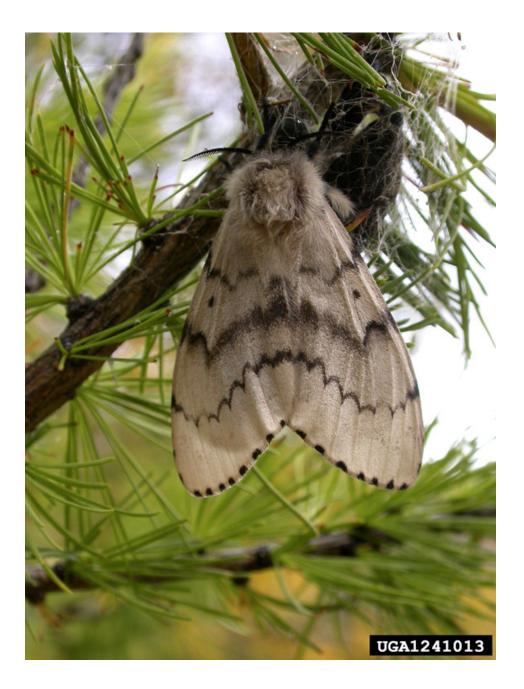
Asian Defoliator Pathway-based Survey Reference



Cover: Adult female of *Lymantria dispar asiatica*. Credit: John H. Ghent, USDA Forest Service, Bugwood.org

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Draft Log

March 2014: The 2014 Version was posted to the CAPS website. October 2012: Draft document posted to CAPS website for review.

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2014 Revisions

1) Revised the length of effectiveness of the Gypsy Moth String Lure in the Introduction and in the datasheets for: *Lymantria albescens*, *Lymantria dispar asiatica*, *Lymantria dispar japonica*, *Lymantria postalba*, and *Lymantria umbrosa*.

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Introduction

Purpose of the Asian Defoliator Survey

The purpose of the Asian Defoliator Survey is to detect new infestations of target species at low population levels. This document provides standardized guidelines for conducting exotic Asian defoliating moth surveys in the United States.

The Asian Defoliator Survey is more inclusive than traditional Asian Gypsy Moth (AGM) surveys conducted through APHIS–PPQ's (Animal and Plant Health Inspection Service–Plant Protection and Quarantine) Agricultural Quarantine Inspection (AQI) program. States without ports and that are further down the Asian Defoliator pathway continuum may be eligible to conduct Asian Defoliator surveys. However, states must work in conjunction with their State Plant Health Director, the National AGM Program Manager, and the Cooperative Agricultural Pest Survey (CAPS) Program Manager to determine which pathways and target species are appropriate for their state.

Background

PPQ has conducted Asian Gypsy Moth surveys because AGM has a strong pathway to the United States and is a serious pest where it is established. AGM has been detected and subsequently eradicated in several states. The goal of this survey is to detect Asian defoliator introductions before the population becomes established.

Additional Asian defoliator species have been added to this survey because they follow similar pathways to AGM, have a similar biology, are capable of similar types of damage, and are capable of spreading within the United States. These similarities are explored more in depth below.

Pathway

The most significant pathway for AGM is via ships and cargo from the Far East. AGM females are attracted to ship and port lighting and lay egg masses on non-host material including ships, shipping containers, timber, rail cars, and automobiles. AGM egg masses are extremely hardy and their tolerance of temperature and moisture extremes enhances the risk of spread. *Dendrolimus sibiricus, Lymantria mathura, L. monacha,* and *L. xylina* also lay egg masses on similar non-host material and can follow the same pathway.

Dendrolimus pini eggs and larvae may be moved through international trade of unprocessed pine logs (Ciesla, 2004). *Dendrolimus punctatus* egg masses or early instar larvae may move through planting material (Ciesla, 2001).

Damage

The target species in this manual severely defoliate trees. This type of damage can have both economic and environmental impacts. All of these target species are considered serious defoliators and pests in their countries of origin. Outbreaks of these species can be large (up to several million hectares); and mortality can be as high as

100% in attacked stands. Attacks to hosts can cause loss of vigor, reduced growth, reduced seed crops, loss of resin production, and reduced yield or total crop loss in fruit crops.

Defoliation by these target species can also affect the health of the forest environment and cause changes to the ecosystem. These species can attack and kill healthy trees over large areas; and outbreaks may lead to a disruption in forest succession and losses of future seed crops. Areas with extensive tree mortality may be more vulnerable to forest fires.

Another cause of environmental damage due to these species is the impact of secondary pests.

Severe defoliation weakens the trees and exposes them to attack by secondary organisms. Defoliation by AGM, *Dendrolimus pini*, and *D. sibiricus* has all been documented as causing additional damage through attack by secondary organisms. Damage caused by *Dendrolimus punctatus* and *Lymantria monacha* may lead to increased susceptibility to bark beetle attack (Ciesla, 2001; Wallner, 2000). In addition, *Dendrolimus punctatus* has been linked to root rot infection of *Pinus elliottii* plantations in southern China (Speight and Wylie, 2001).



Figure 1. AGM infestation. (Image courtesy of USDA-APHIS-PPQ-CPHST Otis Lab.)

Host Range

Another common aspect of these targets that makes them a priority for survey is their wide host range. AGM larvae have been known to feed on more than 600 plant species, covering more than 100 botanical families (USDA-APHIS-PPQ, 2014). AGM

prefer deciduous trees but can develop on conifers. As a result, they have a broader host range than the European subspecies (USDA-APHIS-PPQ, 2014).

Lymantria mathura is a polyphagous pest of taxonomically diverse deciduous trees and can feed on more than 45 genera in 24 families. *Lymantria xylina* has been recorded on 63 species in 29 families and attacks mainly hardwoods. *Lymantria monacha* primarily feeds on conifers, including fir, larch, pine, and spruce but can also attack deciduous trees such as apple, beech, birch, and oak.

Dendrolimus punctatus is a pest of pine species. *Dendrolimus pini* has a moderate host range, feeding primarily on needles of coniferous hosts. *Dendrolimus sibiricus* is polyphagous, damaging over 20 species in five genera in its native range. *Dendrolimus sibiricus* can develop on almost all of the coniferous species found in its native range.

Human Health Impacts

Dendrolimus pini, D. punctatus, and D. sibiricus can negatively impact human health. Exposure to the hairs or secretions of the larvae can cause severe dermatitis and systemic reactions in joints and other parts of the body (FAO, 2007). Dendrolimus sibiricus larvae have stinging hairs that can lead to allergic reactions in individuals living near populations. The hairs of D. punctatus cause reactions in individuals that can include rashes, headaches, dizziness, and localized arthritis if hairs come in contact with exposed skin (Lawson and Lui, 1986).

Spread if Established in the United States

The females of the target species in this survey have the ability to fly from several miles to longer distances, depending on the species. This allows for rapid spread into uninfested areas (USDA APHIS PPQ, 2014). In addition, newly hatched larvae of all of the target species disperse by "ballooning," which consists of climbing a tree or other object, dropping on a silken thread, and becoming wind-borne (Ciesla, 2001, 2004; USDA APHIS PPQ, 2014; Wallner, 2000). Humans may also assist in the spread of these pests through the unintentional movement of egg masses. Eggs can be laid on ships, shipping containers, outdoor furniture, firewood, timber, rail cars, automobiles, and other inanimate objects. Egg masses of the target species are extremely hardy and their tolerance of temperature and moisture extremes enhances the risk of spread (USDA APHIS PPQ, 2014). Once hatched in a new location, the broad host range and the ability of the larvae to disperse enhances their ability to establish (USDA APHIS PPQ, 2014).

Selection of Target Species

The target species of the survey were selected by the National Committee of the Cooperative Agricultural Pest Survey (CAPS) Program, in cooperation with the USDA-APHIS-PPQ Center for Plant Health Science and Technology (CPHST), and the USDA-APHIS-PPQ Asian Gypsy Moth Program. All target species included are exotic pests, not known to occur in the United States.

A note on AGM taxonomy

From the "Asian Gypsy Moth Survey and Response Guidelines" (USDA APHIS PPQ, 2014):

Lymantria dispar is a moth in the family Lymantriidae (some recent classifications consider Lymantriinae to be a subfamily of Noctuiidae; see Pogue and Schaefer, 2007). In a recent review of the genus *Lymantria*, Pogue and Schaefer (2007) recognized three subspecies of *Lymantria dispar*. *L. d. dispar* (L.) (European gypsy moth), *L. d. asiatica* Vnukovskij, and *L. d. japonica* (Motschulsky). For regulatory purposes, the latter two subspecies are both considered Asian gypsy moths (AGM). *L. d. asiatica* occurs in temperate Asia from the Ural Mountains east to China, Korea and the Russian Far East (north of the Himalayans). *L. d. japonica* is found on several major Japanese islands including Honshu, Shikoku, Kyushu, and parts of Hokkaido.

Pogue and Schaefer (2007) also described three new or revised/re-described species that had previously been considered subspecies of *L. dispar*. These are *L. umbrosa* (*Lymantria dispar hokkaidoensis/umbrosa/nesiobia*), *L. albescens* (*L. dispar albescens*), and *L. postalba* (*L. d. postalba/tsushimensis, L. albescens tsushimensis*). All three are native to Japan (though their distributions are generally more limited than that of *L. d. japonica*). All three species, like *L. dispar*, use disparlure as the major, if not sole, component of their sex-attractant pheromone and thus may be caught in gypsy moth monitoring traps.

Pogue and Schaefer indicate that regardless of the changes in nomenclature, "Asian Gypsy Moths" are those that have females capable of flight. Therefore, for regulatory purposes, USDA considers all three newly classified species to be AGM in addition to *L. d. asiatica* Vnukovskij, and *L. d. japonica* (Motschulsky) (Table 1). DNA analysis is used to determine what type of moth is trapped; Section [II, A] covers the submission process of samples.

How to Use This Manual

Introduction

The first section of this manual describes how to conduct Asian Defoliator surveys. This section describes how to select targets and sampling sites. In addition, this section describes trapping, sample processing, and identification protocols.

Pest Datasheets

Pest datasheets have been developed for each target species. Datasheets contain specific information on the biology, ecology, hosts, distribution, survey methods, and identification resources for each target.

Host information (in all Pest Datasheets)

In general, host information in pest datasheets is based on host species present in areas where the target is distributed. These hosts may or may not be present in the United States. Therefore, surveys should be broadened to native species within the host genera or family (i.e., if the pest attacks oaks and none of the host species are found in the United States or within the survey sites, broaden the survey to include native oak species).

Planning a Survey

Choosing Target Species

Asian Defoliator surveys should include the five AGM species/subspecies, as these species present the greatest likelihood of introduction and have the largest host range. Additional targets should be added based on their relevance to the State. Determining which targets species to survey for should be based on 1) the risk of introduction of the target and pathways of introduction; 2) presence of known or potential hosts in your state; 3) climatic suitability of your state for the target; and 4) resources available (financial and staff) for survey and identification of the pest. See **Table 1. Target Insects for Survey** for a list of possible targets.

Scientific Name	Common Name
Dendrolimus pini	Pine-tree lappet
Dendrolimus punctatus	Masson pine moth
Dendrolimus sibiricus	Siberian silk moth
Lymantria albescens	Okinawa gypsy moth
Lymantria dispar asiatica	Asian gypsy moth
Lymantria dispar japonica	Japanese gypsy moth
Lymantria mathura	Rosy moth
Lymantria monacha	Nun moth
Lymantria postalba	White-winged gypsy moth
Lymantria umbrosa	Hokkaido gypsy moth
Lymantria xylina	Casuarina tussock moth

Table 1. Target Insects for Survey

Pathways

When planning surveys, states are encouraged to use a pathway approach when deciding on target species and locations to survey. It is understood that risk factors can be examined along a "risk continuum" beginning at offshore sites (points of origin) to points of potential establishment (commodity production areas, natural lands), and numerous risk points in between (wholesale distribution centers, intermodal sites, rail yards, etc.).

High Risk Sites

Each state may have different high risk sites, depending on the pathways of introduction into the state; the pathways of spread within the state; host types within the state; and the unique types of high risk sites within the state. Below is a list of potential high risk sites. States will need to identify which high risk sites are relevant for their state.

- Ports, including shipping and deep water ports
- Canada or Mexico border crossings
- State border crossings (especially between high risk to low risk states)
- Military household shipments from Japan and Korea
- Nighttime lights
- Inland waterways
- Rivers used for transportation of goods
- Warehouse districts
- Railways
- Inspection stations
- Major highways
- Firework distributors
- Rest areas
- Weigh stations
- City and county parks
- Inland container storage and intermodal sites

The following is a list of possible data types to use to determine high risk sites; it is not all inclusive. Any geospatial data compiled for analysis will likely require assistance from a Geographic Information System (GIS) professional. Please note, this list represents data that has been used in previous state specific AGM analyses; some data layers may not be applicable or available for all states.

- AGM ship interceptions
- Census population types and density
- Commodity import volume
- Container movement volume
- Container volume and duration of storage
- Forest density
- Vegetation types and density
- Wind resource zones

<u>Note:</u> there may be some overlap between the pathways of Asian Gypsy Moth (AGM) and European Gypsy Moth (EGM). However, EGM is established in the Eastern United States and moves primarily on egg masses attached to outdoor items. These items may include: outdoor furniture, firewood, boats, hauling trailers, tents, and recreational vehicles (RVs) (USDA-APHIS-PPQ, 2012). It is not necessarily appropriate to trap for both EGM and AGM in the same locations since the high risk sites for the two species are defined differently. For example, high risk sites for EGM may include campgrounds where residents from the Eastern United States may have inadvertently transported firewood or recreational vehicles with EGM egg masses. Campgrounds would not be considered the highest risk or earliest points of introduction along an AGM pathway. Please review the <u>PPQ Gypsy Moth Program Manual</u> to learn more about EGM high risk sites and human-mediated spread.

For Asian Defoliator surveys, trapping should occur strategically along the AGM pathway and that of the other target species.

Where high risk sites do overlap, trap locations should be coordinated so not to duplicate effort and resources.

Hosts and Climate

The hosts of the target species as well as the climatic suitability of the targets should be considered when planning a survey.

Pest Datasheets

Each pest datasheet within the manual gives specific guidance on the hosts, pathway, and climactic suitability of the target.

NAPPFAST Maps

The North Carolina State University APHIS Plant Pest Forecasting System (NAPPFAST) produces maps to support CAPS and other PPQ surveys. Depending on the level of biological data available, the pest datasheets will include host, risk, or Pareto NAPPFAST maps.

Host Map

The host risk map describes the relative density (on a scale of 1-10) of susceptible hosts. The maps are based on National Agricultural Statistics Service (NASS) and Forest Inventory and Analysis (FIA) data. The scale of one to ten describes the proportion of total host acreage per county: for example a rank of one indicates no host acreage, while a score of ten indicates that 100% of the acres in the county contain suitable hosts for the pest.

Final Risk Map

A final risk map represents the combined host and climatic suitability on a scale of 0-10. The NAPPFAST risk map and the host risk map were multiplied to obtain a

final risk map. A value of one represents low density of susceptible hosts and low likelihood of pest growth and survival. A value of 10 indicates high density of susceptible hosts and a high likelihood of pest growth and survival. A value of zero or the gray area indicates an unsuitable climate for the pest

Pareto Map

The Pareto maps integrate maps of host abundance, climate, and pathway risks into a single risk map. Where no climate map exists, the maps were created from host and pathways only. The risk is rated on a scale of 1-10 based on a series of ordinal risk rankings. The Pareto Risk Map may more accurately reflect the risk potential of a pest than the Final Risk Map because it includes importation pathways.

NAPPFAST Zonal Statistics

States have different levels of hosts, varying environmental conditions, and pest introduction levels represented in the risk maps at county level. Zonal statistics can be used to identify the highest risk pests for an individual state. Files for each state may be viewed on the <u>NAPPFAST</u> page of the CAPS Resource and Collaboration website. If you are unfamiliar with how to analyze and use this data, please contact Dan Borchert for assistance.

For any NAPPFAST-related questions:

Dan Borchert USDA-APHIS-PPQ-CPHST Risk Analyst -Entomologist 1730 Varsity Drive, Suite 300 Raleigh, NC 27606 Phone: 919-855-7545 daniel.m.borchert@aphis.usda.gov

Trap Sites

When choosing a survey site, select a site that contains known or potential hosts and is large enough to hold all of the traps that will be placed there. Some of the lures can inhibit attraction of other target species; therefore, when trapping for more than one species of moth, separate traps for different moth species by at least 20 meters (65 feet).

Trap Types

Several different traps are recommended for the Asian Defoliator targets. Traps are recommended based on the biology of the pest. Refer to <u>Table 2. Asian Defoliator</u> <u>Trap and Lure Combinations</u> for the trap and lure product names as they appear in the IPHIS Survey Supply Ordering System. The three trap types recommended for CAPS Asian Defoliator targets are:

- Milk carton traps,
- Paper delta traps, and
- Wing traps.

Milk carton traps (Fig. 2)

Milk carton traps resemble basic 0.5 gallon (2 quart) paper milk cartons with the addition of small entry ports on each side of the trap. The traps also have a trap hood around the carton which serves as a behavioral stop for males to encourage entry into the ports. The lure hangs inside the carton at approximately the same height as the entry ports. Moths that enter the milk carton trap are killed by an insecticide (DDVP) that is released from a laminated plastic strip which is also hung inside the carton. Milk carton traps can hold as many as 1,000 moths.

Note: the milk carton traps need to be modified for *Dendrolimus pini* and *D. sibiricus*. Consult the specific pest datasheets for detailed instructions.



Figure 2. Milk carton trap. (Image courtesy of William A. Carothers).



Figure 3. Paper delta trap. (Image courtesy of Lee Spaulding, USDA-APHIS-PPQ).



Figure 4. Wing trap. (Image courtesy of Lee Spaulding, USDA-APHIS-PPQ.)

Paper delta traps (Fig. 3)

Paper delta traps are available in many different colors. For this survey, the traps are available in orange, brown, or green. The color of the trap does not affect the efficacy for trapping Asian Defoliator target species. States may choose any one of the three colors. Paper delta traps are available with the adhesive applied to either two or three of the internal sides. Traps with two sticky sides are the approved trap type for this survey.

The traps are prism-shaped and made of plastic-coated cardboard. Moths enter through openings on the triangular ends and are captured on an adhesive that coats two of the three inner surfaces.

The lure should be stapled to one of the non-sticky panels inside the trap.

Wing traps (Fig. 4)

Wing traps are available in either a plastic or paper version. Plastic and paper traps are both equally effective and the State may decide which trap to use. Wing traps have a removable sticky insert. When using a wing trap, the lure (a rubber septum) should be placed inside a lure holder, which is usually included with the trap. The lure holder should be stapled to the underside of the top of the trap on a non-sticky area.

Additional information on how to assemble traps can be found here: <u>http://www.fs.fed.us/r8/foresthealth/programs/gmtrap/trap_assembly.pdf</u>. However, it is important to review each pest datasheet for additional guidance or trap modifications for the specific species.

Trap Density

For AGM, follow the trap density guidance in the AGM Survey and Response Guidelines. For other Asian Defoliator targets, set one trap per each lure combination/target species at each survey site. Note that some lures or lure combinations may be used for more than one species. Use <u>Table 2. Asian Defoliator</u> <u>Trap and Lure Combinations</u> to determine the appropriate trap and lure combination for each species. Traps with different lure combinations should be separated by at least 20 meters (65 feet).

States may have their own Asian Defoliator or AGM survey manual or protocol. Some state-level manuals may prescribe trapping densities that are higher than what is specified here or in the AGM Survey and Response Guidelines. Asian Defoliator surveys will be funded at the minimum trapping densities specified in the AGM Survey and Response Guidelines.

States may survey for Asian Defoliator targets at higher trap densities; however, the additional traps will need to be funded outside of the agreements for the Asian Defoliator surveys.

Table 2: Asian Defoliator Trap and Lure Combinations

Target Species	Lure Product Name	Trap Product Name
Dendrolimus pini Dendrolimus sibiricus	Dendrolimus pini - Dendrolimus sibiricus Lure	Milk Carton Trap
Dendrolimus punctatus	<i>Dendrolimus punctatus</i> Lure	1) Wing Trap Kit, Paper or 2) Wing Trap Kit, Plastic
Lymantria albescens Lymantria dispar asiatica Lymantria dispar japonica Lymantria postalba Lymantria umbrosa	 Gypsy Moth Laminate Lure or Gypsy Moth String Lure 	 Paper Delta Trap, 2 sticky sides, Brown Paper Delta Trap, 2 sticky sides, Green Paper Delta Trap, 2 sticky sides, Orange or Milk Carton Trap
Lymantria mathura	Lymantria mathura Lure	1) Wing Trap Kit, Paper or 2) Wing Trap Kit, Plastic
Lymantria monacha	 Lymantria monacha Lure, 1 Compound or Lymantria monacha Lure, 3 Compound 	 Paper Delta Trap, 2 sticky sides, Brown Paper Delta Trap, 2 sticky sides, Green or Paper Delta Trap, 2 sticky sides, Orange
Lymantria xylina	<i>Lymantria xylina</i> Lure	 Paper Delta Trap, 2 sticky sides, Brown Paper Delta Trap, 2 sticky sides, Green Paper Delta Trap, 2 sticky sides, Orange

Note: When more than one lure or trap option is listed; consult the specific pest datasheet to determine which option is appropriate for your state.

Trapping Season

The trapping period will be the period of expected flight activity of the moths in their native range, extrapolated for the State's climate. Traps should be placed in the field before adult flight activity begins and remain throughout the expected active period. Actual trapping seasons may vary by location and target species. Refer to individual pest datasheets in this document to determine the trapping season for each target. Use NAPPFAST maps and Degree Day information (where available) to extrapolate the target's expected flight season in your state.

Traps for additional Asian Defoliator target species may be added to the same location as the AGM traps as their expected adult activity period occurs.

Ordering Traps and Lures

All traps and lures for the Asian Defoliator Survey should be ordered through the IPHIS Survey Supply Ordering System during the open ordering season (usually September through October, prior to the next year's survey season). By using the ordering system, PPQ can utilize quality assurance procedures that are not available when ordering directly from manufacturers.

All necessary traps and lures needed for the Asian Defoliator Survey are specifically listed in the ordering system. See <u>Table 2. Asian Defoliator Trap and Lure</u> <u>Combinations</u> or the individual pest datasheets for the trap and lure product names. **Note:** every effort is made not to change the CAPS-approved survey methods during a survey season. However, if changes are necessary (i.e., a trap or lure is not available), a notification will be sent out through the Survey Planning forum from the CAPS Resource and Collaboration Site, and a note will be placed on that target's information page on the CAPS Approved Methods page. Please visit the <u>CAPS Approved Methods</u> page for the most current information.

Supplies are shipped via overnight courier, ground transportation, or palletized freight. To avoid problems when receiving supplies, surveyors should specify any particular delivery requirements in the comment box of the order form. For example enter, "Call before shipping in order to arrange for storage, personnel, or equipment to unload the shipment" or "Cannot accept pallets," etc.

Inspect lures upon receiving them from PPQ. Notify the appropriate Regional Program Manager of any lures that are damaged and request replacement lures (see contact information below).

Contact information for trap and lures

For questions about the IPHIS Survey Supply Ordering System or trap and lure quality issues:

Brian Kopper

Program Manager USDA-APHIS-PPQ 920 Main Campus Dr. Raleigh, NC 27606 919-855-7318 Brian.j.kopper@aphis.usda.gov

For technical trap, lure, and survey methodology questions:

Lisa Jackson Biological Scientist USDA-APHIS-PPQ-CPHST 1730 Varsity Dr., Suite 400 Raleigh, NC 27606 919-855-7549 Lisa.d.jackson@aphis.usda.gov

Conducting a Survey

Trap Placement

Milk carton traps and wing traps should be hung using a string, tied to a branch of a host tree. Paper delta traps are most effective when attached directly to the bole of a host tree. The most efficient way of doing this is by stapling a binder clip to the bole of a host tree then clipping the trap in place. However, if a homeowner objects to this method, it can also be hung using a paper clip (see Fig. 5) or by tying a string around the tree and hanging the clip on the string. The traps should be hung at breast height unless vandalism is a problem in which case they should be hung higher (Lance, 2009). If a trap cannot be hung on a host tree, another vertical surface, such as a telephone pole, can be used to hang the trap, preferably within 100 meters (328 feet) of a host. Never hang the traps on branch tips.



Figure 5. Paper delta trap (Image courtesy of USA-APHIS-PPQ).

Lure Handling

Care should be taken to avoid contaminating external surfaces of traps with attractant or cross-contaminating traps with attractants of different species (Lance, 2006). For example:

- Use latex or latex-substitute gloves when handling lures;
- Minimize direct contact with lures;
- Do not touch external portions of traps with gloves that have contacted lures; and
- At a minimum re-glove after handling lures for one species before handling traps or lures for another.

Care must also be taken to avoid cross-contamination via equipment that may come in contact with lures (e.g., staplers) (Lance, 2006). For species listed in this manual, the most critical known cross-contamination issue is ensuring that components of *Lymantria monacha* lures do not contaminate AGM traps, as several of those components are potent inhibitors of male AGM (Lance, 2006). When possible, AGM traps should not be assembled in the same time and space (e.g., same lab and day) as *L. monacha* traps and should not be transported together in the same vehicle (using the same vehicle on different days is acceptable provided that traps are transported in containers such as plastic garbage bags rather than being placed loose in the vehicles (Lance, 2006). This is good general practice in any case) (Lance, 2006).

Lure Storage

For storage, lures should be kept in a freezer or at least in a refrigerator. It is generally acceptable to store lures for different species in the same freezer if they are doubly contained in factory-sealed packages that are, in turn, held separately by species in a secondary closed container such as a glass jar or zip-lock bag (Lance, 2006). Partially used packages of lures can also be stored in the freezer but should be re-sealed using the manufacturer's original method; for example, a heat-sealing device should be used to restore the seal of packets that are constructed of plasticized laminates with impermeable membranes (Lance, 2006).

Checking Traps

- Check traps every two weeks or after bad weather events (rain, strong winds, or snow) which can disturb the sample.
- Examine trap for damage.
- Remove any debris blocking entry ports or funnels, including leaves, twigs, spider webs, etc.
- Ensure that all lures are still in place.
- Remove any suspect specimens from the trap and submit the samples per the sample submission instructions (note, AGM specimens have specific instructions, see <u>Handling and Submission of Suspect AGM Specimens for</u> <u>Identification</u>. It is especially crucial to remove suspect AGM samples every two weeks to in order to reduce the degradation of the specimen's DNA.)
- Change lures per the length of effectiveness for each species (see <u>Table 3:</u> <u>Length of Effectiveness for Asian Defoliator Lures</u>).

Table 3: Length of Effectiveness for Asian Defoliator Lures

Target Species	Lure Product Name	Length of Effectiveness
Dendrolimus pini Dendrolimus sibiricus	Dendrolimus pini - Dendrolimus sibiricus Lure	4 weeks (1 month)
Dendrolimus punctatus	Dendrolimus punctatus Lure	3 weeks
Lymantria albescens Lymantria dispar asiatica Lymantria dispar japonica Lymantria postalba Lymantria umbrosa	 Gypsy Moth Laminate Lure or Gypsy Moth String Lure 	1) 12 weeks (3 months) 2) 24 weeks (6 months)
Lymantria mathura	<i>Lymantria mathura</i> Lure	12 weeks (3 months)
Lymantria monacha	1) <i>Lymantria monacha</i> Lure, 1 Compound or 2) <i>Lymantria monacha</i> Lure, 3 Compound	1) 12 weeks (3 months) 2) 12 weeks (3 months)
Lymantria xylina	<i>Lymantria xylina</i> Lure	4 weeks (1 month)

Sample Processing

Consult the most recent version of <u>Procedures for Submitting Survey Samples to</u> <u>Domestic and Other Identifiers</u> for information on how to process and submit survey samples.

Note: any suspect Asian Gypsy Moth (AGM) samples must be submitted according to the specific instructions for AGM. See <u>Handling and Submission of Suspect AGM</u> <u>Specimens for Identification</u> below.

Handling and Submission of Suspect AGM Specimens for Identification

Specimens that are suspected of being AGM should be submitted to the Center for Plant Health Science and Technology (CPHST) Otis Laboratory for testing (see <u>Asian</u> <u>Gypsy Moth Trapping Submission Guidelines</u>). All specimens collected outside of the EGM quarantine areas will be analyzed. Specimens collected within generallyinfested areas will be analyzed based on sub-samples of total catch because of the large number of insects which can be caught in some areas. It is critical that samples be collected regularly, stored properly, and submitted to the Otis Lab as soon as possible to maintain the integrity of the DNA. If traps cannot be checked regularly, it may be considered to trap when flight is expected rather than spreading resources out across the whole season.

Sample Handling

As a general rule, traps should be checked and samples removed every two weeks in order to reduce the degradation of the specimen's DNA. High temperatures and high humidity speed degradation of specimens and trapping schedules should be adjusted accordingly. If stored unfrozen the specimens should be in containers (paper bags or boxes) which will promote drying. Plastic containers retain moisture that favors the growth of bacteria and fungi, which will quickly degrade the DNA. Specimens should be stored in a freezer if possible (if not, in a cool dry area) and shipped to the Otis Lab as soon as practical. Specimens should not be stored unfrozen for extended periods.

Sample Submission

Milk Carton Traps

- Layer moths loosely between wadded paper towels or tissue paper in a paper bag (brown lunch bag size) to prevent motion and specimen damage during shipment (one bag per trap; if more than one bag is required per trap, label appropriately). Label paper bag clearly with trap numbers matching paperwork.
- Staple or tape paper bag closed.
- Do not attach paperwork to individual bags.
- Do not use plastic bags or paper envelopes as these do not allow moisture release and thus promote fungal growth and decomposition of the moths.
- Do not send traps or paperwork for traps which contain no specimens.

Delta Traps

- Label each trap clearly with trap numbers matching paperwork.
- Package traps to avoid crushing during shipment.
- Do not attach paperwork to individual traps.
- Do not use Styrofoam peanuts or other small packaging materials that could potentially enter the traps.
- Do not disassemble the traps or remove moths from the trap.
- Do not ship traps with sharp staples exposed.

A PPQ Form 305 should be sent with each trap, stating the trap number, collection site, number of specimens (estimates okay), life stage, collection date, and date of last (previous) trap check (to determine maximum time that the moth may have been in the trap prior to the check). Specimens should be shipped via next day delivery for Tuesday through Friday arrival. They should be shipped to:

Molecular Diagnostics Unit USDA, APHIS, PPQ CPHST Otis Laboratory 1398 West Truck Road Buzzards Bay, MA 02542-1329

For questions you can contact John Molongoski at: Email: john.j.molongoski@aphis.usda.gov Phone: 508-563-9303 ext. 218 Fax: 508-564-4398

Asian Gypsy Moth Trapping Submission Guidelines

Specimens trapped in the field can be analyzed for the presence of Asian genetic markers by submitting the specimens to the CPHST Otis Laboratory. All specimens submitted from outside the generally-infested area will be analyzed. Because of the quantity of specimens submitted from within the generally-infested area, only a small fraction can be analyzed. **Collect captured moths a minimum of every two weeks to minimize DNA degradation of the specimens, more frequently in warm climates.**

Store specimens in a cool, dry location (frozen if possible). Ship ASAP after collection

MILK CARTON TRAPS

DO layer loose moths between wadded paper	DO NOT attach paperwork to bags.
towels or tissue paper in paper bag (brown lunch bag size) to prevent motion and specimen damage during shipment.	DO NOT use plastic bags or paper envelopes as these promote fungal growth and do not allow moisture release.
DO label paper bag clearly with trap numbers matching paperwork.	DO NOT send traps or paperwork for traps which contain no specimens.
DO staple or tape paper bag closed.	

DELTA TRAPS

DO label each trap clearly with trap numbers matching paperwork.	DO NOT attach paperwork to traps.
DO package traps to avoid crushing during	DO NOT use Styrofoam peanuts for packaging.
shipment.	DO NOT disassemble the traps or remove moths
	from the trap.

SHIPPING

DO send a PPQ Form 305 for each trap sent.	DO NOT attach paperwork to traps or bags.	
Include: • Trap number • Collection Date • Collection Site • Life Stage • No. of specimens (estimates OK)	DO NOT use Styrofoam peanuts with delta traps.	
DO package moths / traps to prevent crushing or motion during shipping. Moths must be received	DO NOT send traps or paperwork for traps with no specimens.	
whole with antennae and legs attached to body.	SHIP TO:	
DO ship via next day delivery for Tuesday through Friday arrival.	John Molongoski USDA, APHIS, PPQ CPHST Otis Laboratory	
DO ship ASAP after each collection.	1398 West Truck Road Buzzards Bay, MA 02542-1329	
DO keep moths frozen until shipment.	 Voice: (508) 563-9303 ext 218 Fax: (508) 564-4398 	
DO keep specimens dry.	• Email: john.j.molongoski@aphis.usda.gov	

PPQ Form 305 can be obtained from the Otis Lab via phone or email requests. Please do not hesitate to contact us if you have any questions.

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