Anguina tritici

Scientific Name
Anguina tritici (Steinbuch, 1799) Chitwood, 1935

Synonyms
Anguillula tritici, Rhabditis tritici, Tylenchus scandens, Tylenchus tritici, and *Vibrio tritici*

Common Name(s)
Nematode: **Wheat seed gall nematode**

Type of Pest
Nematode

Taxonomic Position
Class: Secernentea, **Order:** Tylenchida, **Family:** Anguinidae

Reason for Inclusion in Manual
Pests of Economic and Environmental Concern Listing 2017

Background Information
Anguina tritici was discovered in England in 1743 and was the first plant parasitic nematode to be recognized (Ferris, 2013). This nematode was first found in the United States in 1909 and subsequently found in numerous states, where it was primarily found in wheat but also in rye to a lesser extent (PERAL, 2015). Modern agricultural practices, including use of clean seed and crop rotation, have all but eliminated *A. tritici* in countries which have adopted these practices, and the nematode has not been found in the United States since 1975 (PERAL, 2015). However, *A. tritici* is still a problem in third world countries where such practices are not widely adapted (SON, n.d.). In addition, trade issues have arisen due to conflicting records of *A. tritici* in the United States (PERAL, 2015).

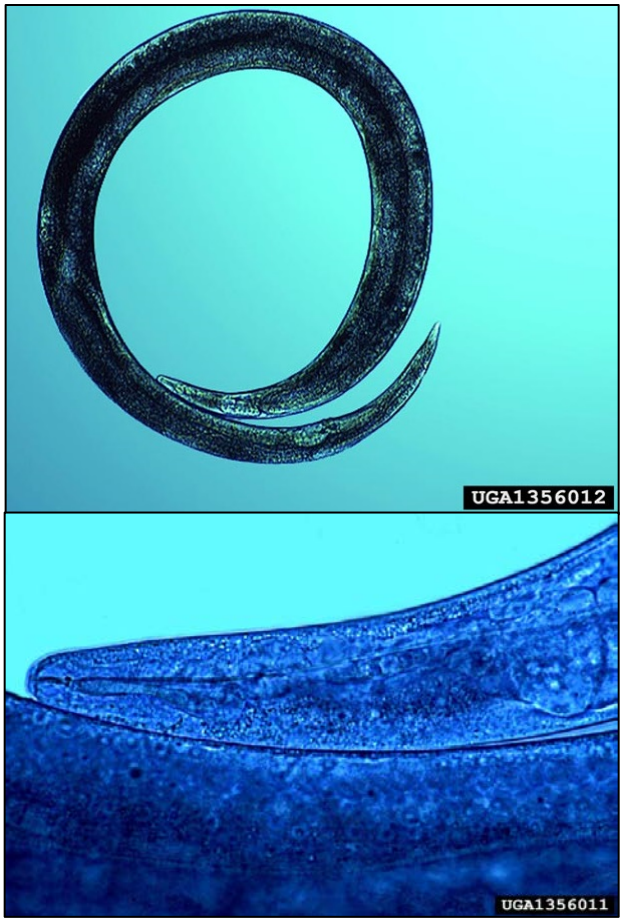

Figure 1: Brightfield light microscope images of an *A. tritici* female as seen under low power magnification. J. D. Eisenback, Virginia Tech, bugwood.org

Figure 2: Wheat seed gall broken open to reveal thousands of infective juveniles. Michael McClure, University of Arizona, bugwood.org

Figure 3: Seed gall teased apart to reveal adult males and females and thousands of eggs. J. D. Eisenback, Virginia Tech, bugwood.org

Pest Description

Measurements (From Swarup and Gupta (1971) and Krall (1991):

Egg: 85 x 38 *µm* on average, but may also be larger (130 x 63 *µm*).

Second-stage juvenile (J2) (n=20): L=0.75 - 0.95 mm (0.77 mm); a=47 - 59 (54); b=4.0 - 6.3 (4.5); c=23 - 28 (26); stylet=10 *µm*.

Third-stage female (n=4): L=1.11 -1.55 mm (1.26 mm); a=28 - 40 (32); b=9.3 -10.2 (9.8); c=20.0 - 22.2 (21.1).

Third-stage male (n=6): L=1.10 - 1.23 mm (1.11 mm); a=26 - 42 (36); b=6.4 - 8.2 (7.6); c=10.2 - 13.4.

Fourth-stage female (n=12); L=1.45 - 1.92 mm (1.86); a=21.0 - 26.5 (22.4); b=9.6 - 18.4 (13.2); c=20.0 - 35.4 (32.3).

Fourth-stage male (n=2): L=1.76, 1.82 mm; a=25.4, 29.1; b=7.5, 9.4; c =15, 20.

Adult female: L=3.0-5.2 mm; a=13-30; b=9.8-25.0; c=24-63; V=70-95; stylet=8-11 *µm.*

Adult male: L=1.9-2.5mm; a=21-30; b=6.3-13.0; c=17-28; stylet 8-11 *µm*; spicules 25-40 *µm;* gubernaculum=*10 µm.*

Other descriptions (as described in CABI, 2015):

"General: Annules very fine, usually visible only in esophageal region; lateral fields with four or more fine incisures, in adults visible only on young specimens. Lip region low and flattened, slightly offset; lips visible as six raised, radial ridges. Procorpus of

esophagus swollen but constricted at junction with median bulb. Esophageal glands forming a roughly pyriform bulb but varying in shape and sometimes showing irregular lobes, not overlapping intestine; cardia small. Tail conoid, tapered to an obtuse or rounded tip. Chromosomes: 2n = 38.

Female: Body obese, spirally coiled ventrally when relaxed by heat. Isthmus of esophagus sometimes swollen posteriorly then offset from glandular region by a deep constriction. Anterior branch of genital tract greatly developed; ovary usually with two or more flexures, with many oocytes arranged about a rachis and ending distally in a cap cell. Spermatheca ± pyriform, its broader end separated from oviduct by a sphincter, its narrower end merging into uterus. Posterior genital branch a simple post-vulval sac. Vulval lips prominent. Several eggs may be present in uterus at one time.

Male: Body sometimes curved dorsally when heat relaxed, i.e. ventral surface outermost. Testis with one or two flexures; spermatocytes arranged about a rachis, ending distally in a cap cell. Vas deferens about 200 *µm* long, separated from testis by a constriction. Spicules paired, stout, arcuate, each having two ventral ridges running from tip to widest part: the head rolled or folded ventrally. Gubernaculum simple, trough-like. Bursa arises just anterior to spicules and ends just short of tail-tip".

Biology and Ecology

Anguina tritici prefers cool conditions in most climates where wheat is grown. The favored micro-habitat for *A. tritici* is within seed galls, where all stages are protected from hostile environmental factors. This nematode also survives on the soil surface, either in or out of galls, and in dry seed storage (CABI, 2015). Second stage juveniles (J2s) within drying galls are capable of entering a cryptobiotic state (anhydrobiosis) to survive during dry conditions (Bird and Buttrose, 1974). Viable juveniles may survive in this state for several decades, and survival for up to 40 years in storage has been documented (Fielding, 1951; Bridge and Starr, 2007). When galls become wet and absorb water, rehydrated juveniles become active (Fielding, 1951).

Nematodes in the infective juvenile stage, J2, emerge from the seed galls on the soil surface and migrate onto newly germinated seedlings, where they enter the plants between leaf sheaths and feed as an ectoparasite. Juveniles likely feed ectoparasitically between compacted leaves until flower primordia form (Ferris, 2013). Later, they penetrate the developing flower ovaries at the time of flower bud initiation. The J2s stimulate formation of galls in place of seed development. Galls can develop from undifferentiated flower ovaries, stamen tissues, and various other tissues (Ferris, 2013). Progression through juvenile stages is then completed inside the galls, and each gall will contain up to 80 adults in a 1:1 sex ratio (Fig. 3) (Ferris, 2013). Reproduction is amphimictic, and females produce up to 2000 eggs per individual over several weeks. Eggs hatch producing J2s, which remain within the galls as the survival stage and perpetuate plant infection as the invasive stage in following years. Dry galls are harvested with developed seeds, and each gall may contain thousands of J2s (Fig. 2) (CABI, 2015). *Anguina tritici* produces one generation per year (Ferris, 2013). Crop

rotation to a non-host for 1-2 years will usually eliminate *A. tritici* from the soil (Ferris, 2013).

Figure 4: Wheat infected (right) compared to healthy wheat (left). J. D. Eisenback, Virginia Tech, bugwood.org

Symptoms/Signs

Ectoparasitic feeding of *A. tritici* may cause leaf rolling, curling, and spiraling (Anwar et al., 2001). Severe infection of young plants can result in stunted plants with distorted, misshapen stems and leaves (Bridge and Starr, 2007). The ear or inflorescence may be absent or, when present, wider and shorter (Fig. 4) (Bridge and Starr, 2007). Plants mature more slowly, and produce smaller seed heads. Yield depressions of up to 50% are possible (Ferris, 2013). Infected wheat heads are reduced with glumes protruding at an abnormal angle exposing the galls to view. This does not occur in rye seed heads (Ferris, 2013).

Infected developing ovaries are transformed into galls that are light brown to nearly black in color (Fig. 5, 6). Galls contain a white mass that consists of dry nematodes in an anhydrobiotic state and becomes powdery if crushed (Bridge and Starr, 2007). Galls appear darker, shorter, and thicker than healthy seeds (Fig. 5, 6). Young galls are short-thick, smooth, light to dark green, turning brown to black with age, 3.5 - 4.5 mm long

and 2 - 3 mm wide. Rye galls are small, buff-colored and longer than wide, 2 - 4.5 mm long by 1 - 2.5 mm wide (Leukel, 1924; Byars, 1920).

Figure 5. *Anguina tritici* seed galls (right) and healthy wheat seeds (left). Ulrich Zunke, University of Hamburg, forestryimages.org

Figure 6: *Anguina tritici* seed galls (left) compared to healthy wheat seeds (right). Michael McClure, University of Arizona, bugwood.org

Pest Importance

Anguina tritici can cause severe crop losses to rye (35-65%) and wheat (20-50%) in third world countries, where suboptimal agricultural practices are common (SON, n.d.). While damage caused by *A. tritici* in modern agricultural systems is insignificant, trade issues with other countries may occur due to historical records showing the presence of the nematode (SON, n.d.). Brazil has halted shipments of U.S. wheat based on general literature describing the nematode as “widespread in the United States” (PERAL, 2015).

Wheat is one of the most commonly grown crops in the United States. In 2015, wheat was grown on over 47 million acres in 42 states, and over 2 billion bushels were produced (USDA-NASS, 2016). Since 1990, the United States has exported an average of 28,762,212 metric tons of unmilled wheat grain per year (PERAL, 2015). Rye was harvested from 360,000 acres in 2015, and 292,115 metric tons were produced (USDA-NASS, 2016).

Anguina tritici is listed as a harmful organism in the following 21 countries: Argentina, Brazil, Chile, Colombia, Ecuador, Egypt, Guatemala, Indonesia, Israel, Jordan, Madagascar, Namibia, Nepal, New Zealand, Paraguay, Peru, South Africa, Taiwan, Thailand, Timor-Leste, and Uruguay (USDA-PCIT, 2016). There may be trade implications with these countries if *A. tritici* is discovered again in the United States.

Known Hosts

Major hosts: *Secale cereale* (rye), *Triticum aestivum* (wheat), *Triticum dicoccum* (emmer wheat), and *Triticum spelta* (spelt).

Minor hosts: *Avena sativa* (oat), *Hordeum vulgare* (barley), *Triticum durum* (durum wheat), and *Triticum turgidum* (rivet wheat) (SON, n.d.; Leukel, 1929; EPPO, 2015; PERAL, 2015).

Although oat is occasionally infested by this nematode in the seedling stage, mature galls of *A. tritici* have not been reported in this crop (Leukel, 1929). In addition, reproduction of *A. tritici* in barley is rare, and this crop is considered to be highly resistant to the nematode (Leukel, 1929). Inoculation experiments on many other grasses were negative, and *A. tritici* is not known to infest any non-cereal grasses (Leukel, 1929).

Known Vectors (or associated insects)

Anguina tritici is not vectored by another species. *Anguina tritici*, however, is the vector of the bacterium *Rathayibacter tritici* (*Clavibacter michiganese* pv. *tritici*), which is the causal agent of a spike blight known as yellow ear rot or 'tundu' of wheat (Bridge and Starr, 2007).

Known Distribution

Africa: Egypt and Ethiopia. **Asia:** Afghanistan, China, India, Iran, Iraq, Israel, Pakistan, Saudi Arabia, South Korea, Syria, and Taiwan. **Europe:** Austria, Azerbaijan, Bulgaria, Croatia, Cyprus, France, Germany, Greece, Hungary, Ireland, Italy, Lithuania, Netherlands, Poland, Romania, Russia, Serbia, Spain, Sweden, Switzerland, Turkey, Ukraine, and the United Kingdom (UK). **Oceania:** Australia and New Zealand (SON, n.d.; Bird and Buttrose, 1974; Mohamedova and Piperkova. 2013; EPPO, 2015).

Anguina tritici has been reported in Brazil and the United States but is considered to be no longer present in either country (EPPO, 2015; PERAL, 2015). This nematode has not been detected in the United States since 1975, despite extensive general surveillance and the distinctive signs (galls) that are easily detected by inspection of grain and seed (PERAL, 2015).

Pathway

The most likely pathway of entry for *A. tritici* is through transport of infested host plant or seed material. The import of *Triticum* spp. seed is allowed from certain countries, some of which are known to have *A. tritici* (USDA, 2015). The import of *Secale* spp. seed is also allowed from all countries (USDA, 2015). While seeds are subject to FSA-A regulations, there are no other import restrictions (USDA, 2015).

Since 2006, there have been shipments of *Triticum aestivum* seed from the following sample of countries where *A. tritici* is present: Germany (68), France (49), China (17), Austria (3), India (2), Croatia (1), South Korea (1), and Russia (1) (AQAS, 2016). Since 2006, there also have been shipments of *Secale cereale* seed from the following countries where *A. tritici* is present: Poland (6), Germany (5), Lithuania (2), and New Zealand (1) (AQAS, 2016). The shipment from New Zealand contained over 19,000 kg of seed (AQAS, 2016).

Since 2006, there have been interceptions of *Triticum aestivum* plant material from the following countries where *A. tritici* is present: India (80), China (23), Turkey (11), Iran (10), Egypt (7), Italy (6), France (4), Hungary (4), New Zealand (4), and Germany (3) (AQAS, 2016). These interceptions occurred in mail, baggage, permit cargo, and general cargo.

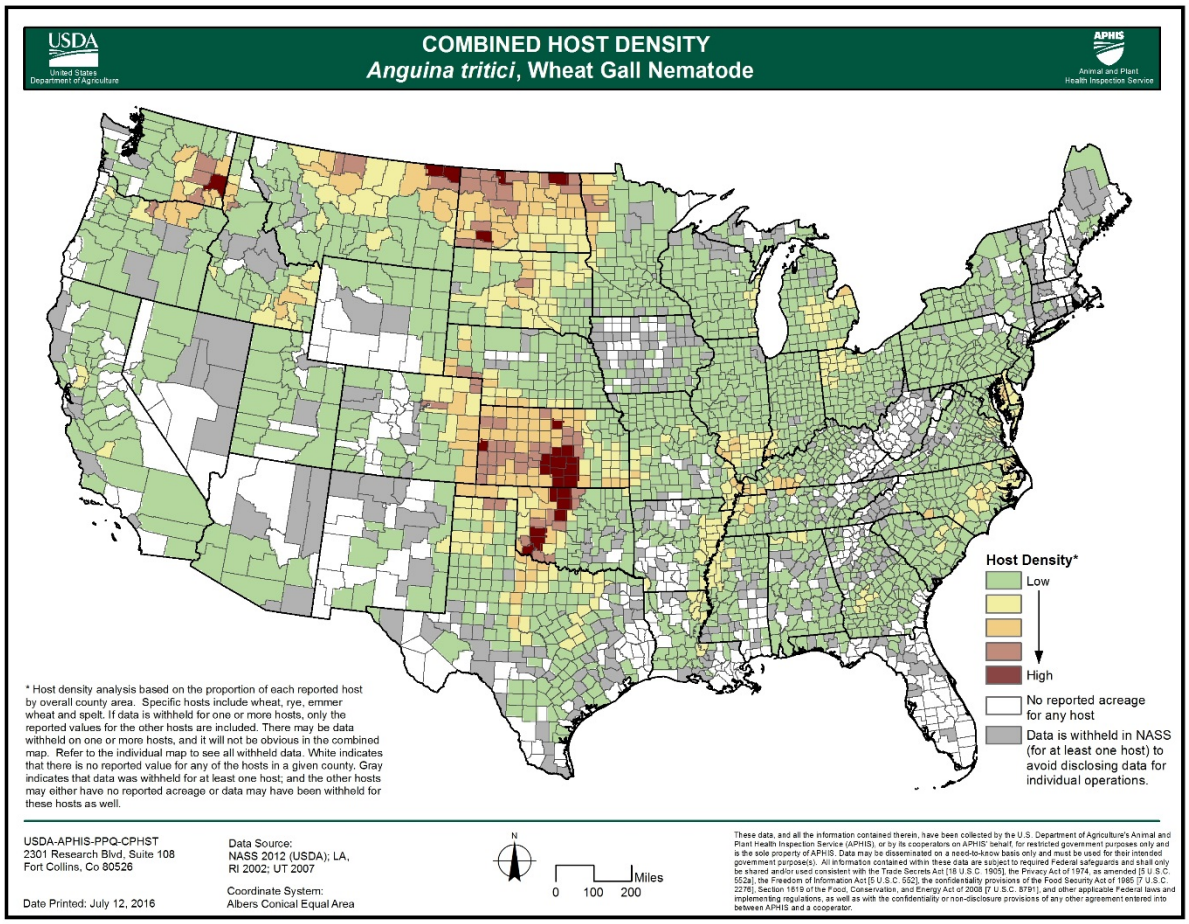

Figure 7. Combined distribution map for *Anguina tritici* within the continental United States. Values represent combined host density low to high (rye, wheat, emmer, and spelt). Map courtesy of USDA-APHIS-PPQ-CPHST.

Potential Distribution within the United States

In the United States, *A. tritici* was previously found in California, Georgia, Maryland, New York, North Carolina, South Carolina, Texas, Virginia, and West Virginia (EPPO, 2015; PERAL, 2015). The states with the most reports of *A. tritici* infestation were Virginia and West Virginia, and the most recent detection of this nematode occurred in Virginia in 1975 (PERAL, 2015). Every reported detection of *A. tritici* in the United States occurred in areas of cultivation (PERAL, 2015).

Wheat is grown throughout the continental United States, particularly in Midwestern states. In 2015, the top ten states for planted wheat acreage were, in order: Kansas, North Dakota, Montana, Oklahoma, Texas, South Dakota, Washington, Colorado,

Minnesota, and Nebraska (USDA-NASS, 2016). The top two rye producing states in 2015 were Georgia and Oklahoma, which together account for over 40% of the U.S. rye harvest (USDA-NASS, 2016).

A recent combined host distribution map for *Anguina tritic*i developed by USDA-APHIS-PPQ-CPHST (Fig. 7) identifies areas of high host acreage based on the combined acreage of the major hosts (rye, wheat, emmer, and spelt). This map illustrates that there are counties in nearly all states with a low level of risk.

Colorado, Idaho, Illinois, Indiana, Kansas, Kentucky, Maryland, Michigan, Minnesota, Missouri, Montana, Nebraska, North Carolina, North Dakota, Oklahoma, Oregon, South Dakota, Tennessee, Texas, and Washington, however, have counties with the highest level of risk (moderate to high) for *A. tritici*, based on host density. The host distribution maps are based on county level data. To combine host data for pest-specific analyses, CPHST normalizes the data by dividing the total host present in a county by overall county area (acres of host in county/ total acres of county). This yields host by county area and allows CPHST to properly combine host distributions without the skewing effects of overall county size. For example, 500 acres of broccoli grown in Tulare County, CA can now be compared to 500 acres of broccoli grown in Scott County, AR. The individual host acreage maps for rye, wheat, and emmer and spelt are provided in the Appendix at the end of the document.

Survey

Approved Method for Pest Surveillance*: The CAPS-approved survey method is the collection of a seed sample coupled with visual survey of the seed for galls and irregular seed.

Baermann funnels and flasks/shakers have been used for nematode emergence/ extraction from the wheat galls in water. The seeds can also be directly dissected to release nematodes into water. Any nematodes that emerge from seed will need to be confirmed using morphological/ molecular methods.

Note: It is possible that surveys for *Anguina tritici* could occur in conjunction with surveys for karnal bunt in wheat.

*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:

“Wheat fields should be inspected for stunted and distorted seedlings. Mature plants should be examined for stunted, irregular spikes with horizontal glumes exposing the galls. Random samples (about 1/2 cup) should be taken from grain in dry storage or vehicles, vessels or aircraft in commerce. Seed lots should be examined for galls or irregular seeds and, if present, they should be included in the 1/2 cup subsamples and their presence noted” (CABI, 2015).

To survey for nematodes in a dry seed lot, salt solution can be used. “Seeds are poured into a 20% salt solution, stirred vigorously, and the debris skimmed from the surface and examined under the microscope for galls” (CABI, 2015). The Baermann funnel method may also be used to separate nematodes from samples (CABI, 2015).

Asaad and Abang (2009) detected *A. tritici* using a funnel separation technique and also by crushing seed samples and staining them with lactophenol-cotton blue solution for microscopic examination.

Diagnosis of ear cockle is based on extraction of nematodes from symptomatic plants, particularly from the apical meristem and by soaking galls. Water reactivates the many thousands of nematodes which are found in the gall tissue (Bridge and Starr, 2007). A scalpel and tweezers can be used to dissect seeds and release nematodes in water (CABI, 2015).

Another way to survey for *A. tritici* is to visit a mill and collect the debris that has been rejected in the milling process (Eisenback, personal communication, 2016).

Key Diagnostics

Approved Method for Pest Surveillance*: Initial diagnosis will be via morphology. All initial diagnoses will need to be confirmed with molecular methods.

A dichotomous key has been developed to identify *Anguina tritici* (Krall, 1991) that uses many morphological and biological characteristics.

Although not officially validated for regulatory purposes, Li et al. (2015) demonstrated PCR primer pairs that target specific areas of the ITS2 region and are able to differentiate *A. tritici* from *A. agrostis*, *A. funesta*, and *A. pacificae* (Li et al., 2015). The PCR assay can be used for single nematodes. The detection of other undescribed *Anguina* species should also be possible by the *Anguina* genus-specific primers included in this assay. This assay can be used for screening *Anguina tritici* morphological suspect samples.

*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:

Powers et al. (2001) described the PCR-RFLP patterns of the internal transcribed spacer region (ITS1) of *A. tritic*i and several other species of *Anguina*. In the study, the restriction enzymes Alu I, Hha I, and Hinf I, used individually, were able to differentiate *A. tritici* from other *Anguina* species. These patterns were different between *Anguina* spp. and allows for the possibility of molecular diagnosis of *A. tritici*.

Easily Confused Species

Anguina tritici is closely related to *Anguina funesta* and *Subanguina wevelli*. The morphological separation of these three species is difficult (SON, n.d.). Recent

molecular diagnostic techniques have facilitated the separation of these three species (Powers et al., 2001).

Anguina tritici is also closely related to *A*. *agropyronifloris*, which is a nematode that occurs in native western wheatgrass and has been used for erosion control in some state road projects (Powers, 2016 personal communication). This nematode could be confused with *A. tritici* during extraction and identification if native grass seed is collected with or is a contaminant in the wheat seed.

Galls caused by *A. tritici* may be confused with symptoms of bunt disease caused by the fungal pathogens *Tilletia indica* (karnal bunt) (EPPO, n.d.), *T. laevis/ T. tritici* (stinking/common bunt) (Burrows, n.d.; Bridge and Starr, 2007). In addition, there are many other pathogens of wheat which are present in fields and in seed lots (Asaad and Abang, 2009), and some plant disease galls resemble nematode galls and may erroneously indicate the presence of *A. tritici* (CABI, 2015).

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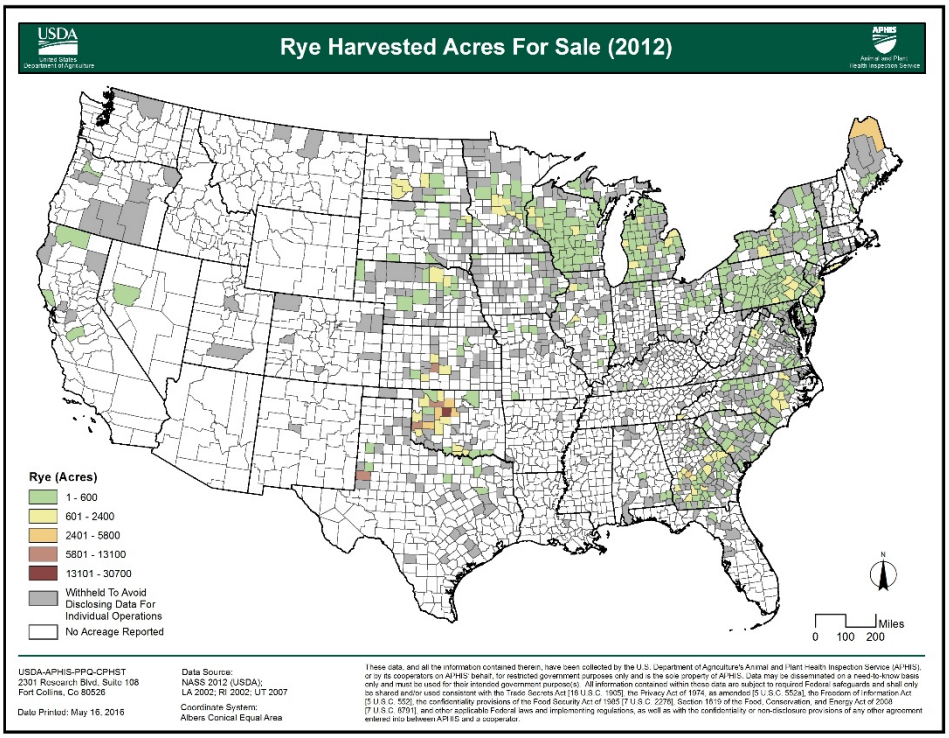
October 2016: Document posted to CAPS Resource and Collaboration site.

Appendix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United States Department of Agriculture
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APHIS
Animal and Plant Health Inspection Service
Rye (Acres)
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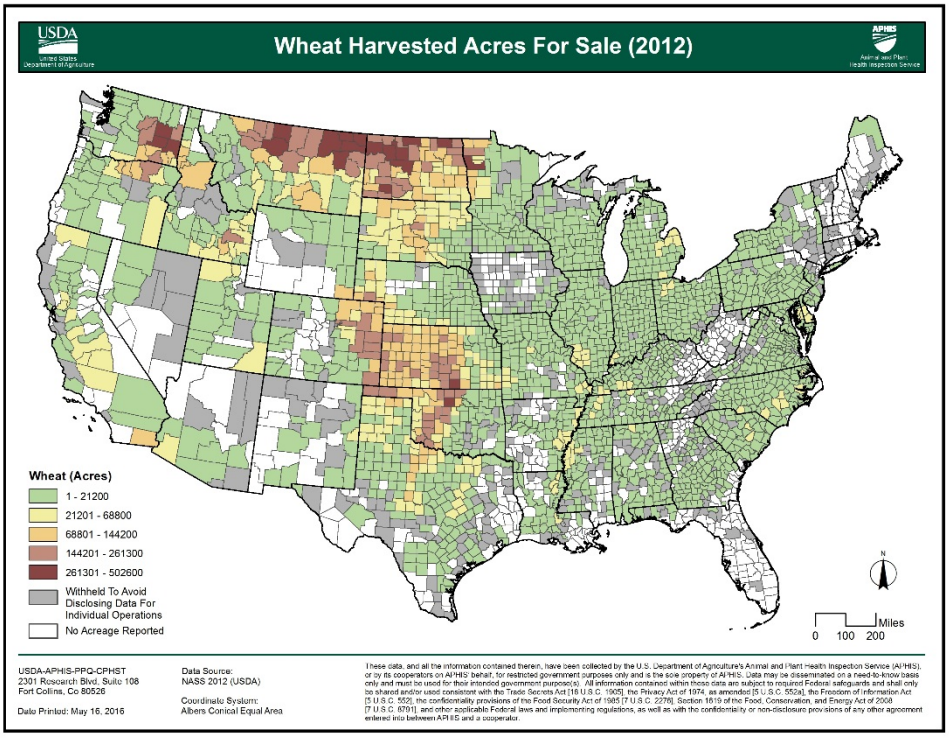
2301 Research Blvd, Suite 108
Fort Collins, Co 80526
Data Printed: May 16, 2016
Data Source:
NASS 2012 (USDA);
LA 2002; RI 2002; UT 2007
Coordinate System:
Albers Conical Equal Area
These data, and all the information contained therein, have been collected by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS), or by its cooperators on APHIS' behalf, for restricted government purposes only and is the sole property of APHIS. Data may be disseminated on a need-to-know basis only and must be used for their intended government purpose(s). All information contained within these data are subject to required Federal safeguards and shall only be shared and/or used consistent with the Trade Secrets Act [18 U.S.C. 1905], the Privacy Act of 1974, as amended [5 U.S.C. 552a], the Freedom of Information Act [5 U.S.C. 552], the confidentiality provisions of the Food Security Act of 1985 [7 U.S.C. 2276], Section 1619 of the Food, Conservation, and Energy Act of 2008 [7 U.S.C. 8791], and other applicable Federal laws and implementing regulations, as well as with the confidentiality or non-disclosure provisions of any other agreement entered into between APHIS and a cooperator.


USDA
United States Department of Agriculture
Wheat Harvested Acres For Sale (2012)
APHIS
Animal and Plant Health Inspection Service
Wheat (Acres)
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Coordinate System:
Albers Conical Equal Area
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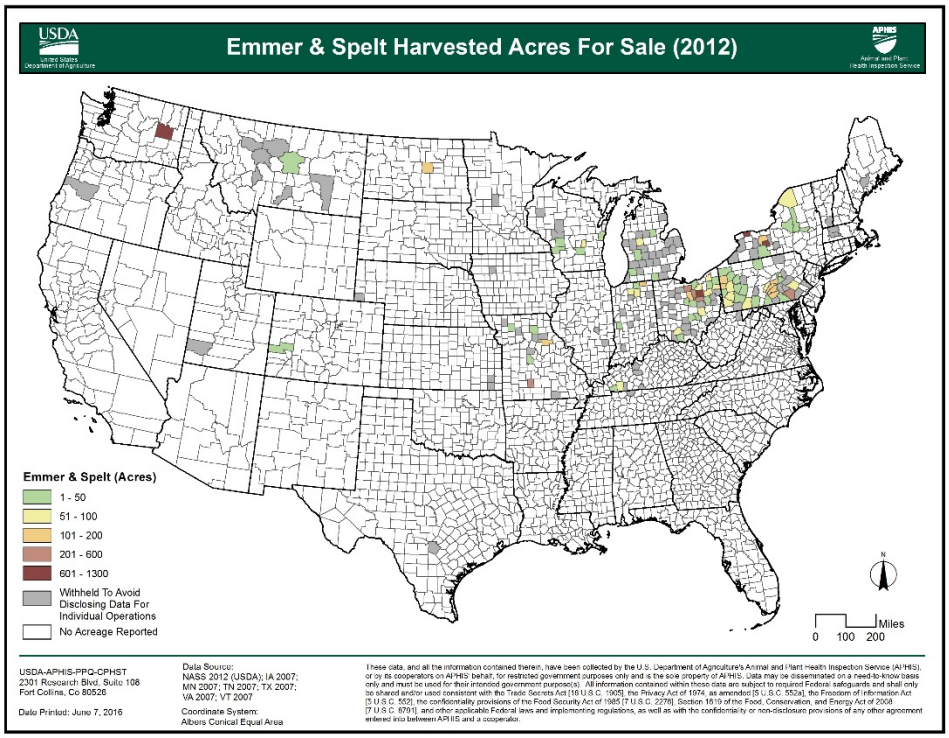
USDA
United States Department of Agriculture
Emmer & Spelt Harvested Acres For Sale (2012)
APHIS
Animal and Plant Health Inspection Service
Emmer & Spelt (Acres)
1 - 50
51 - 100
101 - 200
201 - 600
601 - 1300
Withheld To Avoid Disclosing Data For Individual Operations
No Acreage Reported
N
0 100 200
Miles
USDA-APHIS-PPQ-CPHST
2301 Research Blvd. Suite 108
Fort Collins, Co 80526
Date Printed: June 7, 2016
Data Source:
NASS 2012 (USDA); IA 2007; MN 2007; TN 2007; TX 2007; VA 2007; VT 2007
Coordinate System:
Albers Conical Equal Area
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