bluish dark brown. In field conditions, larvae may be distinguished because (i) the head of *E. ambiguella* is darker than that of *L. botrana*; (ii) *L. botrana* larvae do not carry any protective silk cover; and (iii) the behavior of *L. botrana* when disturbed is quicker and even violent. Moreover, *L. botrana* pupation occurs inside a grayish white cocoon that usually does not incorporate vegetal residues and frass, as occurs in *E. ambiguella* (CABI, 2009).

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Rhagoletis cerasi

Scientific Name
Rhagoletis cerasi (Linnaeus)

Synonyms:
Musca cerasi, Rhagoletis cerasi f. obsoleta, Rhagoletis cerasi fasciata, Rhagoletis cerasi nigripes, Rhagoletis cerasi obsoleta, Rhagoletis obsoleta, Spilographa cerasi, Tephritis cerasi, Trupanea cerasi, *Trypeta signata, Urophora cerasorum*, and *Urophora liturata.*

Common Name(s)
European cherry fruit fly

Type of Pest
Fruit fly

Taxonomic Position
Class: Insecta, **Order**: Diptera, **Family**: Tephritidae

Reason for Inclusion in Manual
Requested by the CAPS community – not being surveyed for regularly with fruit fly funding.

Figure 1. Female *Rhagoletis cerasi* on cherry. Photo courtesy of OPIE/ Rémi Coutin.

Pest Description
Larvae: Larvae are up to 6 mm (0.24 in.) long. The body is whitish and translucent (Alford, 2007).

Pupae: Pupae are 3 to 4 mm (0.12 to 0.16 in.) long; pale yellowish brown (Alford, 2007).

Adult: Average length of female 4.6 mm (0.18 in.), of male 3.4 mm (0.13 in.). Mostly black in color. Head yellowish except posteriorly. Apex of antenna sharply pointed dorsally. Thorax mostly black, postpronotum (= humeral callus) and notopleural stripes whitish. Scutellum mostly whitish except base of sides, with two pairs of marginal bristles. Postnotum black. Legs with femora black, tibiae and tarsi yellowish. Wing slightly longer than body, about 4.8 mm (0.19 in.) in female, about 4.0 mm (0.16 in) in male. Wing crossed by four large and one small (intercalary) dark, distinct bands, the apical and subapical bands fused anteriorly, and the medial band isolated. Abdomen blackish, hind margin of segments yellowish. Female with tubular ovipositor sheath and thin elongate, piercing ovipositor apically. Male with tiny genital complex, coiled aedeagus (USDA, 1983).

Biology and Ecology:
Rhagoletis cerasi adults are found from late May to early July and are active in hot, dry conditions (Alford, 2007). Adults must feed to mature sexually (Boller and Prokopy, 1976) and can be found feeding on honeydew and other sugary excretions from aphids (Alford, 2007). Males establish territories on fruit and defend them while awaiting the arrival of females to mate with (Boller and Prokopy, 1976). Females mate two to three days after emergence and begin ovipositing in 7 to 13 days, mainly on mid and late ripening fruit varieties (Grichanov and Ovsyannikova, n.d.). Females begin laying eggs in mid-June and insert them individually beneath the skin of ripening fruit (Alford, 2007). Females can lay an average of 50 to 60 eggs; usually one per fruit (USDA, 1983). Once a female has laid eggs, she will rub her ovipositor over the fruit surface depositing pheromones on the fruit; the pheromones deter other females from ovipositing on the same fruit (Katsoyannos, 1975). *R. cerasi* adults live an average of two weeks (Bush, 1992).

Figure 2. Damage on cherry caused by larvae exit holes of *R. cerasi.* Photo courtesy of OPIE/ Rémi Coutin)

After one to two weeks, eggs hatch (Alford, 2007). Larvae feed on pulp around the pit for approximately four weeks (USDA, 1983; Alford, 2007). Larvae then move to the soil where they pupate beneath the surface of host trees (Fletcher, 1989; Alford, 2007). One generation occurs annually (Alford, 2007). *R. cerasi* overwinters in the pupal stage, which may last from one to three winters (Alford, 2007). Adult emergence in this genus 'is closely synchronized with the fruiting period of their hosts' (Fletcher, 1989).

R. cerasi has two races that are associated with different host plants. The 'southern' race, found in mainland Europe, is a pest of cherry (*Prunus* spp.). The 'northern' race is found in countries north and east of Switzerland and attacks honeysuckle (*Lonicera* spp.) (Alford, 2007). There is unidirectional sterility between these two races. Matings between males of the 'southern' race and females of the 'northern' race result in low egg hatch; whereas, the reverse ('northern' males crossed with 'southern' females) results in normal fertility levels (Boller, 1989).

Pest Importance
R. cerasi is considered a serious pest of cherry in Europe (USDA, 1983; Alford, 2007). Ripening cherries can be destroyed by this species shortly before harvest (USDA, 1983). If infestations are above tolerated limits for table and canning cherries, they may

be used for distillation, which can tolerate higher limits of infestation (USDA, 1983). However, this can reduce the market prices by up to 50% (USDA, 1983).

From 1983 to 1992, the susceptibility of some sweet cherry cultivars was accessed in Cacak (western Serbia); this species was observed 'causing more damage in mid-early and late sweet cherry cultivars' (Stamenkovic et al., 1996). The percentage of damaged fruits in some late cultivars was as high as 80% (Stamenkovic et al., 1996).

Symptoms/Signs

Fruit damaged by the larvae of *R. cerasi* often rots; heavy infestations can reduce marketable yields (Alford, 2007). Damaged cherries darken and commonly fall off of the tree (Grichanov and Ovsyannikova, n.d.). Mature fruit may have soft spots or an off-color, wilted, or shriveled appearance (USDA, 1983). Exit holes left by mature larvae are visible (Fig. 2) (USDA, 1983). Fruit processors may reject consignments of infested harvested cherries (Alford, 2007).

Adults can be observed resting on or flying around foliage or fruit under sunny conditions (USDA, 1983).

Known Hosts

The major host of *R. cerasi* is cherry, but it will attack other host plants. Cherry hosts include: *Prunus avium* (sweet cherry), *P. cerasus* (sour cherry), *P. cerasus* var. *semperflorens* (allsaints' cherry), *P. fruticosa* (European dwarf cherry), *P. glandulosa* (almond cherry), *P. humilis* (bunge cherry), *P. mahaleb* (mahaleb cherry), *P. padus* (European bird cherry), and *P. serotina* (black cherry) (USDA, 1983).

Other hosts include blood-twig dogwood (*Cornus sanguinea*), several types of *Lonicera* spp. (honeysuckle), *Lycium barbarum* (matrimony vine), *Mahonia aquifolium* (hollyleaved barberry), *Symphoricarpos albus* (snowberry), *S. orbiculatus* (coralberry), *S. rivularis* (garden snowberry), and *Vaccinium myrtillus* (whortleberry) (USDA, 1983).

Known Vectors (or associated organisms)

This pest is not currently known to vector any pathogens or other associated organisms.

Known Distribution

Asia: Iran, Kazakhstan, Kyrgyzstan, Republic of Georgia Tajikistan, Turkey, Turkmenistan, and Uzbekistan. **Europe:** Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Moldova, Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Spain, Sweden, Switzerland, and Ukraine (USDA, 1983; White and Elson-Harris, 1992; Barić et al., 2007; EPPO, 2007; CAB International, 2009).

Potential Distribution within the United States

This genus is found throughout the Holarctic and Neotropic regions of the world (Fletcher, 1989).

Sweet cherry is found in the highest density towards the Pacific Coast (California, Oregon, and Washington) as well as the northeastern United States (Maryland, New York, and Pennsylvania) (USDA CPHST, 2009). This crop is also found at higher densities in other miscellaneous states, including Michigan and Idaho (USDA CPHST, 2009). In general, *Prunus* species are most abundant in the eastern part of the United States (USDA CPHST, 2010). A recent risk analysis by USDA-APHIS-PPQ-CPHST indicates that the United States as a whole has a low risk for establishment of R. cerasi based on available hosts and climate, primarily due to low host density.

This species has been intercepted over 30 times in the last 10 years at U.S. ports of entry, all occurring at airports (AQAS, 2010). All interceptions occurred on *Prunus* spp. with the top four final destinations being Florida, California, Illinois, and Michigan (AQAS, 2010).

Survey

CAPS-Approved Method*: The CAPS-approved method is a trap and lure combination. The trap is a yellow sticky card with the "lure," ammonium acetate and protein hydrolysate, embedded in the adhesive. The trap must be the "Sticky Card, Yellow, Baited" trap available through the PPQ Trap and Lure Ordering Database. This trap is effective for 60 days.

IPHIS Survey Supply Ordering System Product Name:
1) Sticky Card, Yellow, Baited

Lure Placement: Do not place lures for two or more target species in a trap unless otherwise recommended.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: The IAEA Trapping Guidelines for Area-wide Fruit Fly Programmes (2003) recommends trapping male and female *R. cerasi* by using ammonium salts in one of three different traps, yellow panel, Rebell, or red spheres. Trap density per km^2 is given and depends on both type of area (production area, marginal, urban, or points of entry) and scenario (monitoring or detection) (IAEA, 2003).

Katsoyannos et al. (2000) found that the Rebell trap with a slow release formulation of ammonium acetate attached to the lower part of the trap was the most effective of all treatments tested, including the McPhail trap. The Rebell trap is a patented trap that 'consists of two yellow plastic, sticky-coated rectangles (15 by 20 cm; 5.91 x 7.87 in.) that cross each other to form a two dimensional trap' (Katsoyannos et al., 2000).

Key Diagnostics/Identification

CAPS-Approved Method*: Morphological. *R. cerasi* can be distinguished from many of the *Rhagoletis* species present in North America by the combination of its predominantly blackish body and its wing pattern.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: *R. cerasi* can be distinguished from the *Rhagoletis* species present in North America by the combination of its predominantly blackish body and its wing pattern, which includes an intercalary band, a small band on the anterior margin near the midlength, and a complete, unforked apical band. The native cherry-infesting species, including *R. cingulata* (cherry fruit fly), *R. indifferens* (western cherry fruit fly), and *R. fausta* (black cherry fruit fly), lack the intercalary band and have the apical band forked or broken into a posterior branch and an apical spot (USDA, 1983; White & Elson-Harris, 1992; Foote et al., 1993).

Easily Confused Pests

R. cerasi is similar to *R. berberidis,* which is currently not found in the United States; keys to differentiate adults of *R. cerasi* and *R. berberidis* and other Eurasian species can be found in Merz (1994), Korneyev and Merz (1997), and Kutuk and Ozaslan (2006). There are three *Rhagoletis* species found in North America that infest cherries: *R. cingulata* (cherry fruit fly), *R. indifferens* (western cherry fruit fly), and *R. fausta* (black cherry fruit fly) (USDA, 1983). *R. cerasi* adults can be distinguished from these other species by their wing patterns; the three species present in the United States lack the intercalary band and have a forked apical band or an apical spot (USDA, 1983; White & Elson-Harris, 1992; Foote et al., 1993).

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Thaumatotibia leucotreta

Scientific Name
Thaumatotibia leucotreta Meyrick

Synonyms:
Cryptophlebia leucotreta and *Thaumatotibia roerigii*

Common Name(s)
False codling moth, citrus codling moth, orange moth, and orange codling moth.

Type of Pest:
Moth

Taxonomic Position
Class: Insecta, **Order:** Lepidoptera, **Family:** Tortricidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2003 through 2012

Pest Description
False codling moth (FCM), *T. leucotreta,* is a pest of economic importance to many crops throughout sub-Saharan Africa, South Africa and the islands of the Atlantic and Indian Oceans (USDA, 2010). The FCM is an internal fruit feeding tortricid that does not undergo diapause and may be found throughout the year in warm climates on suitable host crops. Larval feeding and development can affect fruit development at any stage, causing premature ripening and fruit drop. *T. leucotreta* is a generalist with respect to host plant selection and has been recorded as feeding on over 50 different plant species. The generalist feeding strategy enables survival in marginal conditions as is necessary due to lack of diapause. Important host crops include avocado (*Persea americana*), citrus (*Citrus* spp.), corn (*Zea mays*), cotton (*Gossypium* spp.), macadamia (*Macadamia* spp.), and peach and plum (*Prunus* spp.) (USDA, 1984, 2010).

Figure 1. Larvae of *T. leucotreta.* Photo courtesy of T. Grove and W. Styn. http://www.bugwood.org.

Eggs: Eggs are flat, oval (0.77 mm (0.03 in.) long by 0.60 mm (0.02 in.) wide) shaped discs with a granulated surface. The eggs are white to cream colored when initially laid.

They change to a reddish color before the black head capsule of the larvae becomes visible under the chorion prior to eclosion (Daiber, 1979a).

Figure 2. Adult male false codling moth. Photo courtesy of Todd Gilligan, Colorado State University

Larvae: First instar (neonate) larvae approximately 1 to 1.2 mm (0.04 to 0.05 in.) in length with dark pinacula giving a spotted appearance, fifth instar larvae are orangey-pink, becoming more pale on sides and yellow in ventral region, 12 to 18 mm (0.47 to 0.71 in.) long, with a brown head capsule and first thoracic segment (Fig. 1). The last abdominal segment bears an anal comb with two to seven spines. The mean head capsule width for the first through fifth instar larvae has been recorded as: 0.22, 0.37, 0.61, 0.94 and 1.37 mm (0.009, 0.0015, 0.024, 0.037, and 0.054 in.), respectively (Daiber, 1979b).

Pupae: Prepupa and pupa form inside a lightly woven silk and soil cocoon created by the fifth instar larvae on ground. Length is 8 to 10 mm (0.31 to 0.39 in.) and sexual determination through morphological differences on pupal case is possible (Daiber, 1979c).

Adult: Adults are grayish brown to dark brown with an average forewing length of 7 to 8 mm (0.28 to 0.31 in.) for males and 9 to 10 mm (0.35 to 0.39 in.) for females. Adult body length 6 to 8 mm (0.24 to 0.31 in.), wingspan of female and male moth is 15 to 20 mm (0.59 to 0.79 in.) and 15 to 18 mm (0.59 to 0.71 in.), respectively. The body is brown with a thorax with a posterior double crest. The male forewing is triangular, with an acute apex, while the female forewing is more elongate with a rounded apex. Male (Fig. 2) is distinguished from female by its large, pale grayish genital tuft, large dense grayish white brush hindlegs, and its heavily tufted hind tibia (Gunn, 1921; Couilloud, 1988; CABI, 2009; Gilligan, 2011). Males also have a semicircular pocket of opalescent scales on the distal end of vein CuA2 on the distal end of vein CuA2 on the hindwing. This character can be used to separate *T. leucotreta* males from all other North American tortricids (Gilligan, 2011).

Forewing pattern can vary between individuals, especially in males where forewing color and pattern expression is not as consistent as in females. Most individuals exhibit a combination of four forewing pattern elements: a small white dot near the end of the

discal cell; a patch of raised, usually rust colored scales near the middle of the wing; a distinct 'question-mark-shaped' band of dark scales along the termen; and a semi-circular band of dark scales in the middle of the costa (Gilligan, 2011).

Male genitalia (Fig. 3) have a rounded tegumen lacking an uncus or socii, large rounded valvae, and a tapered aedeagus that is upcurved distally. Female genitalia (Fig. 4) have a semicircular sterigma, narrow ductus bursae, and large rounded corpus bursae with a pair of thorn-shaped signa (Gilligan, 2011).

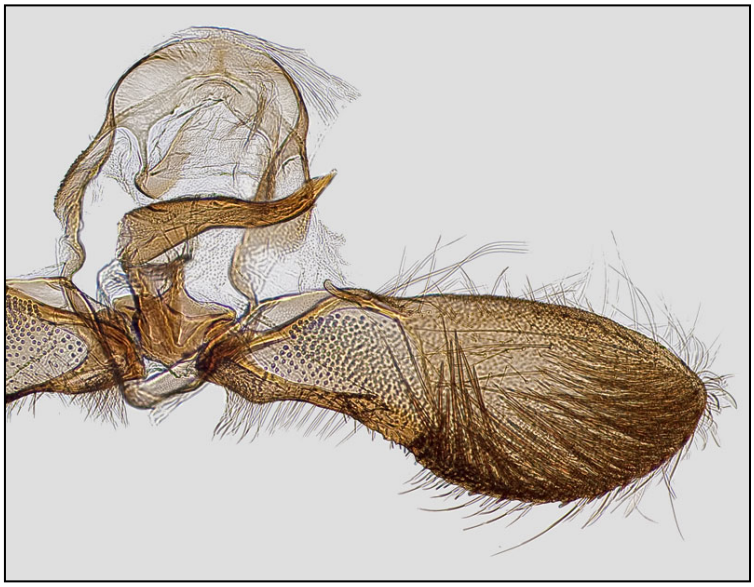

Figure 3. Male genitalia of *T. leucotreta.* Photo courtesy of Todd Gilligan, Colorado State University.

Biology and Ecology

In South Africa, FCM has four to six non-discrete generations per year (Georgala, 1969; Stofberg, 1954). Mated female moths fly at night, depositing eggs, singly or in bunches, on suitable hosts between 5:00 p.m. and 11:00 p.m. Females lay individual eggs (100 to 250 per female) on fruit or foliage (Catling and Aschenborn, 1974; Daiber, 1978). There are reports of females laying up to 800 eggs over their lifespan at optimal temperatures (USDA, 2010). Females lay eggs at random in depressions of the rind of host fruit; on smooth, non-pubescent surfaces; on fallen fruit; or on foliage. Females tend to oviposit on prematurely ripened fruit or wounded fruit when compared to healthy fruit at a normal state of development (Newton and Mastro, 1989).

Figure 4. Female genitalia of *T. leucotreta.* Photo courtesy of Todd Gilligan, Colorado State University.

Egg development requires two to 22 days depending on temperature. Eggs are extremely sensitive to cold temperatures and extended periods of low humidity. Temperatures below freezing over a two to

three day period can kill eggs (Blomefield, 1978; Daiber, 1979a). Daiber (1980) showed that *T. leucotreta* adults live longest at 15°C (59°F) while most eggs were laid at 25°C (77°F). Egg laying at 20 and 25°C (68 and 77°F) increased rapidly soon after the first egg was laid but only gradually at 15°C to reach peak numbers sometime after the initial egg lay. Very few eggs were laid at 10°C (50°F).

Upon hatching, neonate larvae penetrate the fruit where larval development is completed. Larvae wander before gnawing through the rind of the host and make burrows about 1 mm (0.04 in.) in diameter. The entrance is conspicuous due to the presence of frass and discoloration of the surrounding rind. If the host has a hard rind, such as an acorn, entrance is made at the base or attachment to the cup where softer tissue exists. When the host has a soft rind, such as citrus or peach, the larvae will burrow into the rind almost anywhere. Larvae prefer the navel end, or an injured area or cut in the rind. In some rinds, such as avocado, the entrance is marked by formation of a raised crater
(USDA, 2010).

The larval period lasts 12 to 33 days in warm weather and 35 to 67 days in cool weather; there are five instars. Younger larvae feed near the surface; older larvae bore toward the center. Generally, only one to three larvae per fruit survive. Temperature and poor food quality can slow down the rate of larval development (USDA, 2010). By the time the larva is ready to leave the fruit, the fruit might have dropped. Mature larvae leave the fruit and spin cocoons near the soil or in bark crevices. Diapause or a resting stage has not been recorded (USDA, 2010).

Males live 14 to 57 days; females survive 16 to 70 days. Dispersal normally is limited to several hundred meters. Moth activity increases with the onset of host flowering. Females call males through pheromone release starting several hours after dark, peaking 5 hours later, and dropping off rapidly thereafter until daylight. Adults can mate several times per day (USDA, 2010).

Symptoms/Signs

In general, the habit of internal feeding by FCM larvae displays few symptoms. Emerging larvae bore into the albedo and usually feed just below the fruit surface. Cannibalism among young larvae ensures that usually only one caterpillar matures in each fruit. When full-grown the larvae bore their way out of the fruit to seek a site for pupation. The rind around the point of infestation takes on a yellowish-brown color as the tissue decays and collapses. Larval feeding and development can affect fruit development at any stage, causing premature ripening and fruit drop.

Stone Fruit: All stages of stone fruits are vulnerable to attack. False codling moth larvae are capable of developing in hard green fruit before control measures can be started. Once a fruit is damaged, it becomes vulnerable to fungal organisms and scavengers. Larvae damage stone fruits as they burrow into the fruit at the stem end and begin to feed around the stone. Infestations can be identified by the brown spots and dark brown

frass (Daiber, 1976). On peaches, eggs are almost always laid on the upper surface of peach leaves (USDA, 2010).

Avocado: Moths lay eggs superficially on the fruit of avocado. Larvae hatch, develop, and can enter through the skin. Larvae are unable to develop in avocado fruit. However, their entrance creates lesions that lessen the marketability of fruit. Lesions develop into a raised crater on the fruit surface, with an inconspicuous hole in the center where the larva has entered. Granular excreta can also be seen (USDA, 2010).

Citrus: All stages of citrus fruit are vulnerable to attack. FCM larvae are capable of developing in hard green fruit before control measures can be started. Once a fruit is damaged, it becomes vulnerable to fungal organisms and scavengers. There is sometimes a scar visible on infested fruit (USDA, 2010). On oranges, look for a brown patch on the skin, usually with evidence of a hole bored in the center, sometimes with a dark brown frass exuding from the hole. Oranges or other citrus can also drop fruit prematurely.

Cotton: FCM feeds mainly on large green bolls. The younger larvae feed almost entirely inside the boll wall itself, but the older larvae penetrate the inner septum and feed on the developing seeds and lint (Reed, 1974). Larval penetration of cotton bolls facilitates entry of other microorganisms that can rot and destroy the boll. The cultivars Edranol, Hass and Pinkerton were the most susceptible to attack by FCM (USDA, 2010).

Corn: Larvae damage corn by entering the ear from the husk through the silk channel (USDA, 2010). Larvae can be found in the corn stem as well (Reed, 1974). On corn, *T. leucotreta* has been reported laying eggs on the husk of the ear.

Grape: Fresh larval penetration holes in grapes can be seen, but require careful inspection of the fruit. Sometimes a few granules of frass can be found around a fresh penetration hole or a mass of frass may be found around older penetration holes. Other times, however, frass is not visible. The area around the penetration hole can become sunken and brown as damaged tissue decays (Johnson, date not known).

Macadamia: Larvae damage the nuts by feeding on the developing kernel after they pierce the husk and shell. Nuts reaching 14 to 19 mm (0.55 to 0.75 in.) diameter size are at the most risk because nutrient content is the greatest; concurrently, false codling moth reaches the adult stage by this point and is able to oviposit on the nuts (USDA, 2010).

Pest Importance

In Africa, FCM is a major pest of citrus and cotton. In the Citrusdal Valley region of South Africa, FCM caused from 2.9 to 15.2% crop loss on citrus depending on the farm and the pest control program (Schwartz and Anderson, 1983). In Ugandan cotton, boll rotting is a major cause of crop loss. Over 90% of rotten bolls had insect attack symptoms and at least 60% of those were caused by *T. leucotreta* (Reed, 1974).

Approximately 44% of corn cobs examined contained larvae of *T. leucotreta* in Uganda (Reed, 1974).

According to CDFA (2008), commonly grown agricultural hosts in California for FCM include citrus, grapes, peach, plum, cherry, beans, tomato, pepper, persimmon, apricot, olive, pomegranate, English walnut, and corn. Based on its status as a pest in Africa, establishment of FCM in California and/or in other parts of the United States could result in significant economic losses. FCM would likely be a significant production and quarantine issue for numerous agricultural commodities. In California alone, the annual combined gross value of the top ten agricultural commodities which would be directly impacted by this pest is over $7.1 billion, which amounts to 22% of the total agricultural value for the State (USDA NASS, 2007).

Peaches become susceptible to damage about six weeks before harvest. Detecting infested peaches can be difficult if the fruit is still firm and abscission has not occurred; consequently, the danger of selling potentially infested fruit poses a serious threat to the peach industry (Daiber, 1976; USDA, 1984, 2010).

Hofmeyr and Pringle (1998) report resistance in FCM to the chitin synthesis inhibitor trifluron, commonly used for FCM control.

Known Hosts

Major Hosts:
Abelmoschus esculentus (okra), *Abutilon hybridum* (flowering maple), *Abutilon* × *hybridum* (Chinese lantern), *Ananas comosus* (pineapple), *Averrhoa carambola* (carambola), *Camellia sinensis* (tea), *Capsicum* spp. (peppers), *Citrus* spp., *Coffea arabica* (coffee), *Gossypium* spp. (cotton)*, Litchi chinensis* (litchi), *Macadamia spp.* (macadamia)*, Mangifera indica* (mango), *Olea* spp. (olive), *Persea americana* (avocado)*, Prunus armeniaca* (apricot), *Prunus domestica* (plum), *Prunus persica* (peach), *Prunus* spp. (stone fruit), *Psidium guajava* (guava), *Punica granatum* (pomegranate), *Quercus* spp. (oak, acorns), *Ricinus communis* (castor bean), *Sorghum bicolor* (sorghum), and *Zea mays* (corn).

Minor/Wild hosts:
Abutilon spp. (Indian mallow), *Acacia nilotica* (acacia), *Acacia tortilis* (umbrella thorn)*, Annona cherimola* (cherimoya), *Annona glabra* (pond apple), *Annona muricata* (soursop), *Annona reticulata* (Bullock's heart, custard apple), *Annona squamosa* (sugar apple), *Azanza garckeana* (snot apple), *Bauhinia galpinii* (red bauhinia), *Bequaertiodendron magalismontanum* (stamvrug), *Butyrospermum parkii* (shea butter tree), *Caesalpinia pulcherrima* (pride-of-Barbados), *Caesalpinia* spp. (nicker)**,** *Calotropis procera* (sodom apple), *Capparis tomentosa* (African caper), *Carya illinoensis* (pecan), *Cassia petersiana* (monkey pod), *Catha edulis* (khat), *Ceiba pentandra* (kapok), *Chrysophyllum cainito* (star apple), *Chrysophyllum palismontatum* (stamvrugte), *Cola nitida* (bitter cola), *Combretum apiculatum* (apiculatum)**,** *Combretum apiculatum* (rooibos), *Combretum zeyheri* (raasblaar), *Cyphomandra betacea* (tree tomato), *Diospyros mespiliformis* (Jjakkalsbessie), *Diospyros* spp. (persimmon), *Englerophytum*

magalismontanum, Eriobotrya japonica (loquat), *Eugenia uniflora* (Surinam-cherry), *Ficus capensis* (wild fig), *Flacourtia indica* (governor's-plum), *Garcinia mangostana* (mangosteen), *Harpephyllum caffrum* (kaffir-plum), *Hibiscus cannabinus* (kenaf), *Hibiscus spp.* (hibiscus), *Juglans regia* (English walnut), *Juglans* spp. (walnut), *Solanum (Lycopersicon) esculentum* (tomato), *Mimusops zeyheri* (Transvaal red milkwood), *Musa paradisiaca* (banana), *Pennisetum purpureum* (elephant grass), *Phaseolus lunatus* (lima bean), *Phaseolus* spp. (bean), *Physalis ixocarpa* (tomatillo) *Physalis* spp. (groundcherry), *Piper* spp. (pepper), *Podocarpus falcatus* (yellowwood), *Podocarpus* spp. (plum pine), *Pseudolachnostylis maprouneifolia* (kudu-berry)**,** *Royena pallens* (pale-branched Royena), *Saccharum officinarum* (sugarcane), *Schotia* spp. (boerboon), *Sclerocarya birrea* (marula), *Sechium edule* (chayote), *Sida* spp. (fanpetals), *Solanum melongena* (eggplant)**,** *Synsepalum dulcificum* (miraculous berry), *Syzygium cordatum* (waterbessie), *Syzygium jambos* (rose-apple), *Theobroma cacao* (cacao),*Triumfetta* spp. (bur weed), *Vangueria infausta* (wild medlar), *Vigna* spp. (cowpea), *Vitis* spp. (grape), *Xeroderris stuhlmannii* (wing bean), *Ximenia caffra* (suurpruim), *Yucca* spp. (yucca), and *Ziziphus* spp. (jujube).

Known Vectors (or associated organisms)

T. leucotreta is not a known vector and does not have any associated organisms. The wounds produced by *T. leucotreta*, however, can provide an entrance for pathogens and can damage host plants under humid conditions.

Known Distribution

False codling moth is indigenous to Southern Africa and the Ethiopian region. It also occurs on the islands of Madagascar, Mauritius, Reunion, and St. Helena.

Africa: Angola, Benin, Burkina Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Congo, Dahomey, Eritrea, Ethiopia, Gambia, Ghana, Ivory Coast, Kenya, Madagascar, Malawi, Mali, Mauritius, Mozambique, Niger, Nigeria, Nyasaland, Réunion, Rhodesia, Rwanda, Saint Helena, Senegal, Sierra Leone, Somalia, South Africa, Sudan, Swaziland, Tanganyika, Tanzania, Togo, Uganda, Upper Volta, Zaire, Zambia, Zanzibar, and Zimbabwe. **Asia:** Israel.

False codling moth has occasionally been found in Europe, where it was imported with produce from Africa (Bradley et al., 1979; Karvonen, 1983). Border inspections have intercepted false codling moth in Denmark, Finland, Netherlands and United Kingdom; the countries have remained free of the pest (USDA, 2010).

Potential Distribution within the United States

Infestation by FCM generally causes the fruit to drop before harvest. Larval entries, however, can take a few days to become visible. Those that occur near fruit harvest, therefore, are often not detected by the packing house fruit graders and infested fruit can be inadvertently packaged for export.

Increased international trade and tourism between the United States and many African countries in recent years has increased the risk of introduction of this pest. Since 1984,

FCM has been intercepted over 1500 times on 99 plant taxa at 34 U.S. ports of entry. In June 2005, live FCM caterpillars were found at California's border stations inside previously cold treated Clementine citrus from South Africa. Its discovery in California is a new record for the Americas. FCM is not known to be established in California. On June 16, 2005, California Department of Food and Agriculture (CDFA) inspectors found 1 live and 1 dead larva on a shipment of South African clementines at the California border station in Needles. The larvae were identified by both a CDFA lab and the USDA Systematic Entomology Laboratory (SEL) specialist as false codling moth. The fruit had entered the United States in the port of Philadelphia (PA) off the vessel Nova Zembla. Initial review of the cold treatment records did not reveal failures in the treatment. On June 20, a second live larva was intercepted on a separate shipment of South African clementines in California. This shipment came on the vessel Fuji Star on June 14, 2005. This larva was identified by CDFA as FCM. An eradication program would be triggered if two moths were detected within one life cycle and within three miles of each other, or a mated female was found, or any immature stage (egg, larva, or pupa) was found. FCM has not triggered an eradication project in California at this time. Survey using traps and some fruit sampling is continuing around the Ventura County find.

A recent risk analysis by USDA-APHIS-PPQ-CPHST shows that portions of Alabama, Arkansas, California, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Texas are at the greatest risk from *T. leucotreta*. Establishment of *T. leucotreta* is precluded in the northern United Stated based on climate and host range.

Survey

CAPS-Approved Method*: The CAPS-approved method is a trap and lure combination. The approved trap is a wing trap.

Either of the following Trap Product Names in the IPHIS Survey Supply Ordering System may be used for this target:

1) Wing Trap, Paper
2) Wing Trap, Plastic

The Lure Product Name is "*Thaumatotibia leucotreta* Lure." The lure is effective for 56 days (8 weeks).

Trap Spacing: When trapping for more than one species of moth, separate traps for different moth species by at least 20 meters (65 feet).

Method Note: The wing trap and the diamond trap are both effective traps for *Thaumatotibia leucotreta*. In order to standardize data and trap procurement, it is preferable that states use the wing trap. However, if states find reason to use the diamond trap, it is acceptable for negative data reporting. Diamond traps will not be available through the IPHIS Survey Supply Ordering System.

Lure Placement: Placing lures for two or more target species in a trap should never be done unless otherwise noted here.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

For early detection surveys in stone fruit, fields in close proximity to high risk areas such as citrus should be monitored utilizing pheromone traps. The pheromone traps should be placed at a frequency of 1 trap per 4 hectares and traps should be no closer than 150 to 200 m (492 to 656 ft.) to each other. Traps should be inspected weekly. Other primary hosts should also be inspected visually for the presence of FCM during the growing season. The first four rows bordering citrus or stone fruit orchards should be examined carefully.

Trapping: Male *T. leucotreta* are attracted to a two component blend of (*E*)-8-dodecenyl acetate and (*Z*)-8-dodecenyl acetate. These components are most effective when used in a ratio between 70:30 and 30:70 (*E*:*Z*) (Persoons et al., 1977; Venette et al., 2003). More recently, Newton et al. (1993) refined the sex pheromone and reported that a 90:10 ratio was optimal. USDA (2010) recommends utilizing a 50:50 ratio. Burger et al. (1990) showed that 7-vinyldecyl acetate, a by-product of the synthesis of one of the constituents of the pheromone blend, effectively disrupts the attraction of the male moths to virgin females or to synthetic lures.

A loading rate between 0.5 and 1.0 mg per septum was found to attract the greatest number of males. The pheromone blend (1 mg applied to a rubber septum) has been used effectively with Pherocon 1C traps to capture male *T. leucotreta* (Newton et al., 1993). Delta traps have also been used, but these have performed less well than either the Hoechst Biotrap or Pherocon 1C traps. Traps using closed polyethylene vials to dispense pheromones captured more moths than traps using rubber septa (using a 50:50 blend of (*E*)- and (*Z*)-8-dodecenyl acetate). Lures should be replaced every 8 weeks. Traps should be placed approximately 1.5m (5 ft.) high. Hofmeyr and Burger (1995) developed a prototype controlled release dispenser that was capable of releasing sex pheromone without replacement for more than seven months. Pheromone traps (homemade sticky trap with unspecified pheromone blend) have been used to monitor the number of *T. leucotreta* adult males in citrus orchards (Daiber, 1978) and detect the presence of the pest in peach orchards (Daiber, 1981).

Pheromone lures with (*E*)- and (*Z*)-8-dodecenyl acetate may also attract *Cydia cupressana* (native), *Hyperstrotia spp*., *Cydia atlantic*a (exotic), *Cydia phaulomorpha* (exotic) and *Cryptophlebia peltastica* (exotic).

Visual survey: Visual inspections of plant materials may be used to detect eggs, larvae, and adults of *T. leucotreta* (USDA, 1984). Look for plants showing signs of poor growth or rot; holes in fruit, nuts or bolls; adults hidden in foliage; and crawling larvae. Surveys are best conducted during warm, wet weather when the population of the pest increases (USDA, 1984). Eggs will commonly be found on fruits, foliage, and occasionally on branches (USDA, 1984). However, eggs are small and lay singly, which makes them

difficult to detect. On corn, *T. leucotreta* has been reported laying eggs on the husk of the ear.

Fruit should be inspected for spots, mold, or shrunken areas with 1 mm (0.04 in.) exit holes in the center. On citrus fruits and other fleshy hosts, dissections are needed to detect larvae; larvae are likely to be found in the pulp (USDA, 1984). Infested fruits may be on or off the tree. In cotton, older larvae may be found in open bolls and cotton seed (USDA, 1984). Occasionally adults may be observed on the trunk and leaves of trees in infested orchards (USDA, 1984). For field crops, such as corn, the whole plant is the recommended sample unit. Because larvae of *T. leucotreta* have a strongly aggregated spatial distribution among corn plants, a large sample size (>60 plants) is recommended; however at low densities of the pest (<1 larva/plant) sample sizes needed to detect the pest may be prohibitively large.

Soil Sampling: Collect soil samples within 183 meters (200 yards; 600 ft.) of any larval or egg detection and at any spot where dropped, especially prematurely dropped, fruit occur. Soil samples should consist of loose surface soil and any debris. Examine soil for larvae, cocoons and pupae.

Not recommended: Robinson black light traps are ineffective at attracting adult *T. leucotreta* (Begemann and Schoeman, 1999). Therefore, black light traps should not be used. The effectiveness of black light traps may be improved if used in conjunction with pheromone lures (Möhr, 1973). Möhr (1973) speculates that pheromone may provide a long-distant attractant, but that attraction to black light becomes much stronger when moths are in close proximity to light traps.

Key Diagnostics

CAPS-Approved Method*: Morphological. Confirmation of *T. leucotreta* is by morphological identification of adult specimens.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Male *T. leucotreta* can be distinguished from other tortricids by several secondary sexual characters which include a modified hindwing with a circular pocket of opalescent scales and an enlarged apical spur on the hind tibia with a large tuft of scales.

Female *T. leucotreta* lack the male secondary sexual characters and are slightly larger with more elongate forewings. The female genitalia are characterized by a semicircular sterigma, narrow ductus bursae, and large rounded corpus bursae with two thorn-shaped signa.

Mature larvae are similar to those of other Grapholitini, including many species of *Cydia*, *Grapholita*, and *Cryptophlebia*. A tool for identifying larvae of leafrollers and a job aid is provided in Appendix D and Appendix E, respectively, of the New Pest Response

Guideline to False Codling Moth (available at http://www.aphis.usda.gov/import_export/plants/manuals/emergency/downloads/nprg-fcm.pdf). The job aid from Appendix E is also available at http://caps.ceris.purdue.edu/webfm_send/544. Stofberg (1948) provides a detailed description of larval structures that distinguish FCM from other larvae.

See the Padil website for additional FCM images, including diagnostic characters (http://www.padil.gov.au/viewPestDiagnosticImages.aspx?id=314).

A new identification tool, *Tort AI – Tortricids of Agricultural Importance*, is available at http://idtools.org/id/leps/tortai/ from CPHST's Identification Technology Program. This tool contains larval and adult keys, fact sheets, an image gallery, molecular search capacity, and more. *Thaumatotibia leucotreta* is included in this tool.

Easily Confused Pests

Male *T. leucotreta* are unlikely to be confused with any native North American tortricid. Females may appear superficially similar to other Grapholitini and a genitalic dissection may be necessary to confirm identity.

In West Africa, *T. leucotreta* is often found in conjunction with *Mussidia nigrevenella* (pyralid moth). In South Africa, the host ranges for *T. leucotreta, T. batrachopa* (macadamia nut borer), and *Cryptophelbia peltastica* (litchi moth) overlap, most notably on litchi and macadamia (Venette et al., 2003; USDA, 2010). Genitalia or male secondary sexual characters can be used to separate *T. leucotreta* from these other species.

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Secondary Pests of Stone Fruit (Truncated Pest Datasheet)

Aleurocanthus spiniferus

Scientific Name
Aleurocanthus spiniferus Quaintance

Synonyms:
Aleurocanthus citricolus, Aleurocanthus rosae, Aleurocanthus spiniferus var. *intermedia Aleurodes citricola,* and *Aleurodes spinifera.*

Common Name
Orange spiny whitefly, citrus mealy wing

Type of Pest
Whitefly

Taxonomic Position
Class: Insecta, **Order**: Hemiptera, **Family:** Aleyrodidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2006 through 2009; Additional Pests of Concern List- FY2012

Pest Description
Aleurocanthus spiniferus is a whitefly pest that is not known to occur in the continental United States. The pest has been reported to occur in Guam and Hawaii. Although *A. spiniferus* is primarily a pest of citrus and rose, it has been reported to occur on other hosts including peach (*Prunus persica*), black cherry (*Prunus serotina),* pear (*Pyrus* spp.), grape (*Vitis vinifera*), and guava (*Psidium* spp.) (EPPO, 1997; Jeffers, 2009; Gyeltshen et al., 2011).

Figure 1. *A. spiniferus* eggs and nymphs. Nymphal instars 1, 2, and 4 (pupae, 1.2 mm in length). The white, waxy filaments are typical of the species. Photo courtesy of CABI, 2009.

Whiteflies have six developmental stages: egg, crawler (1st instar), two sessile nymphal instars (2nd and 3rd instars), the pupa (4th instar) and adult. Identification of the Aleyrodidae is largely based upon characters found in the pupal (4th instar) stage (Gyeltshen et al., 2011).

Eggs: The elongate-oval eggs (0.2 mm; 0.008 in. long) are yellow when first laid and then darken to charcoal gray or black; each is attached to the leaf by a short pedicel.

Figure 2. Adult *A. spiniferus*. Photo courtesy of M.A. van den Berg. http://www.bugwood.org.

Larvae: The six-legged, dusky, elongate first-instar larvae [0.3 x 0.15 mm (0.01 x 0.006 in.] have two long and several shorter, slender dorsal glandular spines. All subsequent immature stages are sessile, have non-functional leg stubs, and possess numerous, dark dorsal spines on which a stack of exuviae of earlier instars may occur. The second instar [0.4 x 0.2 mm (0.016 x 0.008 in.)] is a dark brown to charcoal convex disc with yellow markings, while the third instar [0.87 x 0.74 mm (0.034 x 0.029 in.)] is usually black with a rounded, greenish spot on the anterior part of the abdomen and obvious dorsal spines. In the fourth immature stage or 'pupa', females are larger (1.25 mm (0.05 in.) long) than males (1 mm (0.04 in.) long). This stage is black, has numerous dorsal spines, and is often surrounded by a white fringe of waxy secretion (Fig. 1). **This is the stage required for identification purposes.**

Figure 3. Sooty mold on citrus leaves. Photo courtesy of M.A. van den Berg. http://www.bugwood.org.

Adults: Winged; the females (1.7 mm (0.07 in.) long) are larger than the males (approximately 1.33 mm (0.05 in.) long). The wings are dark gray at ecdysis (Fig. 2), sometimes developing a metallic blue-gray sheen later; lighter markings on the wings appear to form a band across the insect. The body is orange to red initially; the thorax darkens to dark gray in a few hours. The limbs are whitish with pale yellow markings.

Symptoms/Signs

Both adults and larvae damage plants.

Primarily, orange spiny whitefly affects host plants by sucking the sap but it also causes indirect damage by producing honeydew and subsequently promoting the growth of sooty mold. Sticky honeydew deposits accumulate on leaves and stems and usually develop black sooty mold fungus (Fig. 3), giving the foliage (even the whole plant) a sooty appearance. Sooty mold can lead to reduction of respiration and photosynthesis interfering with normal leaf function (USDA, 1982). In heavy infestations, sooty mold can develop on the fruit, lowering the quality (USDA, 1982). Ants may be attracted by the honeydew.

Inspect for spiral egg masses and larvae on the underside of leaves. The colorful adult may be found on tender terminal growth (USDA, 1982). Infested leaves may be

distorted. The insects are most noticeable as groups of very small, black spiny lumps on leaf undersides.

Heavy infestations can lead to loss of vitality and eventually tree mortality if left unchecked (USDA, 1982).

Survey

CAPS-Approved Method*: The CAPS-approved survey method is a visual survey.

Note: A trap and lure combination has been described in the literature but this method has not been fully evaluated. A sex pheromone has been identified for this species and is used in conjunction with a yellow sticky card for control and monitoring in China (Wang, personal communication; Zhang et al., 2010). The lure components are not described in the literature and the only sources for the lure are Chinese companies (Wang, personal communication). This lure is not currently available for purchase through the PPQ Trap and Lure Ordering Database. The pheromone is being investigated by other PPQ programs. More information will be posted here when it is available.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Surveys should be focused where the greatest risk for establishment occurs. Based on the current distribution of *A. spiniferus* in Africa, Asia, Europe, North America, and Oceania, the pest may be able to establish in U.S. Plant Hardiness Zones 8 to 12 (Jeffers, 2009). The pest could possibly become a pest in heated glasshouses in other areas in the United States (EPPO, 1997). A recent host analysis by USDA-APHIS-PPQ-CPHST indicates that many states in the United States have a low to moderate risk from *A. spiniferus* establishment based on host availability. Areas of Texas and Florida, however, have the greatest risk for establishment. Alabama, Arkansas, Georgia, Louisiana, Mississippi, have the greatest risk of *A. spiniferus* establishment.

Trapping: In China, *A. spiniferus* is trapped for control purposes using a sex pheromone and yellow sticky card (Wang, personal communication; Zhang et al., 2010).

Visual: *Aleurocanthus spiniferus* is most often found on citrus and roses. Examine plants, especially shrubs or trees, closely for signs of sooty mold or sticky honeydew on leaves, stems, and fruit, or for ants running about. A heavy infestation gives trees an almost completely black appearance. Sooty mold can reduce respiration and photosynthesis of plants and can cause plants and fruit to become unsightly and unsalable (EPPO, 1997). Foliage that is badly contaminated may drop while fruit set may be reduced with heavy infestations (EPPO, 1997). Look for distorted leaves with immature stages of *A. spiniferus* on the undersides. Larvae often form dense colonies with up to several hundred larvae on one leaf (EPPO, 1997).

Leaves infested by *A. spiniferus* are mainly found on the lower parts of the host plants with infested leaves tending to be aggregated on the plant (EPPO, 2002). Good light conditions are essential for detection; in poor light, a powerful flashlight is helpful. A large hand lens may be necessary to aid in recognition of the dorsal spines on immature stages (CABI, 2009).

Surveys may be carried out at any time of the year, however adults will not be found during winter (USDA, 1982). Adults may periodically be found on the terminal growth (USDA, 1982), and are active fliers when disturbed (EPPO, 1997).

Movement by this species is dependent on movement of host plants as adult flight is not a major means of long-range dispersal (EPPO, 1997). Spread can occur on movement of nursery stock and infested fruits (Gyeltshen et al., 2011). This species has been intercepted several times over the years at United States ports of entry. Most interceptions occurred on host plant leaves in baggage (AQAS, queried May 5, 2011).

Key Diagnostics/Identification

CAPS-Approved Method*: Confirmation of *A. spiniferus* requires a morphological identification. *A. spiniferus* is similar to many other species within the *Aleurocanthus* genus. Several similar species of *Aleurocanthus* also occur on citrus, including *A. citriperdus* and *A. woglumi*. These species differ from each other only in microscopic characters of the “pupa” (fourth instar) and require expert preparation and identification to distinguish them reliably. The main characteristic difference between orange spiny whitefly and citrus black fly, *A. woglumi* that can be observed in the field, is that the white wax fringe that surrounds their pupal case margins is generally twice as large for the orange spiny whitefly.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:
A diagnostic protocol for positively identifying *A. spiniferus* through examination of the pupal case can be found in EPPO (2002).

Molecular diagnostics to differentiate *A. spiniferus* from closely related whitefly species have recently been studied (Xun et al., 2009).

Additional images are available at
http://www.eppo.org/QUARANTINE/aleurocanthus_spiniferus_IT/first_record.htm.

Easily Confused Pests

A. spiniferus is similar to many other species within the *Aleurocanthus* genus.

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Archips xylosteanus

Scientific Name
Archips xylosteanus Linnaeus

Synonyms:
Archips xylosteana, Cacoecia xylosteana var. *pallens, Phalaena Tortrix xylosteana, Phalaena Tortrix desana*, *Pyralis hybernana, Pyralis obliquana*, *Tortrix characterana*, and *Tortrix westriana.*

Common Name
Variegated golden tortrix, apple leaf roller, brown oak tortrix moth, twist moth forked red barred moth

Type of Pest
Moth

Taxonomic Position
Class: Insecta, **Order:** Lepidoptera, **Family:** Tortricidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2008 through 2012

Pest Description
The larvae of *Archips xylosteanus* feed on the foliage of numerous trees and woody plants, particularly in the Roseaceae, including *Prunus apetala* (wild cherry), *Prunus armeniaca* (apricot), *Prunus avium* (sweet cherry), *Prunus grayana* (wild cherry), *Prunus persica* (peach), and *Prunus verecunda* (wild cherry) (Hrdý et al., 1979; Safonkin, 1998; Konno, 2005; CABI, 2006).

Figure 1. Larva of *Archips xylosteanus*. Picture courtesy of Fabio Stergulc, Università di Udine, http://www.bugwood.org/.

Eggs: Cylindrical, greenish eggs are deposited in oval masses on the trunks or branches of various trees and shrubs and are variable in size (3 x 7 to 4.5 x 10 mm; 0.12 x 0.28 to 0.18 x 0.39 in.).

Eggs are covered with a brown secretion that camouflages them against the bark (Bradley et al., 1973; Schall, 2006).

Larvae: The larvae (Fig. 1) are 16 to 22 mm (0.63 to 0.87 in.) long; abdomen whitish gray varying to dark bluish gray, paler or whitish laterally with black or light gray pinacula. The larvae have a shiny black head. The prothoracic plates are dark brown or black, with a whitish mid-lines and collars; setae whitish; anal plate black or blackish brown; anal combs are present; thoracic legs black; prolegs green dotted with black (Bradley et al., 1973; Meijerman and Ulenberg, 2000; Schall, 2006).

Figure 2. Adult moth of *A. xylosteanus*. Picture courtesy of Milan Zubrik, Forest Research Institute, Slovakia, http://www.bugwood.org/.

Pupae: Pupae are 9 to 12 mm (0.35 to 0.47 in.), although some sources indicate 11 to 12 mm (0.43 to 0.47 in.), in length, dark-brown or black in color with elongate cremasters (Bradley et al., 1973; Beeke and De Jong, 1991; Schall, 2006).

Adults: No clear sexual dimorphism (Bradley et al., 1973; Toimil, 1987). Variation in coloration and forewing markings; forewings whitish ochreous with ochreous brown or reddish brown (Fig. 2), pale edged markings; a black-brown dot at disc; subterminal marking pistol-shaped in males. Hindwings grayish-brown (Meijerman and Ulenberg, 2000). The head and thorax are light purplish-brown and the antennae are simple and filiform (Schall, 2006).

Figure 3. Leaf rolling symptom on oak from *A. xylosteanus*. Picture courtesy of Milan Zubrik, Forest Research Institute, Slovakia, http://www.bugwood.org/.

Male: Wingspan 15 to 21 mm (0.59 to 0.83). Forewing ground color whitish ochreous, partially suffused with olive-gray; markings reddish brown, thinly edged with clear ground color; inner margin of

median fascia sinuate, pre-apical spot semi-ovate, usually contiguous with stria-like marking to tornus (Bradley et al., 1973).

Female: Wingspan 16 to 23 mm (0.63 to 0.91 in.). Forewing ground color as in male; markings less reddish, often darker. Hindwing gray, apical area sometimes tinged with yellow or cupreous (Bradley et al., 1973).

Symptoms/Signs

Archips xylosteanus larvae may cause significant defoliation by feeding on foliage and buds of deciduous trees and shrubs (Spears, 2006). Developing larvae will roll leaves to create protected feeding sites. Larvae may disfigure host plants, although feeding is confined mainly to fully expanded leaves.

Survey

CAPS-Approved Method*: The CAPS-approved method is a trap and lure combination. The approved trap is a wing trap.

Any of the following Trap Product Names in the IPHIS Survey Supply Ordering System may be used for this target:

1) Wing Trap Kit, Paper
2) Wing Trap Kit, Plastic

The Lure Product Name is "*Archips xylosteanus* Lure." The lure is effective for 28 days (4 weeks).

Trap Spacing: When trapping for more than one species of moth, separate traps for different moth species by at least 20 meters (65 feet).

Lure Placement: Placing lures for two or more target species in a trap should never be done unless otherwise noted here.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Surveys should be focused where the greatest risk for establishment occurs. This insect was recently detected in Newfoundland, Canada, a first report for North America (Spears, 2006). Based on the list of countries in Europe and Asia from which the species has been reported, Schall (2006) predicts the species is likely to occur in regions (zonobiomes) with climates characterized as warm-temperate, typical-temperate, arid-temperate, and transitional to cold-temperate or boreal. Consequently, using this approach most of the contiguous United States is predicted to be climatically suitable, with the exception of southern Florida, southern Texas, the desert southwest, and California's coast and Central Valley (Schall, 2006).

A recent risk analysis by USDA-APHIS-PPQ-CPHST indicates that most northeastern, central, and southeastern states have areas with a high risk rating for *Archips xylosteanus* establishment. In the west, California, Colorado, Idaho, Oregon, Utah, and Washington have the highest risk of establishment based on host availability and climate within the continental United States.

Trapping: Male *A. xylosteanus* are attracted to blends of *Z*-11-tetradecenyl acetate and *E*-11- tetradecenyl acetate (El-Sayed, 2006). Ando et al. (1978) were the first to demonstrate that male moths were attracted to a 4:1 mixture of *Z*-11-tetradecenyl acetate: *E*-11-tetradecenyl acetate, but captures with this blend were relatively low (only 13 moths over an unspecified length of time). This mixture also attracted the tortricids *Archippus piceanus similis* and *Pandemis cinnamomeana* (Ando et al., 1978). Frerot et al. (1979, 1983) found that the same two compounds in a 92:8 [*Z*:*E*] mixture captured substantially more male *A. xylosteanus* than any other ratio tested (approximately 150 males over an unspecified length of time). This ratio of these compounds may also be attractive to *Cacaecimorpha pronubana* and *Argyrotaenia pulchellana* (Frerot et al., 1979). Conversely, *A. xylosteanus* may be attracted to pheromone lures for oriental fruit moth, *Grapholita molesta* (93:7 *Z*-8-dodecenyl acetate:*E*-8-dodecenyl acetate + docecanol), red-banded leafroller, *Argyrotaenia velutinana* (2:3 *Z*-11-tetradecenyl acetate:dodecyl acetate), and the oblique banded leafroller, *Choristoneura rosaceana* (*Z*-11-tetradecenyl acetate) (Hrdý et al., 1979). Pheromones produced by *Archips rosana* may interfere with attractants for *A*. *xylosteanus* (Safonkin, 1998).

Pheromone traps should be placed approximately 1.6 meters (5 ft) above the ground and 50 to 100 m (150-300 ft.) apart (Hrdý et al., 1979, Frerot et al., 1983). Pherocon 1C traps are more effective at capturing males than Stuttgart pot traps (Hrdý et al., 1979).

Visual: Individual species of leafrollers are difficult to detect with visual inspections of foliage. Leaf rolling is common among many tortricids, and *A. xylosteanus* may closely resemble related species.

Key Diagnostics/Identification

CAPS-Approved Method*: Confirmation of *A. xylosteanus* is by morphological identification.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Confirmation of *A. xylosteanus* is by morphological identification, so any identification should be confirmed by an appropriately trained entomologist.

A new identification tool, *Tort AI – Tortricids of Agricultural Importance*, is available at http://idtools.org/id/leps/tortai/ from CPHST's Identification Technology Program. This tool contains larval and adult keys, fact sheets, an image gallery, molecular search capacity, and more. *Archips xylosteanus* is included in this tool.

Easily Confused Pests

Archips xylosteanus has a similar appearance to *Archips crataegana* (also not known to occur in the United States) but is generally smaller and more variegated (Bradley et al., 1973). A dichotomous key of common leafroller pests (Tortricidae) in larval and pupal stages is provided by Beeke and De Jong (1991).

Archips xylosteanus is the type species for the Xylosteana group (Razowski, 1997). Other introduced and native members of the Xylosteana group in North America include: *A. argyrospila*, *A. cerasivorana*, *A. eleagnana*, *A. fervidana*, *A. fuscocupreana* (introduced), *A. georgiana*, *A. goyerana*, *A. grisea*, *A. infumatana*, *A. magnoliana*, *A. mortuana*, *A. myricana*, *A. negundana*, *A. nigriplagana*, *A. purpurana*, *A. rileyana*, *A. rosana* (introduced), and *A. semiferana* (Kruse and Sperling, 2002).

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Ceroplastes destructor

Scientific Name
Ceroplastes destructor Newstead

Synonyms:
Gascardia destructor, Gascardia postperlucidus, and *Ceroplastes postperlucidus*.

Note: Numerous misidentifications are mentioned with synonymy in cited literature but are not included here.

Common Name
Soft wax scale, white wax scale, citrus waxy scale, white scale, white waxy scale

Type of Pest
Scale Insect

Taxonomic Position
Class: Insecta, **Order:** Hemiptera, **Family:** Coccidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2005 through 2012

Pest Description
Ceroplastes destructor is a serious pest of more than 150 host plants, including economically important crops such as citrus and coffee. With the exceptions of apple (*Malus* spp.), pear (*Pyrus* spp.), and apricot (*Prunus armeniaca*), most of these plants occur in tropical or semi-tropical areas (Davis et al., 2005). This insect is probably native to Africa but now also occurs in Australasia. At one time, *C. destructor* was reported in the United States (Florida), however this report was likely based on a misidentification of a related indigenous species, *C. dugesii* (CABI, 2009). *Ceroplastes destructor* is not known to occur in the United States.

There are three nymphal instars. The early instars of *C. destructor* are morphologically difficult to distinguish from those of other species of *Ceroplastes*. Wakgari and Giliomee (1998) provide a key to separate different stages of *C. destructor* and detailed descriptions and illustrations of all nymph and adult stages. The following morphology is taken mainly from Wakgari and Giliomee (1998) for the nymph stages and from Qin and Gullan (1994) and Wakgari and Giliomee (1998) for the adult. These descriptions include details visible only in specimens mounted on microscope slides, examined under high magnification.

First-instar nymph: The first-instar nymph is oval, dorsolaterally flat, 0.32 to 0.50 mm (0.01 to 0.02 in.) long; eye-spots heavily pigmented, present dorsolaterally on each side of the head region; marginal setae flagellate, nine to 12 between anterior spiracular

furrows, two to three between anterior and posterior spiracular furrows, seven between posterior spiracular furrow and anal cleft; three spiracular setae present in each spiracular furrow; dorsum without setae or pores; anal plates each with a very long, slender apical seta and three other dorsal setae, one fringe seta and one ventral seta; venter without submarginal setae, one pair of interantennal setae and one pair of prevulvar setae present, a few cruciform pores present in submargin, three quinquelocular pores between spiracle and its corresponding furrow; antennae 6-segmented; legs well developed, without tibiotarsal sclerosis, tarsal digitules equal in size and knobbed, claw denticle absent, claw digitules unequal, one slender and one stout, both apically knobbed.

Second-instar nymph: The second instar is oval, 0.65 to 0.70 mm (~0.03 in.) long, eye-spots pigmented, present dorsolaterally on each side of the head region; marginal setae flagellate, eight to 10 between anterior spiracular furrows, two between anterior and posterior spiracular furrows, six to seven between posterior spiracular furrow and anal cleft; three spiracular setae present in each spiracular furrow; dorsum without setae, with some bilocular pores along submarginal areas; anal plates each with four dorsal setae, two fringe setae and one ventral seta; venter with bristle-shaped submarginal setae, two pair of interantennal setae (one longer one shorter) and one pair of prevulvar setae present, cruciform pores present in submargin, four to seven quinquelocular pores between spiracle and its corresponding furrow; antennae 6-segmented; legs well developed, similar in structure to that of first-instar nymph.

Third-instar nymph: The third instar is oval, 0.85 to 1.20 mm (0.03 to 0.05 in.) long, eye-spots black, present dorsolaterally on each side of the head region; marginal setae flagellate, occasionally clavate to capitate, nine to 14 between anterior spiracular furrows, three to five between anterior and posterior spiracular furrows, seven to 11 between posterior spiracular furrow and anal cleft; six to 10 spiracular setae present in each spiracular furrow; dorsum with one anterior and six lateral dorsal clear areas, setae and pores sparsely distributed over dorsum except lacking in dorsal clear areas, pores bilocular, oval trilocular or triangular trilocular and a few monocular in median area; anal plates each with four dorsal setae, three fringe setae and one ventral seta; venter with bristle-shaped submarginal setae and sparsely distributed ventral setae, two pairs of interantennal setae (one longer, one shorter) and one pair of prevulvar setae present, cruciform pores present in submargin around entire body, 12 to 18 quinquelocular pores between spiracle and its corresponding furrow; antennae 6-segmented; legs well developed, similar in structure to that of first-instar nymph but relatively smaller compared with body size.

Adult female: After Qin and Gullan (1994) and Wakgari and Giliomee (1998). There is some variation in the number of pores and setae recorded by Qin and Gullan (1994) and Wakgari and Giliomee (1998). This is probably due to the fact that Qin and Gullan (1994) used Australian material and their counts included both young and old specimens, while Wakgari and Giliomee (1998) used African material and their counts may only include young specimens.

Adult females are oval, sometimes with some marginal indentation, 2.5 to 6.40 mm (0.1 to 0.25 in.) long, with a strongly sclerotized anal process; eye-spots black, relatively small, present dorsolaterally on each side of the head region; marginal setae bristle-like, eight to 24 between anterior spiracular furrows, four to eight between anterior and posterior spiracular furrows, seven to 12 between posterior spiracular furrow and anal cleft; 37 to 77 spiracular setae present in each spiracular furrow; dorsum derm membranous in young specimens but becoming sclerotized in old specimens, with one anterior and six lateral dorsal clear areas, dorsal setae cylindrical, some with apex slightly expended, evenly distributed over dorsum except lacking in dorsal clear areas, dorsal pores oval trilocular or triangular trilocular and occasionally quadrilocular or bilocular, preopercular pores numbering five to six in single transverse row immediately anterior to anal plates; anal plates each with four dorsal setae, four fringe setae and one ventral seta; venter with submarginal setae and sparsely distributed ventral setae, two pair of interantennal setae (one longer one shorter) and one pair of prevulvar setae present, cruciform pores mostly present in submargin around entire body, sparse in other ventral areas, 65 to 110 quinquelocular (a few with six or seven loculi) pores between spiracle and its corresponding furrow; multilocular pores distributed around vulva and in a band across preceding segment, mostly with 10 loculi, tubular ducts present on abdomen; antennae 6-segmented; legs well developed, similar in structure to that of first-instar nymph but relatively much smaller compared with body size.

The adult male is unknown.

Symptoms/Signs

C. destructor attacks the leaves, branches and stems of host plants, affecting vigor and growth. A large number of young crawlers can be seen on the leaves when the eggs hatch, but these do not persist. They usually settle on the leaf surface along midribs or leaf petioles. Once the crawlers settle down, they start secreting white wax (Fig. 1, 2).

Figure 1. *C. destructor* on coffee. Females are immobile and covered in a white, waxy layer. Photo courtesy of CABI, 2009.

Gimpel et al. (1974) described in detail the process and shapes of the wax produced by wax scales and *C. destructor* produces its wax in a similar way. After three to four days of settlement, the dorsal wax pad appears as a thin, white marking. The wax rays gradually appear around the body margin. The insects move from their original settlement site to the twigs at the beginning of the third instar. At this stage, the wax builds up like a cone and, when more wax is secreted, the late third instar attains its characteristic oval shape. The adults are completely covered

with white wax in irregular shapes. Qin and Gullan (1994) and Wakgari and Giliomee (1998) contain figures that show the wax appearance of the different stages. Sooty mold is usually associated with the scales.

Sooty mold may inhibit photosynthesis, lower fruit quality and reduce yield.

Figure 2. *C. destructor* infestation. Photo courtesy of Rosa Henderson, Landcare Research, New Zealand. http://www.bugwood.org.

Survey

CAPS-Approved Method*: Visual survey is the method to survey for *C. destructor*

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:
Surveys should be focused where the greatest risk for establishment occurs. A recent risk analysis by USDA-APHIS-PPQ-CPHST indicates that most states in the United States have a low to moderate risk rating for *Ceroplastes destructor* establishment based on host availability and climate within the continental United States. Alabama, Florida, Georgia, Louisiana, Mississippi, and Texas have the highest risk of establishment based on host availability and climate.

Visual: Infestations of *C. destructor* on citrus and other hosts are easily detected because the insects are covered by a white wax cover. *C. destructor* can be detected by examining and inspecting plants, especially shrubs or trees, for white wax (adult) covers, or for signs of sooty mold or sticky honeydew on leaves, branches and stems, or for ants running about. First instar nymphs usually settle on the leaf surface along leaf midribs or leaf petioles; while third instar nymphs move to twigs. To be certain about the presence of *C. destructor*, it is necessary to examine slide-mounted specimens under a microscope.

Key Diagnostics/Identification

CAPS-Approved Method*: Morphological: Adult females are very distinct from all U.S. species. Specimens should be slide mounted for confirmation. Immatures are difficult to differentiate from similar-looking species.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Morphological identification of adult females is required to confirm the presence of *C. destructor*.

Easily Confused Pests

Ceroplastes destructor can be confused with other *Ceroplastes* spp. The species of *Ceroplastes* present in the United States include: *Ceroplastes brachyurus*, *C. ceriferus*, *C. cirripediformis*, *C. dugesii*, *C. feltyi*, *C. floridensis*, *C.irregularis*, *C. nakaharai*, *C. rubens*, *C. rusci*, *C. sinensis*, and *C. utilis*.

Ceroplastes destructor has often been confused with closely related species such as *C. ceriferus* and *C. sinensis* in Australia and with *C. dugesii* in the United States and Mexico (Qin, 2000, CABI 2009). *C. destructor* was misidentified as *Ceroplastes ceriferus* in the early literature because of the similarity of the wax test of these two species. However, with microscopic study of slide-mounted specimens, *C. destructor* can be morphologically distinguished from *C. ceriferus* by the absence of tubular ducts on the venter of the head in *C. destructor*. It also differs from other species of *Ceroplastes* by the possession of different-sized claw digitules (one slender and one broad) and a large and round group of spiracular setae (De Lotto, 1965; Williams and Watson, 1990; Qin and Gullan, 1994).

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Ceroplastes japonicus

Scientific Name
Ceroplastes japonicus Green

Synonyms:
Ceroplastes floridensis japonicus. Cerostegia japonica, and *Paracerostegia japonica.*

Common Name(s)
Japanese wax scale, tortoise wax scale

Type of Pest
Scale Insect

Taxonomic Position
Class: Insecta, **Order:** Hemiptera, **Family:** Coccidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2005 through 2012

Figure 1. Nymph(s), second instars (top) and adult females (bottom). Photos courtesy of Giuseppina Pellizzari, Faculty of Agriculture, Dept. Entomology, http://www.bugwood.org.

Pest Description
Ceroplastes japonicus is a significant economic pest of citrus and other fruit crops in Asia and Europe. *C. japonicus* is polyphagous, attacking more than 100 plant species belonging to 40 genera placed in 24 families, including many crop and ornamental plants (Ben-Dov, 1993; Pellizzari and Camporese, 1994; Davis et al., 2005). The most common host plants are citrus, persimmon, holly and ivy. In the Republic of Georgia, it is also common on mulberry and fruit trees, and in Italy, on bay laurel and maple (Pellizzari and Camporese, 1994). *Prunus armeniaca* (apricot), *Prunus avium* (sweet cherry), *Prunus cerasus* (sour cherry), *Prunus laurocerasus* (cherry laurel), *Prunus mume* (Japanese apricot tree),

Prunus persica (peach), and *Prunus yedoensis* (Yoshino cherry) are known hosts of this pest. *Ceroplastes japonicus* is not known to occur in the United States.

Eggs: One female *C. japonicus* may lay up to 2500 eggs. The eggs are less than 0.5 mm (0.02 in.) long (EMPPO, 2003).

Nymphs: Newly hatched nymphs have well developed legs and antennae. *C. japonicus* is characteristically hemispherical in shape and completely covered by a thick layer of oily wax (Masten-Milek et al., 2007). When they find a suitable place for feeding they fix themselves to the surface of the plant and turn into immovable nymphs. The body of the nymph is red and is covered by eight whitish conic wax scales that form a small star (Fig. 1) shape (EMPPO, 2003).

Adult male: The male adult is white, opaque, with a dry wax structure, oblong, star-shaped, with 13 distinct marginal waxy projections plus two small anal plate projections. The total length, including waxy projections is1.7 to 2.0 mm (0.07 to 0.08 in.): width is 1.0 to 1.6 mm (0.04 to 0.06 in.) (Rainato and Pellizzari 2008).

Figure 2. Infestation of laurel (*Laurus* spp.)(top) and trifoliate orange (*Poncirus trifoliata*)(bottom).Photos courtesy of Giuseppina Pellizzari, Faculty of Agriculture, Dept. Entomology, http://www.bugwood.org.

The following morphological description characterizing the adult female is taken from Camporese and Pellizzari (1994), and Pellizzari and Camporese (1994):

Adult female: Mature adult females of *C. japonicus* are grayish to pinkish-white, (Fig. 1) hemispherical and up to 4.0 mm (0.16 in.) in length and 3.5 mm (0.14 in.) in width. They are covered by a thick layer of oily wax. Mounted female oval in shape. 6-segmented antennae. Legs well developed, without tibio-tarsal scleroses. Claw without denticle. Claw digitules of the same shape, broad, with expanded apices.

Margin: Stigmatic setae lanceolate with pointed apices, distributed in two rows: a row with three to four larger setae extending on dorsum, the others distributed along margin. These setae form a continuous row of 111 (97 to 148) setae along the body margin. A few marginal bristle-shaped setae (two to seven) usually mingled with stigmatic setae (rarely contiguous) may help to distinguish between the anterior and posterior group of stigmatic setae. Marginal bristle shaped setae distributed along the body margin except where stigmatic setae are present. There are 26 to 30 marginal setae between eye-spots and 45 to 55 setae from the last stigmatic setae to anal lobe. The last three to four setae on anal lobe are distinctly longer than the others.

Dorsum: Membranous in young female, with one cephalic and six lateral clear areas. Dorsal pores scattered, mostly oval trilocular and triangular trilocular; the oval trilocular predominant over other kinds of pores. Some quadrilocular pores present in medio dorsal region. Irregular bilocular pores mainly distributed in submargins. Minute oval pores with filamentous duct distributed in the submargins (these pores are somewhat difficult to detect). Anal plates with three to four dorsal and one ventral setae. Pre-opercular pores 10 (six to 14) just above the anal plates.

Venter: Tubular ducts with enlarged inner filament form a submarginal band of two to three elements distributed from the eye spot to about the level of the caudal process. Cruciform pores in a submarginal band between the body margin and the band of tubular ducts. Quinquelocular pores in the stigmatic furrow form an irregular band. There are 41 (29 to 66) quinquelocular disc pores in the anterior band and 50 (24 to 72) in the posterior band. Multilocular disc pores numerous around the vulva and on sixth abdominal segment. Several multilocular disc pores arranged in a single row in the remaining abdominal segments (Camporese and Pellizzari, 1994). A few multiocular pores (one to seven) near the base of the coxae and near the stigmatic atrium. Submarginal short setae form a row along the body submargin, interrupted by the bands of stigmatic pores. There is an average of 120 submarginal setae from an anal lobe to the opposite one (Pellizzari and Camporese, 1994).

Authoritative identification involves detailed microscopic examination of newly matured adult females. Morphological descriptions and illustrations of the adult female are given by Pellizzari and Camporese (1994) and the immature female stages are described by Camporese and Pellizzari (1994). A key to the *Ceroplastes* species that occur in the Mediterranean is given by Pellizzari and Camporese (1994).

Symptoms

Infestations of *C. japonicus* occur on the foliage, stems, and branches (Fig. 2). This results in reduced vigor and general debilitation of the host plant. Heavy infestations

may result in chlorotic spotting and premature shedding of leaves, wilting, and dieback of stems. Honeydew deposited on the leaves and fruit serves as a medium for the growth of black sooty molds. The sooty mold results in a reduction of photosynthetic area and lowers the market value of ornamental plants and produce (CABI, 2009).

Survey

CAPS-Approved Method*: Visual survey is the method to survey for *C. destructor. .* Look on leaves, fruit, and stems for 1) thick layer of grayish to pinkish-white, oily wax that contrasts in color with the host plant, 2) signs of sooty mold, and 3) sticky honeydew.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Surveys should be focused where the greatest risk for establishment occurs. A recent host analysis by USDA-APHIS-PPQ-CPHST indicates that the eastern half of the United States has a moderate to high risk for the establishment of *C. japonicus.* The northern coast of California, the coast of Oregon and Washington, and portions of Colorado Idaho, New Mexico, and Utah have moderate levels of risk for the establishment of *C. japonicus* based on host availability within the continental United States.

Visual Survey: Visual inspection of potentially infested plants is the best way of finding colonies of C. *japonicus* on a plant. Inspect host plants with a 10X magnification lens. Look on the leaves and stems of the host plants (Masten-Milek et al., 2007). In Europe, egg hatch occurs in June, the first molt in July, the second molt in August, and adult females are present in September and serve as the overwintering stage. Surveys should be conducted during these times.

Key Diagnostics

CAPS-Approved Method*: Morphological: All stages are similar to other *Ceroplastes* species. *Ceroplastes japonicus* is most commonly confused with *Ceroplastes floridensis*. Adult females can be distinguished based on morphological characters in slide mounted specimens.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:
C. japonicus should be distinguished from the closely related *Ceroplastes floridensis*, which occurs worldwide in tropical and subtropical regions. The main characters used to distinguish the two species are the number and different arrangement of the stigmatic setae along the body margin. In *C. japonicus* the stigmatic setae form an uninterrupted row between the anterior and posterior stigmatic clefts, whereas, in *C. floridensis*, they are interrupted with seven to 12 bristle-shaped marginal setae. *C. japonicus* has an

average of 111 stigmatic setae on each side of the body compared with an average of 60 stigmatic setae in *C. floridensis* (Pellizzari and Camporese, 1994).

The Scale Insects – Identification Tools for Species of Quarantine Significance provides keys and fact sheets that can help identify scale insects, including *Ceroplastes japonicus* (http://www.sel.barc.usda.gov/ScaleKeys/index.html).

Easily Confused Pests

Ceroplastes japonicus can be confused with other *Ceroplastes* spp. including: *C. brachyurus*, *C. ceriferus*, *C. cirripediformis*, *C. dugesii*, *C. feltyi*, *C. floridensis*, *C. irregularis*, *C. nakaharai*, *C. rubens*, *C. rusci*, *C. sinensis*, and *C.utilis*.

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Diabrotica speciosa

Scientific name
Diabrotica speciosa Germar

Synonyms:
Diabrotica amabilis, Diabrotica hexaspilota, Diabrotica simoni, Diabrotica simulans, Diabrotica vigens, and *Galeruca speciosa.*

Common names
Cucurbit beetle, chrysanthemum beetle, San Antonio beetle, and South American corn rootworm

Type of pest
Beetle

Taxonomic position
Class: Insecta, **Order:** Coleoptera, **Family:** Chrysomelidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2010 through 2012

Pest Description
Diabrotica speciosa is considered to be an important pest throughout southern South America (except Chile), but, being highly polyphagous, qualitative reports of its impact on different crops vary in different regions. Adults of this beetle feed on foliage, pollen, flowers, and fruits of many plants. The larvae are pests of roots, especially maize. *D. speciosa* is considered an important pest of maize, cucurbits, and orchard crops throughout its distribution (CABI, 2009). *Prunus domestica* (plum), *Prunus insititia* (damson plum), and *Prunus persica* (peach) are considered minor hosts (Cabrera Walsh, 2003).

Diabrotica speciosa was first described by Germar in 1824, as *Galeruca speciosa*. Two subspecies have been described, *D. speciosa vigens* (Bolivia, Peru and Ecuador), and *D. speciosa amabilis* (Bolivia, Colombia, Venezuela and Panama). These two subspecies differ mainly in the coloring of the head and elytra (Araujo Marques, 1941; Bechyne and Bechyne, 1962).

Eggs: Eggs are ovoid, about 0.74 x 0.36 mm (0.03 x 0.01 in.), clear white to pale yellow. They exhibit fine reticulation that under the microscope appears like a pattern of polygonal ridges that enclose a variable number of pits (12 to 30) (Krysan, 1986). Eggs are laid in the soil near the base of a host plant in clusters, lightly agglutinated by a colorless secretion. The mandibles and anal plate of the developing larvae can be seen in mature eggs.

Larvae: Defago (1991) published a detailed description of the third instar of *D. speciosa*. First instars are about 1.2 mm (0.05 in.) long, and mature third instars are about 8.5 mm (0.33 in.) long. They are subcylindrical; chalky white; head capsule dirty yellow to light brown, epicraneal and frontal sutures lighter, with long light-brown setae; mandibles reddish dark brown; antennae and palpi pale yellow. Body covered by sparse, short, dark setae; light brown irregular prothoracic plate; dark brown anal plate on the ninth segment, with a pair of small urogomphi. A pygopod is formed by the tenth segment, which serves as a locomotion and adherence organ.

Figure 1. Adult *Diabrotica speciosa*. Photo courtesy of Hernan Tolosa.

Pupae: Pupae are 5.8 to 7.1 mm (0.23 to 0.28 in.) long and white. Females with a pair of tubercles near the apex. Mature third instars build an 8 x 4 mm (0.31 x 0.16 in.) oval cell in the soil in which they pupate, and tenerals remain for about 3 days.

Adults: Full descriptions of *D. speciosa* are given by Baly (1886), Araujo Marques (1941), and Christensen (1943). Adults are 5.5 to 7.3 mm (0.22 to 0.29 in.) long; antennae 4 to 5 mm (0.16 to 0.2 in.) (Fig. 1). General color grass-green (USDA, 1957); antennae filiform and dark (reddish-brown to black) and nearly equal to the body in length, first three basal segments lighter; head ranging from reddish brown to black; labrum, scutellum, metathorax, tibiae and tarsi black; elytra each with three large oval transverse spots, basal spots larger and usually reddish toward the humeral callus, the rest yellow. Ventrally, head and metathorax dark brown, prothorax green, mesothorax and abdomen light brown or yellow-green. Pronotum bi-foveate, convex, smooth, shiny, ¼ wider than long. Male antennae proportionally longer than female antennae. Males with an extra sclerite on the apex of the abdomen that makes it look blunt compared with the rather pointed female apex.

Symptoms/Signs

The larval damage resulting from root feeding can cause host death when the host is small, but the larvae will usually only induce stunted growth in larger host plants, due to a reduction in nutrient uptake. Like other *Diabrotica* spp., *D. speciosa* is especially associated with Cucurbitaceae, tolerant of cucurbitacins, and generally feed on pollen-rich plant structures of over 70 plant species. When flowers are scarce, beetles may feed on the tender green parts of other hosts, such as alfalfa, potatoes, corn, bean, soybean, lettuce, and cabbage (EPPO, 2005).

Stone Fruit: Adults feed on stone fruit foliage, flowers, and fruits causing damage.

Corn: Attack on young plants by larvae produces a typical condition known as 'goose neck', in which the plant exhibits stunted growth, reduced vigor, and the first few internodes of the plant grow bent, sometimes to such an extent that the plant actually lies on the ground. On corn, the most economically important stage is the adult, which feeds on the tassels, preventing pollination and kernel number. Adults also cause defoliation and general feeding damage to leaves, flowers, and fruit (EPPO, 2005).

Peanuts/Potatoes: The larvae cause external damage or short bores, similar to those of several other pests such as wireworms and other chrysomelids.

Grape: Adult beetles eat young leaf edges during budding, which usually does not seriously damage the host (Roberto et al., 2001). During the blooming period, however, beetles have been observed on flowers eating the style, stigma, and eventually the ovary. Beetle stigma feeding determines flower aborting and, as a consequence, clusters show low numbers of flowers and fruits. Weedy hosts need to be controlled as beetles can also be observed feeding on and moving into grape from surrounding weeds.

Survey

CAPS-Approved Method*: The CAPS-approved method for *D. speciosa* is visual survey.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Surveys should occur in areas most at risk for establishment of *D. speciosa*. As of 2004, *D. speciosa* has been intercepted over 300 times at ports of entry in the United States, but little is known on its potential distribution within the United States. According to a recent host analysis by USDA-APHIS-PPQ-CPHST, most of the continental United States is at a low to moderate risk from *D. speciosa* establishment based climate and the presence of susceptible hosts. The greatest risk for establishment of *D. speciosa* occurs in portions of Arkansas, Illinois, Indiana, Kansas, Louisiana, Mississippi, Missouri, Oklahoma, and Tennessee. The pest occurs from temperate Argentina to tropical Brazil. The polyphagous nature of *D. speciosa* increases the likelihood of finding hosts and suitable environment if it were introduced into the United States, and is thought to be able to adapt to more temperate climates.

Visual survey: Visual detection of adults is easy, as their feeding period spans from dawn until dusk. Detection of larval damage, on the other hand, is more difficult. First instars are very difficult to sample, and even large infestations can go undetected until the damage caused to the host is extensive. Larger larvae can sometimes be observed feeding on the roots of plants immediately after pulling out of the soil, but methodical

sampling and counting methods have not been developed, as they have been for the North American pest species (Fisher and Bergman, 1986).

Trapping: Adults *D. speciosa* appear to be universally attracted to aromatic compounds from squash blossoms, though the specific compound(s) that attract the beetles varies from species to species. Often, simple blends of two or three compounds are much more potent attractants than any single compound. In addition, female-produced sex attractant pheromones are used for mate location in this genus. In a preliminary trapping test in Brazil, a number of squash volatiles were screened for potential attraction, and 1,4-dimethoxybenzene showed promise as an attractant for *D. speciosa* (Ventura et al., 2000). Marques et al. (2009) also showed that *D. speciosa* is attracted to 1,4-dimethoxybenzene. Traps baited with 1,4-dimethoxybenzene, a volatile substance of *Cucurbita maxima* blossoms captured 29.4 times and 9.4 times more beetles than controls in soybean and common bean fields, respectively (Ventura et al., 2000). Captures of *D. speciosa* in the traps lured with 1,4-dimethoxybenzene analogs did not differ from the control traps, showing that all the structural modifications made on the structure of the natural compound resulted in activity loss (Marques et al., 2009). Results showed that position and nature of the substituents on the aromatic ring played a crucial role in the activity of the natural compound.

Colored traps are being tested for use in trapping adult females and males. Yellow traps attracted more *D. speciosa* female and male beetles than did clear traps. No females and few males were captured by the clear traps (Ventura et al., 2005).

Traps baited with 1,4-dimethoxybenzene captured more beetles than did the unbaited ones in all assessments. Dispensers for the floral volatile attractant 1,4-dimethoxybenze were also compared. Rubber septa dispenser attracted more beetles than did control (dental wicks saturated with acetone). Captures on dental wick, starch matrix, and feminine pad dispensers were intermediate and did not differ from those on rubber septa and unbaited controls (Ventura et al., 2005).

A similar number of beetles were captured using plastic bottle traps (perforated and window with cucurbitacin) and sticky (without cucurbitacin) traps, when baited with the floral attractant. Perforated bottle traps (2000 mL), however, when baited with the floral attractant, caught more beetles than did window bottle traps (both traps contained *Lagenaria vulagaris* powder- a cucurbitacin source - 0.28%). Traps with the insecticide carbaryl captured more beetles than did traps without it at 2 to 4 and 8 to 10 days after trap placement in the field, but not in the remaining periods (0 to 2, 4 to 6 and 6 to 8 days).

The USDA-CPHST laboratory in Otis, MA has applied for funding to manufacture and test potential lures for *D. speciosa*, but has yet to begin work toward this goal.

Key Diagnostics/Identification

CAPS-Approved Method*: Confirmation of *D. speciosa* is by morphological identification. *Diabrotica speciosa* is almost identical to *D. balteata*, which is widely present in the southern United States. Confirmation by a chrysomelid specialist is

required. *D. speciosa* can also be confused with *Diabrotica viridula* (not present in the United States) and other pestiferous *Diabrotica* species in South America.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Confirmation of *D. speciosa* is by morphological identification.

Easily Confused Pests

Diabrotica speciosa somewhat resembles the other main pestiferous *Diabrotica* in South America, *D. viridula*, in coloring, size, biology and host range; but *D. viridula* has dark brown areas toward the cephalic edge of the elytral spots, and distinct humeral plicae. Also, the larvae of *D. viridula* lack urogomphi on the anal plate.

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Eudocima fullonia

Scientific Name
Eudocima fullonia Clerck

Synonyms:
Othreis fullonia, Noctua dioscoreae, Ophideres fullonia, Ophideres obliterans, Ophideres princeps, Othreis phalonia, Othreis pomona, Phalaena fullonica, Phalaena fullonica, Phalaena phalonia, and *Phalaena Pomona*.

Common Names
Fruit piercing moth, fruit-sucking moth

Type of Pest
Moth

Taxonomic Position
Class: Insecta **Order:** Lepidoptera **Family:** Noctuidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2006 through 2012

Pest Description
This species can be a major pest of *Citrus* spp. in many areas, but it will also attack other fruits including banana, fig, guava, kiwifruit, longan lychee, mango, stone fruit, persimmon and ripening papaw (Astridge and Fay, 2005).

Eggs: Hemispherical, just over 1 mm (0.04 in.) in diameter, and greenish-white to creamy-yellow when laid. Delicate surface sculpturing can be seen with the aid of a microscope. The brownish head capsule of the developing larva becomes obvious beneath the shell a few hours before hatching.

Figure 1. Larva of *E. fullonia*. Photo courtesy of CABI, 2009.

Larvae: The newly hatched larvae are 4 to 5 mm (0.16 to 0.2 in.) long, a bright translucent green in color, and inconspicuously banded by brown spots and hairs. The head capsule is 0.5 mm (0.02 in.) wide. Second instars are a uniform dull black, with two developing, paired, lateral orange eyespots. Larvae molt four or five times during development. Final instars can reach about 60 mm (2.36 in.) in length, with a head capsule of 4.5 mm (0.18 in.). Mature larvae are a velvety brown to black (Fig. 1) or pale yellow to green. There are

fine powdery white spots along the entire length of the body and two conspicuous, paired, lateral eyespots on the second and third abdominal segments. In dark larvae, the eyespots are peripherally white (above) and orange (below), with a central black area surrounding a pale blue core. When resting, larvae hold the posterior part of the body upwards, while the anterior part is curled with the head tucked under (Fig. 1). If disturbed, larvae may rear and 'spit' digestive juices. Larvae move with a semi-looping action.

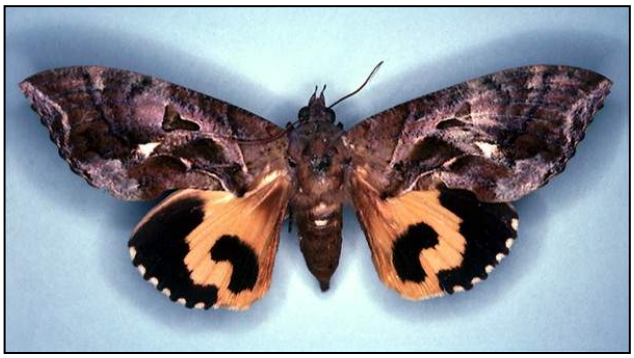

Figure 2. Adult female fruit piercing moth. Photo courtesy of CABI, 2009.

Pupae: The post-feeding larva forms a silken cocoon among the leaves of the larval host plant and attaches itself within the cocoon at the anal end. The pupa is about 30 mm (1.18 in.) long, a glistening brown-black, and can be sexed at this stage using differences in the position of the genital grooves.

Adults: Adults have 7 to 10 cm (2.76 to 3.94 in.) wingspans, with mottled brown, gray, green, or silvery white forewings. The color patterns of the forewings are sexually dimorphic. Males have leaf-like forewings of red-brown to purplish-brown. There is an inconspicuous, irregular spot centrally placed near the anterior margin. In females, the forewings are more variegated and striated than in males. The color varies between purplish-brown and grayish-ochre, often flecked with green and white. There is a distinct dark, roughly triangular mark in a similar position to the spot in the male forewing. The hind wings are characterized by the orange-yellow color, extensively bordered by a black and hatched area and a central black mark (kidney shaped or round). These are often exposed when the moth is feeding. The thorax is a purplish-brown and the abdomen orange-yellow. An individual moth can spend several hours feeding from the one fruit, but would generally attack a number of fruit on a single night. Adults rest with the forewings held tent-like over the body. When feeding, the forewings are held out exposing the bright hindwings (Fig. 3).

Figure 3. Male (left) and female (right) *E. fullonia* on a green mandarin fruit. Photo courtesy of CABI, 2007.

Symptoms/Signs

For most moth pests, the larvae are the damaging stage. **The fruit piercing moth differs in this aspect, because it is the adult moth that is the damaging stage, and the larvae are essentially not harmful.** The mouth parts of the moth are about 2.5 cm (1 in.) long and strong enough to penetrate through tough-skinned fruit. Once the moth

has punctured the skin of the fruit, a process that usually takes a few seconds, it feeds upon the juices of the fruit (Fig. 3). The proboscis is pushed deeper as the juices become exhausted in that particular site; normally only ripened or well matured fruits are attacked unless these are unavailable (Baptist, 1944). Feeding occurs at night. Fruit flesh damaged by this moth becomes soft and mushy differing from fruit damaged by fruit flies, which is more liquid. On pear, Cave and Lightfield (1997) state that *E. fullonia* is considered an external feeder.

Figure 4. A damaged fruit showing fruit rot around the piercing moth feeding site. Photo courtesy of CABI, 2009.

A round, pinhole-sized puncture is made in fruits. The hole serves as an entry point for pathogens and can result in early fruit drop. The latter is an obvious sign of fruit piercing moth activity in citrus. A small cavity is left in the fruit in the feeding site. The area of the fruit around the cavity will be dry and spongy. It may be bruised beneath the skin (Astridge and Fay, 2005). The fruit piercing moth is a known vector of *Oospora citri*, a fungus that rots the fruit and has a penetrating odor that attracts this moth. Other microorganisms that gain entrance into the fruit and cause rotting include *Fusarium* spp., *Colletotrichum* spp., and several types of bacteria. When moths are abundant, green fruit is attacked, causing premature ripening and dropping of fruits. On oranges, a green fruit turns yellow at the site of the piercing and fungi soon develop within the wound.

Survey

CAPS-Approved Method*: The CAPS-approved survey method is visual survey.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Surveys should be focused where the greatest risk for establishment occurs. A recent host analysis by USDA-APHIS-PPQ-CPHST indicates that most states in the United States are at low risk for *E. fullonia* establishment based strictly on host availability. Portions of California, Florida, Maryland, Massachusetts, New Jersey New York, Pennsylvania, Vermont, and West Virginia have a moderate risk, however.

Visual survey: Adult moths are likely to be found on mature fruit several weeks before harvest. The most effective way to monitor for fruit piercing moths is to inspect the crop by flashlight after sundown beginning a few weeks before harvest (Davis et al., 2005).

Moths are most active in the first few hours of the night. The large, red-glowing eyes of the moths are easily seen. Check trees/vines in the two outer rows of an orchard, particularly on the leeward side. Most damage occurs in the peripheral rows. Surveys were typically initiated 30 minutes after sundown and lasted one hour.
Foliage of host plants may be inspected for larvae and other life stages (Davis et al., 2005).

In some fruits, such as lychees, detection of fruit piercing moth damage can be difficult. The slightest sign of weeping can be an indication, and when the fruit is squeezed, the juice will squirt out. The damage site will be flaccid and flattened in appearance and lack the firm, rounded flesh of intact fruit. Many farmers opt to place freshly picked fruit in a cool store at high humidity, which facilitates detection of damage after one day.

Survey site and selection: Areas where host material is found should be targeted for surveys; these can include orchards, nurseries, residential areas and other areas where host plants are used as ornamentals.

Time of year to survey: In Fiji, generations are continuous throughout the year (Martin Kessing and Mau, 1993). The larval populations increase in June and peak in August (Davis et al., 2005). After this, adults emerge with females laying eggs from June to October (Kumar and Lal, 1983).

Trapping: No pheromones or semiochemicals have been identified for *E. fullonia*. Combinations of attractants have been developed and incorporated into sugared-agar baits. Field trials with a range of these experimental baits in Clementine and hybrid mandarins in north Queensland showed many more attacks on the best baits (85% of moths) than on fruit up to the early harvest phase. Baits were less competitive, but still very attractive, when nearly all fruit reached the ripe stage. Recent studies have resulted in the selection of a suitable toxicant to incorporate in the baits, while the means to present the attractants so that they have a practical field life are still being pursued.

In an experiment by Reddy et al. (2007), researchers found that *E. fullonia* was significantly more attracted to fruit puree with agar and phytogel than to fruit puree with agarose. Fruit baits were ranked with banana being the most preferred, followed by banana and orange. These were significantly more attractive than kiwi, apple, pineapple, pear, papaya, mango, grapefruit, tomato, and green grape. The least attractive were star fruit, plum, and sour sop which were no more attractive than the controls (Reddy et al., 2007).

This species is attracted to ultraviolet lights making blacklight traps a potential trapping method (Martin Kessing and Mau, 1993). However, this method is not specific to this species.

Key Diagnostics/Identification

CAPS-Approved Method*: Confirmation of *E. fullonia* requires a morphological identification.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Confirmation of *E. fullonia* requires a morphological identification.

A short description of the adult can be found in Baptist (1944). Maddison (1982) also has short descriptions of the life stages.

Easily Confused Pests

Adults of *E. fullonia* closely resemble species such as *Eudocima homaena* and *Eudocima jordani*. All species of *Eudocima* cause similar damage. Separation of species involves detailed microscopic examination. Fruit discoloration could be attributed to fruit fly damage, but the size of the hole left at the damage site will clarify whether fruit-piercing moths were involved.

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Eutetranychus orientalis

Scientific Name
Eutetranychus orientalis Klein

Synonyms:
Anychus orientalis, Anychus ricini, Eutetranychus monodi, Eutetranychus sudanicaus, Eutetranychus anneckei, Anychus latus, and *Eutetranychus latus.*

Common Name(s)
Citrus brown mite, oriental mite, oriental red mite, oriental spider mite, and Lowveld citrus mite

Type of Pest
Mite

Taxonomic Position
Class: Arachnida, **Order:** Acarina, **Family:** Tetranychidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2006 through 2009

Pest Description
The citrus brown mite is principally a pest of citrus, but has also been reported to attack a wide variety of other plants, including banana, cassava, castor bean, cotton, fig, frangipani, maize, mulberry, oleander, peach, plum, rose, squash, grape, pawpaw, pear, quince, and walnut (Jeppson et al. 1975; Gupta, 1985; Meyer, 1987).

The genus *Eutetranychus* is characterized by its empodium, which is reduced to a small protuberance (Avidov and Harper, 1969). The life cycle of *E. orientalis* is completed in four active (larva, protonymph, deutonymph, and adult) and three quiescent stages (nymphochrysalis, deutochrysalis, and teleochrysalis) (Lal, 1977).

Eggs: The eggs of *E. orientalis* are oval or circular (Fig. 1) and flattened, coming to a point dorsally, but lacking the long dorsal stalk of other spider mites. Newly laid, the eggs are bright and hyaline, but later they take on a yellow, parchment-like color (Smith-Meyer, 1981). Diameter of the eggs is 0.14 mm (0.006 in.)

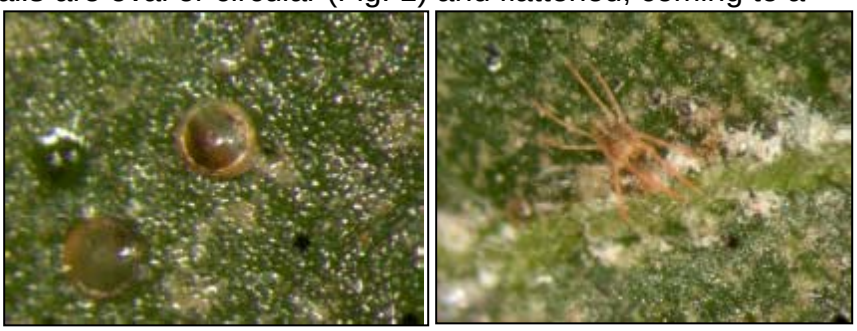

Figure 1. Eggs (left) and adult (right) of *E. orientalis*. Photos courtesy of Pedro Torrent Chocarro.

(Avidov and Harper, 1969).

Larvae: Average size of the larva of *E. orientalis* is 190 x 120 µm. The abdomen of female larvae and nymphs is greenish brown, while the abdomen of male larvae is reddish brown. The protonymph is pale-brown to light-green, with legs shorter than the body, average size 240 x 140 µm. The deutonymph is pale-brown to light-green, average size 300 x 220 µm.

Adults: Adult female *E. orientalis* are broad, oval, and flattened. They vary in color from pale brown through brownish-green to dark green with darker spots within the body. The legs are about as long as the body and are yellow-brown (Fig. 1). Average size is 410 x 280 µm. Females have the dorsal striae of the prodosoma more or less parallel and slightly but distinctly lobed. The dorsal setae of the body are set on small tubercles, and the lateral setae of the body are moderately slender and spatulate.

Technical description: Empodia lacking on all tarsi; true claws slender, padlike, each with pair of tenent hairs; duplex setae of tarsi loosely associated, not paired as in other spider mites; 2 pairs of anal setae; 3 pairs of dorsal propodosomal setae, and 10 pairs of dorsal hysterosomal setae, all setae stout, serrate; dorsal striae of hysterosoma form V-pattern between setae D1 and E1, and setal bases E1 and F1 form a square; setal cout (solenidia or sensory rodlike setae in parentheses) of legs (Meyer, 1974). L coxa 2-1-1-1, trochanter 1-1-1-1, femur (8-6-3/4-1/2), genu (5-5-2-2), tibia 9(1/4)-6(0/2)-6(0/1)-7, and tarsus 15(3)-13(1/2)-10(1)-10(1).

Adult male *E. orientalis* are much smaller than the females. They are elongate and triangular in shape with long legs (leg about 1.5 x body length). Usually males have a higher solenidia count.

Short setae are found on legs and body of both sexes at all stages. The body setae are short, however, and cannot be seen with a 10x lens (Smith-Meyer, 1981; Dhooria and Butani, 1984).

The outstanding characteristic in the adult is that the legs are equal to, or longer than the body length (Avidov and Harper, 1969).

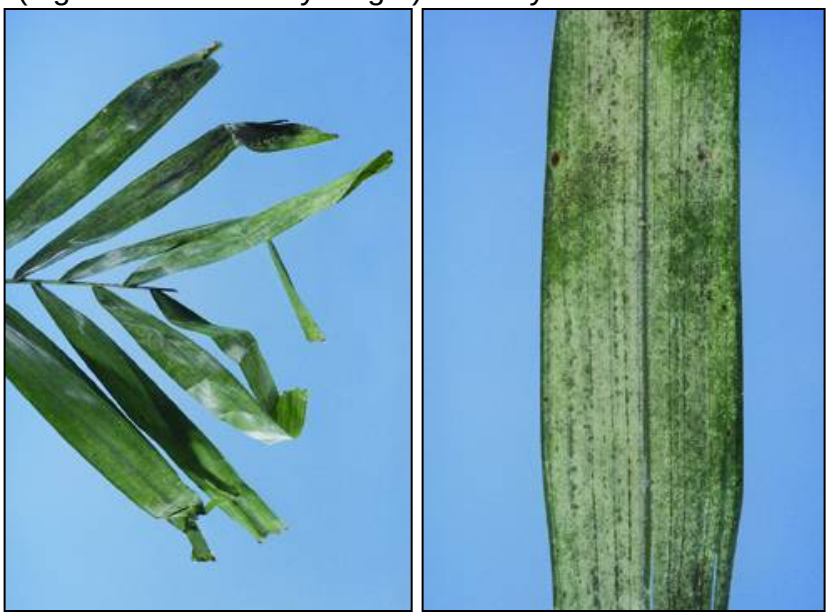

Figure 2. *Eutetranychus* feeding damage on *Ptychosperma* palm. Photos courtesy of http://www.pestalert.org/viewArchPestAlert.cfm?rid=62.

Symptoms/Signs

E. orientalis begins feeding on the upper side of the leaf along the midrib and then

spreads to the lateral veins, causing the leaves to become chlorotic. Pale yellow streaks develop along the midrib and veins (Fig. 2) initially, which later progress to a grayish or silvery appearance of the leaves. At times, the leaves appear to be covered in a layer of fine dust. When damaged, the younger, tender leaves show margins that are twisted upwards. Usually, little webbing is produced but can occur. In heavier infestations, the mites feed and oviposit over the whole upper surface of the leaf. Very heavy infestations on citrus cause leaf fall and die-back of branches, which may result in defoliated trees. Lower populations in dry areas can produce the same effect.

Survey

CAPS-Approved Method*: Visual survey is the method to survey for *E. orientalis.*

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Surveys should be focused where the greatest risk for establishment occurs. A recent risk analysis by USDA-APHIS-PPQ-CPHST (Fig. 3) indicates that in most states in the continental United States pest establishment is unlikely. Risk for *E. orientalis* establishment based on climate and host availability is low in Arizona, California, and Nevada, Texas, and Utah. Risk is low to moderate in Florida.

Visual survey: The presence of *E. orientalis* can be detected by discoloration of the host leaves and pale-yellow streaks along the midribs and veins. Eggs, immature stages, and adults may be observed visually on the upper leaf surface. Adult females are larger than the males. They are oval and flattened and are often pale brown through brownish-green to dark green. Webbing is possible (often dust colored), providing protection for the eggs. The spread of the mite is windborne, and new infestations commonly occur at the field perimeters. Field perimeters should, therefore, be scouted, especially field perimeters facing prevailing winds. Studies indicate that alfalfa plays a role in dispersing tetranychid mites to other crops (Osman, 1976). Fields near alfalfa should be targeted for survey. Shake leaves above white paper or cloth, and use a hand lens to observe mites.

Hall (1992) discusses sampling strategies for spider mites in orange groves. The author's sampling method consisted of examining 16 leaves per tree, five trees within a small area of trees, and three areas per block. The leaves were collected by gently pulling four leaves from each of the north, east, south, and west sides of a tree. The leaves from each side of the tree were placed into separate plastic bags. The bags were placed in a cold ice chest, taken to the laboratory, and examined under a microscope to count the number of spider mites present per leaf (both surfaces).

Gilstrap and Browing (1983) recommend using a liquid sampling procedure for leaf collecting of mites, where leaves are placed in a jar filled with 0.5% liquid dishwashing soap and 0.5% standard bleach (5% NaCl) (each % by volume) in a solvent of distilled water. The liquid soap is used to break up surface tension; while the bleach is used to

dissolve any webbing. The author showed that the liquid sampling procedure collected more mites than the 'normal procedure'. In the 'normal procedure', leaves are placed in a paper bag and a mite brushing machine is used to dislodge mites from the samples when processed the next day. Dhorria and Butani (1984) collected forty random leaves (10 leaves per tree) from each almond variety at different heights and all sides of the plants to assess mite resistance. A mite brushing machine was used to dislodge the mites from the leaves on to counting disks.

Key Diagnostics/Identification

CAPS-Approved Method*: Confirmation of *E. orientalis* is by morphological identification. The mite can only be identified by examination of the adult male.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.
Literature-Based Methods: According to a NAPPO pest alert, the only form of *E. orientalis* that can be identified is the adult male. Conflicting information states that identification of *E. orientalis* requires examination of cleared and mounted female specimens by transmitted light microscopy.

Mite experts agree that though it may be possible to identify a specimen with a slide mounted female, one can never be 100% sure without a male for confirmation.

Walter et al. (1995) provide a key to differentiate known Australian species of *Eutetranychus*, including *E. orientalis*.

Easily Confused Pests

E. orientalis can be easily mistaken for the Texas citrus mite (*E. banksii*). Similarity of the female *E. orientalis* with other tetranychid mites such as the two-spotted mite (*Tetranychus urticae*) can make identification difficult.

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Helicoverpa armigera

Scientific Name
Helicoverpa armigera Hübner

Synonyms:
Bombyx obsoleta, Chloridea armigera, Chloridea obsoleta, Helicoverpa commoni, Helicoverpa obsoleta, Heliothis armigera, *Heliothis conferta, Heliothis fusca, Heliothis obsoleta*, *Heliothis pulverosa*, *Heliothis rama*, *Heliothis uniformis*, *Noctua armigera*, and *Noctua barbara.*

Common Name
Old world bollworm, scarce bordered straw worm, corn earworm, cotton bollworm, African cotton bollworm, tobacco budworm, tomato grub, tomato worm, and gram pod borer.

Type of Pest
Moth

Taxonomic Position
Class: Insecta, **Order:** Lepidoptera, Family: Noctuidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2003 through 2012

Pest Description
For more information, see Common (1953), Dominguez Garcia-Tejero (1957), Kirkpatrick (1961), Hardwick (1965, 1970), Cayrol (1972), Delattre (1973), and King (1994).

Eggs: Yellowish-white when first laid (Fig. 1), later changing to dark brown just before hatching. Eggs are gum drop-shaped and 0.4 to 0.6 mm (~0.02 in.) in diameter. The top is smooth, otherwise the surface contains approximately 24 longitudinal ribs. The eggs then change to dark or gray black a day before hatching (Bhatt and Patel, 2001; CABI, 2009).

Figure 1. Eggs of *Helicoverpa armigera.* Photo courtesy of BASF Corp.

Larvae: Larval color darkens with successive molts for the six instars typically observed for *H. armigera*. Coloration can vary considerably due to diet content (Fig. 2 A, B). Coloration ranges from bluish green

to brownish red (Fowler and Lakin, 2001). Freshly emerged first instar larvae are translucent and yellowish-white in color. The head, prothoracic shield, supra-anal shield, and prothoracic legs are dark-brown to black, as are also the spiracles and raised base of the setae, which give the larvae a spotted appearance (Fig. 2A, B) due to sclerotized setae, tubercle bases, and spiracles (King, 1994; Bhatt and Patel, 2001). Second instar larvae are yellowish green in color with black thoracic legs. Five abdominal prolegs are present on the third to sixth, and tenth abdominal segments. The full grown larvae are brownish or pale green with brown lateral stripes and distinct dorsal stripe; long and ventrally flattened but convex dorsally. Larval size in the final instar ranges from 3.5 to 4.2 cm (1.38 to 1.65 inches) in length (King, 1994).

Pupae: Dark tan to brown (Fig. 2C), 14 to 22 mm (0.55 to 0.87 in.) long and 4.5 to 6.5 mm (0.18 to 0.26 in.) in width. Body is rounded both anteriorly and posteriorly, with two tapering parallel spines at posterior tip. Pupae typically are found in soil.

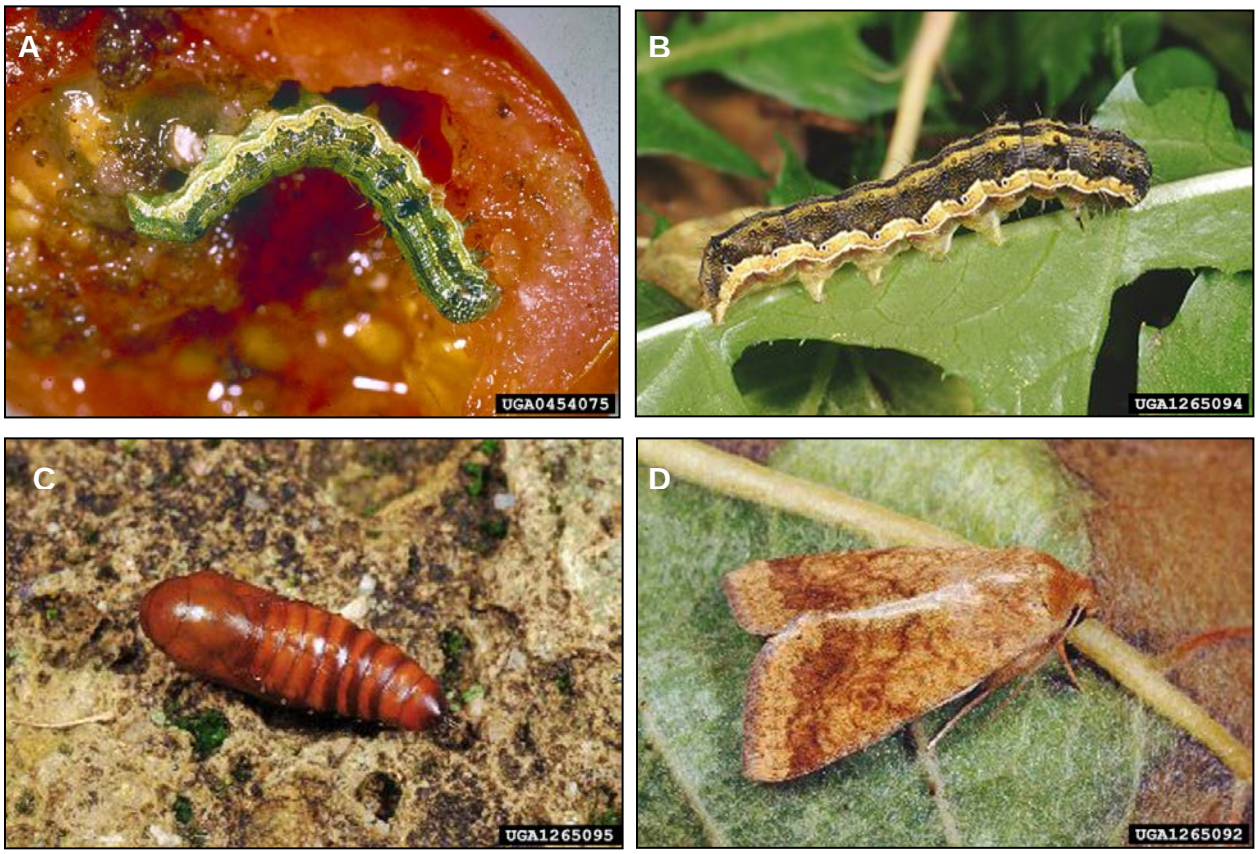

Figure 2. Life stages of *Helicoverpa armigera* (images not to scale): (A, B) larva, (C) pupa, and (D) adult. Photos courtesy of Central Science Laboratory, Harpenden Archive, British Crown and Paolo Mazzei http://www.bugwood.org.

Adults: A stout-bodied moth with typical noctuid appearance, with 3.5 to 4 cm (1.38 to 1.57 inches) wing span; body, 14 to 19 mm (0.55 to 0.75 in.) long. Color is variable, but male usually greenish-gray and female orange-brown (Fig. 2D). Forewings have a line of seven to eight blackish spots on the margin and a broad, irregular, transverse brown

band. Hind wings are pale-straw color with a broad dark-brown border that contains a paler patch; they have yellowish margins and strongly marked veins and a dark, comma-shaped marking in the middle.

Symptoms/Signs

H. armigera larvae prefer to feed on reproductive parts of hosts (flowers, flower buds, and fruits), but may also feed on foliage and shoots (EPPO, 2003). Larvae feed internally in fruits and vegetables and can be difficult to detect (EPPO, 2003). Larvae may also be found near bore-holes in fruits and flowers. Later instars feed externally and are easier to detect (EPPO, 2003). Larval feeding results in holes bored into reproductive structures and feeding damage within the plant. Many times larvae will move from one structure to another without completely consuming the first (Mustafa, 2004).

Fruit trees may be attacked early on in the growing season, damaging fruit buds, blossoms, and young shoots (Kriegler, 1961). Attacks are usually restricted to fruitlets once they are formed (Kriegler, 1961). Attacks on fruit, specifically peaches, can lead to malformed fruit that has large corky depressions due to feeding that occurs on and below the fruit surface (Kriegler, 1961).

It may be necessary to cut open the plant organs to detect the pest. Secondary pathogens (fungi, bacteria) may develop due to the wounding of the plant. Frass may occur alongside the feeding hole from larval feeding within. Eggs are laid on or near floral structures. Plants in flower are preferred to those that are not in flower (Firempong and Zalucki, 1990). Depending on the quality of the host, *H. armigera* may also lay eggs on leaf surfaces.

Female moths tend to choose pubescent (hairy) surfaces for oviposition rather than smooth leaf surfaces (King, 1994). Taller plants also tend to attract heavier oviposition than shorter plants (Firempong and Zaluski, 1990).

Survey

CAPS-Approved Method*: The CAPS-approved method is a trap and lure combination.

Any of the following Trap Product Names in the IPHIS Survey Supply Ordering System may be used for this target:

1) Plastic bucket trap
2) *Heliothis* trap
3) Texas (Hartstack) trap

The Lure Product Name is "*Helicoverpa armigera* Lure." The lure is effective for 28 days (4 weeks).

Trap Spacing: When trapping for more than one species of moth, separate traps for different moth species by at least 20 meters (65 feet).

Method Notes: The Plastic Bucket Trap is also known as the unitrap. The trap has a green canopy, yellow funnel, and white bucket and is used with a dry kill strip. See Brambila et al. (2010) for instructions on using the plastic bucket trap. The Texas (Hartstack) trap is not available commercially. See Hartstack et al. (1979) or Johnson and McNeil (1994) for images and trap design.

Lure Placement: Placing lures for two or more target species in a trap should never be done unless otherwise noted here.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:
Surveys should be focused on areas where this pest is most likely to establish. According to Fowler and Lakin (2001), it is probable that *H. armigera* could establish in every state in the continental United States based on habitat and host suitability and would probably pose the greatest economic threat to the following states: Alabama, Arizona, Arkansas, California, Georgia, Illinois, Iowa, Kansas, Louisiana, Michigan, Minnesota, Mississippi, Nebraska, New Mexico, North Carolina, Ohio, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Virginia, and Wisconsin. A recent risk analysis by USDA-APHIS-PPQ-CPHST, however, indicates that areas of Alabama, Arkansas, Arizona, Florida, Georgia, Louisiana, Mississippi, Oklahoma, South Carolina, and Texas have the greatest risk for *H. armigera* establishment based on host availability and climate within the continental United States. Areas of most states, however, have a low to moderate risk for *H. armigera* establishment.

Survey site and selection: *Helicoverpa armigera* is a major insect pest of both field and horticultural crops in many parts of the world (Fitt, 1989). *H. armigera* has been reported causing serious losses throughout its range, in particular to cotton, tomatoes, and corn. Surveys should be focused on areas that have abundant host material like agricultural crops and nurseries.

Time of year to survey: Moths emerge in May to June depending on latitude. In Australia, *H. armigera* begin emerging in the spring (Duffield and Steer, 2006). In Pakistan, moths are active in June and July when ambient temperatures fall (Mustafa, 2004). Adults can be present at different times of the year as this pest can have multiple generations per year.

Trap Placement: Traps should be hung at or slightly above crop height. This can range from 1.5 to 1.8 m (5 to 6 ft) in height (Gauthier et al., 1991; Greg and Wilson, 1991), depending on the crop. The Texas trap should be placed directly above the crop canopy (Greg and Wilson, 1991).

Trapping: (From Venette et al., 2003). Pheromone traps using (*Z*)-11-hexadecenal and (*Z*)-9-hexadecenal in a 97:3 ratio have been used to monitor populations of *H. armigera*

(Pawar et al., 1988; Loganathan and Uthamasamy, 1998; Loganathan et al., 1999; Visalakshmi et al., 2000; Zhou et al., 2000). Of three pheromone doses tested in the field (0.75, 1.0, and 1.25 mg/septum), 1 mg attracted the most males (Loganathan and Uthamasamy, 1998); the trap type was not specified. Rubber septa impregnated with these sex pheromone components (1 mg/septum) were equally effective in capturing males for 11 days in the laboratory (Loganathan et al., 1999). Captures of *H. armigera* in the field were significantly lower with 15-day-old lures than with fresh lures, and the authors recommend replacing lures every 13 days (Loganathan et al., 1999). Similar observations were reported by Pawar et al. (1988). Males responded to the pheromone during dark hours only, commencing at 6:00 PM and terminating at 6:00 AM. The highest response was between 11:00 PM and 4:00 AM (Kant et al., 1999).

Trap design has a significant impact on the number of male *H. armigera* moths that will be captured with pheromone lures. Funnel traps and Texas traps are substantially more effective than sticky traps (Kant et al., 1999). Hartstack *(i.e.,* hollow cone) traps have also been used to effectively monitor densities of adults (Walker and Cameron, 1990). Cone traps are significantly more effective than water-pan traps (Sheng et al., 2002). Traps have been placed approximately 1.8 meter (6 feet) above the ground (Kant et al., 1999; Zhou et al., 2000), and have been separated by a distance of at least 50 meters (160 feet) (Kant et al., 1999). Aheer et al. (2009), however, installed traps at a height of 1.5 meters (4.9 feet) and were separated by a distance of about 10 meters (33 feet). For routine monitoring of pests, pheromone traps are deployed at a density of 5 traps per hectare (Sidde Gowda et al., 2002).

Adults of both sexes can be captured in black light traps.

Visual survey: Visual inspections of plants for eggs and/or larvae are frequently used to monitor and assess population sizes for *H. armigera*. Females lay several hundred eggs on the leaves (top 20 cm (7.87 in.)), flowers and fruits (Duffield and Chapple, 2000). The lower leaf surface is a preferred oviposition site. Eggs may hatch in less than 3 days at an optimum temperature of 27 to 28°C (81 to 82°F). The feeding larvae can be seen on the surface of plants but they are often hidden within plant organs (flowers, fruits, etc.). Bore holes and heaps of frass (excrement) may be visible, but otherwise it is necessary to cut open the plant organs, especially damaged fruit, to detect the pest (Bouchard et al., 1992). In temperate regions, *H. armigera* overwinters as a pupa buried several cm in the soil. Adults appear in April to May and can be observed until October, because of the long migration period.

This pest may move through ornamental plants and cut flowers in international trade (EPPO, 2003).

In vegetative Australian cotton and irrigated soybean, a minimum of 60 whole plants per 100 hectare commercial field are examined for the presence of *H. armigera* eggs or larvae; when plants begin to produce squares, only the upper terminal (approximately 20 cm (7.87 inches)) of a plant is inspected (Brown, 1984; Dillon and Fitt, 1995; Duffield and Chapple, 2000). In experimental plots, visual inspections for *H. armigera* in pigeon

pea were restricted to the upper third of whole plants (4 sets of five plants in a 30 x 30 meter (98 x 98 foot) plot) (Sigsgaard and Ersbøll, 1999).

Leaves of tomato plants are more attractive than flowers or fruits as *H. armigera* oviposition sites, but use of a single-leaf sample unit (with a sample size of 30 plants per field) has proven ineffective in detecting low densities of *H. armigera* (Cameron et al., 2001). On some tomato cultivars, leaves in the upper half of the plant are preferentially selected for oviposition (Saour and Causse, 1993).

Helicoverpa armigera is capable of long-distance migratory flights (King, 1994; Zhou et al., 2000; Casimero et al., 2001; Shimizu and Fujisaki, 2002; CABI, 2009). Adults can disperse distances of 10 km (6.2 miles) during 'non-migratory flights' and hundreds of kilometers (up to 250 km (155 miles)) when making 'migratory flights', which occur when host quality or availability declines (Saito, 1999; Zhou et al., 2000; Casimero et al., 2001; Fowler and Lakin, 2001).

Key Diagnostics/Identification

CAPS-Approved Method*: Confirmation of *Helicoverpa armigera* is by morphological identification. *H. armigera* can be visually screened to some degree, but definitive screening and identification requires dissection. *Helicoverpa armigera* and the native, abundant species, *Helicoverpa zea* are very similar looking. Final identification is by dissection of (adult) male genitalic structures.

Screening aids and instructions for dissecting *H. armigera* are available at:

Brambila, J. 2009. Helicoverpa armigera Screening Aids. http://caps.ceris.purdue.edu/webfm_send/552.

Brambila, J. 2009. Dissection instructions for identifying male *Heliocoverpa amigera* and *H. zea*. http://caps.ceris.purdue.edu/webfm_send/551.

Passoa, S. 2007. Identification guide to larval Heliothinae (Lepidoptera: Noctuidae) of quarantine significance. http://caps.ceris.purdue.edu/webfm_send/109.

*For the most up-to-date methods for survey and identification and additional resources, see the Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

H. armigera belongs to a complex of similar species. Adults may be identified by distinct differences in genitalia (Common, 1953; Kirkpatrick, 1961; Hardwick, 1965; EPPO, 2003). Differentiation between *H. armigera* and *H. zea,* which is present in the United States, is very difficult; identification is by dissection of internal structures of adult males (Pogue, 2004). A morphological study of *H. assulta, H. punctigera,* and *Heliothis virescens* (formerly *H. rubrescens*) compares similarities and differences between species; a key is provided for identifying adults (Kirkpatrick, 1961).

A diagnostic protocol for *H. armigera* has been developed by the EPPO (2003). This protocol includes identification sections on the last instar larvae as well as the adult. It also includes a comparison between the male genitalia of *H. armigera* and similar species, including *H. zea* and *H. punctigera* (EPPO, 2003).

Immunological tests are available to differentiate *H. punctigera* and *Heliothis virescens* in egg or larval stages (Ng et al., 1998).

The LepTon test, an Enzyme Linked Immunosorbent Assay (ELISA) based approach, has been developed to distinguish between *H. armigera* and *H. punctigera* in the egg and larval stages (Trowell et al., 1993). Cahill et al. (1984) provide morphological information to distinguish third/fourth and sixth instar larvae of *H. armigera* and *H. punctigera*.

Agusti et al., (1999) developed sequence amplified characterized region (SCAR) markers to detect *H. armigera* eggs in the gut of predators. It may be possible to adapt this procedure to detect *H. armigera in planta*.

Easily Confused Pests

Helicoverpa zea is native to the United States and is attracted to the same lure as *H. armigera. H. zea* is very similar looking to *H. armigera* and is encountered frequently in *H. armigera* traps. Additional noctuid species that can be confused easily with *H. armigera*, include *H. assulta* (not known in the United States), *H. punctigera (*not known in the United States), and *Heliothis virescens* (present in the United States) (Kirkpatrick, 1961; CABI, 2009).

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Oxycarenus hyalinipennis

Scientific Name
Oxycarenus hyalinipennis Costa

Synonyms:
Aphanus hyalinipennis and *Aphanus tardus* var. *hyalipennis*

Common Name(s)
Cotton seed bug, cotton stainer, dusty cotton stainer, dusky cotton bug, dusky cottonseed bug, Egyptian cotton seed bug

Type of Pest
Bug

Taxonomic Position
Class: Insecta, **Order:** Hemiptera, **Family:** Lygaeidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2008 through 2012

Pest Description
Eggs: Oval 0.28 x 0.95 mm (0.01 x 0.04 in.), longitudinally striated, pale yellow becoming pink.

Nymphs: Head and thorax brownish-olivaceous, abdomen pinkish. Fifth instar darker brown on head and thorax, wingpads distinct, extending to at least third abdominal segment.

Adults: (Fig. 1) Newly emerged individuals are pale pink, but rapidly turn black. Length of male about 3.8 mm (0.15 in.); female 4.3 mm (0.17 in.). Male abdomen terminates in round lobe, while the female's is truncate. The insects have three tarsal joints and a pair of ocelli. Second antennal segments are usually in part pale yellow. Hemelytra hyaline and usually whitish; clavus, base of corium, and costal vein more opaque than rest. Setae of 3 different types: More or less erect, stiff setae,

Figure 1. *Oxycarenus hyalinipennis* adult, dorsal and side view. Photos courtesy of Natasha Wright, Florida Department of Agriculture and Consumer Services, http://www.bugwood.org/.

which are blunt at tip and terminate in four to seven small teeth; normal, straight, tapering setae; and very thin, curved, flat-lying setae (USDA, 1983).
Oxycarenus hyalinipennis begins feeding, mating, and egg laying when the seeds of its host become available. Resting adults leave their shelters, move to young cotton plants, and wait for the bolls to ripen. Females lay eggs in the lint of the open bolls. Adults and nymphs generally feed on the seeds of plants in the family Malvaceae. The last generation undergoes aestivation until seed material is available the next growing season (NPAG, 2003).

Symptoms/Signs

Oxycarenus hyalinipennis is a seed feeder, with primary hosts occurring within the Malvaceae family, specifically *Gossypium* spp. (cotton). Currently there are 40 hosts reported in the literature from the Malvales order on which *O. hyalinipennis* is capable of reproduction. On cotton, the lint in which the bugs have been present is stained pinkish, sometimes with a trace of green, and contaminated with crushed fragments of the insect. Cotton seeds appear undamaged on the outside; internally, the embryos are shriveled and discolored (USDA, 1983).

O. hyalinipennis has been reported on several other hosts, including stone fruit, in 11 different families. The ability of *O. hyalinipennis* to reproduce on these hosts and the level of feeding damage, however, is not known on these additional hosts (Holtz, 2006).

O. hyalinipennis has been observed sucking the fruits of grapes (Avidov and Harpaz, 1969) as well as several types of fruit trees (plum, pear) (USDA, 1983). In Israel, *O. hyalinipennis* has been recorded causing damage to dates, figs, avocado, and persimmon (Nakache and Klein, 1992). Avidov and Harpaz (1969) state that infestations in Israel can occur in apricot, peach, and persimmon, and to a lesser extent in apple, pear, quince, and grapevine. Damage can be due to feces, pungent odors (caused by crushing of adults or nymphs) or toxic saliva (Avidov and Harpaz, 1969; Nakache and Klein, 1992; Sweet, 2000). The feeding damage can appear as greasy spots that exude light colored gum (Avidov and Harpaz, 1969).

Larvae are only known to complete development if a host plant within the order Malvales is present (USDA-APHIS, 2010). Fruits are not known to be true hosts, but they can still be damaged by adults looking for moisture (USDA-APHIS, 2010). Sweet (2000) suggests that *O. hyalinipennis* feeds on these other plants to obtain moisture so the true hosts will not be damaged by their toxic saliva. This way, the true hosts will continue to develop more valuable food seeds later on (Sweet, 2000).

Survey

CAPS-Approved Method*: Visual survey is the approved method to survey for *O. hyalinipennis.*

The CPHST Pest Datasheet includes a detailed visual survey protocol for this pest in cotton. This information is also available in the cotton commodity-based reference manual.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: *O. hyalinipennis* has been intercepted a few times each year at U.S. ports of entry. All interceptions occurred at airports, mostly in baggage; no interceptions were recorded from preferred malvaceous hosts. Interceptions of *O. hyalinipennis* have occurred on fruits and vegetables including apple, avocado, corn, dates, figs, grapes, peach, okra, pineapple, and pomegranate, as well as hibiscus (USDA, 2009). These interceptions point to the risk of *O. hyalinipennis* moving on commodities that are not its reproductive hosts (NPAG, 2003). *O. hyalinipennis* was recently found in Puerto Rico and Florida, but its current distribution is unknown. There is no pheromone or lure currently available for use in trapping *O. hyalinipennis*.

Surveys should be focused where the greatest risk for establishment occurs. According to Holtz (2006), *O. hyalinipennis* could potentially complete four to seven generations per year in all areas where U.S. cotton is produced. Based on a probability map, California, Arizona and Texas may be most vulnerable to *O. hyalinipennis*. A recent risk analysis by USDA-APHIS-PPQ-CPHST, however, indicates that most states in the United States have areas that are at low to moderate risk for *O. hyalinipennis* establishment based on climate and host availability. Surveys should pay particular attention to Florida and states along the Gulf Coast, as *O. hyalinipennis* is present in the West Indies (Slater and Baranowski, 1994) and the Bahamas (Randall Smith and Brambila, 2008). This pest was recently found in 2010 in Monroe County, Florida (FDACS, 2010) and also in Puerto Rico and the U.S. Virgin Islands (USDA-APHIS, 2010). The pest was only ever detected at two locations in the lower Keys and has been identified nowhere else throughout Monroe County, Florida in spite of repeated surveys.

Survey site selection: Surveys should be conducted in high risk areas. "In Florida, this may include cultivated or wild cotton fields in southern Florida closer to the Caribbean islands where it is currently known to be established. Areas with regular traffic from countries with known infestations that may carry hitchhiker bugs should also be targeted for regular surveys" (USDA-APHIS, 2010).

Time of year to survey: Surveys should be carried out when the host plants are in seed. Surveyors for cotton should examine crops when host plants have newly matured bolls and dry seeds (Derksen et al., 2009). For early detection surveys, surveying during the quiescent period of the host is not recommended. This is due to the cryptic nature of *O. hyalinipennis* (USDA-APHIS, 2010).

A sampling protocol has been developed for surveying for this pest in cotton. There is also another set of survey procedures developed by APHIS that is found in the New Pest Response Guidelines for *O. hyalinipennis* (USDA-APHIS, 2010). This manual

includes information on detection surveys as well as delimiting and monitoring surveys for if the pest is found in the United States.

Visual survey: Visual inspection is the only survey method available at this time. Samy (1969) observed adult clusters on leaves of mango, guava, and citrus. For cotton, cotton bolls can be tapped or torn open and examined for evidence of *O. hyalinipennis*. Sweep netting is not recommended unless the pest has a high likelihood of being found (USDA-APHIS, 2010). Adults prefer crevices in such resting sites as tree trunks, undersides of leaves on trees, pods of legumes, dried flower heads, roots of grasses, under sheath leaves of corn and sugarcane, telephone poles or wooden posts, old nests of *Polistes* spp. (paper wasps), and crevices between strands of barbed wire (Kirkpatrick, 1923).

Trees that adults can commonly be found on include *Ficus*, *Acacia*, and some *Eucalyptus*. Adults prefer rougher bark to smoother bark. Adult colonies can be found from near ground level to 6 to 7 m (19.7 to 23.0 ft.) (Kirkpatrick, 1923).

In *O. hyalinipennis*, the metathoracic glands appear similar in males and females, but a day or so after emergence the tubular glands of both sexes undergo a dramatic change from synthesizing aliphatics to the synthesis of sesquiterpenes, principally (*Z,E*)-α-farnesene (Olagbemiro and Staddon, 1983; Knight et al., 1984). Although many explanations for this phenomenon have been proposed, it appears that the metathoracic scent glands may have evolved sexual roles in this lygaeid species.

Ultraviolet lights: "UV-light traps are not recommended for surveying for the cotton seed bug except in cases where there is a need to confirm eradication or enhance detection of a known population. UV-light traps are not pest specific, and consequently are cumbersome and time-consuming for sampling and identification purposes. In addition, it is unclear whether or not UV-light traps would be an effective monitoring tool for the cotton seed bug. Kirkpatrick (1923) demonstrated positive phototropism in laboratory experiments; however, when Kirkpatrick placed light traps at night in the direct path that the cotton seed bug was known to use between a tree and nearby field where they were coming from, no individuals were captured. It was concluded that the cotton seed bug did not migrate at night, and was not attracted to light at night" (USDA-APHIS, 2010). "Conversely, Nakache and Klein (1992) noted that the cotton seed bug was strongly attracted to light at night in Israel. Additional research regarding the efficacy of UV-light traps is needed" (USDA-APHIS, 2010).

Key Diagnostics/Identification

CAPS-Approved Method*: Morphological examination of adults is needed to confirm identification. A field screening aid is available for *O. hyalinipennis* on the CAPS website at http://caps.ceris.purdue.edu/webfm_send/529. Final identification should be confirmed by dissecting and examining adult male internal structures (Brambila, 2010).

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: The key diagnostic involves morphological examination of adults.

A technical description of *O. hyalinipennis* can be found in Samy (1969). The egg and nymph stage are described in Sweet (2000).

Easily Confused Pests

A similar oxycarenid, *Metopoplax ditomoides*, is exotic to the United States but currently found in Oregon (Lattin and Wetherill, 2002; USDA-APHIS, 2010). The anterior part of the head of *M. ditomoides* is more rounded versus acute (USDA-APHIS, 2010).

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Spodoptera litura

Scientific Name
Spodoptera litura Fabricius

Synonyms:
Mamestra albisparsa, Noctua elata, Noctua histrionica, Noctua litura, Prodenia ciligera, Prodenia declinata, Prodenia evanescens, Prodenia glaucistriga, Prodenia litura, Prodenia subterminalis, *Prodenia tasmanica, Prodenia testaceoides*, *Prodenia littoralis,* and *Spodoptera littoralis.*

Common Name(s)
Cotton cutworm, rice cutworm, armyworm, taro caterpillar, tobacco budworm, cotton leafworm, cluster caterpillar, cotton worm, Egyptian cotton leafworm, tobacco caterpillar, tobacco cutworm, tobacco leaf caterpillar, common cutworm

Type of Pest
Moth

Taxonomic Position
Class: Insecta, **Order:** Lepidoptera, **Family:** Noctuidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List- 2009 through 2012

Pest Description
The two Old World cotton leafworm species, *Spodoptera litura* and *S. littoralis*, are allopatric, their ranges covering Asia and Africa, Europe and the Middle East, respectively. Many authors have regarded them as the same species, but they have been differentiated based on adult genitalia differences (Mochida, 1973; CABI, 2009).

Eggs: Spherical, somewhat flattened, sculpted with approximately 40 longitudinal ribs, 0.4 to 0.7 mm (0.016 to 0.028 in.) in diameter; pearly green, turning black with time, laid in batches covered with pale orange-brown or pink hair-like scales from the females body (Pearson, 1958; CABI, 2009).

Larva: Newly hatched larvae are tiny, blackish green with a distinct black band on the first abdominal segment. Fully grown larvae are stout and smooth with scattered short setae. Head shiny black, and conspicuous black tubercules each with a long hair on each segment. Color of fully grown larvae not constant, but varies from dark gray to dark brown, or black, sometimes marked with yellow dorsal and lateral stripes of unequal width. The lateral yellow stripe bordered dorsally with series of semilunar black marks. Mature larvae are 40 to 50 mm (1.57 to 1.97 in.). Two large black spots on first and eight abdominal segments (Hill, 1975; USDA, 1982; CABI, 2009).

Figure 1. Egg mass (left), larva (center), and adult (right). Photos courtesy of CABI, 2009.

Pupa: Reddish brown in color, enclosed inside rough earthen cases in the soil, 18 to 22 mm (0.71 to 0.87 in.) long, last abdominal segment terminates in two hooks (USDA, 1982; CABI, 2009).

Adult: Body whitish to yellowish, suffused with pale red. Forewings dark brown with lighter shaded lines and stripes. Hind wings whitish with violet sheen, margin dark brown and venation brown. Thorax and abdomen orange to light brown with hair-like tufts on dorsal surface. Head clothed with tufts of light and dark brown scales. Body length 14 to 18 mm (0.55 to 0.71 in.), wing span 28 to 38 mm (1.1 to 1.50 in.) (Hill, 1975; USDA, 1982).

See Schmutterer (1969), Cayrol (1972), and Brown and Dewhurst (1975) for additional information.

Symptoms/Signs

Specific symptoms on stone fruit are not available. On most crops, damage arises from extensive feeding by larvae, leading to complete stripping of the plants. Larvae are leaf eaters but sometimes act as a cutworm with crop seedlings.

Spodoptera litura feeds on the underside of leaves causing feeding scars and skeletonization of leaves. Early larval stages remain together radiating out from the egg mass. However, later stages are solitary. Initially there are numerous small feeding points, which eventually spread over the entire leaf. Because of this pest's feeding activities, holes and bare sections are later found on leaves, young stalks, bolls, and buds. Larvae mine into young shoots. In certain cases, whole shoot tips wilt above a hole and eventually die (Hill, 1975; USDA, 1982). Feeding damage can also occur as tunnels in compact foliage such as cabbage hearts (Waterhouse and Norris, 1987).

On cotton, leaves are heavily attacked and bolls have large holes in them from which yellowish-green to dark-green larval excrement protrudes. In tobacco, leaves develop irregular, brownish-red patches and the stem base may be gnawed off. The stems of corn are often mined and young grains in the ear may be injured (CABI, 2009). Damage is mainly to foliage, however, fruit can also be damaged (Waterhouse and

Norris, 1987). If heavy feeding on a young plant occurs, it may lead to stunted development and fruit may be small or late to develop (USDA, 2005).

On grape, larvae scrape the leaf tissue and cause 'drying of the leaves' (Balasubramanian et al., 1978). The larvae damage the growing berries and cause defoliation. Balikai et al. (1999) also showed that later instar larvae cut the rachis of grape bunches and petioles of individual berries during the night hours leading to fruit drop. The stems of corn are often mined and young grains in the ear may be injured (CABI, 2009).

Large batches of up to 300 eggs may be found on the underside of the host leaves (USDA, 2005); while pupae can be found underground (Waterhouse and Norris, 1987).

Survey

CAPS-Approved Method*: The CAPS-approved method is a trap and lure combination. The trap is Plastic Bucket Trap. The lure is effective for 84 days (12 weeks).

The Lure Product Name is "*Spodoptera litura* Lure".

Trap Spacing: When trapping for more than one species of moth, separate traps for different moth species by at least 20 meters (65 feet).

Method Notes: This trap is also known as the unitrap. The trap has a green canopy, yellow funnel, and white bucket and is used with a dry kill strip. For instructions on using the trap, see Brambila et al. (2010).

Lure Placement: Placing lures for two or more target species in a trap should never be done unless otherwise noted here.

Lure Notes: Place *S. litura* and *S. littoralis* lures in different traps and separate at least 20 meters (65 feet).

Though the lures for *Spodoptera littoralis* and *S. litura* are composed of the same two compounds (Z,E,9,11-14:AC and Z,E,9,12-14:AC), the compounds are loaded into the lure dispensers in different amounts depending on the target species. Therefore, it is necessary to use the specific lure for each of the two targets.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:
Surveys should occur in areas with the greatest risk of pest establishment. The pest has been present in Hawaii since 1964 (CABI, 2009). *S. litura* was identified in a sample from a Miami-Dade County, Florida nursery in April 2007. Pheromone traps have been placed over a nine square mile area and have yielded no additional finds.

A recent risk analysis by USDA-APHIS- PPQ-CPHST shows that portions of Alabama, Arkansas, California, Florida, Georgia, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, and Texas are at the greatest risk from *S. litura*. Establishment of *S. litura* is unlikely in many areas of the United States.

Survey site and selection: This species is highly polyphagous. The host range of *S. litura* covers at least 120 species (Venette et al., 2003). Economically important crop species include alfalfa, beans, mustards (*Brassica* spp.), peppers (*Capsicum* spp.), corn, cotton, cucurbits, eggplant, grape, peanuts, potatoes, rice, soybeans, sweet potatoes, and tobacco. Surveys should be conducted in areas where host plants are abundant. This can be in agricultural settings, nursery settings, or around ports of entry.

Time of year to survey:
Four generations occur between June and October in Japan (Nakasuji, 1976). In the seasonal tropics, several generations occur during the rainy season with the dry season survived by the pupal stage (EPPO, n.d.).

Trap Placement: Traps should be placed approximately 1.2 m (4 ft) off of the ground. Traps should not be placed under trees. When checking traps, make sure that the funnel is open and the entrance is unblocked.

Trapping: The identification of a male sex pheromone of *S. litura*, (Z,E)-(9,11)-tetradecadienyl acetate and (Z,E)-(9,12)-tetradecadienyl acetate by Tamaki (1973) has enabled effective monitoring of this species for several years. One milligram of a 10:1 mixture of these two compounds in a rubber septum attracted a comparable number of males as 10 caged virgin females in the field (Yushima et al., 1974). The compounds are most effective in a ratio (A:B) between 4:1 to 39:1 (Yushima et al., 1974). The two components in a ratio of 9:1 are available commercially as Litlure in Japan (Yushima et al., 1974). For early detection sampling, traps should be placed in open areas with short vegetation (Hirano, 1976). Krishnananda and Satyanarayana (1985) found that trap catches at 2 m (6 ft.) above the ground level caught significantly more male *S. litura* than those placed at higher of lower heights (ranging from 0.5 m to 4.0 m; 1.6 to 13.1 ft.). Ranga Rao et al. (1991) suggest trap placement at 1 m (3.3 ft.).

Visual survey: Visual survey can be used to determine the presence of *S. litura*. The presence of newly hatched larvae can be detected by the 'scratch' marks they make on the leaf surface. Particular attention should be given to leaves in the upper and middle portion of the plants (Parasuraman, 1983). The older larvae are night-feeders, feeding primarily between midnight and 3:00 am and are usually found in the soil around the base of plants during the day. They chew large areas of the leaf, and can, at high population densities, strip a crop of its leaves. In such cases, larvae migrate in large groups from one field to another in search of food. *S. litura* may be detected any time the hosts are in an actively growing stage with foliage available, usually spring and fall. Check for 1st and 2nd instar larvae during the day on the undersurface of leaves and host plants. Watch for skeletonized foliage and perforated leaves. If no larvae are obvious, look in nearby hiding places. Third instar larvae rest in upper soil layers during

the day. Sweep net for adults and larvae at dawn or dusk. Look for external feeding damage to fruits. Watch near lights and light trap collections for adult specimens. Submit similar noctuid moths in any stage for identification (USDA, 1982).

Not recommended: Light traps have been used to monitor *S. litura* populations (Vaishampayan and Verma, 1983). Capture of *S. litura* moths was affected by the stage of the moon, with the traps being least effective during the full moon and most effective during the new moon (Parasuraman and Jayaraj, 1982).

Key Diagnostics/Identification

CAPS-Approved Method*: Confirmation of *S. litura* is by morphological identification. It is difficult to distinguish *S. litura* from *S. littoralis* without close examination of the genitalia; consult appropriate keys by Todd and Poole (1980) and Pogue (2002). To separate from other noctuids, use the key developed by Todd and Poole (1980).

Screening aids to help identify *S. litura* in the field:
Brambila, J. 2008. Rice Cutworm, Field Diagnostics - *Spodoptera litura.* http://caps.ceris.purdue.edu/webfm_send/555.

Brambila, J. 2008. Rice Cutworm, Wing Diagnostics - *Spodoptera litura*. http://caps.ceris.purdue.edu/webfm_send/556.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Wing coloration has been used to separate the sexes of *S. litura* (Singh et al., 1975). *S. litura* can be easily confused with *S. littoralis*. Adults are similar, and they can be distinguished only through examination of genitalia. On dissection of the genitalia, ductus and ostium bursae are the same length in female *S. littoralis,* different lengths in *S. litura*. The shape of the juxta in males is very characteristic, and the ornamentation of the aedeagus vesica is also diagnostic. The larvae of the two species are not easily separable, but some distinguishing criteria are used for the 6th instar. Mochida (1973) provides information on morphological discrimination between the adult, pupal and larval stages of the two species.

For additional images, including photos of host damage see http://www.padil.gov.au/viewPestDiagnosticImages.aspx?id=418.

Easily Confused Pests

Adult *S. litura* closely resemble *Spodoptera ornithogali* (yellowstriped armyworm), a pest in the United States. However, the hindwings of female *S. litura* are darker than those of *S. ornithogalli*. It is also similar in appearance to *S. dolichos*, *S. pulchella* and other *Spodoptera* species found in the United States. *S. litura* is similar to *S. littoralis* which is not currently present in the United States.

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Plant Pathogens

Primary Pests of Stone Fruit (Full Pest Datasheet)

Candidatus Phytoplasma prunorum

Scientific Name
Candidatus Phytoplasma prunorum Seemuller and Scheider, 2004

Synonyms:
Apricot chlorotic leaf roll, plum leptonecrosis, peach yellows, peach decline, apricot dieback, peach rosette and peach vein clearing.

Note: Peach rosette is a distinct disease from the disease caused by the North American peach rosette phytoplasma.

Common Name(s)
European Stone Fruit Yellows (ESFY)

Type of Pest
Phytoplasma

Taxonomic Position
Class: Mollicutes, **Order:** Acholeplasmatales, **Family:** Acholeplasmataceae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List - 2010 through 2011; Stone fruit survey

Pest Description
Mollicutes are prokaryotes that have small genomes (530 bp to 1350 kbp), lack a cell wall, are pleomorphic, and have a low G + C content (23-29 mol%). Phytoplasmas belong to the class Mollicutes and are the proposed causative agents of diseases in several hundred plant species (McCoy et al., 1989). Phytoplasmas reside in the phloem tissue of the infected plant host and are transmitted primarily by insect vectors, principally leafhoppers and planthoppers, although psyllids have been shown to vector these organisms as well (Carraro et al., 1998b; White et al., 1998). Although phytoplasmas have been detected in affected plant tissues and insects with the use of technologies based on the transmission electron microscope, antibodies, and nucleic acids, they are unable to be cultured *in vitro*. Phytoplasmas cannot be morphologically or ultrastructurally distinguished from one another using either electron or light microscopy (CABI, 2009). *Candidatus* in scientific classification is a formal word that is placed before the genus and species name of bacteria that cannot be maintained in a Bacteriology Culture Collection. *Candidatus* status may be used when a species or genus is well characterized but unculturable.

European stone fruit yellows (ESFY) is a severe disease of stone fruit caused by a phytoplasma in the apple proliferation group of phytoplasmas. The group/cluster also includes phytoplasmas associated with other perennial fruit tree diseases present in Europe, including apple proliferation (*Candidatus* Phytoplasma mali) and pear decline (*Candidatus* Phytoplasma pyri). In contrast, phytoplasmas infecting stone fruit in North America (X-diseases) are members of the Western-X disease group (Poggi Pollini et al., 2001).

Diseases of stone fruits associated with phytoplasmas, including apricot chlorotic leaf roll, plum leptonecrosis, peach yellows, and peach decline, were found to have a common etiology and a single name of European stone fruit yellows was proposed (Lorenz et al., 1994). The disease is present in Europe, particularly in the Mediterranean basin.

Nemeth (1986) described the morphological features of the ESFY phytoplasma, referred to as apricot chlorotic leafroll phytoplasma, as pleomorphic (varying in size and shape) bodies. Bacilliform (rod-shaped) particles were also found. Round-shaped or spherical intravascular bodies can be found in young and lightly infested phloem cells. Bodies in old and heavily infested cells are compressed and degenerated (Nemeth, 1986).

Biology and Ecology

ESFY is an epidemic disease, characterized by rapid and widespread movement when conditions are favorable for host-plants and vectors (Carraro and Osler, 2003). The phytoplasma is graft-transmissible in plum and has been transmitted from apricot to *Vinca rosea* (Madagascar periwinkle) by dodder (*Cuscuta subinclusa*) (Carraro et al., 1992).

Cacopsylla pruni (plum psyllid) is the primary vector of the disease (Carraro et al., 1998b). The psyllid vector completes one generation per year and overwinters as an adult on shelter plants (conifers) (Carraro et al., 2001a). At the end of winter, *C. pruni* moves from shelter plants to stone fruit trees for oviposition (egg-laying). From May till the beginning of July, the new generation feeds on stone fruit. As soon as adult development is complete, *C. pruni* abandons the stone fruit trees (Carraro et al., 2001a). Fialova et al. (2004) noted that *C. pruni* prefers blackthorn (*Prunus spinosa*) for its development. The insect is strictly oligophagous on *Prunus* species.

Figure 1. *Cacopsylla pruni*. Photo courtesy of W. Jarausch. Agroscience.

The psyllid transmits the ESFY phytoplasma in a persistent manner: the minimum acquisition period is 2-4 days; the minimum latent period is 2-3 weeks; and the minimum inoculation period is 1-2 days. The retention of infectivity of *C. pruni* lasts through the winter and the following spring. When the overwintering insects reach the stone fruit trees, they are already infected and infective (Carraro et al., 2001a). The natural transmission period lasts as long as the vector is present on *Prunus* species (Carraro et al., 2004a). In areas with high infection pressure, the natural infectivity of *C. pruni* reaches levels greater than 10% (mean individual transmission potency) (Carraro et al., 2002), and the annual rate of newly infected plants was 20% (Carraro et al., 1992).

Figure 2. Leaf rolling symptom of ESFY in apricot. Photo courtesy of G. Morvan. EPPO.

In contrast to the Carraro et al. (1992, 2002) studies, Theubaud et al. (2009) found that immature and mature *C. pruni* were hardly infectious (0.6%) despite effective phytoplasma acquisition and multiplication. Immature vectors born on infected plants were able to reach their maximum phytoplasma load (10^7 genomes per insect) only after migrating to conifers. After a life-long retention of phytoplasma, their transmission efficiency was every high (60%) at the end of the winter (when they migrate back to their *Prunus* host). The authors concluded that most transmissions occur only after an effective latency of 8 months, following vector migrations and overwintering in conifers in mountainous regions in France (Thebaud et al., 2009).

Figure 3: Apricot tree showing symptoms of yellowing, leaf curl, and decline. A symptomless shoot is shown in the foreground. Image from Davies and Adams (2000).

Carraro et al. (1998b) found that it took 4-5 months for *Prunus* plants to show typical ESFY symptoms (referred to as incubation period) after vector transmission. Seemuller et al. (1998) found that the ESFY phytoplasma can persist in the stem of *Prunus* taxa in the dormant (winter) season, which is in sharp contrast to the apple proliferation and pear decline phytoplasmas in this group.

Carraro et al. (2002) demonstrated the important role played by wild *Prunus* species, such as *P. spinosa* (blackthorn) and *P. cerasifera* (cherry plum). These plants are hosts for the vector and the ESFY phytoplasma in the epidemic cycle of the disease. The phytoplasma can, therefore, survive and persist in nature independently of the presence of cultivated and susceptible plants. It should also be noted that some cultivated *Prunus* spp. are completely tolerant, and these plants can act as sources of inoculum for the spread of ESFY.

Figure 3. Reddening of Japanese plum leaves affected by ESFY (right) compared to an unaffected leaf (left). Photo courtesy of Dr. B. Schneider, BBA.

Jaraush et al. (2001) detected the ESFY-phytoplasma in *Celtis australis* (European hackberry), *Fraxinus excelsior* (European ash), and *Rosa canina* (dog rose) growing in the surroundings of infected apricot orchards. Sanchez-Capuchino et al. (1976) found the phytoplasma in *Convolvulus arvensis* (field bindweed) and *Cynodon dactylon* (bermudagrass). Varga et al. (2000) detected the ESFY phytoplasma in grapevine in Hungary. The exact role played by these non-*Prunus* species in the epidemiology of the disease is not yet clear. Jarausch et al. (2001) speculated that they may be end-hosts of the phytoplasma.

Symptoms/Signs

Ca. Phytoplasma prunorum is associated with European stone fruit yellows (ESFY) disease, which primarily includes diseases of apricot, Japanese plum, and peach. Symptoms of ESFY are influenced by species, cultivar, root stock, and environmental factors. There are many tolerant hosts that do not show any symptoms of disease but can harbor infections.

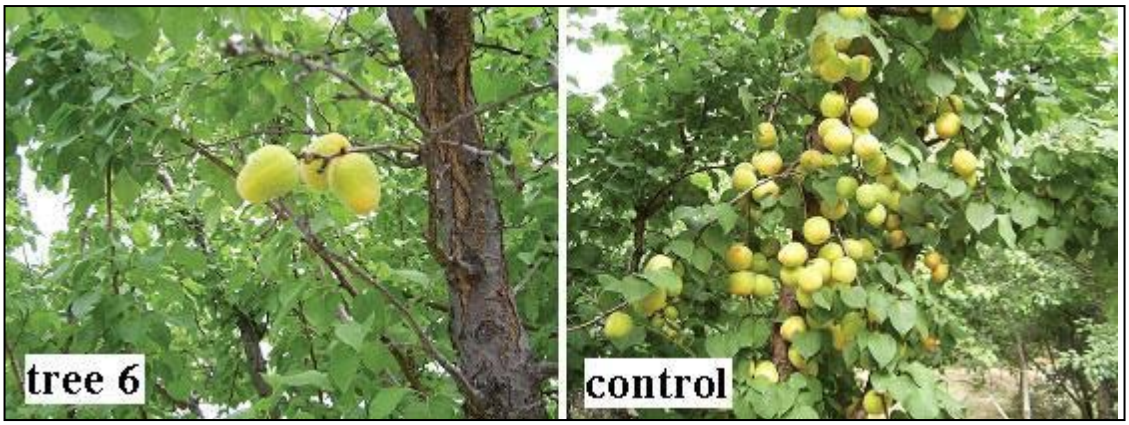

Figure 4: Fruit set of *Ca.* Phytoplasma prunorum infected tree compared with the control (non-infected) tree. Image from Gazel et al. (2009).

Apricot and Japanese plum trees in general show typical 'yellows' symptoms accompanied by leaf roll (Fig. 1, 2) followed by leaf reddening (Fig. 3), reduction, or suppression of dormancy with the consequent risk of frost damage, severe and progressive necrosis, decline, and eventual death of the tree (Morvan, 1977).. Peaches exhibit early leaf reddening, severe upward longitudinal rolling of leaves, abnormal thickening and suberization of the midribs and primary veins, autumnal growth of latent buds which produce tiny chlorotic leaves and sometimes flowers, and early phylloptosis (leaf fall) (Poggi Pollini et al., 2001). The leaves also tend to be 'more brittle' than normal. Symptoms first appeared in late summer in Italy with latent bud production occurring in September (Poggi Pollini et al., 2001).

ESFY affects tree flowers and shoots in winter, which leads to lack of fruit production (Fig. 4) and chlorosis of the leaves later in the growing season. The early break in dormancy increases the susceptibility of affected trees to frost, which can cause damage to the phloem (Fig. 5). Disease often starts with only a few branches affected but the whole tree may become affected as the disease progresses. Infected shoots are typically shorter and bear smaller, deformed leaves. Leaves can drop prematurely. Shoots may die back. Yield is reduced. Fruit on affected branches develops poorly and may fall prematurely.

Figure 5. Phloem necrosis in a *Prunus* spp. affected by ESFY (right. Photo courtesy of Dr. B. Schneider, BBA.

Pest Importance

The ESFY phytoplasma induces economically important disorders of apricot (Desvignes and Cornaggia, 1982), Japanese plum (Dosba et al., 1991), and peach (Marcone et al., 1996). Within the most sensitive cultivars of apricot and Japanese plum, 100% of the infected plants can die (Carraro and Osler, 2003). Production can be totally lost (Carraro and Osler, 2003). In Turkey, susceptible young apricot and plum trees infected with *Ca.* Phytoplasma prunorum die quickly (within 1 to 2 years after infection), and the pathogen also causes yield and quality losses on trees older than five years (Gazel et al., 2009).

Known Hosts

Major Hosts: *Prunus armeniaca* (apricot), *Prunus salicina* (Japanese plum), and *Prunus persica* (peach) (Carraro et al., 1992; Jarausch et al., 2000a; Carraro and Osler, 2003).

Note: The level of susceptibility and symptom expression varies significantly among the 'other' hosts.

Other Hosts: *Celtis australis* (European hackberry), *Convolvulus arvensis* (field bindweed), *Cynodon dactylon* (bermudagrass), *Fraxinus excelsior* (European ash), *Prunus americana* (American plum), *P. amygdalus* (sweet almond), *P. avium* (sweet cherry), *P. bokhariensis* (Indian, Persian gum), *P. brigantina* (Briançon apricot), *P. cerasifera* (cherry plum), *P. cerasus* (sour cherry), *P. coccomilia* (coccomilia), *P. consociiflora* (Chinese wild peach), *P. dasycarpa* (purple, black apricot), *Prunus domestica* (European plum), *Prunus dulcis* (almond), *P. hollywood* (Hollywood cherry plum), *Prunus serrulata* (flowering cherry), *P. laurocerasus* (cherry laurel), *P. mahaleb* (Mahaleb cherry), *P. maritime* (beach plum), *P. mexicana* (Mexican plum), *P. mume* (Japanese apricot), *Prunus orthosepal*, *P. padus* (European bird cherry), *P. salicina* x *cerasifera* (methley, cherry plum), *P. serrulata* (Japanese flowering cherry), *P. simonii* (apricot plum), *P. spinosa* (blackthorn), *P. subcordata* (Klamath plum), *P. tomentosa* (Nanking cherry), *Rosa canina* (dog rose), and *Vitis vinifera* (grape) (Morvan and Castelain, 1972; Sanchez-Capuchino et al., 1976; Giunchedi et al., 1982; Poggi Pollini et al., 1995; Jarausch et al., 1998; Jarausch et al., 1999a; Jarausch et al., 2000a; Jarausch et al., 2000b; Varga et al., 2000; Jaraush et al., 2001; Kison and Seemuller, 2001; Carraro et al., 2002; Carraro et al., 2004b; Fiavola et al., 2004; Pignatta et al., 2008).

The ESFY phytoplasma also causes infection of *Prunus* rootstocks: *Prunus besseyi* x *P. hortulana. P. cerasifera*, *P. domestica*, *P. domestica* x *P. cerasifera*, *P. mariana*, *P. persica* x *P. cerasifera,* and *P. salicina* x *P. spinosa* (Jarausch et al., 1998; Jarausch et al., 2000b; Kison and Seemuller, 2001). The susceptibility and sensitivity of the rootstocks to ESFY varies according to the different genotypes: some are highly sensitive, *i.e.* apricot seedlings and Rubira peach; others, such as Bromptom, are tolerant (Kison and Seemuller, 2001).

Japanese plum (*P. salicina*) and apricot (*P. armeniaca*) are the most susceptible and sensitive hosts. European plum (*P. domestica*) is susceptible but generally tolerant to ESFY (Carraro et al., 1998a). Despite a healthy appearance, European plum can be infected with ESFY phytoplasma and be important reservoirs of the pathogen. Some cultivars, however, can show weak symptoms but low mortality (Jarausch et al., 2000a).

Carraro et al. (2004b) demonstrated that all twelve *Prunus* species evaluated were hosts for the ESFY phytoplasma. *Prunus armeniaca* (apricot) and *P. salicina* (Japanese plum) showed high susceptibility and high sensitiveness (severe symptoms/leaf roll, small chlorotic leaves); *P. persica* (peach/nectarine) and *P. tomentosa* (Nanking cherry) showed high susceptibility and low sensitiveness (mild symptoms/yellowing only); *P. cerasifera* (cherry plum), *P. domestica* (European plum), and *P. spinosa* (blackthorn) showed high showed high susceptibility and tolerance (no symptoms); *P. amygdalus* (sweet almond), *P. avium* (sweet cherry), *P. laurocerasus* (cherry laurel), *P. mahaleb* (Mahaleb cherry), and *P. padus* (European bird cherry) showed low susceptibility and tolerance.

Ferrini et al. (2002) observed similar results to the Cararro et al. (2004b) study with the exception of the reaction of *P. tomentosa* (Nanking cherry), which ranged from being tolerant to showing mild symptoms in the two studies, respectively. The authors showed that *P. cerasifera* (cherry plum), *P. mehaleb* (Mahaleb cherry), *P. padus* (European bird cherry), *P. spinosa* (blackthorn), and *P. tomentosa* (Nanking cherry) were highly tolerant to the disease and the presence of specific symptoms is the exception. Jarausch et al. (1999a) also showed that *P. avium* (sweet cherry) demonstrated a high level of resistance to ESFY. Morvan and Castelain (1972) showed that *P. americana* (American plum) and *P. coccomilia* (coccomilia) were symptomless carriers.

Known Vectors (or associated insects)

Cacopsylla pruni is the primary vector of the disease (Carraro et al., 1998b). Poggi Pollini et al. (1996) found that the leafhoppers *Anaceratogallia* and *Euscelis* were infected by 16SrX-B phytoplasma. Pastore et al. (2003, 2004) indicated the detection of the ESFY phytoplasma in *Empoasca decendens* and *Empoasca* spp. and the ability of these leafhoppers to transmit ESFY to apricot.

Known Distribution

This pest occurs in Europe.

Africa: Tunisia (Khalifa and Fakhfakh, 2011). **Europe:** Albania, Austria, Azerbaijan, Belgium, Bosnia-Herzegovina, Bulgaria, Czech. Republic, England, France, Germany, Greece, Hungary, Italy, Netherlands, Romania, Serbia and Montenegro, Slovenia, Spain, Switzerland, and Turkey (Jarausch et al., 1998; Carraro et al., 1998a; Davies and Adams, 2000; Jarausch et al., 2000b; Topchiiska et al., 2000; Machado et al., 2001; Navratil et al., 2001; Carraro and Osler, 2003; Myrta et al., 2003; Ramel and Gugerli, 2004; Sertkaya et al., 2005; Delic et al., 2007; Fialova et al., 2007; Mehle et al., 2007; Polak et al., 2007; Ambrozic Turk et al., 2008; Jarausch et al., 2008; CABI, 2009; Gazel et al., 2009; Verbeek, 2009; Balkishiyeva et al., 2010).

The incidence of disease is different in each country. ESFY is a serious problem in countries bordering the Mediterranean Sea (Spain, France, Italy, Balkans), where the cultivation of susceptible and sensitive *Prunus* species (apricot and Japanese plum) is widespread. The record from South Africa is considered invalid (CABI, 2009).

Potential Distribution within the United States

A host analysis by USDA-APHIS-PPQ-CPHST shows that portions of California are at the greatest risk from this phytoplasma based on host availability. Most of the continental United States has a low level of risk for *Ca.* Phytoplasma prunorum establishment. The areas most of risk would be apricot, Japanese plum, and peach growing areas.

Survey

CAPS-Approved Method*:

The CAPS-approved survey method is to collect symptomatic plant tissue by visual survey. Sensitive stone fruit species (apricot and Japanese plum) can indicate the

presence of ESFY in a given area; thus particular attention should be paid to these hosts.

Follow instructions in Phytoplasma sample submission for Cooperative Agricultural Pest Survey (CAPS) Program and Farm Bill Goal 1 surveys FY 2014.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:
Visual surveys are typically conducted for ESFY phytoplasma (Jarausch et al., 2008). Delic et al. (2007) carried out surveys during autumn (October) and spring (April). Several hectares of orchards were visually inspected, and stone fruit trees were checked for symptoms of phytoplasma infection. The symptoms considered were leaf roll, yellowing, and phloem necrosis. Fialov et al. (2004) also conducted visual surveys in apricot and peach orchards, experimental plantings, and private gardens for symptoms of ESFY phytoplasma. Twigs from symptomatic stone fruit trees exhibiting yellows, leaf rolling, or decline were cut for phytoplasma tests during vegetative growth stages. Myrta et al. (2003) collected leaf samples from symptomatic samples and assayed the mid-ribs and petioles by PCR (polymerase chain reaction).

Insect vectors were shaken from *Prunus domestica* and *P. salicina* trees onto an underlying net and grouped using an aspirator (Carraro et al., 2004a). The population of the vector in the orchards was high, however, in this site. Fialova et al. (2007) used sweep-netting to capture psyllids during the vegetative season (April through mid-July). Fialova et al. (2004) used a limb-jarring technique; while Jarausch et al. (2008) used a beat-tray method.

Key Diagnostics/Identification

CAPS-Approved Method*:
Molecular: Follow instructions in Phytoplasma sample submission for Cooperative Agricultural Pest Survey (CAPS) Program and Farm Bill Goal 1 surveys FY 2014.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:
Culture: The phytoplasma that causes European stone fruit yellows is obligate and cannot be cultured on microbiological growth media.

Biological Indexing: Greenhouse indexing, which consists of graft-transmission onto a woody indicator, often using peach GF 305 as a test plant, is a time-intensive method (Desvignes and Cornaggia, 1982). Waterworth and Mock (1999) and Polak et al. (2007) found that this method is not as reliable as a nested PCR for detection of phytoplasmas. The best woody indicators for fast diagnostic detection of the ESFY phytoplasma in the Czech Republic were Tomcot, Leskora, LE-2927 and Bergeron (strongest visual

symptoms); weak appearance of symptoms was found in genotypes Velkopavlovicka, Veecot, and M-LE-1 (Necas and Krska, 2006).

Fluorescence Microscopy: For large scale diagnosis, the DAPI (4', 6'-diamidino-2-phenilindole, 2HCl) staining method (Seemuller, 1976) can be used, although the percentage of false negative can reach high levels. False negatives generally occur when phytoplasma colonization of plants is poor or uneven. This test detects fluorescence of phytoplasmas in the sieve tubes of the leaf veins.

Biological indexing and DAPI staining are time-consuming and do not often allow specific identification of phytoplasmas (Poggi Pollini et al., 2001).

Molecular: Seemuller and Scheider (2004) offer a summary of the molecular studies conducted on the apple proliferation, European stone fruit yellows, and pear decline phytoplasmas. The authors conclude that the phytoplasmas are coherent and discrete taxa and can be distinguished as distinct species with the proposed names *Ca.* Phytoplasma mali (apple proliferation), *Ca.* Phytoplasma prunorum (European stone fruit yellows), and *Ca.* Phytoplasma pyri (pear decline). A chromosome map of the ESFY phytoplasma is available (Marcone and Seemuller, 2001).

Necas and Krska (2005, 2006) found that DNA isolated from phloem gave a more reliable reaction than that isolated from leaf-stalks. The best time to collect phloem samples was June and September in the Czech. Republic; while August was the worst month. Jarausch et al. (1999b) found that colonization of trees by the ESFY phytoplasma was systemic from July until leaf fall, and that the ESFY phytoplasma could be detected in off-season grown leaves during winter until March. Almost no phytoplasma could be detected in normally grown leaves in April and May (Jarausch et al.,1999b).

Kirkpatrick et al. (1987), Ahrens and Seemuller (1992), and Maixner et al. (1995) developed a procedure to enrich DNA of phytoplasmas. Most authors working with the ESFY phytoplasma used these procedures or some modification (*e.g.*, Malisono et al., 1996) of these procedure (Marcone et al., 1996; Kison et al., 1997; Carraro et al., 1998a,b; Jarausch et al., 1998; Davies and Adams, 2000; Kison and Seemuller, 2001; Fialov et al., 2004; Sertkaya et al., 2005; Bertolini et al., 2007; Delic et al., 2007). The method of Doyle and Doyle (1990) was employed for isolating DNA from the insect vector (Carraro et al., 1998b; Carraro et al., 2001a,b, Carraro et al., 2004a; Delic et al., 2007). Green et al. (1999) developed an 'easy and efficient' DNA extraction method from woody plants for detection of phytoplasmas by PCR. Maskova (2009) evaluated four methods for DNA extraction of the ESFY phytoplasma, including the Ahrens and Seemuller (1992) method, and found that none of the methods provided the consistent quality and quantity of DNA necessary for ESFY phytoplasma detection. The authors, however, do not offer an alternative strategy.

PCR amplification is now widely used for the sensitive and reliable diagnosis of phytoplasmas in fruit trees. Due to the close genetic relatedness of the apple proliferation group of phytoplasmas, specific identification often requires the digestion of

the amplicons with various endonucleases and subsequent RFLP analysis (Ahrens and Seemuller, 1992; Deng and Hiruki, 1991; Gundersen and Lee, 1996; Lee et al., 1995; Schneider et al., 1995; Smart et al., 1996; Kison et al., 1997; Gibb et al., 1999; Jarausch et al., 2000b; Heinrich et al., 2001).

Gundersen and Lee (1996) showed that nested PCR using two universal primer pairs for phytoplasmas increased the detection sensitivity 100-fold and readily detected phytoplasmas from all the woody hosts and insect vectors tested. Torres et al. (2004) used nested PCR with 16SrX group specific primers and were able to detect the ESFY phytoplasma in 50% of asymptomatic trees that showed symptoms the following year. Ambrozic Turk et al. (2008) used PCR and nested PCR to detect the ESFY phytoplasma in 100% of Japanese plums, 70% of apricots, 13% of peaches/nectarines, 0% of cherries, and 51% of European plum (asymptomatic) trees sampled in Slovenia. Poggi Pollini et al. (1997, 2001) used immunoenzymatic detection of PCR products to detect phytoplasmas, including ESFY. Bertolini et al. (2007) developed a co-operational PCR coupled with dot blot hybridization for detection of *Ca.* Phytoplasma mali, *Ca.* Phytoplasma prunorum, and *Ca.* Phytoplasma pyri. The sensitivity of this method was at least one hundred times greater than conventional PCR and similar to that achieved by nested PCR and real-time PCR.

A primer pair (ECA1/ ECA 2), designed from conserved chromosomal sequences, showed no cross reaction in PCR amplification with other phytoplasmas of the apple proliferation group and proved to be highly specific for ESFY phytoplasma (Jarausch et al., 1998). Rubio-Cabetas and Sancho (2009) evaluated nested PCR with group-specific primers followed by RFLP and direct PCR with specific primers for *Ca.* Phytoplasma prunorum from Jarausch et al. (1998). Rubio-Cabetas and Sancho (2009) recommend the nested PCR followed by RFLP analysis for routine diagnosis rather than the direct PCR.

Real-time PCR: Torres et al. (2005) developed a real-time PCR that detects *Ca.* Phytoplasma mali, *Ca.* Phytoplasma prunorum, and *Ca.* Phytoplasma pyri (three phytoplasmas in apple proliferation group of quarantine importance). Martini et al. (2007) and Yvon et al. (2009) developed a specific PCR and real-time PCR assay for *Ca.* Phytoplasma prunorum in plants and insect vectors. Pignatta et al. (2008) developed a specific multiplex real-time PCR procedure that allows the simultaneous detection of ESFY phytoplasma and host DNA, in order to avoid false negatives due to PCR inhibition.

Easily Confused Pests

The ESFY phytoplasma is phylogenetically closely related to the apple proliferation (AP) and pear decline (PD) phytoplasmas. The peach yellow leaf roll (PYLR) phytoplasma from California was found by Kison et al. (1997) to also be closely related to AP, PD, and ESFY. The PYLR agent could clearly be distinguished from the AP and ESFY phytoplasmas by Southern blot hybridization with DNA fragments from the AP phytoplasma and by RFLP analysis of ribosomal DNA employing *SSp*I, *Bsa*AI, and *Rsa*I

restriction endonucleases. The PYLR phytoplasma, however, was indistinguishable from the PD phytoplasma by PCR-amplified ribosomal DNA (Kison et al., 1997).

Aldaghi et al. (2007) developed a real-time PCR protocol for *Ca.* Phytoplasma mali. This probe could distinguish a single mismatch between *Ca.* Phytoplasma mali and *Ca.* Phytoplasma prunorum, but late fluorescent curves were obtained from European stone fruit yellows isolates. Aldaghi et al., (2008) developed a new probe and adapted the original procedure to eliminate the late fluorescent curves.

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Monilia polystroma

Scientific Name
Monilia polystroma (anamorph) G.C.M. van Leeuwen, 2002

Synonyms:
Monilinia fructigena (Japanese isolates)

Common Name(s)
Asiatic brown rot, twig blight, twig canker

Type of Pest
Fungal pathogen

Taxonomic Position
Class: Leotiomycetes, **Order:** Helotiales, **Family:** Sclerotiniaceae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2012 through 2014

Background
The genus *Monilinia* is in the family Sclerotiniaceae and is characterized by the production of conidial and stromatal anamorphs (asexual stage), apothecial ascomata, and ascospores (Byrde and Willetts, 1977). The genus *Monilia* is the anamorph.

Monilinia spp. are well-known pathogens causing brown rot of fruit trees in many fruit production regions of the world. Three species of *Monilinia*, *M. fructicola*, *M. fructigena*, and *M. laxa*, are particularly important with regard to fruit trees and ornamentals, because they cause serious blossom and twig blight and brown rot of fruits (Petróczy et al., 2012). In 2002, a new species (described based solely on the anamorph), *Monilia polystroma*, was distinguished from *M. fructigena* based on morphological and molecular characteristics of isolates from Japan (van Leeuwen et al., 2002). This work confirmed the earlier work of Fulton et al. (1999), which showed the isolates of *M. fructigena* from Japan, on the basis of ITS sequence data, were distinct from European isolates and could possibly be regarded as a separate species.

Monilinia laxa and M. fructigena are the main agents of brown rot in Europe and are widespread. *M. fructicola* is widespread in the United States, North America, South America, South Africa, and Australia, and it's also present in at least six countries in Europe (Bosshard et al., 2006; Petróczy and Palkovics, 2006; Duchoslavova et al., 2007; Pellegrino et al., 2009; De Cal et al., 2009; Hilber-Bodmer et al., 2010; Hinrichs-Berger and Muller, 2010). *M. laxa* is also known to occur in the United States, primarily in the Pacific Northwest. *M. fructicola* is particularly problematic in the United States due to fungicide resistance and increased adaptability and variability due to the frequent occurrence of the sexual stage (Fulton and Brown, 1997). *Monilia polystroma* is not

known to occur in the United States and to date has been reported from Japan, China, Czech Republic, Hungary, Poland, Serbia, and Switzerland (van Leeuwen et al., 2002; Petróczy and Palkovics, 2009; Zhu and Guo, 2010; EPPO Reporting Service, 2011; Hilber-Bodmer et al., 2010, 2012; Poniatowska et al., 2013; Vasic et al., 2013). The color of the pustules on infected plant tissue is buff for *Monilia polystroma* and *Monilinia fructigena* and grayish-brown for *M. fructicola* and *M. laxa* (Byrde and Willetts, 1977; van Leeuwen and van Kesteren, 1998).

Hu et al. (2011) discuss the existence of two additional *Monilinia* species in China. China is also known to have the four species discussed previously. *Monilinia mumecola*, previously isolated in Japan from *Prunus mume* and causing brown rot of papaya in China, was found on peaches/nectarines in China. A new species, *M. yunnanensis*, was also found on peaches/nectarines in China and recently described by Hu et al. (2011).

Pest Description

Colonies on potato dextrose agar (PDA) reach 50 to 60 mm in diameter after 6 days at 22°C under a 12 hr. light/12 hr. dark cycle. Colony margin even, sporogenous tissue slightly elevated above the colony surface (1 to 2 mm), color buff/pale luteous. Stromatal initials formed 10 to 12 days after inoculation at 22°C (71.6°F) under 12 hr. light/12 hr. dark cycle; mature, black stromatal plates first discrete, later coalescing. Macroconidia globose, ovoid or limoniform, smooth measuring 12 to 21 x 8 to 12 µm, average 16.4 x 10.1 µm (distilled water) when grown on cherry agar (CHA) at 22°C under NUV, and 11 to 20 x 8 to 11 µm, average 14.9 x 9.1 µm on pear fruit at 15°C (59°F). On fruit, a thick hyphal layer of stroma appearing after the fruit is colonized; conidial tufts buff to brownish gray. The authors were unable to induce the formation of apothecia and thus only describe the anamorphic stage (van Leeuwen et al., 2002).

In the lifecycle of *Monilia polystroma*, like *M. fructigena*, the teleomorph (sexual stage) hardly plays a role. Apothecia are seldom found in the field (Willetts and Harada, 1984; Batra and Harada, 1986). Harada (1977) managed to obtain apothecia *in vitro* with *M. polystroma* isolates (referred to at that time as *Monilinia fructigena*).

Biology and Ecology

Due to the recent species description of *Monilia polystroma*, very little is known about the biology and ecology of the pathogen. It is expected, however, that the biology and ecology will be similar to other brown rot fungi, particularly *Monilinia fructigena*. Casals et al. (2010) evaluated the effect of temperature (0 to 38°C; 32 to 100.4°F) and water activity (a_w: 0.87 to 0.99) on the percentage of conidial germination over time for *Monilinia laxa*, *M. fructicola*, and *M. fructigena*. The three species of *Monilinia* studied were able to germinate over a wide temperature range (0 to 35°C; 32 to 95°F) at 0.99 a_w , but no germination occurred at 38°C (100.4°F) for any of the tested isolates. The optimum temperature for germination occurred after four hours of incubation and was in the range 15 to 30°C (59 to 86°F) for the studied species. Isolates of *M. fructicola* and *M. fructigena* reached 85 to 99% germination after two hours of incubation at 25°C (77°F) at 0.99 a_w; while *M. laxa* needed four hours.

Conidia of brown rot fungi, in general, overwinter in fruit mummies or cankerous lesions. These conidia serve as a primary inoculum source in the spring. Under unfavorable climatic conditions, infections can remain latent in immature fruit until conditions become favorable for disease development later in the season (Gell et al., 2008).

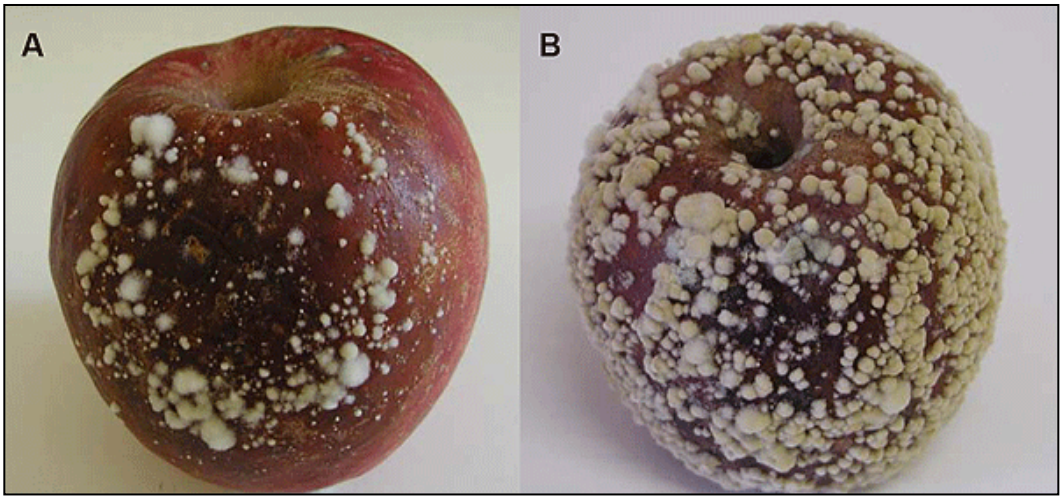

Figure 1. Apples naturally infected with *Monilinia fructigena* at; a) 5 days incubation and b) 14 days incubation. Photo courtesy of DAFF (Department of Agriculture, Fisheries, and Forestry-Australia).

Infection of *Monilinia fructigena* takes place via cracks and wounds in the fruit skin (Xu and Robinson, 2000) and also via fruit-to-fruit contact (Michailides and Morgan, 1997). Wind, water, insects, birds, and man are responsible for the dispersal of *Monilinia* conidia in pome and stone fruit orchards (Byrde and Willetts, 1977; Bannon et al., 2009). Splash dispersal is important for short range spread within a tree (Bannon et al., 2009). Lack (1989) reported spread by insects. Kable (1965) discovered that airborne conidia ensured a wide dispersal of conidia within an orchard. Van Leeuwen et al. (2002b) observed that late infected fruits in one season can contribute to primary inoculum *of M. fructigena* in the next spring, and in early summer dropped fruit (such as fruit on the ground from very late thinning) can contribute to infection on the tree. Disease incidence can be controlled by avoiding fruit wounds caused by biotic (insects, birds, man) and abiotic (frost, hail) agents.

Figure 2. Apples infected with *Monilinia fructigena*. Photo courtesy Radek Sotalar – Czech. Republic.

Monilia polystroma may colonize infected fruit of some cultivars slightly faster than *Monilinia fructigena* (van Leeuwen et al., 2002). In addition, van Leeuwen et al. (2002) speculate that the abundant stroma formed by *Monilia polystroma* may enhance the survival of the species by inhibiting decomposition of infected fruits, possibly increasing the amount of primary inoculums produced in the next season compared with *Monilinia fructigena*.

Symptoms/Signs

Damage will be similar to those caused by *Monilinia fructigena* (van Leeuwen et al., 2002). Symptoms include twig and leaf blights, stem cankers, and brown fruit rots (Fig. 1 to 3).

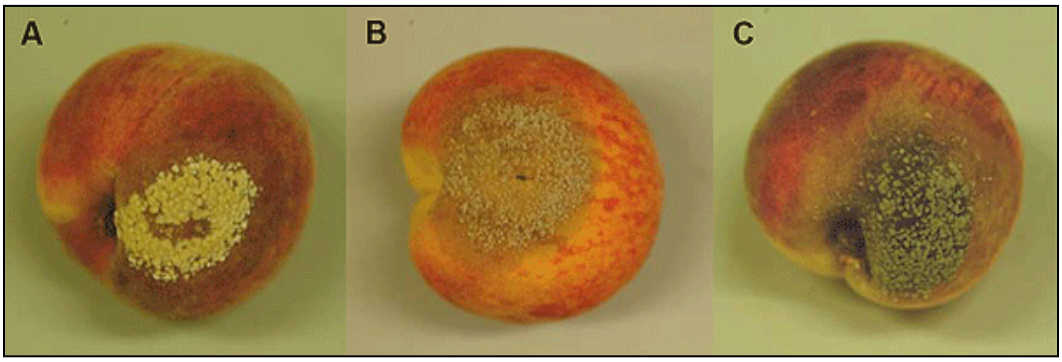

Figure 3. Peaches inoculated with a) *Monilinia fructigena*; b) *M. fructicola*; and c) *M. laxa*. **Note:** *Monilia polystroma* will be similar to *Monilinia fructigena* shown in panel A. Photo courtesy of DAFF (Department of Agriculture, Fisheries, and Forestry-Australia).

The primary and most frequent symptom is fruit rot **(**Fig. 1 to 3). Initial fruit lesions are brown, circular, and firm (Fig. 2). Eventually, the whole fruit decays and turns brown. Tufts of mycelium and conidia (cream-white to buff colored) sprout from the skin of the infected fruit (Fig. 1, 3), often arranged in concentric rings (Fig. 2) (Byrde and Willetts, 1977). When the relative humidity is low and/or when the fruits are not ripe, no mycelium and very few or no conidial tufts develop. Rotted fruits may either fall to the ground or dry out on the tree, leaving a hard, shriveled ‘mummy’. Mummified fruit hang on branches of trees until spring or fall to the ground where they remain throughout the winter months, partly or completely buried beneath the soil or leaf litter (Byrde and Willetts, 1977). Infection of fruit can take place at any time during fruit development, but the disease is only severe in ripe or ripening fruit.

Specific symptoms of *Monilia polystroma* in apple from Hungary (Fig. 4) include brownish dieback on the leaf petioles and laminas and on small fruits and fruit pedicels. Infected areas are covered with yellowish exogenous stromata (a compact mass of mycelium (with or without host tissue) that supports fruiting bodies or in which fruiting bodies are embedded) (Petróczy and Palkovics, 2009).

Figure 4. Brownish dieback symptoms and yellowish stromata on apple cv. 'Ashton Bitter' from Hungary. Photo courtesy of Tibor Szabo.

Pest Importance

Brown rot of stone fruits is an extremely destructive disease. The pathogens that cause brown rot of stone fruit also occur on apple and pear fruit trees. The disease may destroy or seriously reduce a crop by rotting mature fruit, either on the tree or after harvest.

Monilia polystroma causes severe fruit rot of fruit trees and is closely related to *Monilinia fructigena*, a regulated pest in the United States. Impacts of *Monilia polystroma* are likely to be similar to the impacts of *Monilinia fructigena*, which causes losses of apple and stone fruits, both before and after harvest. Twigs and shoots can also be infected, albeit less frequently. Crops may be severely reduced or destroyed due to the infection.

In general, *M. fructigena* is less damaging than *M. fructicola* or *M. laxa*. The severity of the disease varies from year to year depending upon environmental and storage conditions. *M. fructigena* is highly infectious and is reported to cause considerable losses in Europe during summer when warm temperatures are favorable to disease development (Scopes and Ledieu, 1983). The greatest losses are often observed in apple and plum fruits. Losses of between 7 and 36% have been reported in European apple orchards and between 0.2 and 1.5% in stored fruits (Jones and Aldwinckle, 1990; van Leeuwen et al., 2000). Latent infections can also occur, with symptoms developing after fruit ripening.

Monilinia polystroma (a synonym for *Monilia polystroma*) is listed as a harmful organism in Canada (USDA-PCIT, 2013). There would be trade implications with Canada if this pest were found in the United States.

Known Hosts

Cydonia (quince), *Malus* (apple), *Prunus* (stone fruit), and *Pyrus* (pears) (van Leeuwen et al., 2002).

Known Vectors (or associated insects)

Monilia polystroma is not known to be a vector, is not known to be vectored by another organism, and does not have any associated organisms. Insects may play a role in the dispersal of conidia, like in *Monilinia fructigena* (Lack, 1989), but this has not been studied specifically for *Monilia polystroma*.

Known Distribution

Asia: China and Japan. **Europe:** Czech Republic, Hungary, Poland, Serbia, and Switzerland (van Leeuwen et al., 2002; Petróczy and Palkovics, 2009; Zhu and Guo, 2010; EPPO Reporting Service, 2011; Hilber-Bodmer et al., 2010, 2012; Poniatowska et al., 2013; Vasic et al., 2013).

Isolates of *Monilinia fructigena* from other areas of east Asia should be examined to determine whether some isolates actually belong to *Monilia polystroma* (van Leeuwen et al., 2002).

Pathway

There have been 69 shipments of *Pyrus* sp. propagative material from known host countries since January, 2003 (AQAS, queried July 30, 2013). During the same timeframe, there have been 51 shipments of *Malus* sp., 47 shipments of *Prunus* sp., and 1 shipment of *Cydonia* sp. (all propagative material) from known host countries. Shipment sizes ranged from 1 gram to 9110 plant units. These shipments are likely comprised of a mixture of seed, plants, and cuttings based on the units of measure used.

Recently, the import of potential host plant material (with the exception of seeds), including all known host genera of *M. polystroma*, has been more tightly controlled to prevent the spread of the Citrus Longhorned Beetle (CLB) and Asian Longhorned Beetle (ALB). Import of *Malus* sp. plants for planting is allowed from several countries in Europe (Belgium, France, Germany, Netherlands), which are geographically close to the known host countries. Import of *Prunus* sp. plants for planting material are allowed from Netherlands. Import of *Pyrus* sp. propagules is prohibited from all countries except Canada. Effective May 11, 2011, import of *Cydonia* sp. plants for planting is prohibited from all countries. Due to these regulations, the potential pathway from shipment of host plant material is lowered (USDA, 2013).

Transport of *Monilia polystroma* host plant material from known host countries into the United States is common and creates a large potential pathway for this pest. For example, there have been 363 interceptions of *Pyrus* sp. plant material destined for propagation or consumption from known host countries since 2003. A significant portion of these interceptions was fruit intended for consumption. Interceptions were made in shipping cargo, airline baggage, and mail. There were also interceptions of *Cydonia* sp. (3), *Malus* sp. (311), and *Prunus* sp. (90) plant material since 2003. (AQAS, 2013).

Potential Distribution within the United States

Susceptible hosts are present in the United States. According to a recent host analysis by USDA-APHIS-PPQ-CPHST, the eastern half of the continental United States has a moderate to high level of risk of *Monilia polystroma* establishment based solely on the presence of susceptible hosts. Most areas of the western United States have a low risk; while portions of California, Washington, and Oregon have a moderate risk.

Survey

CAPS-Approved Method*: Visual survey is the approved survey method for *M. polystroma.* For visual survey, collect symptomatic plant material.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Survey for *Monilia polystroma* consists of visual inspection for symptoms, tissue sampling, and pathogen isolation.

Key Diagnostics/Identification

CAPS-Approved Method*: Morphological. Identification of brown rot fungi is commonly based on morphology and colony characteristics. This is the CAPS-Approved method until molecular methods can be validated for regulatory use.

Identification of the three main *Monilinia* species *(fructicola*, *laxa*, and *fructigena*) is commonly based on morphology and colony characteristics. Identification is possible by combining cultural characteristics, such as growth rate, growth pattern and color, with morphological data, such as conidial dimensions and the length of the germ tube (van Leeuwen and van Kesteren, 1998; De Cal and Melgarejo, 1999). Most of these characters are quantitative and overlap, so the identification has to be conducted under standardized conditions and starting from pure cultures. Lane (2003) also provides information for distinguishing the three main *Monilinia* spp. based on cultural characteristics (*M. fructigena*, *M. fructicola*, and *M. laxa*). *Monilia polystroma* can be distinguished from *M. fructigena* based on morphological and molecular characteristics of isolates (van Leeuwen et al., 2002).

Hu et al. (2011) discuss two additional *Monilinia* spp. in China: *Monilinia mumecola* and *M. yunnanensis*.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Culture/Isolation: For isolation, the standard procedure is to place pieces of infected material (with or without surface sterilization) on slightly acidic agar medium (pH 4-4.5) (EPPO, 2009). Isolation of *Monilinia* spp. from stone fruit and pome fruit surfaces is difficult, however, due to the presence of several fast-growing fungal species such as *Rhizopus*, *Alternaria*, and *Penicillium* spp. It is also possible to have mixed *Monilinia* infections. Phillips and Harvey (1975) tested a medium containing pentachloronitrobenzene (PCNB), canned strained peaches, neomycin, streptomycin, agar, and distilled water and found that though it was not totally selective that it could be used to estimate spore density of *Monilinia* spp. on the surface of fruit. Amiri et al. (2009) developed a selective medium for recovery and enumeration of *Monilinia* species that may be useful for *Monilia polystroma*. This selective medium, referred to as APDA-F500, is composed of acidified potato dextrose agar (pH 3.6) amended with fosetyl aluminum (fosetyl-AL) at 500 µg/ml. Holb and Chauhan (2005) showed that the best carbohydrate sources for mycelia growth of *Monilia polystroma* were glucose, fructose, and saccharose; while the best nitrogen source was peptone.

Molecular: Several molecular methods have been developed to distinguish *Monilinia* species. Fulton and Brown (1997) and Snyder and Jones (1999) established a PCR-based method of targeting to distinguish *M. fructigena* from *M. fructicola* and *M. laxa* based on the group I intron in the gene for the ribosomal subunit. Subsequent studies, however, showed that these methods were not reliable because some isolates of *M. fructicola* lack a group I intron in their nuclear rDNA small subunit (Förster and Adaskaveg, 2000; Fulton et al., 1999; Hughes et al., 2000; Cote et al., 2004b). Other PCR primers and protocols for *M. fructicola* were published by Förster and Adaskaveg (2000), Boehm et al. (2001), and Ma et al. (2003). However these methods discriminate *M. fructicola* from *M. laxa* but have not been validated for distinguishing *M. fructicola* from *M. fructigena*. Fluorescent AFLP fingerprinting and inter-simple sequence repeat analysis has been used to examine the genetic diversity of *M. fructicola* (Fan et al., 2010; Gril et al., 2010).

Ma et al. (2005) developed a pair of PCR primers specific to *M. laxa* on the basis of the differences in the DNA sequence of the intron 6 of *β*-tubulin gene from *M. laxa*, *M. fructicola* and other fungal species.

Ioos and Frey (2000) designed species-specific primer pairs for *Monilinia fructigena*, *M. fructicola*, and *M. laxa* based on the ribosomal internal transcribed spacer (ITS) region. This method, while testing for all three *Monilinia* species, produces PCR amplicons of the same size (356 bp), so three separate PCR reactions have to be performed in order to identify the species. Hughes et al. (2000) also developed species-specific primers for *Monilinia fructigena*, *M. fructicola*, and *M. laxa.* An internal control based universal PCR protocol was developed for *Monilinia* spp., and species-specific primers were designed by using SCAR makers (Gell et al., 2007). Miessner and Stamler (2010) and Hily et al.

(2010) developed a primer/primers based on difference in the intron-exon of the cytochrome b gene to distinguish *Monilinia fructigena*, *M. fructicola*, and *M. laxa.* Cote et al. (2004) developed a multiplex PCR that can distinguish *Monilinia fructigena*, *M. fructicola*, *M. laxa*, and *Monilia polystroma* on inoculated and naturally infected apple and stone fruit. This PCR method uses a common reverse primer (MO 368-5) and three species specific forward primers (MO 368-8R, MO 368-10R, and Laxa – R2) to differentiate the three *Monilinia* species. In this assay, a 402-bp PCR product for *M. fructigena*, a 535-bp product for *M. fructicola*, and a 351-bp product for *M. laxa* are produced. Furthermore, another specific 425-bp PCR product was amplified, enabling the identification of isolates of *Monilia polystroma*. Malvarez et al. (2001) were able to use the Cote et al. (2004) primers (prior to their publication) to identify species of *Monilinia* in Uruguay. Upon comparing the *M. fructigena* and *M. polystroma* sequences with the genomic sequence of unknown function previously described by Cote et al. (2004). Petroczy et al. (2012) revealed insertions and substitutions in the *M. polystroma* sequences. Repetitive sequence motifs were identified, which can be used for differentiation between *M. fructigena* and *M. polystroma.*

According to EPPO (2009), the PCR method of Hughes et al. (2000), Ioos and Frey (2000), and Cote et al. (2004) have been shown not to give cross-reaction with *Monilia polystroma*.

Real-time PCR methods have been developed by Luo et al. (2007) and van Brouwershaven et al. (2010). The Luo et al. (2007) method, which is based on the Ma et al. (2003) primer for *M. fructicola*, is a SYBR Green assay and has been tested only against *M. fructicola*, *M. laxa*, *Botrytis cinerea*, *Botryosphaeria dothidea*, and *Alternaria alternata*. The van Brouwershaven (2010) method is a Taq man assay and has been validated against *Monilinia fructigena*, *M, laxa*, *M. fructicola,* and *Monilia polystroma*; a FAM-labeled probe will detect *M. fructicola* while a VIC-labeled probe will detect *M. fructigena*, *M. laxa*, and *Monilia polystroma* as a group. Since the United States currently has both *M. fructicola* and *M. laxa,* at present these real-time methods may be of limited utility for the detection of exotic *Monilinia* or *Monilia* species.

Seven different PCR methods were tested by Hu et al. (2011) to differentiate *Monilinia* spp. None of the six molecular tools alone were able to distinguish all five *Monilinia* species (*M. fructigena, M. fructicola, M. laxa, M. yunnanensis, and M. mumecola*) (Ioos and Frey 2000; Ma et al. 2003, 2005; Cote et al., 2004; Gell et al., 2007; Miessner and Stammler, 2010; Hily et al., 2010). Note: The authors didn't test *Monilia polystroma*.

M. fructigena, M. fructicola, and *M. laxa* were reliably differentiated by the methods of Ioos and Frey (2000), Miessner and Stammler (2010), and Hily et al. (2010). However, neither of these methods was able to distinguish *M. fructigena* from *M. yannanensis*. Likewise, the methods developed by Ioos and Frey (2010), Ma et al. (2003, 2005) did not distinguish between *M. mumecola* and *M. laxa*. The method developed by Hily et al. (2010) did not distinguish *M. mumecola* from *M. fructicola*. Additionally, the methods of Miessner and Stammler (2010) and Hily et al. (2010) did not distinguish between *M. yunnanensis* and *M. laxa*.

Hu et al. recently (2011) developed an additional multiplex PCR to distinguish *M. fructicola* from *M. mumecola*, *M. yunnanensis* in China. Additional work needed to see if these primers distinguish *M. fructigena, Monilinia laxa*, and *Monilia polystroma*, because the authors did not find these species in China and did not present any specific data for these species.

Easily Confused Pests

Monilia polystroma could easily be confused with other brown rot fungi, particularly *Monilinia fructicola*, *fructigena*, and *laxa*). *Monilia polystroma* was first classified as a *Monilinia fructigena*. *Monilinia laxa* is considered to be more a pathogen of blossoms and twigs than of fruit and primarily occurs on *Prunus* spp. *M. fructigena* is mainly a fruit pathogen and primarily occurs on apple, pear, and other pome fruit trees, although it is also found on *Prunus* spp. (USDA ARS, 2011). *M. fructicola* is a pathogen of blossoms, twigs, and fruits and mainly affects stone fruits but can occur on apples, pears, and other pome fruits (USDA ARS, 2011). The color of the pustules on infected plant tissue is buff for *M. fructigena* and grayish-brown for *M. fructicola* and *M. laxa* (van Leeuwen and van Kesteren, 1998).

Monilia polystroma is quite similar to *Monilinia fructigena* but differences do exist. *Monilia polystroma* forms a large number of dark/black stromata in agar culture (van Leeuwen et al., 2002). *Monilinia fructigena* has the largest macroconidia where the conidia of *Monilia polystroma* are slightly smaller. Colonies of *Monilia polystroma* are similar to those *of M. fructigena*, but black stromatal plates occur on the colonies after incubation for 10 to 13 days, and *Monilia polystroma* isolates grow faster than *M. fructigena* isolates under the same conditions (van Leeuwen et al., 2002).

Other fungi can cause rots with similar symptoms to *Monilia polystroma* (*Penicillium* spp., *Mucor* spp.). Avoid collecting fruits with blue, green, or yellow colored molds or fruit that are ‘leaking’ fluid.

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Monilinia fructigena

Scientific Name
Monilinia fructigena Honey, 1945

Synonyms:
Acrosporum fructigenum, Monilia fructigena, Oidium fructigenum, Oidium wallrothii, Oospora candida, Oospora fructigena, Sclerotinia fructigena, Stromatinia fructigena, Torula fructigena

Preferred Common Name
Brown rot

Other Common Names
Apple brown rot, Asian/European brown rot of Rosaceae**,** brown fruit rot, fruit canker, fruit rot, Monilinia brown rot, spur blight, spur canker, twig blight, twig canker, wither tip

Type of Pest
Fungal pathogen

Taxonomic Position
Class: Leotiomycetes, **Order:** Helotiales, **Family:** Sclerotiniaceae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2014

Background
Monilinia fructigena is an Ascomycete fungus. The primary morphological character that distinguishes members of the Ascomycota is the ascus (plural asci), a sac-like cell containing the ascospores cleaved from within by free cell formation after karyogamy and meiosis. Eight ascospores typically are formed within the ascus but this number may vary from one to over a thousand according to the species. Asci are typically formed in an ascocarp (*i.e.,* a perithecium, pseudothecium, apothecium, or cleistothecium). Ascomycetes may have two distinct reproductive phases, one sexual (teleomorph) involving the formation of the asci and ascospores, and the other asexual (anamorph), with spore/conidia production occurring at different times on the same mycelium. The genus *Monilinia* is in the family Sclerotiniaceae and is characterized by the production of conidial and stromatal anamorphs (asexual stage), apothecial ascomata, and ascospores (Byrde and Willetts, 1977). The genus *Monilia* is the anamorph.

Monilinia spp. are well-known pathogens causing brown rot of fruit trees in many fruit production regions of the world. Three species of *Monilinia*, *M. fructigena, M. fructicola*, and *M. laxa*, are particularly important with regard to fruit trees and ornamentals, because they cause serious blossom and twig blight and brown rot of fruits (Petroczy et al., 2012). In 2002, a new species (described solely based on the anamorph), *Monilia polystroma*, was distinguished from *M. fructigena* based on morphological and molecular characteristics of isolates from Japan (van Leeuwen et al., 2002). This work confirmed the earlier work of Fulton et al. (1999), which showed the isolates of *M. fructigena* from Japan, on the basis of ITS sequence data, were distinct from European isolates and could possibly be regarded as a separate species.

Monilinia fructigena and *M. laxa and* are the main agents of brown rot in Europe and are widespread. *M. fructicola* is widespread in the United States, North America, South America, South Africa, Australia, and occurs in at least six countries in Europe (Bosshard et al., 2006; Petroczy and Palkovics, 2006; Duchoslavova et al., 2007; Pellegrino et al., 2009; De Cal et al., 2009; Hilber-Bodmer et al., 2010; Hinrichs-Berger and Muller, 2010). *M. laxa* is also known to occur in the United States, primarily in the Pacific Northwest. *M. fructicola* is particularly problematic in the United States due to fungicide resistance and increased adaptability and variability due to the frequent occurrence of the sexual stage (Fulton and Brown, 1997). *Monilia polystroma* is not known to occur in the United States and to date has only been reported from China, Czech Republic, Hungary, Japan, Poland, Serbia, and Switzerland (van Leeuwen et al., 2002; Petroczy and Palkovics, 2009; Zhu and Guo, 2010; Hilber-Bodmer et al., 2012; Poniatowska et al., 2013; Vasic et al., 2013). The color of the pustules on infected plant tissue is buff for *Monilia polystroma* and *Monilinia fructigena* and grayish-brown for *M. fructicola* and *M. laxa* (Byrde and Willetts, 1977; van Leeuwen and van Kesteren, 1998).

Hu et al. (2011) discuss the existence of two additional *Monilinia* species in China. China is also known to have the four species discussed previously. *Monilinia mumecola*, previously isolated from Japan from *Prunus mume* and causing brown rot of papaya in China, was found from peaches/nectarines in China. A new species, *M. yunnanensis*, was also recently described by Hu et al. (2011) from peaches/nectarines in China.

Pest Description

In a study by van Leeuwen et al., (2002) using six different *M. fructigena* isolates from throughout Europe, mean colony growth rate was 5mm/day on potato dextrose agar (PDA) at 22°C (71.6°F) under a 12 hr. light/12 hr. dark cycle. Aerial mycelium rose 4-5 mm above the colony surface, and the color of sporogenous tissue was buff/pale luteous. Stromata formed on only four out of six test colonies 21 days after inoculation with a mean size of 0.4cm^2 and a range of 0-0.9cm^2. Macroconidia are globose, ovoid or limoniform, smooth measuring, on average,19 x 11.5 µm (distilled water) when grown on cherry agar (CHA) at 22°C (71.6ºF) and 21.5 µm x 13 µm on pear fruit at 15°C (59°F). The authors were unable to induce the formation of apothecia and thus only described the anamorphic stage.

Biology and Ecology

Casals et al. (2010) evaluated the effect of temperature (0 to 38°C; 32 to 100.4°F) and water activity (a_w: 0.87 to 0.99) on the percentage of conidial germination over time for *Monilinia fructigena*, *M. fructicola*, and *M. laxa*. The three species of *Monilinia* studied were able to germinate over a wide temperature range (0 to 35°C; 32 to 95°F) at 0.99 a_w, but no germination occurred at 38°C (100.4°F)for any of the tested isolates. The optimum temperature for germination occurred after four hours of incubation and was in the range 15 to 30°C (59 to 86°F) for the studied species. Isolates of *M. fructicola* and *M. fructigena* reached 85 to 99% germination after two hours of incubation at 25°C (77°F) at 0.99 a_w; while *M. laxa* needed four hours.

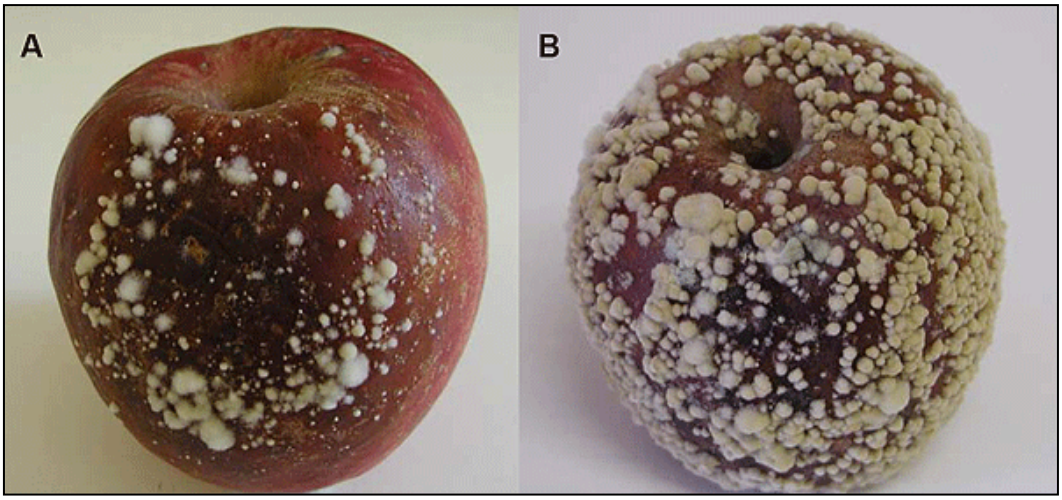

Figure 1. Apples naturally infected with *Monilinia fructigena* **at; a) 5 days incubation and b) 14 days incubation**. Photo courtesy of DAFF (Department of Agriculture, Fisheries, and Forestry-Australia).

Conidia of brown rot fungi, in general, overwinter in fruit mummies or cankerous lesions. These conidia serve as a primary inoculum source in the spring. Under unfavorable climatic conditions, infections can remain latent in immature fruit until conditions become favorable for disease development later in the season (Gell et al., 2008).

Figure 2. Apples infected with *Monilinia fructigena*. Photo courtesy Radek Sotalar – Czech. Republic.

Infection of *Monilinia fructigena* takes place via cracks and wounds in the fruit skin (Xu and Robinson, 2000) and also via fruit-to-fruit contact (Michailides and Morgan, 1997). Wind, water, insects, birds, and man are responsible for the dispersal of *Monilinia* conidia in pome and stone fruit orchards (Byrde and Willetts,

1977; Bannon et al., 2009). Splash dispersal is important for short range spread within a tree (Bannon et al., 2009). Lack (1989) reported spread by insects. Kable (1965) discovered that airborne conidia ensured a wide dispersal of conidia within an orchard. Van Leeuwen et al. (2002b) observed that late infected fruits in one season can contribute to primary inoculum *of M. fructigena* in the next spring, and in early summer dropped fruit (such as fruit on the ground from very late thinning) can contribute to infection on the tree. Disease incidence can be controlled by avoiding fruit wounds caused by biotic (insects, birds, man) and abiotic (frost, hail) agents.

Symptoms/Signs

Symptoms include stem cankers, twig and leaf blights, and brown fruit rots (Fig. 1 to 3).

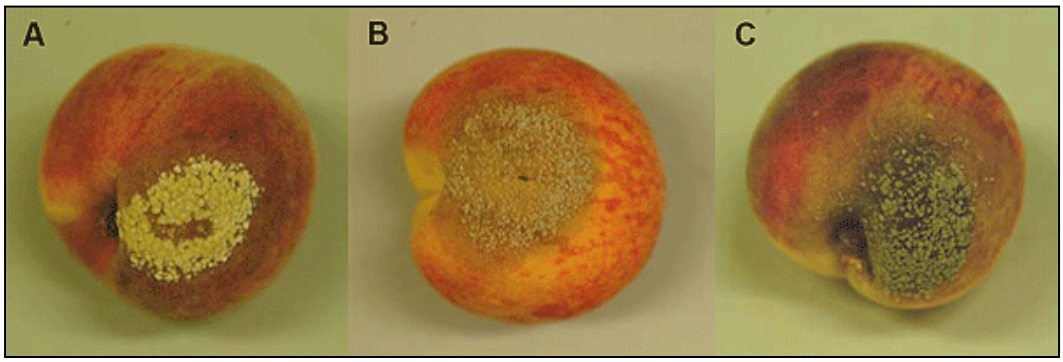

Figure 3. Peaches inoculated with a) *Monilinia fructigena*; b) *M. fructicola*; and c) *M. laxa*. Photo courtesy of DAFF (Department of Agriculture, Fisheries, and Forestry-Australia).

The primary and most frequent symptom is fruit rot (Fig. 1 to 3). Initial fruit lesions are brown, circular, and firm (Fig. 2). Eventually the whole fruit decays and turns brown. Tufts of mycelium and conidia (cream-white to buff colored) sprout from the skin of the infected fruit (Fig. 1, 3), often arranged in concentric rings (Fig. 2) (Byrde and Willetts, 1977). When the relative humidity is low and/or when the fruits are not ripe, no mycelium and very few or no conidial tufts develop. Rotted fruits may either fall to the ground or dry out on the tree, leaving a hard, shriveled 'mummy'. Mummified fruit hang on branches of trees until spring or fall to the ground where they remain throughout the winter months, partly or completely buried beneath the soil or leaf litter (Byrde and Willetts, 1977). Infection of fruits can take place at any time during fruit development, but the disease is only severe in ripe or ripening fruits.

Pest Importance

Brown rot of stone fruits is an extremely destructive disease. The pathogens that cause brown rot of stone fruit also occur on apple and pear fruit trees. The disease may destroy or seriously reduce a crop by rotting mature fruit, either on the tree or after harvest.

Monilinia fructigena, a regulated pest in the United States, causes severe fruit rot of fruit trees. This pest causes loss of apple and stone fruits, both before and after harvest.

Twigs and shoots can also be infected, albeit less frequently. Crops may be severely reduced or destroyed due to the infection.

In general, *M. fructigena* is less damaging than *M. fructicola* or *M. laxa*. The severity of the disease varies from year to year depending upon environmental and storage conditions. *M. fructigena* is highly infectious and is reported to cause considerable losses in Europe during summer when warm temperatures are favorable to disease development (Scopes and Ledieu, 1983). The greatest losses are often observed in apples and plum fruits. Losses of between 7 and 36% have been reported in European apple orchards and between 0.2 and 1.5% in stored fruits (Jones and Aldwinckle, 1990; van Leeuwen et al., 2000). Latent infections can also occur, with symptoms only developing after fruit ripening.

Monilinia fructigena is listed as a harmful organism in the following countries: Argentina, Canada, Chile, Ecuador, Egypt, Jordan, New Zealand, Peru, Syria, and Taiwan (USDA-PCIT, 2013). If this pest were found in the United States, there are potential trade implications with these countries.

Known Hosts

Cydonia spp. (quince), *Malus* spp. (apple), *Prunus* spp. (stone fruit), and *Pyrus* spp. (pear) (van Leeuwen et al., 2002).

Other hosts:
Actinidia arguta* (kiwi)**, ***Amelanchier canadensis (shadbush), ***Amygdalus communis*** (almond), ***Armeniaca vulgaris*** (apricot), ***Azalea* spp. (azalea)**, ***Berberis* spp.** (barberry), ***Capsicum* spp.** (pepper), ***Cerasus* spp.** (cherry), ***Chaenomeles* spp.** (flowering quince), ***Corylus* spp.** (hazelnut), ***Cotoneaster* spp.** (cotoneaster), ***Crataegus laevigata*** (hawthorn), ***Crataegus oxyacantha*** (English hawthorn), ***Diospyros* spp.** (persimmon), ***Elaeagnus macrophylla*** (maruba-gumi), ***Eriobotrya* spp.** (loquat), ***Ficus* spp.** (fig), ***Fragaria* spp.** (strawberry), ***Mespilus germanica*** (medlar), ***Psidium* spp.** (guava), ***Rhododendron* spp.** (rhododendron), ***Rosa* spp.** (rose), ***Rubus* spp.** (blackberry), ***Solanum lycopersicum*** (tomato), ***Sorbus* spp.** (rowan), ***Vaccinium* spp.** (blueberry), ***Vitis spp.*** (grape) (Mackie & Kumar, 2005; Petroczy et al., 2005; Amiri et al., 2009; USDA-ARS, 2005; CABI, 2013; EPPO, 2013).

Known Vectors (or associated insects)

Insects play a role in the dispersal of *Monilinia fructigena* (Lack, 1989). According to this study, insects from the order Diptera and Hymenoptera played the largest role among insects in spreading this pest.

Known Distribution

Africa: Egypt and Morocco. **Asia:** Afghanistan, Belarus, China, India, Iran, Israel, Japan, Kazakhstan, Korea (North), Korea (South), Lebanon, Nepal, Russia, Taiwan, Turkey, and Uzbekistan. **Europe:** Armenia, Austria, Azerbaijan, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Georgia, Greece, Hungary, Ireland, Italy (Including Sicily), Latvia, Lithuania, Luxembourg,

Moldova, Montenegro, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, and United Kingdom (UK) (CABI, 2013; EPPO, 2013).

North America: *M. fructigena* was found in Beltsville, Maryland, United States, in 1979, but it was successfully eradicated (CABI, 2013; EPPO, 2013).

This pathogen was reported in Brazil, Chile, and Uruguay, but these reports proved to be erroneous (EPPO, 2013). There have also been unconfirmed reports in Canada (Ginns, 1986), Cuba (Arnold, 1986), and New Caledonia (Huguenin, 1986).

It is unclear at this time if this fungus is present in Mexico. There were two interceptions of *M. fructigena* on *Prunus persica* var. *nucipersica* and *Malus* sp. fruit imported from Mexico (Paul Larkins, personal communication; AQAS, 2013). These interceptions suggest that this pathogen may be present in Mexico even though it has not been officially confirmed there.

Isolates of *Monilinia fructigena* from other areas of East Asia should be examined to determine whether some isolates actually belong to *Monilia polystroma* (van Leeuwen et al., 2002).

Pathway

Monilinia fructigena has been intercepted 33 times at U.S. entryways since 1984 (AQAS, 2013). Of those interceptions, 32 of them were found on contaminated fruit and the other on infected seed. All of the fruit was either *Malus sp*. or *Prunus* sp. In general, interceptions of *Malus* sp. or *Prunus* sp. propagative material are common. For example, there were 186 interceptions of *Malus* sp. propagative material and 56 interceptions of *Prunus* sp. propagative material from host country China in the past ten years. During the same timeframe there were 1,216 interceptions of *Malus* sp. and 203 interceptions of *Prunus* sp. (propagative material) from European countries. *M. fructigena* is located in at least 29 different European countries.

In addition to Europe and China, *M. fructigena* is found in at least 21 other countries. It is also possibly in Mexico (Paul Larkins, personal communication). This fungus also has many other known hosts in addition to *Malus* sp. and *Prunus* sp. (CABI, 2013). A wide host range coupled with a broad diversity of known hosts lead to the creation of many possible pathways into the United States.

Potential Distribution within the United States

There is a high potential for distribution of *Monilinia fructigena* in the United States if it becomes established. According to a recent host analysis by USDA-APHIS-PPQ-CPHST for *Monilia polystroma,* a pest whose known hosts are also known hosts of *M. fructigena*, the eastern half of the continental United States has a moderate to high level of risk of establishment. This map is based solely on the presence of susceptible hosts. Most areas of the western United States have a low risk; while portions of California, Washington, and Oregon have a moderate risk.

Since *M. fructigena* has many more known hosts than *M. polystroma*, the potential for distribution of *M. fructigena* in the United States is likely much higher and far reaching than this map would indicate.

Survey

CAPS-Approved Method*: Visual survey is the approved survey method for *Monilinia fructigena .* For visual survey, collect symptomatic plant material.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Survey for *Monilinia fructigena* consists of visual inspection for symptoms, tissue sampling, and pathogen isolation.

Key Diagnostics/Identification

CAPS-Approved Method*: Morphological. Identification of brown rot fungi is commonly based on morphology and colony characteristics. This is the CAPS-Approved method until molecular methods can be validated for regulatory use.

Identification of the three main *Monilinia* species *(fructigena, fruticola*, and *laxa*) is commonly based on morphology and colony characteristics. Identification is possible by combining cultural characteristics, such as growth rate, growth pattern and color, with morphological data, such as conidial dimensions and the length of the germ tube (van Leeuwen and van Kesteren, 1998; De Cal and Melgarejo, 1999; van Leeuwen et al., 2002). Most of these characters are quantitative and overlap, so the identification has to be conducted under standardized conditions and starting from pure cultures. Lane (2003) also provides information for distinguishing the three main *Monilinia* spp. based on cultural characteristics (*M. fructigena*, *M. fructicola*, and *M. laxa*). *M. fructigena* can be distinguished from *Monilia polystroma* based on morphological and molecular characteristics of isolates (van Leeuwen et al., 2002).

Hu et al. (2011) discuss two additional *Monilinia* spp. in China: *Monilinia mumecola* and *M. yunnanensis*.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Culture/Isolation: For isolation, the standard procedure is to place pieces of infected material (with or without surface sterilization) on slightly acid agar medium (pH 4-4.5) (EPPO, 2009). Isolation of *Monilinia* spp. from stone fruit and pome fruit surfaces is difficult, however, due to the presence of several fast-growing fungal species such as *Rhizopus*, *Alternaria*, and *Penicillium* spp. It is also possible to have mixed *Monilinia* infections. Phillips and Harvey (1975) tested a medium containing pentachloronitrobenzene (PCNB), canned strained peaches, neomycin, streptomycin,

agar, and distilled water and found that though it was not totally selective that it could be used to estimate spore density of *Monilinia* spp. on the surface of fruit. Amiri et al. (2009) developed a new selective medium (acidified potato dextrose agar (PDA) with fosetyl-Al) for recovery and of enumeration of *Monilinia* spp. from stone fruit.

Molecular: Several molecular methods have been developed to distinguish *Monilinia* species. Fulton and Brown (1997) and Snyder and Jones (1999) established a PCR-based method of targeting to distinguish *M. fructigena* from *M. fructicola* and *M. laxa* based on the group I intron in the gene for the ribosomal subunit. Subsequent studies, however, showed that these methods were not reliable because some isolates of *M. fructicola* lack a group I intron in their nuclear rDNA small subunit (Förster and Adaskaveg, 2000; Fulton et al., 1999; Hughes et al., 2000; Cote et al., 2004b). Other PCR primers and protocols for *M. fructicola* were published by Förster and Adaskaveg (2000), Boehm et al. (2001), and Ma et al. (2003). However these methods discriminate *M. fructicola* from *M. laxa* but have not been validated for distinguishing *M. fructicola* from *M. fructigena*. Fluorescent AFLP fingerprinting and inter-simple sequence repeat analysis has been used to examine the genetic diversity of *M. fructicola* (Fan et al., 2010; Gril et al., 2010).

Ma et al. (2005) developed a pair of PCR primers specific to *M. laxa* on the basis of the differences in the DNA sequence of the intron 6 of *β*-tubulin gene from *M. laxa*, *M. fructicola* and other fungal species.

Ioos and Frey (2000) designed species-specific primer pairs for *Monilinia fructigena*, *M. fructicola*, and *M. laxa* based on the ribosomal internal transcribed spacer (ITS) region. This method, while testing for all three *Monilinia* species, produces PCR amplicons of the same size (356 bp), so three separate PCR reactions have to be performed in order to identify the species. Hughes et al. (2000) also developed species-specific primers for *Monilinia fructigena*, *M. fructicola*, and *M. laxa.* An internal control based universal PCR protocol was developed for *Monilinia* spp., and species-specific primers were designed by using SCAR makers (Gell et al., 2007). Miessner and Stamler (2010) and Hily et al. (2010) developed a primer/primers based on difference in the intron-exon of the cytochrome b gene to distinguish *Monilinia fructigena*, *M. fructicola*, and *M. laxa.* Cote et al. (2004) developed a multiplex PCR that can distinguish *Monilinia fructigena*, *M. fructicola*, *M. laxa*, and *Monilia polystroma* on inoculated and naturally infected apple and stone fruit. This PCR method uses a common reverse primer (MO 368-5) and three species specific forward primers (MO 368-8R, MO 368-10R, and Laxa – R2) to differentiate the three *Monilinia* species. In this assay, a 402-bp PCR product for *M. fructigena*, a 535-bp product for *M. fructicola*, and a 351-bp product for *M. laxa* are produced. Furthermore, another specific 425-bp PCR product was amplified, enabling the identification of isolates of *Monilia polystroma*. Malvarez et al. (2001) were able to use the Cote et al. (2004) primers (prior to their publication) to identify species of *Monilinia* in Uruguay. Upon comparing the *M. fructigena* and *M. polystroma* sequences with the genomic sequence of unknown function previously described by Cote et al. (2004). Petroczy et al. (2012) revealed insertions and substitutions in the *M. polystroma*

sequences. Repetitive sequence motifs were identified, which can be used for differentiation between *M. fructigena* and *M. polystroma.*

According to EPPO (2009), the PCR method of Hughes et al. (2000), Ioos and Frey (2000), and Cote et al. (2004) have been shown not to give cross-reaction with *Monilia polystroma*.

Real-time PCR methods have been developed by Luo et al. (2007) and van Brouwershaven et al. (2010). The Luo et al. (2007) method, which is based on the Ma et al. (2003) primer for *M. fructicola*, is a SYBR Green assay and has been tested only against *M. fructicola*, *M. laxa*, *Botrytis cinerea*, *Botryosphaeria dothidea*, and *Alternaria alternata*. The van Brouwershaven (2010) method is a Taq man assay and has been validated against *Monilinia fructigena*, *M, laxa*, *M. fructicola,* and *Monilia polystroma*; a FAM-labeled probe will detect *M. fructicola* while a VIC-labeled probe will detect *M. fructigena*, *M. laxa*, and *Monilia polystroma* as a group. Since the United States currently has both *M. fructicola* and *M. laxa,* at present these real-time methods may be of limited utility for the detection of exotic *Monilinia* or *Monilia* species.

Seven different PCR methods were tested by Hu et al. (2011) to differentiate *Monilinia* spp. None of the six molecular tools alone were able to distinguish all five *Monilinia* species (*M. fructigena, M. fructicola, M. laxa, M. yunnaensis, and M. mumecola*) (Ioos and Frey 2000; Ma et al. 2003, 2005; Cote et al., 2004; Gell et al., 2007; Miessner and Stammler, 2010; Hily et al., 2010). Note: The authors didn't test *Monilia polystroma*.

M. fructigena, M. fructicola, and *M. laxa* were reliably differentiated by the methods of Ioos and Frey (2000), Miessner and Stammler (2010), and Hily et al. (2010). However, neither of these methods was able to distinguish *M. fructigena* from *M. yannanensis*. Likewise, the methods developed by Ioos and Frey (2010), Ma et al. (2003, 2005) did not distinguish between *M. mumecola* and *M. laxa*. The method developed by Hily et al. (2010) did not distinguish *M. mumecola* from *M. fructicola*. Additionally, the methods of Miessner and Stammler (2010) and Hily et al. (2010) did not distinguish between *M. yunnanensis* and *M. laxa*.

Hu et al. recently (2011) developed an additional multiplex PCR to distinguish *M. fructicola* from *M. mumecola*, *M. yunnanensis* in China. Additional work needed to see if these primers distinguish *M. fructigena, Monilinia laxa*, and *Monilia polystroma*, because the authors did not find these species in China and did not present any specific data for these species.

Easily Confused Pests

Monilinia fructigena can easily be confused with other brown rot fungi, particularly *M. fructicola*, M. *laxa, and Monilia polystroma*). *Monilia polystroma* was originally classified as *Monilinia fructigena*. *M. laxa* is considered to be more a pathogen of blossoms and twigs than of fruit and primarily occurs on *Prunus* spp. *M. fructigena* is mainly a fruit pathogen and primarily occurs on apple, pear, and other pome fruit trees, although it is also found on *Prunus* spp. (USDA ARS, 2005). *M. fructicola* is a pathogen of blossoms,

twigs, and fruits and mainly affects stone fruits but can occur on apples, pears, and other pome fruits (USDA ARS, 2005). The color of the pustules on infected plant tissue is buff for *M. fructigena* and grayish-brown for *M. fructicola* and *M. laxa* (van Leeuwen and van Kesteren, 1998).

Monilinia fructigena is quite similar to *Monilia polystroma* but differences do exist. For example, *Monilia polystroma* forms a large number of dark/black colored stromata in agar culture (van Leeuwen et al., 2002). *Monilinia fructigena* has the largest macroconidia where the conidia of *Monilia polystroma* are slightly smaller. Colonies of *Monilinia fructigena* are similar to those of *Monilia polystroma*, but black stromatal plates occur on *M. polystroma* colonies after incubation for 10 to 13 days, and *Monilia polystroma* isolates grow faster than *M. fructigena* isolates under the same conditions (van Leeuwen et al., 2002).

Other fungi can cause rots with similar symptoms to *Monilia polystroma* (*Penicillium* spp., *Mucor* spp.). Avoid collecting fruits with blue, green, or yellow colored molds or fruit that are 'leaking' fluid.

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Potyvirus Plum pox virus

Scientific Name
Potyvirus Plum pox virus (PPV)

Common Name(s)
Plum pox virus, plum pox, sharka

Type of Pest
Plant pathogenic virus

Taxonomic Position
Class: RNA Virus, **Family:** *Potyviridae*
Genus: *Potyvirus*

Reason for Inclusion in Manual
Program Pest

Pest Description
Plum pox virus (PPV) is a RNA potyvirus with flexuous filamentous particles approximately 750 nm in length x 15 nm in diameter. PPV is composed on one molecule of RNA (positive sense, ssRNA) and a protein envelope. The genome is expressed as a 350 kDa polyprotein precursor that is proteolytically processed by viral and host proteases into ten smaller functional proteins (Garcia et al., 1994; Lopez-Moya et al., 2000; Schneider et al., 2011). PPV is the causal agent of plum pox disease. PPV strains can infect all cultivated stone fruit species including plum, peach, nectarine, apricot, almond, and cherry, as well as wild and ornamental *Prunus* species.

Seven strains of PPV (D, M, El-Amar, C, W, T, and Rec) (Kerlan and Dunez, 1979; Crescenzi et al., 1997a; Bodin et al., 2003; James et al., 2003; James and Varga, 2005; Glasa et al., 2005; Serce et al., 2009), have been identified worldwide based on their biological,

Figure 1. PPV symptoms on a plum leaf and fruit. Photos courtesy of Dr. Laszlo Palkonvics, Corvinus University, Budapest, Hungary.

serological and molecular properties to date (Table 1). PPV-M and PPV-D are the most widespread. All occurrences in the United States have been identified as strain D (PPV-D) (Damsteegt et al., 2001; Schneider et al., 2011); whereas strains D, W, and Rec have been reported from Canada (Rochon et al., 2003; Thompson et al., 2009). Strain D naturally infects peach, nectarine, apricot and plum; almond and cherry are not natural hosts, although they can be infected artificially (Damsteegt et al., 2007). Epidemics of PPV-D progress slowly in peach and this virus strain is not seed-transmitted.

Table 1: Strains or Serotypes of *Plum pox virus*

Strain	Originally Described From	Notes
PPV-M (Marcus)	Greece (peach)	Present in many European countries but absent from the Americas. Causes rapidly spreading epidemics in peach, but less frequently found in plums. Efficiently transmitted by aphids.
PPV-D (Dideron)	France (apricots)	Present in all areas where PPV has been reported, including the United States. Infrequently found in peach. PPV-D isolates cause slower spreading epidemics and are less efficiently transmitted by aphids than PPV-M.
PPV-Rec (Recombinant)	Not known	Recognized only recently through the use of improved strain typing methods. A group of isolates from a single homologous recombination event between PPV-M and PPV-D (*Nlb* gene). Widespread in several central and eastern European countries. Recently found in Turkey. Frequently associated with plums, and efficiently transmitted by aphids.
PPV-EA (El Amar)	Egypt (apricots)	Not reported outside of Egypt at this time.
PPV-C (Cherry)	Moldova (sour cherry)	Reported in Moldova in 1980's. Reported and eradicated in Italy. Sporadically present in central and eastern European countries. PPV-C isolates are the only isolates to infect cherry systemically. Able to infect other *Prunus* spp. under experimental conditions.
PPV-W (Winona)	Canada (plum)	Reported and eradicated from two infected plum trees in Canada.
PPV-T (Turkey)	Turkey (apricot)	Recognized only recently through the use of improved strain typing methods. These isolates have a recombination event in the *HC-Pro* gene. To date this strain is only known to occur in the Ankara region of Turkey.

Figure 2: Fruit deformation caused by plum pox virus infection in sensitive plum. Photo courtesy of P. Gentit, Ctifl, France.

Plum pox is a major viral disease of *Prunus* species and is the most important and destructive viral disease of stone fruit trees in Europe and the Mediterranean region (Roy and Smith 1994). Plum pox virus (PPV) is an aphid-transmitted disease and was first reported from plums in Bulgaria around 1915 (some sources say 1917). Its viral nature was not known until 1932 (Atanasoff, 1932). Also widely known around the world by its Slavic name, sharka, the virus spread slowly through eastern Europe, reaching western Europe in the 1970's. In Canada, plum pox was found in Ontario and Nova Scotia in 2000 (Thompson et al., 2001). In the United States, the disease was recorded in Pennsylvania in 1999 (Levy et al., 2000a), followed by New York and Michigan in 2006 (Gottwald, 2006; Snover-Clift et al., 2007). The disease has since been eradicated in Michigan and Pennsylvania (NAPPO Phytosanitary Pest Alert, 2009) and Nova Scotia (Ministry of Agriculture, 2010). Eradication efforts are continuing in New York and Ontario.

Biology and Ecology

Short distance spread of PPV is the result of aphid transmission in a non-persistent manner (Cornell University, 2008). Aphids test leaf and fruit surfaces by probing them. When an aphid test probes a leaf or a fruit cell, the aphid's sap-sucking mouthpart, a stylet, penetrates the tissue and draws up cell contents. Test probes last as little as 30 seconds. During probing of an infected host, virus particles can be pulled into the stylet and stick to the lining of the food canal. Once acquired, PPV remains in the stylet for up to three hours. During this time the virus can be transferred to healthy trees when viruliferous (virus carrying) aphids expel their stylet contents during new probes. The virus does not persist in the aphid after it has been expelled into new tissue (Cornell University, 2008).

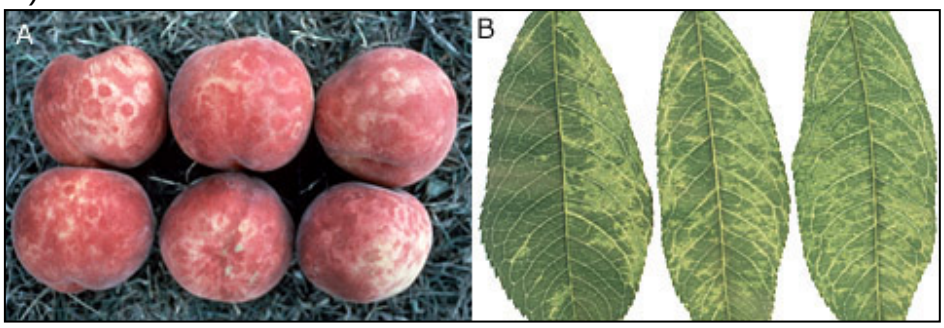

Figure 3: Symptoms of plum pox virus. **A)** Chlorotic ring patterns in peach fruit; **B)** Chlorotic blotches in peach leaves. Photos courtesy of P. Gentit, Ctifl, France.

Spring aphid flights are important for spread within and between orchards. Several aphid species can transmit PPV but only a few of them are considered important vectors in the northeastern United States: the black bean aphid (*Aphid fabae*), the spirea aphid (*Aphid spiraecol*), the black peach aphid (*Brachycaudus persicae*), and the green peach aphid (*Myzus persicae*) (Gildow et

al., 2004). *Toxoptera citricida* (brown citrus aphid) is also an efficient vector, but does not occur in major stone-fruit growing areas (Gildow et al., 2004). All infected trees, even when not showing symptoms, are sources of possible PPV transmission to healthy trees. Aphids can spread PPV from several yards to a few miles and is unlikely to occur over long-distance as the lifespan of the virus within an aphid is generally less than an hour.

Long-distance spread of PPV over several miles occurs primarily by movement of infected plants or plant parts. Virus infection can spread through infected nursery stock or infected buds collected from infected trees. Spatial analysis of PPV-infected trees in orchards suggests a preferential virus spread several tree spaces away from infected trees, rather than to neighboring trees (Dallot et al., 2003). Thus, secondary infections can be widely scattered from the original infection site if the primary virus sources are not controlled.

Figure 4: Yellow rings caused by PPV on a yellow-fleshed peach cultivar (top), color break symptoms induced by PPV in peach flowers. Photos courtesy of European and Mediterranean Plant Protection Organization Archive, http://www.bugwood.org and P. Gentit, Ctifl, France.

The presence of other viruses, such as *plum dwarf virus*, *Prunus necrotic ringspot virus*, and *apple chlorotic leaf spot virus*, can increase the severity (synergistic effect) of plum pox symptoms. Capote et al. (2006) inoculated Japanese plum plants with either PPV-D or PPV-M and then one year later challenge inoculated with the other strain. The presence of PPV-D did not cross-protect the tree against PPV-M infection. In PPV-D-infected plants, the PPV-M strain used as challenge inoculation behaved differently depending upon the plum cultivar assayed. In cv. Black Diamond, PPV-M invaded the plant progressively, displacing the previous PPV-D population; whereas in cv. Sun Gold, both PPV isolates coexisted in the plant. In contrast, the PPV-D isolate was unable to infect plants of both cultivars in which a PPV-M population was already established.

There are no effective control measures against plum pox virus. The use of certified planting material, the removal of wild hosts, and the control of aphid vectors will help to prevent any outbreaks of the disease and reduce the risk of the disease spreading.

Symptoms/Signs

Symptoms of PPV can be conspicuous or very subtle on stone fruit trees. Symptoms vary in type and severity with the strain of the virus, host, cultivar, environmental factors, and the timing of infection. Diagnostic symptoms occur mainly on leaves and fruits in the United States. In general, leaf symptoms include vein yellowing or light green to yellow rings. Foliar symptoms may develop during the cooler temperatures of spring and fall but fade during the host summer months. Symptoms of PPV occur sporadically and often are not apparent until three or more years after infection. Newly infected trees are rarely symptomatic. It is critical that symptomless trees be regarded very seriously as they will act as a silent virus source for further infections.

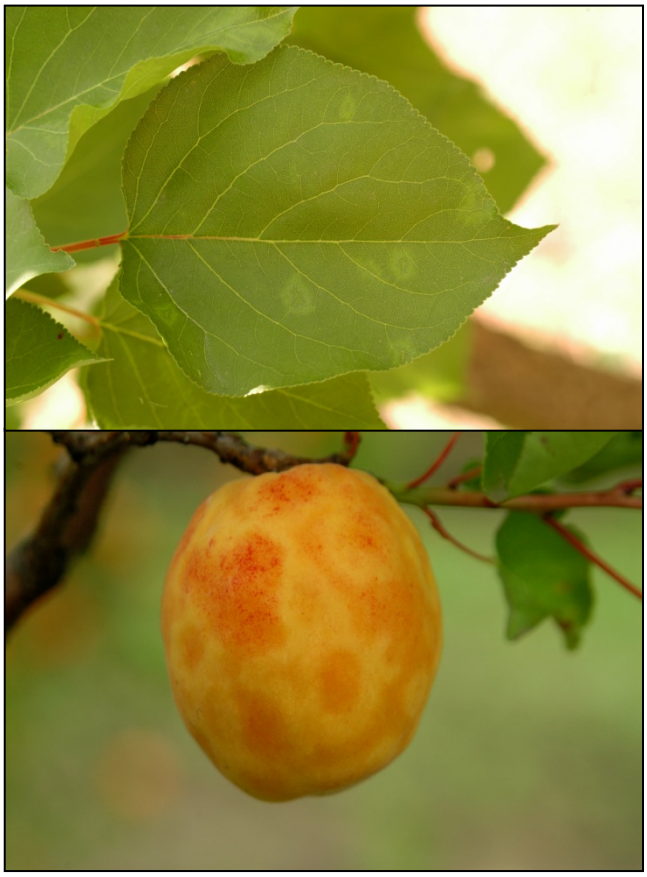

Figure 5. PPV symptoms on an apricot leaf and fruit. Photos courtesy of Dr. Laszlo Palkonvics, Corvinus University, Budapest, Hungary.

Plums: Pale green or light yellow chlorotic spots, blotches, bands, rings, or line patterns (Fig. 1) may occur on the leaves. They are difficult to see in the bright sunlight. Leaf symptoms are most easily seen on the fully expanded leaves from late May/early June. These symptoms are often irregularly distributed and may appear on only a few branches or leaves. Plum fruit symptoms depend on the original color of the fruit. Dark-skinned fruits show bluish, necrotic rings, which may be sunken (Fig. 1). Pale-skinned fruit show uneven ripening, blotching, and rings. Necrotic tissue may extend through the flesh to the stone, on which a reddish necrotic ring may develop. Plum fruits are often deformed (Fig. 2). Also, some plum cultivars can drop fruit prematurely

Figure 6: Rings on the stone of apricot caused by PPV. Photo courtesy of Biologische Bundesanstalt für Land- und Forstwirtschaft Archive, http://www.bugwood.org.

Peach: The leaf symptoms of PPV on peach are distinctive. Affected leaves are distorted when they first unfold, having a wavy edge and a slight twist, and the veins

show pale green or bright yellow flecks or lines (Fig. 3). These symptoms disappear as the leaves mature. Peach fruit may develop lightly pigmented rings (Fig. 3, 4) or line patterns that result from the convergence of several rings. Peach fruit, however, may have paler colored rings and lines than those found in plums. Peaches are generally more susceptible to damage from the disease than plums. Flowers on PPV-infected peach trees may exhibit color breaking (Fig. 4) but only on cultivars with large showy flowers. Color-breaking appears as darker pink stripes on the flower petals.

Almonds: Show few leaf symptoms. Infection is often symptomless.

Apricot: Show lighter symptoms than plum or peach (Fig. 5) Apricot fruits may be misshapen, turn brown or become necrotic and may have rings (Fig. 6) on the surface of the seed.

Cherry: Pale green patterns and rings appear on the leaves. Fruits are slightly deformed with chlorotic and necrotic rings, notched marks, and premature fruit drop**. Note: PPV strain D, which occurs in the United States, is not known to naturally cause infection in cherry.**

The visual symptoms accompanying the reduction in sugar content make the affected fruit unmarketable.

Pest Importance

PPV is the most widespread disease of stone fruits in Europe. This virus reduces fruit yield and quality. It also shortens the productive lifespan of orchards and can render stone fruit trees useless for fruit production. Even symptomless trees produce reduced quantities of fruit. The economic impact of PPV to the peach, plum, and apricot industry worldwide is estimated to $600 million per year. Nemeth (1986) estimated that plum pox losses as high as 80 to 100% were possible.

Plum pox is economically important, because it causes fruit to be unmarketable, it weakens infected trees, and it decreases fruit yield. The presence of PPV can also enhance the damaging effects and increase the economic losses caused by other endemic viruses infecting various species of the genus *Prunus.* These include the *Prune dwarf virus*, *Prunus necrotic ringspot virus* (causes browning), and *Apple chlorotic leaf spot virus* (causes yellowing).

In southeastern France, the newly identified strain of PPV, PPV-Rec induces severe necrosis, resulting in early leaf drop and tree decline (even in the absence of endemic *Prunus* viruses). The severity of the disease depends on the strain of the virus present and the susceptibility of the infected *Prunus* cultivars and species. A wide-scale outbreak of PPV could lead to a decrease in stone fruit exports and higher prices for domestic consumers.

Known Hosts

Plum pox virus has a broad experimental host range, although it has a rather restricted natural host range within the genus *Prunus* (Damsteegt et al., 2007). Wild and weedy *Prunus* spp. can serve as reservoirs of the virus in European countries (Polak, 2004, 2006). This has not been shown, however, in Canada or the United States (Stobbs et al., 2005). Virus isolates vary in their reaction to different hosts, and not all strains or isolates infect the same hosts.

Natural hosts (all strains): *P. amygdalus* (almonds), *Prunus armeniaca* (apricot), *P. avium* (sweet cherry), *P. blireana* (blireana flowering plum), *P. cerasifera* (Myrobalan plum), *P. cerasus* (sour cherry), *P. domestica* (plum), *P. glandulosa* (dwarf flowering almond, cherry almond), *P. insititia* (damson plum), *P. japonica* (Korean cherry/ Japanese bush cherry), *P. mume* (Japanese apricot), *P. nigra* (Canada plum), *P. persica* (peaches/nectarines), *P. salicina* (Japanese plum), *P. serotina* (black cherry), *Prunus spinosa* (blackthorn), and *P. tomentosa* (Nanking cherry) (Nemeth, 1986; Polak, 1997; Labonne et al., 2004; Polak, 2004; Stobbs et al., 2005; James and Thompson, 2006; Polak, 2006; Damsteegt et al., 2007; Maejima et al., 2010).

Prunus species that have been proven to be hosts to Pennsylvania PPV-D strains in nature or by aphid and/or graft inoculation trials (most followed by back transmissions) (Damsteegt et al., 2007) are given in Table 2.

Table 2: *Prunus* susceptibility to Pennsylvania isolates of *Plum pox virus* (PPV-D) as assessed by either or both aphid and graft inoculation*

Species	Common Name	Visual Symptoms**	ELISA***	PCR (aphid, graft)****
P. Americana	American plum	(8/23)	(11/23)	(+, +)
P. andersonii	Desert peach	(0/9)	(1/9)	(+, +)
P. angustifolia	Chickasaw plum	(14/21)	(9/21)	(+, +)
P. armeniaca	Apricot	(15/31)	(11/31)	(+, +)
***P. avium* 'Mazzard'**	Sweet cherry	(10/54)	(11/54)	(+, +)
P. cerasifera	Cherry plum	(12/14)	(8/14)	(+, NA)
***P. cerasifera* 'Myrobalana'**	Myrobalan plum	NA	NA	(NA, +)
***P. cerasifera* 'Thundercloud'**	Myrobalan plum	NA	NA	(NA, +)
P. cerasus	Sour (tart) cherry	(0/19)	(0/19)	(-, NA)
P. cistena	Purple leaf sand cherry	(0/65)	(3/65)	(+,+)
P. davidiana	David's peach	(0/13)	(5/13)	(+, +)
***P. domestica* 'Brompton'**	Garden plum	(2/2)	(2/2)	(+, +)
P. domestica* subsp. *insititia	Bullace plum	(1/2)	(1/2)	(NA, NA)
***P. dulcis* 'Butte' and 'Mission'**	Almond	(3/30)	(17/30)	(+, NA)
P. emarginata	Bitter cherry	(4/32)	(7/32)	(+, +)

P. fruticosa	European dwarf cherry	NA	NA	(NA, +)
***P. glandulosa* 'Rosea Plena'**	Dwarf flowering almond	NA	NA	(NA, +)
P. hortulana	Wild goose plum	NA	NA	(NA, +)
P. humilis	Humble bush cherry	(10/18)	(12/18)	(+, NA)
P. ilicifolia	Holly leaf cherry	NA	NA	(NA, +)
***P. incam* 'Okame'**	Flowering cherry	(0/13)	(2/13)	(-, +)
***(P. incam* 'Okame') – OP 'Dream Catcher'**	Flowering Cherry	NA	NA	(NA, +)
P. incise		NA	NA	(NA, +)
***P. laurocerasus* 'Otto Luyken'**	'Otto Luyken' cherry laurel	NA	NA	(NA, +)
***P. laurocerasus* 'Schipkaensis'**	'Schipkaensis' cherry laurel	(0/29)	(3/29)	(+, NA)
P. lyonii	Catalina Isl. Cherry	NA	NA	(NA, +)
P. maackii	Manchurian cherry	NA	NA	(NA, +)
P. mahaleb	Mahaleb cherry	(6/74)	(19/74)	(+, +)
P. maritima	Beach plum	(3/3)	(3/3)	(+, +)
P. mexicana	Mexican plum	NA	NA	(NA, +)
P. mume	Japanese apricot	(12/12)	(12/12)	(+, NA)
P. nigra	Canadian plum	(0/3)	(1/3)	(+, +)
P. padus	European bird cherry	(4/45)	(14/45)	(+, +)
P. pensylvanica	Pin cherry	(2/44)	(13/44)	(+, +)
P. pumila* var. *besseyi	Western sand cherry	(6/39)	(14/39)	(+, +)
P. pumila* var. *depressa	Eastern sand cherry	(0/35)	(22/35)	(+, +)
P. salicina	Japanese plum	(3/21)	(5/21)	(+, NA)
P. sargentii	Sargent's cherry	NA	NA	(NA, +)
P. serotina	Black cherry	(11/78)	(35/78)	(+, +)
P. serrulata	Japanese flowering cherry	(9/15)	(9/15)	(+, NA)
***P. serrulata* 'Kwansan'**	Kwansan cherry	(0/13)	(0/13)	(+, +)
***P.* × 'Snofozam' (Snow Fountains)**	Snow Fountain cherry	(0/17)	(0/17)	(-, +)
P. spinosa	Blackthorn, sloe	(1/1)	(1/1)	(NA, NA)
***P. subhirtella* 'Pendula'**	Equinox cherry	NA	NA	(NA, +)
P. tenella	Dwarf Russian almond	NA	NA	(NA, +)
P. triloba	Flowering almond	(3/5)	(3/5)	(+, +)
P. virginiana	Chokecherry	(10/35)	(11/35)	(+, +)
P. virginiana* var. *demissa	Western chokecherry	(3/21)	(4/21)	(+, +)

P. yedoensis	Yoshino flowering cherry	NA	NA	(NA, +)

* Data from Damsteegt et al. (2007)
** Visual symptoms: number of plants with symptoms/total number of plants
*** ELISA: number of plants with 405 nm absorbance levels 4 x higher than negative controls/total plants
**** PCR: NA=not attempted, + = positive, - = negative

Experimental hosts (all strains):
The virus has been transmitted to many *Prunus* species including: *Prunus americana* (American plum), *P. armeniaca ansu* (ansu apricot), *P. besseyi* (western sandcherry), *P. besseyi* x *munsoniana* x *salicina*, *P. brigantina* (alpine, Briancon apricot), *P. cerasifera* x *munsoniana* x *angustifolia*, *P. cerasifera* x *spinosa*, *P. cistena* (purple leaf sand cherry), *P. cocomilia* (Italian plum), *P. kurdica*, *P. dasycarpa* (black apricot), *P. davidiana* (David's peach), *P. holosericea* (apricot), *P. hortulana* (wild goose plum/Hortulan plum), *P. laurocerasus* (cherry laurel), *P. mahaleb* (mahaleb cherry), *P. mandshurica* (Manchurian apricot), *P. maritima* (beach plum), *P. mexicana* (Mexican plum), *P. microcarpa* (Japanese apricot), *P. munsoniana* (wild goose plum), *P. munsoniana* x *triloba*, *P. pensylvanica* (pin cherry), *P. pseudoarmeniaca* (Italian plum), *P. pumila* (sand cherry), *P. serrulata* (Japanese flowering cherry), *P. sibirica* (Siberian apricot), *P. simonii* (apricot plum), and *P. triloba* (flowering almond) (Hamdorf, 1975; Nemeth, 1986; Polak, 2001; Labonne et al., 2004).

Many non-*Prunus* species, in at least sixteen plant families, have been infected artificially with one or more strains of the *Plum pox virus*, and in some cases found to be naturally infected (**shown in bold**) in the field. Herbaceous hosts infected experimentally by Pennsylvania isolates of PPV are shown in green (Schneider et al., 2011). Most of these are herbaceous annuals but a few are perennial or woody and could serve as overwintering sources of the virus. Hosts include: *Agrostemma githago* (common corncockle), *Ajuga genevensis* (blue bugleweed), *Arabidopsis thaliana* (arabidopsis), *Amni majus* (laceflower), *Borago officinalis* (common borage), ***Campanula rapunculoides*** (rampion bellflower), *Capsella bursa-pastoris* (shepherd's purse), *Celosia* spp. (cock's comb), *Chenopodium amaranticolor* (lambsquarters), *Chenopodium foetidum* (lambsquarters), *Chenopodium murale* (nettleleaf goosefoot), *Chenopodium quinoa* (quinoa), *Chenopodium* spp. (lambsquarters), *Chrysanthemum* spp. (chrysanthemum), *Cichorium* spp. (chicory, endive), *Cirsium arvense* (Canada thistle), *Clematis* spp. (clematis/virgin's bower), *Convulvulus arvense* (field bindweed), *Coreopsis* spp. (beggarticks/tickseed), *Cyamopsis tetragonoloba* (guar), *Digitalis lanata* (Grecian foxglove), ***Dimorphotheca aurantiaca*** (cape marigold), *Emilia sagittata* (tasselflower), ***Euonymus europea*** (euonymous), *Galeopsis segetum* (downy hempneedle), *Gladiolus* spp. (gladiolus), *Gomphrena globosa* (common globe amaranth), *Humulus lupulus* (hop), *Hyoscyamus niger* (black henbane), *Lactuca serriola* (prickly lettuce), ***Lamium album*** (white deadnettle), ***L. amplexicaule*** (henbit, deadnettle), ***L. purpureum*** (purple deadnettle), *Lathyrus odoratus* (sweet pea), *Linaria cymbalaria* (Kenilworth ivy), ***Ligustrum vulgare*** (European privet), *Lithospermum arvense* (corn gromwell), ***Lupinus albus*** (white lupine), *Lupinus luteus* (European yellow lupine), *Lycium barbarum* (matrimony vine), *L. halimifolium* (matrimony vine), ***Medicago lupulina*** (black medic), ***Melilotus officinalis*** (yellow sweet clover), *Meliotus*

spp. (sweet clover), *Mimulus variegates* (monkey flower), *Nicandra physaloides* (shoo-fly plants/apple of Peru), *Nicotiana benthamiana* (tobacco), *N. megalosiphon* (tobacco), *N. occidentalis* #37 B (tobacco), *N. tabacum* (tobacco), *Nicotiana* spp. (tobacco), ***Oenothera biennis*** (evening primrose), *Papaver somniferum* (opium poppy), *Passiflora foetida* (fetid passionflower), *Petunia hybrid* (petunia), *Pisum sativum* (pea), *Pisum* spp. (pea), *Physalis* spp. (groundcherry), ***Ranunculus acer*** (buttercup), ***R. arvensis*** (buttercup), *R. repens* (buttercup), *Ranunculus* spp. (buttercup), *Rorippa sylvestris* (creeping yellow cress), *Rumex crispus* (curled dock), *Senecio* spp. (groundsel), *Sesbania exaltata* (bigpod sesbania), *Sesbania vulgaris* (Colorado river hemp), ***Silene inflata*** (maidenstears), ***Silene vulgaris*** (maidenstears), ***Solanum dulcamara*** (climbing nightshade), *Solanum lycopersicon* (tomato), *Solanum* spp. (nightshades), *Sonchus* spp. (sowthistle), ***Sorbus domestica*** (service tree), *Stachys recta* (stiff hedgenettle), *Stellaria media* (common chickweed), *Symphitum officinale* (common comfrey), *Taraxacum officinale* (common dandelion), *Trifolium incarnatum* (crimson clover), ***T. pretense*** (red clover), *T. repens* (white clover), *Trigonella foenum-graecum* (fenugreek), *Torenia fournieri* (bluewings), *Verbena officinalis* (herb of the cross, prostrate verbena), *Veronica* spp. (speedwell), *Vicia* spp. (vetch), ***Zinnia elegans*** (elegant zinnia), and *Z. violacea* (zinnia) (Sutic, 1972; Nemeth,1986; Polak, 2001; Llacer, 2006; Wang et al., 2006; Schneider et al., 2011).

Baumgartnerova (1997) found walnut (*Juglans regia*) to be a new host of PPV. This conclusion, however, has not been confirmed by two independent laboratories and Polak (2006) suggested that this species should be removed from the list of natural hosts of PPV.

Known vectors (or associated organisms)

Plum pox virus has been transmitted by at least 20 aphid species, although only four to six are considered important vectors (Table 3). The efficiency of transmission is dependent on the virus strain, host cultivars, age of the host cultivars, aphid species, and time of year. The most important aphid vectors reported from several countries are *Brachycaudus cardui, B. helichrysi, Myzus persicae,* and *Phorodon humuli.* Although reports vary from country to country, the natural virus spread is low in July and August but high in spring and autumn. Spring flights of *B. helichrysi, M. persicae,* and *P. humuli* are most important for spread within and between orchards (Levy et al., 2000b). Analysis of spatial distribution of PPV by Gottwald et al. (1995) suggest a lack of movement by aphid vectors to immediately adjacent trees and a preference for movement several tree spaces away.

Aphids can acquire the virus in probes as short as 30 seconds, and can transmit for up to one hour. Aphids that have been starved before feeding can transmit for up to three hours after acquisition. There is no correlation between the ability to transmit PPV and the ability to colonize *Prunus*. PPV can be spread in orchards by transient aphids as efficiently as aphids colonizing *Prunus* (Labonne et al., 1995).

Aphids were found to transmit PPV within 100 to 120 meters (328 to 394 ft.) of the source plants, but they have been shown to carry the virus on their stylets for several kilometers if starved during flight.

Table 3: Aphid species shown to be vectors of *Plum pox virus*.

Aphid Species	Colonizes *Prunus*	Host
Aphis arbuti	No	*Arbutus unedo*
A. craccivora*	No	Polyphagous
A. fabae	No	Polyphagous
A. gossypii*	No	Polyphagous
A. hederae	No	*Hedera helix*
A. spiraecola*	Occasionally	Polyphagus; Apple; Citrus
Brachycaudus cardui	Yes	*Prunus;* Compositae
B. helichrysi**	Yes	*Prunus;* Compositae
B. persicae*	Yes	*Prunus*
Dysaphis plantaginea	No	Apple; Plantago
D. pyri	No	Pear; *Gallium*
Hyalopterus pruni*	Yes	*Prunus; Fragmites*
Macrosiphum rosae	No	*Rosa;* Dipsaceae
Megoura rosae	No	Leguminoseae
Myzus persicae**	Yes	Polyphagous
M. varians	Yes	Peach; *Clematis*
Phorodon humuli**	Yes	*Prunus; Hop*
Rhopalosiphum padi	No	*Prunus padus;* Gramineae
Sitobion fragariae	No	*Rosa;* Gramineae
Ureleucon sonchi	No	*Lactuca; Sonchus*

*Recognized aphid vectors, ** Most important vectors. Data from Levy et al. (2000b).

Known Distribution

Africa: Egypt, South Africa, and Tunisia. **Asia:** China, India, Iran, Japan, Jordan, Kazakhstan, Pakistan, Syria, and Turkey. **Europe:** Albania, Austria, Azores, Bosnia-Herzegovina, Bulgaria, Croatia, Cyprus, Czech. Republic, Denmark, France, Germany, Greece, Hungary, Italy, Lithuania, Luxembourg, Moldova, Norway, Poland, Portugal, Romania, Russia, Serbia and Montenegro, Slovakia, Slovenia, Spain, Ukraine, and the United Kingdom. **North America:** Canada and the United States. **South America:** Argentina and Chile (Staniulis et al., 1998; Reyes et al., 2003; Boulila et al., 2004; Spiegel et al., 2004; Navratil et al., 2005; Dal Zotto et al., 2006; Kollerová et al., 2006; Mumford, 2006; Candresse et al., 2007; Papayiannis et al., 2007; Maejima et al., 2010; Kamenova et al., 2011).

The disease has been found in Switzerland, Belgium, Estonia, and the Netherlands, but did not establish or is no longer found (Roy and Smith, 1994; Levy et al., 2000b). Although thought to be eradicated from Denmark, PPV was recently found to be present in five nurseries (IPPC, 2011).

Potential Distribution within the United States

In the United States, the disease was first recorded in Pennsylvania in 1999, followed by reports from New York and Michigan in 2006. The disease is now considered to be eradicated in Pennsylvania and Michigan. It has the potential to occur wherever susceptible hosts are grown. A recent host analysis by USDA-APHIS-PPQ-CPHST indicates that most of the eastern United States and portions of Washington, Oregon, and California have a moderate risk rating for *Plum pox virus* establishment based on host availability within the continental United States.

Survey

CAPS-Approved Method*: The CAPS-approved survey method is to collect symptomatic plant tissue by visual survey. The hierarchical sampling method is recommended PPV (Hughes et al., 2002).

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Hughes et al. (2002) and Gottwald (2006) discuss the use of hierarchical sampling in the surveillance program for *Plum pox virus* in the United States. The method was adopted by the Pennsylvania Department of Agriculture, as well as Canada, to survey for PPV. Virus incidence may be assessed by sampling groups of orchard trees, recording the groups as 'virus-positive' (one or more infected trees) or 'virus-negative' (no infected trees), and then calculating disease incidence at the individual tree scale by means of a formula involving incidence at the group scale and the number of trees per group. Differences in spatial aggregation characteristics of various pathosystems can be accounted for by adjusting the apparent group size in the formula to predict disease incidence more accurately at the individual plant scale from incidence at the group scale.

Following the confirmation of PPV infection in Adams County, an initial survey was conducted by the Pennsylvania Department of Agriculture in autumn 1999. In this survey, a number of orchard blocks with visual symptoms of PPV infection were located before leaf fall made further sampling impossible (Gottwald, 2006). Plots consisting of 400 trees in a 20 by 20 rectangular pattern were established in nine of these blocks (*i.e.,* all those of sufficient size). In these plots, the location and PPV status, determined by ELISA using 5B-IVIA monoclonal antibodies (Cambra et al., 1994), of each tree were recorded in the form of a 'map'. Missing trees were also recorded. More orchard blocks with visual symptoms of PPV infection were located when the survey was continued in the spring and summer of 2000.

In the hierarchical sampling scheme, the sample covers 25% of the trees in a block. The sampling unit is a group of four trees in a two-by-two rectangular arrangement. In practice, three to four leaves are taken from each tree; one leaf from each main unit is kept as a bulked sample. For subsequent laboratory assay, this bulked sample is divided into two subsamples of six to eight leaves each. If neither of the subsamples provides a PPV-positive ELISA result, the group is recorded as PPV-negative; otherwise the group is recorded as PPV-positive. Since only PPV incidence

at the group scale is assessed in this way, PPV incidence at the scale of the individual tree is then calculated from the equation:

$$\tilde{p}_{low} = 1(1 - \check{p}_{\text{high}})^{\frac{1}{\check{v}}}$$

Where:

$\check{p}_{\text{high}}$ = the probability that the group contains at least one PPV-positive tree

$\tilde{p}_{low}$ = the probability that an individual tree is positive.

$\check{v}$ = effective sample size

Where the disease has been reported previously, surveys are centered at and near locations where positive trees were found the previous year. Within a one mile radius of a site that tested positive, all trees have one sample taken (a sample consists of eight leaves per tree). Because distribution of PPV in infected trees is not uniform, each sample consists of eight leaves taken from multiple locations on a tree. This strategy increases the probability that the virus, if present, will be detected. Out to five miles from former sites of infection, every tree is sampled at four leaves per tree, two trees combined per sample. Beyond five miles, all the orchards are sampled and each orchard has 25% of the trees sampled at four leaves per tree. Leaf samples are placed in plastic bags, barcode labeled, and stored on ice until shipped to a laboratory. Once orchard and homeowner samples are delivered, they are scanned into a database and tracked by their barcode number. No information other than the barcode is available to technicians (blind testing procedure).

Key Diagnostics

CAPS-Approved Method*: The approved screening protocol for the field is the PPV Enzyme-Linked ImmunoSorbent Assay (ELISA). The work instruction is available upon request from Renee.M.Devries@aphis.usda.gov. The work instruction describes detection of PPV using the ELISA kit from Agdia Inc., which detects six known PPQ strains/subgroups: PPV-C, PPV-D, PPV-EA, PPV-M, PPV-Rec, and PPV-W in leaves, fruit, and flowers.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Indicator Hosts: Indicator hosts are given in Table 4 with diagnostic symptoms (Bernhard et al., 1969; Damsteegt et al., 1997; Kegler et al., 2001; Glasa and Candresse, 2005; Gentit, 2006).

Table 4: Plum pox virus indicator host plants.

Indicators	Symptoms
Woody Plants:	
***Prunus persica* cv. GF305**	Vein clearing and distortion of leaves

P. tomentosa	Chlorotic mottle, vein chlorosis, leaf deformation, and necrotic spots
***Prunus marianna* cv. GF8.1**	Diffuse chlorotic spots on leaves
***Prunus instititia* cv. St. Julien no.2**	Chlorotic spots and rings
***Prunus domestica* K4 (Kirke x Persikovaja)**	Hypersensitive hybrid, pale green leaf mottling, necrotic leaf spots, shoot tip necrosis and/or eventual decline, depending on the isolate
Herbaceous Plants:	
Chenopodium foetidum	Chlorotic, chloro-necrotic, or necrotic spots depending on the viral isolate
Nicotiana benthamiana	Stunting, chlorotic mosaic with dark green islands, leaf puckering
***N. clevelandii* or *N. clevelandii* x *N. glutinosa* hybrid**	Chlorotic or necrotic local lesions, systemic chlorotic mottling – some isolates induce very mild or no symptoms
***Pisum sativum* cv. Colmo, Express Genereux or Serpette d' Auvergne**	Light green mosaic, chlorotic mottling

Staniulis et al. (1998) inoculated plants of *Chenopodium foetidum* (chlorotic local lesions) and *Pisum sativum* cvs. Rainiai and Citron (mottling). Damsteegt et al. (1997) reported that *P. tomentosa* was generally useful as a diagnostic indicator of PPV and showed different symptoms when infected with PPV-D or PPV-M (serotype specificity).
Antibodies: Monoclonal antibody 5B-IVIA (Cambra et al., 1994) allows for the universal detection of PPV. Strain-specific monoclonal antibodies have also been developed for both the D and M strains/serotypes (Cambra et al., 1994; Boscia et al., 1997). These enzymes, however, are not suited for differentiating other serotypes (*e.g.*, El-Amar and Cherry) (Candresse et al., 1998). Polyclonal and monoclonal antibodies have also been produced for the PPV-C and PPV-EA strain (Boscia et al., 1997; Crescenzi et al., 1997b; Myrta et al., 1998; Myrta et al., 2000).

ELISA: Clark and Adams (1977) developed the first ELISA test and included *Plum pox virus* as an application of this technology to plant viruses. Strain-specific antibodies are available (see section above).

Individual state Department of Agriculture laboratories work in conjunction with USDA-APHIS to screen *Prunus* trees for the presence of *Plum pox virus* (PPV). The protocol described below is used in New York State and is provided as an example of how PPV is detected. The PPV Lab in Geneva, NY, tests approximately 2,500 to 3,000 leaf samples daily during their survey operations period.

Upon delivery to the testing lab, leaf samples are unpacked, scanned into a database, and tracked by a barcode assigned to them by the collection team. Duplicate barcode labels are then printed and stored with the samples in a cold room until processing. Leaf samples are stacked with the petioles aligned and 0.5 grams of tissue is cut from the base of the leaves, avoiding petioles and midribs. Leaf tissue weights are checked every 10 samples or whenever leaf types change or a new *Prunus* species is tested.

Excised leaf material is placed in a plastic bag, which is labeled with the corresponding barcode. Grinding buffer (5 ml) is added to the bag and the sample is ground with a tissue homogenizer. Crude leaf extracts are then tested for PPV by ELISA) in microtiter plates using specific antibodies. Remaining leaf material is stored in a cold room until the sample has gone through the entire ELISA procedure with no indication of PPV infection.

For ELISA, microtiter plates consist of 96 wells, six of which are designated as controls (positive: PPV-infected material, negative: healthy plant material and grinding buffer). Every sample is replicated, so that a total of 45 samples can be run on one microtiter plate. Sample testing is a three-day process. On the first day, 96-well microtiter plates are coated with an antibody specific to PPV and incubated overnight in a cold room, allowing the antibodies to adhere to the surface of the wells. On the second day, microtiter plates are rinsed, loaded with ground leaf samples, and incubated overnight in a cold room. If PPV is present in the leaf sample to be tested, virus particles will adhere to the antibodies coated on the microtiter plate wells. On the third day, microtiter plates are rinsed and treated with an antibody specific to PPV that has an enzyme tag. If the coating antibody captured PPV, the second antibody will adhere to it, sandwiching the PPV particle between the two antibodies. Microtiter plates are rinsed and a solution that reacts colorimetrically with the enzyme tag on the secondary antibody is added. After one hour of incubation in the dark, any well, in which the virus is present, will turn yellow. In contrast, wells that do not contain the virus will remain colorless. Plates are scanned on a microplate reader and any sample that reads 2.0 times higher than the negative control is flagged as a positive suspect.

Lateral Flow Device: Mumford et al. (2001) describe a lateral flow device for on-site detection of PPV. The on-site kit, which contains a one-step lateral flow device and a simple, bottle extraction system, can give a result in three minutes.

Immunochromatographic Assay: Byzova et al. (2010) raised two monoclonal antibodies that recognized strains PPV-D, M, and C. The authors developed a 10-minute immunochromatographic assay for PPV with a detection limit of 3 ng/ml. The assay demonstrated good compatibility with the data obtained via ELISA.

Molecular: Sequence analysis of PCR fragments corresponding to the C-terminal part of the PPV coat protein gene has allowed identification of a molecular polymorphism correlated to serotype of the PPV isolates (Candresse et al., 1994, 1995). Initial results have indicated that an *Rsa*I restriction fragment length polymorphism (RFLP) located in this region could be used for PCR amplification, to discriminate between D and M serotypes of PPV (Bousalem et al., 1994; Wetzel et al., 1991). More recently, a cluster of non-coding, third-base mutations on five consecutive codons located around the *Rsa*I RFLP site was found to show excellent correlation with the viral serotype (Candresse et al., 1995). This observation was used as a basis for direct PCR typing of isolates belonging to the D and M serotypes of PPV (Candresse et al., 1994). Levy and Hadidi (1994) utilized a simple and rapid procedure for processing PPV infected plant tissue (Gene Releaser) for use with a specific 3' non-coding region RT-PCR assay. The 3'

non-coding region was used, because *Asian Prunus latent potyvirus*, a newly identified latent potyvirus in *Prunus* spp., may react positively with PPV-coat protein primers, in Southern blot hybridization with a PPV coat protein clone, and in ELISA with PPV polycolonal antiserum (Hadidi and Levy, 1994).

Immunocapture PCR(IC-PCR), reverse transcriptase PCR (RT-PCR), RT-PCR with RFLP, PCR-ELISA, print-capture PCR, and integrated RT-PCR/nested PCR have been used to detect PPV and to type strains/serotypes of PPV (Wetzel et al., 1991; Wetzel et al., 1992; Olmos et al., 1996; Olmos et al., 1997; Olmos et al. 1999; Poggi Pollini et al., 1997; Hammond et al., 1998; Nemchinov et al., 1998; Staniulis et al., 1998; Szemes et al., 2001; Glasa et al., 2005; Papayiannis et al., 2007; Olmos et al., 2008). Szemes et al., (2001) developed a RT-PCR/nested PCR technique for the simultaneous detection of PPV-D, M, EA, and C.

Faggioli et al. (1998) compared three different techniques to prepare PPV viral RNA for RT-PCR: 1) an immunocapture technique using a specific antiserum, 2) a silica-capture method using a non-specific matrix, and a simple and rapid RNA extraction. All three techniques allowed for the successful amplification and detection of PPV, but the silica capture method was less effective.

Candresse et al. (1998) compared an indirect double antibody sandwich ELISA using monoclonal antibodies for PPV-D and PPV-M with specific PCR assays or RFLP analysis of PCR fragments. Overall, the authors found an excellent correlation between the results of the ELISA and PCR assays for PPV-D and PPV-M. Adams et al. (1999) compared the detection effectiveness of immunocapture PCR (IC- PCR) and ELISA in dormant plum trees. IC-PCR has been shown to be about a thousand times more sensitive than ELISA (Candresse et al., 1995). ELISA was shown to be effective for the detection of PPV in bark samples throughout the winter; 71-80% of samples were ELISA positive compared to 85-86% in the same samples by IC-PCR. In one-year old shoots taken from infected branches of orchard trees, 66-81% were positive by ELISA compared with 81-87% by IC-PCR. It was not recommended that sampled be bulked for ELISA detection, because only 38-65% were positive by ELISA compared with 92-100% by IC-PCR due to the uneven distribution of the virus in plant tissues.

Sanchez-Navarro et al. (2005) developed a multiplex RT-PCR for the detection of eight stone fruit viruses: *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Plum pox virus* (PPV), *Apple mosaic virus* (ApMV), *American plum line pattern virus* (APLPV), *Apple chlorotic leaf sport virus* (ACLSV), *Apricot latent virus* (ApLV), and *Plum bark necrosis stem pitting associated virus* (PBNSPaV). Jarosova and Kundu (2010) used a single-tube multiplex RT-PCR to detect PPV, PDV, and PNRSV. Both methods included an internal control.

Real-Time PCR: Schneider et al. (2004) developed a real-time, fluorescent, RT-PCR reaction assay for the detection of PPV in the Smart Cycler (Cepheid). Varga and James (2005) developed real-time multiplex assay utilizing SYBR Green technology to detect and differentiate PPV-D and PPV-M types in woody and herbaceous plants.

Olmos et al. (2005) used Taqman technology in real-time assay for the universal detection and quantification of PPV in plant material and aphid vectors. The sensitivity of the real-time RT-PCR assay was 100 times higher than nested RT-PCR and 1000 times higher than ELISA and conventional RT-PCR.

Easily Confused Pests

PPV symptoms are sometimes difficult to distinguish from other diseases and may be confused with rusty spot (*Podosphaera* spp.) of peaches and nectarines and bacterial canker as well as insect-related problems such as damage from thrips, white apple leafhopper, and San Jose scale. Nutritional deficiencies and pesticide damage can also be confused with PPV symptoms.

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Secondary Pests of Stone Fruit (Truncated Pest Datasheet)

Candidatus Phytoplasma mali

Scientific Name
Candidatus Phytoplasma mali Seemuller & Schneider, 2004

Synonyms:
Phytoplasma AP-MLO, *Phytoplasma mali*

Common Name(s)
Apple proliferation and apple witches' broom

Type of Pest
Phytoplasma

Taxonomic Position
Class: Mollicutes, **Order:** Acholeplasmatales, **Family:** Acholeplasmataceae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2010 through 2014

Pest Description
Mollicutes are prokaryotes that have small genomes (530 to 1350 kbp), lack a cell wall, are pleomorphic, and have a low G + C content (23 to 29 mol%). Phytoplasmas belong to the class Mollicutes and are the proposed causative agents of disease in several hundred plant species (McCoy et al., 1989). 'Phytoplasma', formerly known as mycoplasma-like organism, has been adopted to collectively name the wall-less, non-helical prokaryotes that reside in the phloem tissue of the infected plant host and are transmitted primarily by insect vectors. The vectors are principally leafhoppers and planthoppers, although psyllids have been shown to vector these organisms as well (Carraro et al., 1998; White et al., 1998; IRPCM, 2004). Although phytoplasmas have been detected in affected plant tissues and insects with the use of technologies based on transmission electron microscopy, antibodies, and nucleic acids, they are unable to be cultured *in vitro*. Phytoplasmas cannot be morphologically or ultrastructurally distinguished from one another using either electron or light microscopy (CABI, 2009). *Candidatus* in scientific classification is a formal word that is placed before the genus and species name of bacteria that cannot be maintained in a Bacteriology Culture Collection. *Candidatus* status may be used when a species or genus is well characterized but unculturable (IRPCM, 2004).

Apple proliferation (AP) is a severe disease of apple caused by a phytoplasma in the apple proliferation group of phytoplasmas (Seemuller et al., 1994). The pathogen can also affect stone fruit (Lee et al., 1995; Navratil et al., 2001; Paltrinieri et al., 2001;

Mehle et al., 2006; Cieslinska and Morgas, 2011). The group/cluster also includes phytoplasmas associated with other perennial fruit tree diseases present in Europe, including European stone fruit yellows (*Candidatus* Phytoplasma prunorum) and pear decline (*Candidatus* Phytoplasma pyri) (Seemuller et al., 1994). In contrast, most phytoplasmas infecting stone fruit in North America (X-diseases) are members of the Western-X disease group (Poggi Pollini et al., 2001).

Candidatus Phytoplasma mali is found in the sieve tubes of the current season's phloem. The phytoplasma is highly pleomorphic, approximately 200 to 800 nm in diameter. The phytoplasma is bounded with a trilaminar cytoplasmic membrane but lacks a rigid cell wall (Seemuller, 1990). The AP phytoplasma is transmitted by the insect vectors *Cacopsylla picta*, *C. melanoneura*, and *Fieberiella florii.*

Analyses of a non-ribosomal DNA fragment, composed of three putative open reading frames (ORFs) (in particular ORF2 coding for a protein significantly homologous with a bacterial nitroreductase), proved the existence of at least three different AP phytoplasma subtypes named AT-1, AT-2, and AP-15 (Jarausch et al., 1994, 2000). Molecular characterization of the genes coding the ribosomal proteins L22 and S3 revealed the presence of higher genetic heterogeneity within isolates of *Ca.* Phytoplasma mali and led to the proposal of four subtypes rpX-A, rpX-B, rpX-C, and rpX-D (Martini et al., 2005, 2008). Analyses of ribosomal and non-ribosomal DNA fragments of *Ca*. Phytoplasma mali populations from northwestern Italy revealed the presence of three AP phytoplasma subtypes (AT-1, AT-2, and AP-15), and reported the identification of at least two phytoplasmal genetic lineages, designated AT-1a and AT-1b, among the AP phytoplasma isolates of the AT-1 subtype (Casati et al., 2010).

Figure 1: Wilting (top) and dying (bottom) cherry trees infected with the AP phytoplasma. Images from Mehle et al. (2006).

Symptoms/Signs

Trees infected by apple proliferation often occur in clusters, and these clusters grow (expand) year by year (Bliefernicht and Krczal, 1995). Symptoms are unevenly distributed on the plants. Additionally, there is considerable variability in virulence in *Ca.* P. mali. Based on symptomatology, the

phytoplasma strains can be defined as avirulent to mildly, moderately, or highly virulent; and the trees can be simultaneously affected by more than on strain of the apple proliferation phytoplasma (Seemuller and Schneider, 2007; Seemuller et al., 2010).

Stone Fruit:

Cherry: Symptoms of AP in cherry include wilting, dying, and floral and phloem necrosis (Fig. 1, 2) (Mehle et al., 2006).

Apricot: Symptoms of AP in apricot include stem necrosis and leaf wilting (Mehle et al., 2006).

Plum: The primary symptom of AP in plum is late blooming (Mehle et al., 2006).

Apple: Trees affected by the AP phytoplasma, in general, lack vigor. Trunk circumference and crown diameter are reduced compared to healthy trees. Shoots are thin and the bark, which is sometimes fluted lengthwise, has a reddish-brown color. Necrotic areas appear on the bark and some branches may wither. Diseased trees may die, but often recover if adequately fertilized (EPPO, 1997).

Late growth of terminal buds in the autumn is usually the first noticeable symptom. A rosette of terminal leaves, which often become infected with powdery mildew, sometimes develops late in the season in place of the normal dormant bud. A more reliable symptom, however, is the premature development of axillary buds, which give rise to secondary shoots/shoot proliferation (witches' brooming). These abnormal secondary shoots are usually numerous near the apex of the main shoot, whereas normal laterals of healthy trees arise nearer the base of the shoots. The angle between these secondary shoots and the main shoots is abnormally narrow on infected trees (Bovey, 1963). The witches' brooms do not develop repeatedly on the same branch. They may appear successively on

Figure 2: Floral (top) and phloem necrosis (bottom) in cherry trees infected with the AP phytoplasma. Images from Mehle et al. (2006).

various parts of the tree, or all at once over the whole tree, but usually develop only during the first two or three years following infection.

Leaves appear earlier than normal. Leaves of infected plants roll downward and become brittle, they are finely and irregularly serrated and are smaller than normal (Fig. 2). They also tend to turn red in autumn in contrast to the yellow coloration of healthy plants. Summer leaves are chlorotic. Early defoliation may occur.

Stipules are abnormally enlarged (long); while petioles are rather short (an important symptom in nursery surveys). Leaf rosette may appear on the shoot ends or the shoot tips may die (an important symptom in nursery surveys). Flowering is delayed, sometimes until late summer or autumn, but most blossoms on infected trees are normal. In some cases, flowers show numerous petals and the peduncles are abnormally long and thin. The calyx end and peduncular cavities are shallower and broader, giving the fruit a flattened appearance. Fruit fail to set and may stay on the tree for a long period. Fruit are reduced in size with incomplete coloration and poor flavor. Seeds and seed cavities are smaller.

Root weight is reduced; the fibrous root system of infected trees forms compact felt-like masses of short roots so that the larger ones are unable to develop (a fine hairy root system).

Dahlia: Symptoms of AP in dahlia include bushy growth accompanied by shoot proliferation, narrowed leaves, and flower bud deficiency (Kaminska and Sliwa 2008a). **Note:** plants in this study were co-infected with apple proliferation and aster yellows phytoplasmas.

Rose: Symptoms of AP in rose include dieback, witches' broom, bud proliferation, stunted growth, leaf and flower malformation, and shoot and flower proliferation (Kaminska and Sliwa, 2004). **Note:** plants in this study were co-infected with apple proliferation and aster yellows phytoplasmas.

Lily: Symptoms of AP in lily include leaf scorch/leaf burn, leaf malformation and necrosis; flower bud abscission (Kaminska and Sliwa 2008b).

CAPS-Approved Method*:

The CAPS-approved survey method is to collect symptomatic plant tissue by visual survey. The best time to sample aboveground tissue is in late summer to early fall, because phytoplasma population is highest at this time. At least five samples per plant need to be collected due to the low titer and erratic distribution of the pathogen in the phloem of the plant. Phytoplasmas are present in the roots of infected plants year around.

Follow instructions in Phytoplasma sample submission for Cooperative Agricultural Pest Survey (CAPS) Program and Farm Bill Goal 1 surveys FY 2014.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Delic et al. (2007) carried out surveys during autumn (October) and spring (April). Several hectares of orchards were visually inspected, and stone fruit, apple, and pear trees were checked for symptoms of phytoplasma infection. The symptoms considered were witches' broom, small leaves, enlarged stipules, and reddening. Fialova et al. (2003) visually examined apple trees from intensive orchards, nurseries, and private gardens for the presence of proliferation symptoms and enlarged stipules. Samples were taken during the vegetative season from all trees with symptoms. Three shoot samples per tree were used for the molecular analyses. Ermacora et al. (2008) conducted two field inspections annuals in September and December to identify AP symptoms. In September, plants were monitored for witches' brooming, small leaves with enlarged stipules, and reddish leaves. In December, only witches' brooming was monitored.

Rekab et al. (2010) sampled trees at the beginning of June (spring), the end of July (summer), and the beginning of October (fall). Each tree was subdivided into three or four homogeneous sampling zones, depending on tree size, and from each zone three samples were taken. Each sample was composed of 8 leaf disks, about 1.4 cm (0.55 in.) in diameter (0.35 mg total on average), including the middle of leaf midribs. Phytoplasma presence in the trees was uneven. Samples collected in mid-summer had significantly lower phytoplasma concentrations, as measured by real-time PCR, than those collected in the spring or fall. Samples collected in the fall were less variable than those collected in other sampling dates and a lower number of samples were negative. The experiment also showed that a sample of 8 leaf disks is too small, but a sample of 24 leaf disks, particularly if collected in the fall, may provide a reliable means to estimate differences in phytoplasma concentration, which were two orders of magnitude different in these experiments. If less pronounced differences are observed, the sample size required would be much larger (Rekab et al., 2010).

Avinent and Llacer (1995) took bark samples from diseased trees and used bark from one or two year old branches for phloem extraction of the phytoplasmas occurring in fruit trees in Spain.

Due to the large percentage of latent infections, observation of symptoms will not always reveal the 'true disease status" of an orchard. Baric et al. (2007) sampled each tree from eight adjacent rows representing one-third of the orchard by collecting at least three pencil-thick root pieces. The AP phytoplasma was detected using the Baric and Dalla Via (2004) real-time PCR assay.

Insect vectors, *Cacopsylla picta* overwintered adults and nymphs, were shaken from apple trees onto an underlying net and grouped using an aspirator (Carraro et al., 2008). The population of the vector in the orchards was high, however, in this site. Yellow sticky traps, sweep netting, and a beat tray methodology have also been used to

sample insect vectors (Tedeschi et al., 2002; Galetto et al., 2005; Fialova et al., 2008; Tedeschi et al., 2009; Casati et al., 2010).

Key Diagnostics/Identification

Molecular: Follow instructions in Phytoplasma sample submission for Cooperative Agricultural Pest Survey (CAPS) Program and Farm Bill Goal 1 surveys FY 2014.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Serological: ELISA is available. Loi et al. (2002) developed monoclonal antibodies against apple proliferation phytoplasma. Brzin et al. (2003) showed that an ELISA procedure was very sensitive and reliable compared to PCR. The phytoplasma could be reliably detected in samples of leaves, shoots, and roots during the growing season and also in dormant bud-wood and roots.

This DAS-ELISA is commercially available from Bioreba AgG (http://www.bioreba.ch/files/Product_Info/ELISA_Reagents/ApP_DAS_ELISA.pdf). Some isolates of the AT-1 subtype, however, may not be recognized with this test (Martini et al., 2005).

Culture: The phytoplasma that causes apple proliferation is obligate and cannot be cultured on microbiological growth media.

Biological Indexing: Greenhouse indexing, which consists of graft-transmission onto a woody indicator is a time-intensive method. For apple proliferation phytoplasma, this process can take up to two years. The best woody indicator for fast diagnostic detection of the AP phytoplasma is *Malus* x *dawsoniana* (EPPO, 1997). If the very sensitive indicator *Malus* x *dawsoniana* is grafted directly in June on the scion, it develops a leaf reddening during the following autumn and bark splitting and scaling during the next spring (EPPO, 1997).

Fluorescence Microscopy: For large scale diagnosis, the DAPI (4', 6'-diamidino-2-phenilindole, 2HCl) staining method (Seemuller, 1976) can be used, although the percentage of false negative can reach high levels. False negatives generally occur when phytoplasma colonization of plants is poor or uneven. This test detects fluorescence of phytoplasmas in the sieve tubes of the leaf veins.

Biological indexing and DAPI staining are time-consuming and do not often allow specific identification of phytoplasmas (Poggi Pollini et al., 2001).

Molecular: Seemuller and Schneider (2004) offer a summary of the molecular studies conducted on the apple proliferation, European stone fruit yellows, and pear decline phytoplasmas. The authors conclude that the phytoplasmas are coherent and discrete taxa and can be distinguished as distinct species with the proposed names *Ca.*

Phytoplasma mali (apple proliferation), *Ca.* Phytoplasma prunorum (European stone fruit yellows), and *Ca.* Phytoplasma pyri (pear decline). A chromosome map of the apple proliferation phytoplasma is available (Lauer and Seemuller, 2000). Seemuller et al. (2010) used single-strand conformation polymorphism (SSCP) and sequence analysis to examine strain similarity of *Ca.* Phytoplasma mali.

DNA extraction and enrichment: Kirkpatrick et al. (1987), Ahrens and Seemuller (1992), and Maixner et al. (1995) developed a procedure to enrich DNA of phytoplasmas using axial phloem tissue of apple trees. Most authors working with the AP phytoplasma used these procedures or some modification (*e.g.,* Malisano et al., 1996) of these procedures (Jarausch et al., 1994; Avinent and Llacer, 1995; Fialova et al., 2003; Baric and Dalla Via, 2004; Delic et al., 2007; Carraro et al., 2008). The method of Doyle and Doyle (1990) was also employed for isolating DNA from the insect vector (Carraro et al., 2008) and plant samples (Firraro et al., 1994; Kison et al., 1994; Delic et al., 2007; Bisognin et al., 2008). Green et al. (1999) developed an 'easy and efficient' DNA extraction method from woody plants for detection of phytoplasmas by PCR.

PCR: PCR amplification is now widely used for the sensitive and reliable diagnosis of phytoplasmas in fruit trees. Due to the close genetic relatedness of the apple proliferation group of phytoplasmas, specific identification often requires the digestion of the amplicons with various endonucleases and subsequent RFLP analysis or sequencing (Deng and Hiruki, 199; Ahrens and Seemuller, 1992; Gundersen and Lee, 1996; Lee et al., 1993; Lee et al., 1995; Schneider et al., 1995; Smart et al., 1996; Kison et al., 1997; Gibb et al., 1999; Jarausch et al., 2000; Heinrich et al., 2001). Firrao et al. (1994) developed a ‘rapid’ PCR protocol for the apple proliferation organism without the need for a restriction digestion or hybridization step.

Restriction analysis of P1A/P7a amplicons using *Hpa*II and *Fau*I endonucleases allows us to 1) distinguish AT-1 from AT-2 and AP-15; 2) differentiate the genetic lineages AT-a1 and AT-1b, and 3) discriminate AT-1 isolates from Italy and Germany (Casati et al., 2010).

Nested PCR: Nested PCR has been employed for the detection of phytoplasmas both in plants and psyllids using universal primers (Deng and Hiruki, 1991; Gundersen and Lee, 1996) and/or 16SrX phytoplasma group specific primer pairs (Lee et al., 1995; Lorenz et al., 1995).

Immunocapture PCR (IC-PCR): Rajan and Clark (1995) use immunocapture-PCR to detect apple proliferation in apple bark. They used rabbit polyclonal antibodies to capture the phytoplasma and then amplified with universal PCR primers.

Real-time PCR: Jarausch et al. (2004) developed a quantitative real-time PCR for apple proliferation phytoplasma in plants and insects from a nitroreductase gene sequence. Galetto et al. (2005) developed an apple proliferation specific real-time PCR assay from the same nitroreductase gene sequence. These authors also developed a universal assay for detection of phytoplasmas belonging to groups 16Sr-V, 16Sr-X, and 16 Sr-XII.

Torres et al. (2005) developed a real-time PCR that will detect *Ca.* P. mali, *Ca.* P. prunorum, and *Ca.* P. pyri (three pytoplasmas in apple proliferation group of quarantine importance).

Baric and Dalla-Via (2004) developed a real-time PCR for apple proliferation phytoplasma in apple plant material. The assay also amplified a host gene from apple as an internal control. Baric et al. (2006) compared the Baric and Dalla Via (2004) real-time PCR with four conventional PCR assays. The real-time procedure had the highest sensitivity and specificity and was not susceptible to PCR inhibition. The one downfall was the high cost of the procedure.

Aldaghi et al. (2007) developed a real-time PCR protocol for *Ca.* Phytoplasma mali. This probe could distinguish a single mismatch between *Ca.* P. mali and *Ca.* P. prunorum, but late fluorescent curves were obtained from European stone fruit isolates. Aldaghi et al. (2008) developed a new probe and adapted the original procedure to eliminate the late fluorescent curves.

Easily Confused Pests

The apple proliferation (AP) phytoplasma is phylogenetically closely related to the European stone fruit yellows (ESFY) and pear decline (PD) phytoplasmas. These three phytoplasmas belong to the 16SrX group and have nearly identical 16S rDNA sequences (Seemuller and Schneider, 2004).

The peach yellow leaf roll (PYLR) phytoplasma from California was found by Kison et al. (1997) to also be closely related to AP, PD, and ESFY. The PYLR agent could clearly be distinguished from the AP and ESFY phytoplasmas by Southern blot hybridization with DNA fragments from the AP phytoplasma and by RFLP analysis of ribosomal DNA employing *SSp*I, *Bsa*AI, and *Rsa*I restriction endonucleases. The PYLR phytoplasma, however, was indistinguishable from the PD phytoplasma by PCR-amplified ribosomal DNA (Kison et al., 1997).

Aldaghi et al. (2007) developed a real-time PCR protocol for *Ca.* Phytoplasma mali. This probe could distinguish a single mismatch between *Ca.* Phytoplasma mali and *Ca.* Phytoplasma prunorum, but late fluorescent curves were obtained from European stone fruit yellows isolates. Aldaghi et al., (2008) developed a new probe and adapted the original procedure to eliminate the late fluorescent curves.

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Phellinus noxius

Scientific Name
Phellinus noxius (Corner) G. Cunn.

Synonyms:
Corticum spp., *Fomes noxius*, *Hymenochaete noxia*, *Hymenochaete noxius*, *Phellinidium noxium*, and *Poria setulalsocrocea.*

Common Name
Brown root rot, brown cocoa root rot, brown root, brown tea root disease, collar rot, stem rot

Type of Pest
Fungus

Taxonomic Position
Class: Basidiomycetes, **Order:** Hymenochaetales, **Family:** Hymenochaetaceae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2006 through 2009

Pest Description
Phellinus noxius has a very wide host range; it affects over 200 species of woody plants, including a variety of forest, plantation, orchard, and landscape trees and shrubs. *Phellinus noxius* is a significant pathogen of *Araucaria cunninghamii* (colonial pine), *Camellia sinensis* (tea), *Coffea* spp. (coffee), *Elaeis guineensis* (African oil palm), *Hevea brasiliensis* (rubber), *Tectona grandis* (teak), and *Theobroma cacao* (cocoa) and is widely found in southeast Asia, Oceania, Central and South America, and Africa. *P. noxius* has been reported to occur on peach (*Prunus persica*), Japanese plum (*Prunus mume*), and Taiwan cherry (*Prunus campanulata*).

Figure 1. Mycelia (left) and arthrospores (right) of *P. noxius*. Photos courtesy of Pao-Jen Ann.

The basidiocarp, also referred to as a sporocarp or conk, is perennial, solitary or imbricate, sessile with a broad basal attachment, commonly resupinate. **Basidiocarps are not always produced in nature but can be induced in the laboratory** (Bolland et al., 1984). Pileus 5 to 13 x 6 to 25 x 2 to 4 cm (1.97 to 5.1 x 2.4 to 9.8 x 0.79 to 1.57 in.), applanate, dimidiate or appressed-reflexed; upper surface deep reddish-brown to umbrinous, soon blackening, at first tomentose, glabrescent, sometimes with narrow concentric zonation, developing a thick crust; margin white then concolorous, obtuse. Context up to 1 cm (0.30 in.) thick, golden brown, blackening with KOH, silky-zonate fibrous, woody. Pore surface grayish-brown to umbrinous; pores irregular, polygonal, 6 to 8 mm (0.24 to 0.31 in.), 75 to 175 µm diameter, dissepiments 25 to 100 µm thick, brittle and lacerate; tubes stratified, developing 2 to 5 layers, 1 to 4 mm (0.04 to 0.16 in.) to each layer, darker than context, carbonaceous.

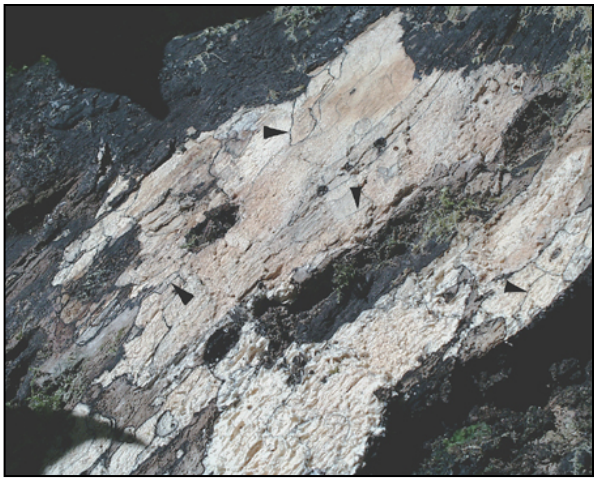

Figure 2. Dry, honeycombed, white wood rot caused by *P. noxius*. Photo courtesy of Fred Brooks.

Figure 3. Mycelial crust of *P. noxius* on multi-trunked tree in the rainforest. Photo courtesy of Fred Brooks. http://www.bugwood.org.

Basidiospores approximately 4 x 3 µm, ovoid to broadly ellipsoid, hyaline, with a smooth, slightly thickened wall, and irregular guttulate contents. Basidia 12 to 16 x 4 to 5 µm, short clavate, 4-spored. Setae absent. Setal hyphae present both in the context and the dissepiment trama. Context setal hyphae radially arranged, up to 600 x 4 to 13 µm, unbranched or rarely branching, with a thick dark chestnut brown wall and capillary lumen; apex acute to obtuse, occasionally nodulose. Tramal setal hyphae diverging to project into the tube cavity, 55 to 100 x 9 to 18 µm, with a thick dark chestnut-brown wall (2.5 to 7.5 µm thick) and a broad obtuse apex. Hyphal system dimitic with generative and skeletal hyphae, non-agglutinated in the context, but strongly agglutinated in the dissepiments. Generative hyphae 1 to 6.5 µm diameter, hyaline or brownish, wall thin to somewhat thickening, freely branching, simple septate. Skeletal hyphae 5 to 9 µm diameter, unbranched, of unlimited growth, with a thick

reddish-brown wall (up to 2.5 µm thick) and continuous lumen, non-septate (Pegler and Waterson, 1968).

Symptoms/Signs

Symptoms of brown root rot are similar to those caused by other root rot pathogens: slow plant growth, yellowing and wilting of leaves, defoliation, branch dieback, and plant death (Brooks, 2002). Although dead wood is initially discolored reddish brown, it later becomes white, dry, and crumbly.

Signs of the pathogen, unlike the symptoms, are distinctive for this disease. *P. noxius* forms a thick, dark brown to black crust of mycelium around infected roots and lower stems (Fig. 3), which gives the disease its name. The leading edge of the crust is creamy white, glistens with drops of clear, brownish exudate, and is usually noticeable even in the dark understory of the rainforest. Patches of white mycelium are present between the bark and sapwood. As colonization progresses, white, soft, crumbly wood becomes laced with reddish strands of fungus hyphae that turn black with age (Fig. 4). Basidiocarps, or fruiting bodies, are purplish brown bracts (conks) with yellow-white growing margins and concentric blackish zones towards the edges (CABI, 2009). The basidocarps are gray to brown on the spore-forming surface (Brooks, 2002). Unlike other similar fungi, there are no rhizomorphs. Spread is by physical contact with the root encrustations.

Figure 4. With age, reddish hyphae in wood agglutinate (stick together), turn black and brittle. Photo courtesy of Fred Brooks.

Survey

CAPS-Approved Method*: Collect symptomatic plant material via visual survey.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Surveys should be focused in areas with the greatest risk of pathogen establishment. *P. noxius* has a broad host range and would undoubtedly find numerous suitable hosts in North America, but would most likely be restricted to tropical or near tropical regions (Hodges, 2005).

ThisA recent host analysis shows that portions of the Great Lake states, the northeastern United States, and Colorado, Florida, Idaho, and Utah have the greatest risk of *P. noxius* establishment based on host availability. Most of the remaining states have counties that are considered at low to moderate risk.

Visual survey: Visual survey is the most common method used to survey for *P. noxius.* A dark brown mycelial mat or sleeve on the surface of the roots and up to the base of the stem is used reliably for field identification of *P. noxius*. Soil is scraped away around the collar and the main roots and the distinctive mycelial sleeve is often present (Nandris et al., 1987). Particular attention should be paid to trees that appear wilted or dead. *P. noxius* tends to be a problem in cleared forests converted to agricultural land (tree farms) or in disturbed areas and surveys should be conducted in these areas.

Baiting: Early detection of the pathogen before typical wilt symptoms are visible is very difficult and time consuming. Baiting out the pathogen by placing sticks of a susceptible host in the soil and retrieving for laboratory examination after three weeks is also conducted, particularly in virgin forests to detect parasites on the root system of wild trees (Nandris et al., 1987; CABI, 2009). According to Nandris et al. (1987), the area around the root collar can be mulched for three weeks to provide a damp zone that allows the superficial mycelium to progress from the roots onto the trunk of rubber trees. When the mulch is removed, the mycelial filaments of the pathogen can be observed.

Key Diagnostics/Identification

CAPS-Approved Method*: Confirmation of *P. noxius* is via morphological identification.

A. Surface sterilized diseased root tissues are plated on potato dextrose agar amended with ampicillin and benomyl or Chang (1995) medium.

B. The cultural characteristics of the fungus are examined and compared to photos in Ann et al. (2002) and Brooks et al. (2002).

C. The Key of the Polyporaceae described by Cunningham (1965) is then used for identification of the fungus.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: According to Ann et al (1999), after plating surface sterilized diseased root tissue on potato dextrose agar amended with ampicillin and benomyl, the cultural and morphological characteristics are the fungus are examined and compared. The Key of the Polyporaceae described by Cunningham (1965) is then used for identification of the fungus. In culture, mycelia are initially white and then brown with irregular dark brown lines or patches. In addition, staghorn-like hyphae and arthrospores, but no clamp connections are commonly observed (Sahasi et al., 2007).

Chang (1995) developed a selective medium for *P. noxius* using malt extract agar as a basal medium amended with benomyl, dicloran, ampicillin, and gallic acid. Tergitol NP-7 was added for isolation from soil.

Bolland et al. (1984) developed a method to induce sporulation in basidiocarps of *P. noxius* to obtain single spore isolates.

A PCR to detect *P. noxius* was developed in Taiwan to detect specific regions of the ITS (Tsai et al., 2007).

Easily Confused Pests

P. noxius basidiocarps are sometimes confused with *P. lamaensis*, another tropical *Phellinus* species. *P. lamaensis* sporocarps have short, reddish-brown, cone-shaped cells called hymenial setae growing into their pores, however, *P. noxius* does not.

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Appendix A: Diagnostic Resource Contacts

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Appendix B: Glossary of Terms

Abcission: The shedding of leaves or other plant parts as the result of physical weakness in a specialized layer of cells (the abscission layer) that develops at the base of the structure.

Acquisition Period: The period of time for a vector to acquire a pathogen (e.g., a virus).

Acrostichal Setae: A row of setae/bristles on the top surface of the middle part of a fly's thorax (the mesothorax). The bristles run longitudinally, that is, in a line from the head-end in the direction of the rear of the insect.

Aedeagus: In male insects, the penis or intromittent organ, situated below the scaphium and enclosed in a sheath.

Aestivation: Dormancy in summer during periods of continued high temperatures, or during a dry season.

Agglomerate: To form or collect into a rounded mass.

Airborne: Transported by air.

Albedo: White or whitness – reflective power, reflected light. The spongy white tissue on the inside of the rind of citrus fruit.

Allopatric: Occurring in separate, non-overlapping geographic areas. Often used to describe populations of related organisms unable to crossbreed because of geographic separation.

Anal plate: 1) Lepidoptera larvae: The shield–like covering of the dorsum of the last segment; 2) Embryonic larvae: tergum XI, 3) Cocciids: a pair of triangular or semicircular sclerites at the cephalic end of the caudal cleft.

Anamorph: The imperfect or asexual stage of a fungus.

Annual: A plant that completes its life cycle and dies within one year (see perennial).

Antibody: A specific protein formed in the blood of warm-blooded animals in response to the presence of an antigen.

Antigen: Any foreign chemical (normally a protein) that induces antibody formation in warm-blooded animals.

Apex: The top or highest part of something.

Apical: At, near, or pertaining to the apex of any structure.

Apothecia: Open, cuplike or saucerlike, ascus-bearing fungal fruiting body (ascocarp), often supported on a stalk.

Applanate: Flattened out or horizontally expanded.

Appressed: Closely flattened down or pressed against a surface.

Arid: Having little or no rain; too dry or barren to support vegetation.

Arthrospore: A fungal spore resulting from the fragmentation of a hyphae.

Bacilliform: Shaped like short rods with rounded ends.

Basal: Pertaining to the base or point of attachment to or nearest the body.

Basidiocarp: Sexual fruiting body of a basidiomycetous fungus.

Basidiospores: Haploid (1N) sexual spore produced on a basidium.

Basidium (**pl. basidia; adj. basidial):** Specialized cell or organ, often club-shaped, in which karyogamy and meiosis occur, followed by production of externally-borne basidiospores (generally four) that are haploid. There are several types of basidia.

Blight: Sudden, severe, and extensive spotting, discoloration, wilting, or destruction of leaves, flowers, stems, or entire plants.

Bolls: The spherical shaped fruits of cotton and flax.

Boreal: Relating to or characteristic of the climatic zone south of the Arctic, esp. the cold temperate region dominated by taiga and forests of birch, poplar, and conifers.

Brittle: Hard but liable to break or shatter easily.

Buff: A pale yellow-brown color.

Calyx: The outer-most group of leaves surrounding the flower; the external-most part of the flower.

Cambium: A cellular plant tissue from which phloem, xylem, or cork grows by division, resulting (in woody plants) in secondary thickening

Camouflage: To hide or disguise the presence of.

***Candidatus*:** In scientific classification is a formal word that is placed before the genus and species name of bacteria that cannot be maintained in a Bacteriology Culture Collection. *Candidatus* status may be used when a species or genus is well characterized but unculturable.

Canes: The hollow, jointed stem of a tall grass or plant.

Canker: A plant disease characterized (in woody plants) by the death of cambium tissue and loss and/or malformation of bark, or (in non-woody plants) by the formation of sharply delineated, dry, necrotic, localized lesions on the stem; "canker" may also be used to refer to the lesion itself, particularly in woody plants.

Carbonaceous: Consisting of, containing, relating to, or yielding carbon.

Cephalopharyngeal: Head region.

Chaetotaxy: The arrangement of bristles.

Chorion: The outer shell or covering of the insect egg.

Chlorotic (chlorosis): Abnormal condition of plants in which the green tissue loses its color or turns yellow as a result of decreased chlorophyll production due to disease or lack of light.

Cilia: Fine hair-like projections from certain kinds of cells.

Ciliate: Having cilia.

Cisanal setae: In coccids, the shorter and further two of the four setae (commonly known as hairs) near the caudal ring.

Clamp connection: A bridge- or buckle-hyphal protrusion in basidiomycetous fungi, formed at cell division and connecting the newly divided cells.

Clypeus: A broad plate at the front of an insect's head

Cocoon: A silken case inside which the pupa is formed.

Collar: The portion of the seedling or plant near the surface of the soil; in grafted woody plants, the scion portion of the plant near the soil surface.

Color-breaking: Darker pink stripes on the flower petals.

Concave: Curving inward.

Concentric: Of or denoting circles, arcs, or other shapes that share the same center, the larger often completely surrounding the smaller.

Concolorous: Colored the same throughout.

Conidium (pl. conidia): An asexual, nonmotile fungal spore that develops externally or is liberated from the cell that formed it.

Conidiophores: Simple or branched hypha on which conidia are produced.

Conk: A shelf-like, typically hardened basidiocarp of a wood decaying fungus, usually a polypore.

Convex: Curving or bulging outward.

Cornuti: Literally, the horned ones. Sclerotized structures on male genetalia in some insects; spines.

Corpus bursae: A dilated membranous sac at the anterior end of the bursa copulatrix.

Crawlers: One that crawls, especially an early form of certain insect larvae.

Cremaster: 1) The apex of the last segment of the abdomen; 2) the terminal spine or hooked process of the abdomen of subterranean pupa, which is used to facilitate emergence from the earth; 3) an anal hook by which some pupae are suspended.

Cross Protection: The process whereby a normally susceptible host is infected with a less virulent pathogen (usually a virus) and thereby becomes resistant to infection by a second, usually related, more virulent pathogen.

Cruciform: Cross-shaped.

Cryptic: Serving to conceal, hide.

Cucullus: A hood-shaped organ, resembling a cowl or monk's hood, as certain concave and arched sepals or petals; resembling a hood.

Cucurbitacins: Toxic tetracyclic triterpenes found in plants of the family Cucurbitaceae, *e.g.*, squash, pumpkin, cucumber, melons.

Cupreous: Of or like copper.

Debris: The scattered remains of something broken or destroyed; rubble or wreckage.

Defoliation: Loss of leaves from a plant, whether normal or premature.

Degree Days: Development of poikilothermic ("cold-blooded") organisms such as insects, fungi, and plants, is regulated by environmental temperatures. Development to particular stages in the life cycles of these organisms is largely controlled by how much heat they experience, where heat is considered as a function of temperature and time. Degree-days are an estimate of the amount of heat accumulated over a 24-hr period. They are calculated using lower and upper developmental thresholds unique to a particular organism and, typically, some approximation of the 24-hour temperature pattern derived from minimum and maximum daily temperatures (which are commonly available from local weather-recording stations). Only those temperatures falling

between the lower and upper thresholds are included in the calculations. Degree-day values may be positive or equal zero (all temperatures above or below thresholds), but are never negative. Degree-days are calculated for each day and are then summed to provide cumulative (total) degree-days. Starting points for calculating cumulative degree-days are usually arbitrary, typically January 1 but often later (e.g. April 1) in areas with cold winter temperatures. Based on experimental data, cumulative degree-days are linked to specific development events of interest (e.g. adult insect emergence). Thus, a pest manager can anticipate or predict an event of interest based on local temperature data and an appropriate degree-day based developmental model.

Desiccated: The state of extreme dryness, or the process of extreme drying.

Deutonymph: The third instar of a mite.

Diapause: A period of arrested development and reduced metabolic rate, during which growth, differentiation, and metamorphosis cease; a period of dormancy not immediately referable to adverse environmental conditions.

Dieback: Progressive death of shoots, leaves, or roots, beginning at the tips.

Digitus: Having appendages of the feet (as found in member of the family Coccidae), which may be either broadly dilated or knobbed hairs; tenent hairs, empodial hairs.

Dimidiate: Having only one half developed.

Dimitic: A fungus contains generative hyphae and just one of the other two types; usually have generative and skeletal hyphae.

Discocellular: Of or pertaining to the discal cell in the wings of lepidopterous insects.

Discoid: Shaped like a disc.

Disease: Abnormal functioning of an organism

Disease Incidence: Number of plants affected by a disease within a population.

Disease Severity: The measure of damage done by a disease.
Dissection: To cut into parts; consisting of many lobes or segments, as some leaves.

Dissepiment: A partition in a part or organ; a septum.

DNA (abbr. for deoxyribonucleic acid): The double-stranded, helical molecule that contains genetic code information. Each repeating unit, or nucleotide, is composed of deoxyribose (a sugar), a phosphate group, and a purine (adenine or guanine) or a pyrimidine (thymine or cytosine) base

Dormancy: A condition of suspended growth and reduced metabolism of an organism, generally induced by internal factors or environmental conditions as a mechanism of survival.

Dorsal: On the upper surface.

Drupe: The fruit of Prunus species. A type of fruit in which an outer fleshy part surrounds a shell (a pit or stone) of hardened exocarp with a seed inside.

Ductus bursae: The duct in female Lepidoptera extending from the ostium to the bursa copulatrix.

Dusky: Dark in color.

Ecdysis: Molting; the process of shedding the exoskeleton.

Eclosion: The emergence of an insect from the pupa case, or of a larva from the egg.

ELISA (Enzyme-Linked ImmunoSorbent Assay): A serological test in which the sensitivity of the reaction is increased by attaching an enzyme that produces a colored product to one of the reactants.

Elliposoid: A geometric surface, all of whose plane sections are either ellipses or circles.

Embryo: A minute rudimentary plant contained within a seed or an archegonium.

Embryonic: Of or relating to an embryo; in a rudimentary stage with potential for further development.

Emergence: The process of coming into being, or of becoming important or prominent. The process of coming into view or becoming exposed after being concealed.

Entries: Holes extending deeper than 3 mm into the fruit.

Envelope: Virology: a protein covering that packages the virus's genetic information.

Epidemic: Prevalent and spreading rapidly among many individuals in a community at the same time.

Etiology: The study of the causes of diseases.

Excrement (excreta): Waste matter discharged from the bowels; feces.

Exogenous: Derived or originating externally.

Exotic: Originating in or characteristic of a distant foreign country.

Exudate: Liquid excreted or discharged from diseased tissues, from roots and leaves, or by fungi.

Exuvia: The cast skin of an arthropod.

Eye spot: Spots of color that look like eyes; usually on the wings of butterflies and moths.

Facultative: Capable of changing life-style.

Facultative diapause: May or may not need to diapause; not required for development.

Femora (pl. for Femur): The third leg segment, located between the trochanter and the tibia.

Fecundity: The number of offspring per number of potential offspring (*e.g.*, eggs).

Filiform: Thread-like or hair-like.

Flaccid: Lacking in strength or firmness or resilience.

Flagellomere: A segment of the antennal flagellum. Male acuelate Hymenoptera have eleven flagellomeres, females have ten.

Forewing: Either of the anterior pair of wings on an insect that has four wings.

Frass: Plant fragments made by a wood-boring insect usually mixed with excrement; solid larval insect excrement.

Fruiting Body: Any of various complex, spore-bearing fungal structures.

Furled: Rolled or folded up and secured neatly.

Gena: The cheek; that part of the head on each side below the eyes, extending to the gular suture; in Odonata the area between the eyes and clypeus and mouth parts; in Diptera the space between the lower border of the eye and oval margin, merging into the face at the front and limited by the occipital margin behind.

Germ tube: Hypha resulting from an outgrowth of the spore wall and cytoplasm after germination.

Glabrescent: Lacking hair or a similar growth or tending to become hairless.

Glomerule: Webbing spun around bud clusters or flowers.

Gnathos: A mid-ventral plate on the ninth tergum in lepidopterans.

Graft: A shoot or bud of one plant or tree inserted or to be inserted into the stem or trunk of another where it continues to grow becoming a permanent part of the tree.

Granular: Resembling or consisting of small grains or particles.

Gravid: Pregnant; carrying eggs or young.

Greenhouse Indexing: Graft-transmission onto a woody indicator.

Gum: Gelatinous, sugary aggregate that is synthesized and exuded by plant tissues.

Gummosis: A gummy substance exuding from wounds or entrance holes.

Hemispherical: Shaped like the half of a globe or sphere.

Hierarchical: Of the nature of a hierarchy; arranged in order of rank.

Hind wing: Either of the posterior wings of a 4-winged insect.

Honeydew: Sugary ooze or exudate, often from aphids, and a characteristic symptom of some fungi.

Hyaline: Like glass, transparent and colorless.

Hymenial: Pertaining to a hymenium.

Hymenium: Continuous, spore-bearing layer of a fungus fruiting body.

Hypha (pl. hyphae): Single, tubular filament of a fungal thallus or mycelium; the basic structural unit of a fungus.

Imbricate: Arrange (scales, sepals, plates, etc.) so that they overlap like roof tiles.

Incubation period: The time between penetration of a host by a pathogen and the first appearance of disease symptoms.

Indigenous: Originating or occurring naturally in a particular place; native.

Indicator host: A plant species that gives characteristic symptoms to a specific virus or pathogen.

Inflorescence: A characteristic arrangement of flowers on a stem; a flower cluster.

Inoculum: Pathogen or its parts, capable of causing infection when transferred to a favorable location.

Instar: An insect or other arthropod between molts.

Intercalary: Formed or situated somewhere between apex and base of a given structure.

Internode: A part of a plant stem between two of the nodes from which leaves emerge.

Involuted: Rolled inward, spirally.

Iridescent: A display of lustrous rainbow-like colors.

Irroration: Tips of scales.

Katepisternal: Lower part of episternum, which is the anterior part of the mesopleuron.

Labial: Of or relating to lips.

Lanceolate: Lance or spear shaped, oblong tapering to the end.

Larvae (pl. for larva): An early, free living immature form of any animal that changes structurally when it becomes an adult usually by complex metamorphosis.

Latent Period: The time between infection and the production of new inoculum; the time after a vector has acquired a pathogen and before it can be transmitted.

Lenticular: Shaped like a lentil; Of or relating to a lens.

Lesions: Localized diseased area or wound.

Limoniform: Shaped like a lemon.

Lint: The cotton fibers separated from the seed.

Longevity: The duration of life.

Lure: A synthetic chemical which acts as the natural lure (pheromone) for one sex of an insect species. Placed in traps to attract a particular insect.

Luteous: Egg-yellow or clay yellow; (of yellow) having a light to medium greenish tinge.

Maxillary: Of or relating to the maxillae of an arthropod; of or attached to a jaw or jawbone, esp. the upper jaw.

Micropyle: A very small opening in the outer coat of an ovule, through which the pollen tube penetrates; the corresponding opening in the developed seed; one of the minute openings in the insect egg, through which spermatozoa enter in fertilization.

Microtrichium (pl. microtrichia): Small, sclerotized, and non-innervated cuticular projects on the body and wings of insects; which are also found on the tracheae.

Mine: Damage from leaf miners due to the larva, which lives and feeds for a part of all of its time between the epidermal layers of a leaf.

Mollicute: One of a group of prokaryotic organisms bounded by flexuous membranes and lacking cell walls (phytoplasmas and spiroplasmas).

Molt: A process of shedding the exoskeleton, ecdysis.

Monandrous: Having only one male mate at a time.

Moniliform: Resembling a string of beads.

Mortality: Death.

Mottle: Disease symptom comprising light and dark areas in an irregular pattern, usually caused by a virus; often used interchangeably with mosaic.

Multivoltine: Pertaining to organisms with many generations in a year or season.

Mummies: A dried, shriveled fruit; plant part or organ partially or completely replaced with fungal structures.

Mycelium: Mass of hyphae constituting the body (thallus) of a fungus.

Necrotic (Necrosis): Death of cells or tissue, usually accompanied by black or brown darkening.

Neonate: A recently born larva.

Nocturnal: Belonging to or active during the night.

Nodulose: Having minute nodules.

Notopleural: In Diptera, a depression, more or less triangular, situated immediately before the transverse suture and behind the humeri.

Nymphs: The immature stage (following hatching) of an insect that does not have a pupal stage; the immature stage of Acari (mite) that has eight legs.

Obligate: Restricted to a particular set of environmental conditions, without which an organism cannot survive (e.g., an obligate parasite can survive only by parasitizing another organism).

Obtuse: Not sharp, acute, or pointed; blunt in form; rounded at the extremity.

Ocellar: Referring to area around the ocelli such as ocellar bristles, ocellar triangle etc.

Ocelli: A simple eye of an insect or other arthropod.

Ochreous: Yellow with a slight tinge of brown.

Oligophagous: Feed on a restricted range of food.

Olivaceous: Of a dusky yellowish green color; olive green.

Opalescent: Showing varying colors as an opal does; having a milky iridescence

Opaque: Without any surface luster; not transparent.

Ovate: Having an oval outline or ovoid shape, like an egg.

Overwinter: Live through the winter.

Ovicide: Insecticides that are designed to kill eggs

Oviposit (oviposition): To deposit or lay eggs or ova. The act of depositing eggs.

Ovoid: Egg-like in shape or appearance.

Palpus (pl. palpi): Finger-like, usually segmented appendage of the maxilla (maxillary palp) and labium (labial palp).

Parasitoid: A parasitoid is an organism that spends a significant portion of its life history attached to or within a single host organism which it ultimately kills (and often consumes) in the process.

Parenchyma: The primary tissue of higher plants, composed of thin-walled cells and forming the greater part of leaves, roots, the pulp of fruit, and the pith of stems.

Pathosystem: A subsystem of an ecosystem that is defined by the phenomenon of parasitism. A plant pathosystem is one in which the host species is a plant. The parasite is any species in which the individual spends a significant part of its lifespan inhabiting one host individual and obtaining nutrients from it. The parasite may thus be an insect, mite, nematode, parasitic plant, fungus, bacterium, mycoplasma, virus, or viroid.

PCR (acronym for polymerase chain reaction): A technique used to amplify the number of copies of a specific region of DNA in order to produce enough of the DNA for use in various applications such as identification and cloning.

Pedicel: Small slender stalk; stalk bearing an individual flower, inflorescence, or spore.

Peduncle: The stalk bearing a flower or fruit, or the main stalk of an inflorescence.

Perforate: Pierce and make a hole or holes in.

Periderm: The corky outer layer of a plant stem formed in secondary thickening or as a response to injury or infection.

Peritreme: That part of the integument of an insect which surrounds the spiracles.

Perennial: Something that occurs year after year; plant that survives for several to many years (see annual).

Persistent transmission (syn. circulative transmission): A type of virus transmission in which the virus is acquired and transmitted by the vector after relatively long feeding times and remains transmissible for a prolonged period while in association with its vector

Petiole: The stalk that joins a leaf to a stem; leafstalk.

Pheromone: A substance given off by one individual that causes a specific reaction by other individuals of the same species; such as sex attractants, alarm substances etc.

Phloem: The vascular tissue in vascular plants, that conducts and distributes sugars and other dissolved foods from the places the food is produced to the places the food is needed or stored.

Photoperiod: The physiological reaction of organisms to the length of day or night.

Phototropism: The orientation of a plant or other organism in response to light, either toward the source of light (positive phototropism) or away from it (negative phototropism).

Phylloptosis: Leaf fall.

Phytophagous: Plant-eating.

Phytoplasma: Plant-parasitic pleomorphic mollicute (prokaryote with no cell wall) found in phloem tissue; cannot yet be grown on artificial nutrient media.

Pinaculum: In caterpillars, an enlarged seta-bearing papilla forming a flat plate.

Pinprick: Flask-shaped holes about 3 mm deep in fruit.

Pleomorphic: Able to assume various shapes (and perhaps sizes); pertaining to a life cycle in which an organism has more than one distinct form.

Polygonal: Having many sides or relating to a surface marked by polygons.

Polygynic: Phenotypic trait whose expression is controlled by, or associated with, more than one gene; mating with multiple males/females.

Polyphagous: Eating many kinds of food.

Polyprotein: A protein translated from an entire viral genome, which is then cleaved by proteases into the active protein products.

Polyvoltine: Having many different broods per season.

Pome fruits: A fleshy fruit, such as an apple, pear, or quince, having several seed chambers and an outer fleshy part largely derived from the hypanthium. Also called false fruit.

Postpronotal (Postpronotum): The posterior region of the pronotum.

Primary inoculum: Inoculum, usually from an overwintering source, that initiates disease in the field, as opposed to inoculum that spreads disease during the season (see secondary inoculum).

Proboscis: An elongated sucking mouthpart that is typically tubular and flexible.

Prokaryote: An organism without internal membrane-bound organelles, lacking a distinct nucleus, such as bacteria and mollicutes.

Prolegs: 1) Any process or appendage that serves the purpose of a leg; 2) specifically, the pliant, non-segmental abdominal legs of caterpillars and some sawfly larvae. Not true segmented appendages.

Proliferation: To grow or multiply by rapidly producing new tissue.

Propagative transmission (syn. circulative propagative transmission): Pathogen transmission characterized by a long period of acquisition of the pathogen (usually a mollicute, e.g. phytoplasma or spiroplasma, and sometimes a virus) by a vector (typically an insect), a latent period before the vector is able to transmit the pathogen, and retention of the pathogen by the vector for a long period because the pathogen reproduces or replicates in the vector.

Proteases: Protein-degrading enzymes.

Proteolytically: The hydrolysis of proteins into simpler compounds by the action of enzymes.

Protonymph: Second instar of a mite.

Protuberance: Something, such as a bulge, knob, or swelling, that protrudes.

Pulp: The soft moist part of fruit.

Pupa (pl. pupae): The stage between the larva and adult in insects with complete metamorphosis, a nonfeeding and usually inactive stage.

Pygopod: Foot-like appendages that may be used in locomotory functions.

Quiescent: Quiet and at rest, but not necessarily dormant and having the potential for resumed activity; can apply to non-meristematic cells.

Race: Subgroup or biotype within a species or variety, distinguished from other races by virulence, symptom expression, or host range, but not by morphology.

Rachis: Floral thorn or point.

Real Time PCR: A laboratory technique based on polymerase chain reaction, which is used to amplify and simultaneously quantify a targeted DNA molecule. It enables both detection and quantification (as absolute number of copies or relative amount when normalized to DNA input or additional normalizing genes) of a specific sequence in a DNA sample. The procedure follows the general principle of polymerase chain reaction; its key feature is that the amplified DNA is quantified as it accumulates in the reaction in *real time* after each amplification cycle.

Resin: Any of numerous clear to translucent yellow or brown, solid or semisolid, viscous substances of plant origin, such as copal, rosin, and amber, used principally in lacquers, varnishes, inks, adhesives, synthetic plastics, and pharmaceuticals.

Resinous: Like resin.

Resistant (n. resistance): Possessing properties that prevent or impede disease development (see susceptible).

Restriction fragment length polymorphism (RFLP): A variation in DNA sequence that is easily recognized because it occurs at a site where a restriction enzyme cuts a specific sequence, producing DNA fragments of varying lengths. RFLP's often serve as genetic markers.

Resupinate: Upside down.

Reticulate: Descriptive of surface sculpture, usually the insect’s integument, which is covered with net-like lines.

Retinaculum: A loop on the underside of the forewing of some moths. Along with the frenulum, a spine at the base of the forward or costal edge of the hindwing, it forms a coupling mechanism for the front and rear wings of the moth.

Ribonucleic acid (abbr. RNA): Several nucleic acids composed of repeating units of ribose (a sugar), a phosphate group, and a purine (adenine or guanine) or a pyrimidine (uracil or cytosine) base; transcribed from DNA and involved in translation to proteins.

Sclerites: Any of the hard chitinous plates that make up the exoskeleton of an arthropod.

Sclerotized: Hardened.

Scutellum: A sclerite of the thoracic notum; the mesoscutellum appearing as a more or less triangular sclerite behind the pronotum, especially in Hemiptera.

Scutum: The middle division of a thoracic notum, just anterior to the scutellum.

Secondary Infection or Secondary Spread: Infection resulting from the spread of infectious material produced after a primary infection or from secondary infections without an intervening inactive period.

Secondary inoculum: Inoculum produced by infections that took place during the same growing season (see primary inoculum).

Septa (pl. of septum): A part that separates two cavities or two masses of tissue.

Serology: A method using the specificity of the antigen-antibody reaction for the detection and identification of antigenic substances and the organisms that carry them.

Serotype: A subdivision of virus strains distinguished by protein or a protein component that determines its antigenic specificity.

Sessile: Not supported on a stem or footstalk; immobile.

Setae: Bristles; commonly known as hairs.

Sexual dimorphism: Sexes are different in form or color in the same species; may be seasonal or geographic; male and female look different by color, form, etc.

Sieve Tube: An element of phloem tissue consisting of a longitudinal row of thin-walled elongated cells with perforations in their connecting walls through which food materials pass.

Sign: Indication of disease from direct observation of a pathogen or its parts (see symptom).

Single stranded, positive sense RNA: Also known as a sense-strand RNA virus, a virus whose genetic information consists of a single strand of RNA that is the positive (or sense) strand which encodes mRNA (messenger RNA) and protein. Replication in positive-strand RNA viruses is via a negative-strand

Skeletonize: To remove leaf tissue between the veins, leaving the network of veins intact.

Solitary: Done or existing alone.

Sooty mold: Ascomycete fungi that grow from the sugary honeydew secreted by plants and insects (aphids, scales, whiteflies) that suck sap from their host plants.

Spatulate: Rounded and broad at the top, attenuate at base. Shaped like a spoon, with a narrow end at the base.

Spiracles: Breathing pores; in the plural the lateral openings on the segments of the insect body through which air enters the trachea.

Spore: A specialized reproductive body in fungi (and some other organisms), containing one or more cells, capable of developing into an adult.

Sporocarp: Spore-bearing fruiting body.

Sporogenous: Producing spores or reproducing by means of spores.

Stag Head: Defoliated, dead, or dying major branches in the crown of a tree, usually resulting from inadequate water uptake or translocation.

Sterigma: A spore-bearing projection from a cell.

Strain: A distinct form of an organism or virus within a species, differing from other forms of the species biologically, physically, or chemically.

Stroma (pl. stromata): Compact mass of mycelium (with or without host tissue) that supports fruiting bodies or in which fruiting bodies are embedded.

Stunting: Reduction in height of a vertical axis resulting from a progressive reduction in the length of successive internodes or a decrease in their number.

Stylet: A stiff, slender, hollow feeding organ of plant-parasitic nematodes or sap-sucking insects, such as aphids or leafhoppers.

Suberization: To convert into cork tissue.

Superficial: Occurring at or on the surface.

Susceptible: Prone to develop disease when infected by a pathogen (see resistance).

Symptom: Indication of disease by reaction of the host, e.g. canker, leaf spot, wilt (see sign).

Synergism (Synergistic): Greater than additive effect of interacting factors.

Systemic: Of or affecting the entire organism or bodily system; any of a group of pesticides that are absorbed into the tissues of plants, which in consequence become poisonous to insects etc. that feed on them.

Tactile: Of or connected with the sense of touch; perceptible by touch or apparently so.

Tarsus: The leg segment immediately beyond the tibia, consisting of one or more segments or subdivisions.

Tegumen: Lepidoptera: the tergum in male genitalia. A structure shaped as a hood or inverted trough, positioned dorsal of the anus; the uncus articulates with its caudal margin, derived from the ninth abdominal tergum.

Teleomorph: The sexual or so-called perfect growth stage or phase in fungi.

Temperate: Free from extremes; mild.

Tenerals: The period when the adult insect is newly emerged from the pupal case or nymphal skin. During the teneral period, the insect's exoskeleton has not hardened or darkened, leaving it vulnerable.

Tergite: A dorsal sclerite or part of a segment, especially when such part consists of a single sclerite.

Termen: The outer margin of a wing, between the apex and the posterior or anal angle.

Tolerance (adj. Tolerant): Ability of a plant to endure an infectious or noninfectious disease, adverse conditions, or chemical injury without serious damage or yield loss; (of pesticides) the amount of chemical reside legally permitted on an agricultural product entering commercial channels, usually measured in parts per million (ppm).

Tomentose: Covered with densely matted filaments.

Transient: One who stays for only a short time.

Transmit (n. transmission): To spread or transfer, as in spreading an infectious pathogen from plant to plant or from one plant generation to another.

Transparent: Allowing light to pass through so that objects behind can be distinctly seen.

Trilocular: Having three chamberlike divisions or cavities.

Tubercles: A small rounded projection or protuberance, esp. on a bone or on the surface of an animal or plant.

Tufted (Tuft): Growing in small dense clumps or tufts.

Umbrinous: Umber (a natural pigment darker than ocher, normally dark yellowish-brown in color (raw umber) or dark brown when roasted (burnt umber) in color.

Uncus: Any hook-shaped process or part.

Univoltine: Having only one generation per year.

Urogomphi: Fixed or mobile processes found on the terminal segments of certain larvae; variously termined styli, cerci, pseudocerci, corniculi.

Vector: Literally a bearer; specifically a host of a disease transmissible to another species of organism.

Ventral: Pertaining to the under surface of the abdomen.

Vesica: Lepidoptera: the penis, or terminal part of the aedeagus. The vesica is membranous and eversible, typically held within the tubular part of the aedeagus, but everted and inflated during copulation.

Vigor: Strength, growth, and overall good health.

Virus: A submicroscopic, intracellular, obligate parasite consisting of a core of infectious nucleic acid (either RNA or DNA) usually surrounded by a protein coat.

Viruliferous: A term used to describe a vector that has acquired and carries a virus, and can transmit the virus to a healthy plant.

Vitta (pl. vittae): A broad longitudinal stripe.

Wilt: Drooping of leaves and stems from lack of water (inadequate water supply or excessive transpiration); vascular disease that interrupts normal water uptake.

Witches' Broom: Disease symptom characterized by an abnormal, massed, brushlike development of many weak shoots arising at or close to the same point.

Zonation: Distribution in zones or regions of definite character.

Definitions taken from:

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