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Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

Proposed Release of Three Parasitoids for the Biological Control of the Emerald Ash Borer (*Agrilus planipennis*) in the Continental United States

Environmental Assessment, April 2, 2007

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I. Background and Introduction

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) and Forest Service (FS) are proposing to release three insect parasitoid[1] species for the biological control (biocontrol) of the nonindigenous emerald ash borer (EAB) (*Agrilus planipennis*). This environmental assessment[2] (EA) has been prepared, consistent with USDA, APHIS' National Environmental Policy Act (NEPA) implementing procedures (Title 7 of the Code of Federal Regulations (CFR), part 372). It examines the potential effects on the quality of the human environment that may be associated with the release of these biocontrol agents to control infestations of EAB within the continental United States. This EA considers the potential effects of the proposed action and its alternatives, including no action.

The EAB is an invasive wood-boring beetle from Asia threatening North America's ash trees (*Fraxinus* spp.). It was introduced into the Detroit, Michigan, area, probably sometime in the 1990s, and was identified as the cause of ash mortality in the area in 2002 (Haack et al., 2002). EAB larvae feed on ash phloem, cutting off the movement of resources within the tree and killing the tree in 4-5 years. EAB is now considered established in natural ecosystems throughout the lower peninsula of Michigan and contiguous areas of Ohio, Indiana, and Ontario, Canada. Separate infestations are also found in the upper peninsula of Michigan, Illinois, and Maryland. EAB appears well suited for climatic conditions in North America and destroys entire stands of ash. It is predicted that EAB will continue to disperse along various continuous corridors of ash now present in natural and urban environments due to the widespread use of ash as a landscape tree.

[1] In this case, small, stingless wasps that during their development, live in the body or egg of a single host individual, eventually killing that individual.

[2] Regulations implementing the National Environmental Policy Act of 1969 (42 United States Code 4321 *et seq.*) provide that an environmental assessment "[shall include brief discussions of the need for the proposal, of alternatives as required by section 102(2)(E), of the environmental impacts of the proposed action and alternatives, and a listing of agencies and persons consulted." 40 CFR § 1508.9.

II. Purpose and Need for the Proposed Action

APHIS and FS propose to release three parasitoids into the environment of the continental United States for the purpose of reducing EAB populations. These parasitoids are known to attack EAB consistently in its native range in China. The biocontrol agents include one larval ectoparasitoid, *Spathius agrili* (Hymenoptera: Braconidae) (Yang et al., 2005), one species of egg parasitoid, *Oobius agrili* (Hymenoptera: Encyrtidae) (Zhang et al., 2005), and one species of larval endoparasitoid, *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) (Liu et al., 2003; Yang et al., 2006). Initial releases of each parasitoid are planned for summer of 2007. Post-release monitoring, including impacts on EAB and non-target wood-boring beetles and spread and establishment of each parasitoid species, will be conducted.

There is a need to control EAB, an invasive wood-boring beetle that is spreading rapidly and poses a serious threat to ash trees in the United States if not controlled. Despite state and federal quarantines designed to contain EAB, the lack of effective methods to detect EAB-infested trees and the large size of the infestation has resulted in a shift by regulatory agencies from a strategy of area-wide eradication to one focused on eradication in outlying areas and containment in the core infestation area (GAO, 2006). In the United States, EAB eradication efforts involved the removal of all ash trees within a circle of specified radius around known infestations (typically ½ mile). By the time an infestation was discovered and treated, however, EAB had usually already dispersed outside the eradication zone. The bronze birch borer, *A. anxius* Gory, a native species closely related to EAB, is known to spread at a rate of 10 to 20 miles per year, and this has been proposed as an estimate for EAB's natural dispersal rate. Besides natural dispersal, the spread of EAB has been accelerated through human-assisted movement of infested ash firewood, timber, solid-wood packing materials, and nursery stock. This resulted in the spread of EAB from Michigan to Maryland and Virginia in 2003. As EAB spreads throughout North America, regulatory agencies, land managers, and the public are seeking sustainable management tools such as biological control to reduce EAB population densities and to slow its spread (Cappaert et al., 2005; GAO, 2006; Poland and McCullough, 2006).

APHIS has responsibility for taking actions to exclude, eradicate, and/or control plant pests, including EAB, under the Plant Protection Act (7 United States Code (U.S.C.) 7701 *et seq.*). APHIS has been delegated the authority to administer these statutes and has promulgated Quarantines and Regulations (7 CFR 319) which regulate the importation of commodities and means of conveyance to help protect against the introduction and spread of harmful pests. The underlying strategy of the proposed program is to reduce EAB population densities in infested areas and slow its spread into to new areas.

III. Alternatives

APHIS considered two alternatives in response to the need to control EAB and contain infestations: (1) no action and (2) biological control by the release of the three parasitoids, *O. agrili, T. planipennisi,* and *S. agrili* (the preferred action). Both alternatives are described briefly in this section and the potential impacts of each are considered in the following section.

A. No Action

Under the no action alternative, APHIS and FS would not release the three parasitoids, *O. agrili, T. planipennisi,* and *S. agrili,* for the management of EAB. APHIS, in cooperation with the appropriate State Departments of Agriculture, would continue the current program that includes survey, quarantine, and eradication of EAB in outlying areas and containment in the core infestation area. Some control measures could be taken by other Federal or non-Federal entities; those actions would not be under APHIS' control or funded by APHIS. Local business owners and area residents could attempt to control damage from EAB infestations by removing the infested trees from their properties. The lack of effective measures to prevent the spread of EAB from infested areas (occurring via natural dispersal or artificial spread from movement of infested ash products) will likely lead to an increase in EAB populations, increase its range of distribution within the United States, and further economic and environmental damage.

B. Biological Control Action (Preferred Alternative)

Under this alternative, APHIS and FS would release three parasitoids for biocontrol of EAB. The biocontrol agents include one species of egg parasitoid, *Oobius agrili* (Hymenoptera: Encyrtidae) (Zhang et al., 2005), one species of larval endoparasitoid, *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) (Liu et al., 2003; Yang et al., 2006), and one larval ectoparasitoid, *Spathius agrili* (Hymenoptera: Braconidae) (Yang et al., 2005). Initial releases of each parasitoid are planned for the summer of 2007. Releases of these EAB parasitoids are expected to reduce the population of EAB and slow the spread rate in the United States. This alternative would reduce but not eradicate EAB in the United States.

Measures that are occurring under the "no action" alternative would likely continue even if the three parasitoids are released into the environment; however, these biocontrol agents would serve as another tool to assist in the protection of ash trees in North America.

1) *Spathius agrili*:

Spathius agrili is a gregarious larval ectoparasitoid; adult wasps lay multiple eggs on e surface of EAB larvae after paralyzing the host EAB during oviposition (egg laying) and stopping further development of the host.

Taxonomy: *Spathius agrili* Yang (Hymenoptera: Braconidae). No synonymy or common names.

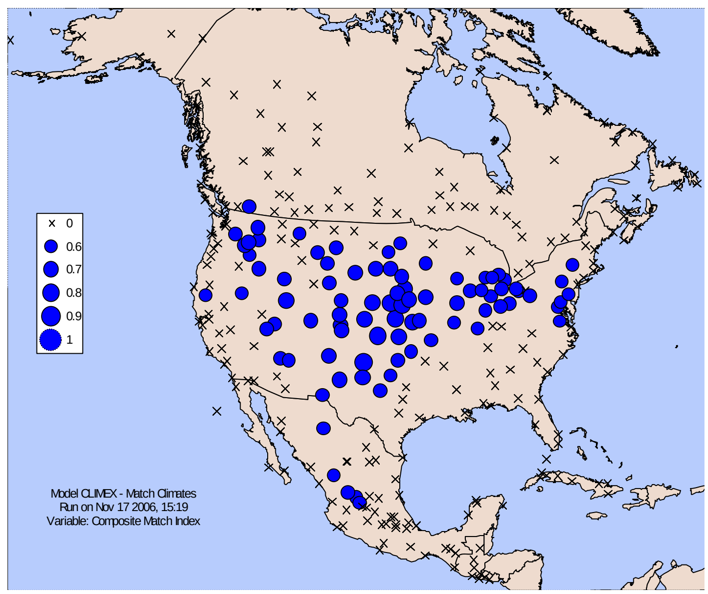
Methods used to identify *S. agrili*. Yang et al. (2005) give a detailed description of *S. agrili*. A key to *Spathius* of North America was developed that includes information on how to separate *Spathius agrili* from the native species (Marsh and Strazanac, in press).

Location of voucher specimens. Insect Museum, Chinese Academy of Forestry, Beijing, China; Nationaal Natuurhistorisch Museum, Leiden, the Netherlands; U.S. National Natural History Museum, Washington, DC; and West Virginia University Arthropod Collection, Morgantown, WV.

Natural geographic range, other areas of introduction, and expected attainable range in North America (also habitat preference and climatic requirements of *S. agrili*). *Spathius agrili* has been collected from Tianjin, China and Changchun, Jilin Province, China. This species is much easier to collect in Tianjin, which would be the likely source of insects released in the United States. The climatic conditions that are most conducive to population growth of *S. agrili* are not explicitly known; therefore a climate matching analysis was conducted to determine how similar Tianjin is to the upper Midwest. The climate matching feature of the climate analysis software CLIMEX (Herne Scientific Software Pty Ltd, Melbourne, Australia) was used. The model calculated a Climate Match Index (CMI) that is a composite of six variables: maximum temperature, minimum temperature, total rainfall, rainfall pattern, relative humidity, and soil moisture. A CMI of 1 indicates an exact climate match. Because these insects do not live in the soil, soil moisture was not included in the model. Adult *Spathius* are only present in the summer, so the model was run for the adult stage only for June through September, and because adults are free living outside of the tree, relative humidity was included as a factor. For larvae, which live under bark, relative humidity was not included in the model, and the entire year was included.

For larvae, the CMI for Lansing MI, which is representative of locations in the upper Midwest, was 0.63 (Figure 1) when comparisons were made with Tianjin, China. CMI values were similar throughout the central part of the United States (Figure 1). The CMI for adults was 0.60. Although the CMI for adults was higher for Changchun (0.74) the value for larvae was similar (0.66). This indicates that there is not a great advantage to attempting to collect *S. agrili* from Changchun rather than Tianjin, where collections are much easier to make. Although the climate in Tianjin does not perfectly match that in the upper Midwest, it is sufficiently similar that *S. agrili* could establish successfully.

Figure 1: Composite Match Index generated by CLIMEX climate software showing the similarity in yearly climate between Tianjin, China and North America for *S. agrili* larvae.



The cold hardiness of EAB and *S. agrili* were tested in the laboratory. The super-cooling point of EAB was -22.36ºC, while that of *S. agrili* was -26.28 (Wu et al., in press), indicating that *S. agrili* should be able to withstand cold temperatures in the more northerly range of EAB.

Source of the culture/agent in nature (name of collector, name of identifier). Dr. Yang Zhong-qi collected the *Spathius* in Dagong, Tianjin, China (38º56'N, 117 º29'E) and described it as a new species (Yang et al., 2005).

Life history (including dispersal capability and damage inflicted on EAB). *Spathius agrili* is well synchronized with its host, emerging in the spring over 1½ months after adult EAB when third- and fourth- instar EAB larvae are present. Females search the trunks of infested trees, ovipositing on the EAB larvae through the bark. The EAB is paralyzed and from 2-18 eggs are laid per larva. In the laboratory, a female lays an average of 23 eggs during her lifetime, which averages 29 days. Because *S. agrili* females only seem to attack EAB larvae that are feeding and close to the surface of the tree, fecundity in the lab may be lower than in the field because of rearing methods. Sample percentage parasitism in the field in China increases from August to October from an average of 12% to an average of 42% (Yang et al., in press). Although data are not available on the dispersal capability of *S. agrili*, they fly strongly and are long lived, indicating that dispersal capabilities may be high.

Known host range of *S. agrili* based on valid literature records, host data from museum specimens, and unpublished records. This species has not been collected or described from any other host. It was described as a new species attacking EAB in 2005 (Yang et al., 2005). In preparing his manuscript, Dr. Yang reviewed several collections of Braconid wasps in China and Russia and conducted molecular analyses on described species, comparing them to *S. agrili*, before concluding that *S. agrili* was a previously undescribed species.

History of past use of *S. agrili*. *Spathius agrili* has never been used as a biocontrol agent.

Pathogens, parasites, hyperparasitoids of *S. agrili* and how to eliminate them from a culture of the agent. No pathogens, parasitoids, or hyperparasitoids have been observed attacking *S. agrili*, either in China or in specimens shipped to the United States. A small subset of the parasitoids collected in China will be reared and identified by Dr. Yang Zhong-qi, an expert on braconid parasitoids. This will ensure that the established colony contains only *S. agrili* and no other braconids or hyperparasitoids. Further observations will be made on parasitoids emerging in quarantine in the United States. Only healthy *S. agrili* will be used to establish a colony. Any diseased organisms will be sent to insect pathologists for identification and hyperparasitoids will be eliminated from the colony. Insects that are subsequently reared in the United States will be maintained in pure culture and monitored for disease.

Other closely related genera, sibling species or closely-similar species in North America. The "Nomina Insecta Nearctica; a check list of the insects of North America" (Poole, 1997) lists 25 species in the genus *Spathius*. Some of these species are known to attack borers in the genus *Agrilus*, and *Spathius floridanus* has been observed attacking EAB larvae in Michigan at very low rates.

2) *Oobius agrili:*

Oobius agrili is an egg parasitoid. Adult wasps lay an egg inside EAB eggs, that are laid between bark layers and crevices on ash trunks and branches. Larval wasps consume and kill host eggs to complete their devlopment.

Taxonomy: *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) (Zhang et al., 2005). No synonymy or common names.

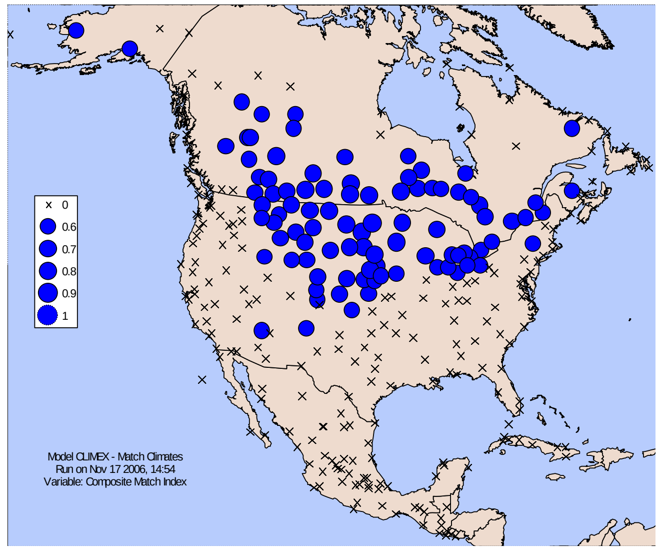
Methods used to identify *Oobius agrili*. *Oobius agrili* is distinguished from the other *Oobius* spp. according to morphological characteristics described by Zhang et al. (2005). Although no egg parasitoids are known from EAB in Michigan (Bauer et al., 2005), encyrtid parasitoids are reported from eggs of *Agrilus anxius* including *Ablerus* sp., *Avetianella* sp., *Ooencyrtus* sp., and *Thysanus* sp. (Nash et al., 1951; Barter, 1957; Loerch and Cameron, 1983).

Location of voucher specimens. Institute of Zoology, Chinese Academy of Sciences, Beijing, China; U.S. National Natural History Museum, Washington, D.C.; USDA Forest Service, Northern Research Station, E. Lansing, MI; Entomology Museum, Michigan State University, E. Lansing, MI; Museum of Zoology, Insect Division, University of Michigan, Ann Arbor, MI.

Natural geographic range, other areas of introduction, and expected attainable range in North America (also habitat preference and climatic requirements of *Oobius agrili*). *Oobius agrili* was discovered in 2004 in the vicinity of Changchun in Jilin Province, China. This species may have a wider distribution in China, however, no further exploration for EAB egg parasitoids has been conducted in China due to limited funding and the difficulty in finding EAB eggs. To estimate climatic conditions conducive to establishment of *O. agrili* in North America, Jilin Province was compared to North America using the climate matching feature of the climate analysis software CLIMEX (Herne Scientific Software Pty Ltd, Melbourne, Australia). Because these insects do not live in the soil, soil moisture was not included in the model. Adult *O. agrili* are only present in the summer, so the model was run for adults only for June through September, and because adults are free living outside of the tree, relative humidity was included as a factor.

For larvae, the CMI for Lansing MI, which is representative of all the locations in the upper Midwest, was 0.66 (Figure 2) when comparisons were made with Changchun in Jilin Province, China. CMI values were similar throughout the central part of the United States (Figure 2).

Figure 2. Composite Match Index generated by CLIMEX showing the similarity in yearly climate between Changchun, Jilin Province, China and North America for *O. agrili* larvae.



Source of the culture/agent in nature (name of collector, name of identifier). EAB eggs are collected near Changchun City (43º54'N, 125º12'E) in Jilin Province, China by Dr. Tonghai Zhao, Chinese Academy of Forestry, Beijing and shipped from this area of China to Dr. Leah Bauer's quarantine laboratory in E. Lansing, Michigan. The eggs are held for up to 8 months until emergence of *O. agrili* is complete.

EAB/ *Oobius agrili* interaction (e.g., parasitoid, pathogen, parasite, competitor, antagonist, etc.) *Oobius agrili* is a solitary egg parasitoid. No entomopathogens or hyperparasitoids have been discovered infecting or attacking *O. agrili* in shipments received from China.

Life history (including dispersal capability and damage inflicted on EAB). Based on data from field-collected eggs from China, *O. agrili* spends the winter as mature larvae in EAB eggs and peak emergence occurs when EAB begin ovipositing in mid-June, although some adults remain in diapause until July and August. The sex ratio of *O. agrili* reared from field-collected EAB eggs in China is 14.5:1 (female:male). *O. agrili* is thelytokous parthenogenic (females produce females without mating). Much of the knowledge of this parasitoid's biology is based on laboratory studies performed at 25ºC with females held in a plastic cup with a streak of honey, moist cotton ball, and EAB eggs laid on a small ash twig. *Oobius agrili* females prefer to oviposit in 0- to 6-day-old EAB eggs and live an average of 22.5 days. Their average lifetime fecundity is approximately 24 progeny per female, and each generation requires about three weeks (Table 1). Based on monthly field collections in China during

2005, it was confirmed that *O. agrili* completes at least two generations per year with parasitism reaching almost 62% in August. These attributes of *O. agrili*, including female-biased sex ratio, parthenogenesis (females produce females without mating), short generation time, and high rates of parasitism and fecundity, are characteristics of successful biocontrol agents (Kimberling, 2004). No data are available on the dispersal capability of *O. agrili,* but despite their small size (about 0.9 mm) they are relatively long-lived (approximately three weeks) and are active jumpers and fliers in the laboratory. Dispersal, however, may be facilitated by selecting release sites with connecting corridors of ash, such as along riparian areas. Moreover, since *O. agrili* reproduces through parthenogenesis, dispersal is facilitated because females do not need to find a male and mate before parasitism of EAB can occur.

Table 1. Biology of *O. agrili* in the laboratory reared in EAB eggs at 25ºC (Bauer, 2007a).

Stage	Egg-pupal	Adult	
		Female	Male
Duration (d)	22.5 ± 2.1	13.6 ± 6.8	12.0 ± 0
Fecundity (parthenogenic)	23.6 ± 11.4 (average)		
	62 (lifetime maximum)		
	5 (daily maximum)		

Known host organisms based on valid literature records, host data from museum specimens, and unpublished records. This egg parasitoid was discovered in China in 2004. Specimens were sent to Drs. Zhang and Huang, Chinese Academy of Science, Beijing, China for taxonomic description as *Oobius agrili* (Zhang et al., 2005).

History of past use of *Oobius agrili*. *Oobius agrili* has never been used as a biocontrol agent.

Pathogens, parasites, hyperparasitoids of *Oobius agrili* and how to eliminate them from a culture of the agent. No pathogens, parasitoids, or hyperparasitoids have been observed attacking *O. agrili*, either in observations in China or in specimens shipped to the United States. Insects used to establish colonies or to release in the field will be allowed to emerge as adults in quarantine and only healthy, unparasitized adults will be used.

Other closely related genera, sibling species or closely-similar species in North America. There are eight described *Oobius* spp., all from the Palearctic region and southern Africa. Of those, five species parasitize eggs of the insect family Buprestidae (four are specific to *Agrilus* spp.), one parasitizes a cerambycid, one is reported from eggs of an asilid (which may be a case of mistaken identity), and the host is unknown for the last species (Trjapitzin, 1963; 1989; Annecke, 1967; Prinsloo, 1979; Zhang et al., 2005).

3) *Tetrastichus planipennisi:*

Tetrastichus planipennisi is a larval endoparasitoid. Adults wasps lay one or more eggs inside EAB larvae; larval wasps consume and kill host larvae to complete their development.

Taxonomy: *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae). No synonymy or common names.

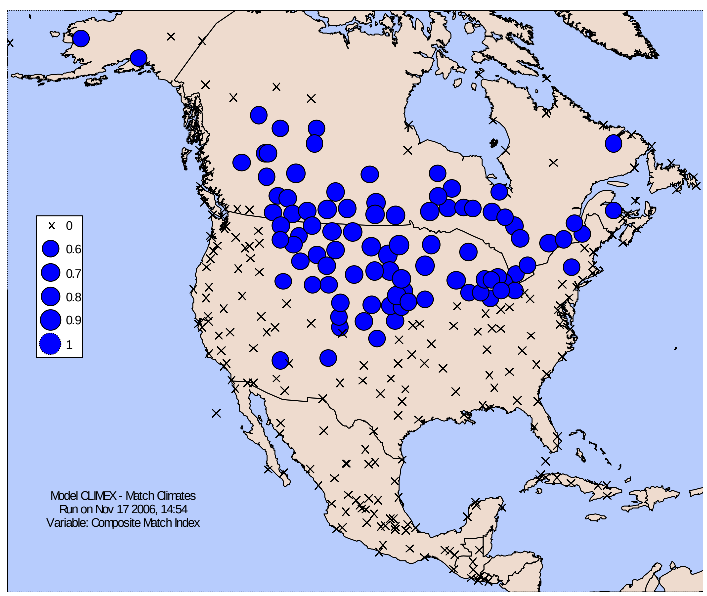
Methods used to identify *T. planipennisi*. *T. planipennisi* can be distinguished from the other *Tetrastichus* spp. according to morphological characteristics described by Yang et al. (2006). No *Tetrastichus* spp. have been found parasitizing EAB in North America, although other species parasitize *Agrilus* spp. in North America including *T. rugglesi* Roh., which parasitizes bronze and red-necked caneborers, *A. rubicola* Abeille and *A. ruficollis* (F.), in New York (Mundinger, 1941) and an undescribed *Tetrastichus* sp. reared *A. anxius* in Pennsylvania (Loerch and Cameron, 1983; UCD, 2006). Additional species reported parasitizing *Agrilus* spp. in Europe include *T. agrili* (Crawford, 1914), *T. agrilocidus* (Graham, 1991), and *T. heeringi* (TSalbukov, 1983); and *T. jinzhouicus* in China (Liao, 1987).

Location of voucher specimens. Institute of Zoology, Chinese Academy of Sciences, Beijing, China; Insect Museum, Chinese Academy of Forestry, Beijing, China; U.S. National Natural History Museum, Washington, D.C.; USDA Forest Service, Northern Research Station, E. Lansing, MI; Entomology Museum, Michigan State University, E. Lansing, MI; Museum of Zoology, Insect Division, University of Michigan, Ann Arbor, MI; Canadian National Collection of Insects, Agriculture and Agri-Food Canada, Ottawa, Canada.

Natural geographic range, other areas of introduction, and expected attainable range in North America (also habitat preference and climatic requirements of *T. planipennisi*). *Tetrastichus planipennisi* was discovered in 2003 in Jilin and Liaoning Provinces of China (Liu et al., 2003) and later in Heilongjiang Province (Yang et al., 2006). This species may have wider distribution in China, however, no further exploration for EAB egg parasitoids have been done in China due to limited funding for foreign exploration. To estimate climatic conditions conducive to establishment of *T. planipennisi* in North America, Jilin Province was compared to North America using the climate matching feature of the climate analysis software CLIMEX (Herne Scientific Software Pty Ltd, Melbourne, Australia). Because these insects do not live in the soil, soil moisture was not included in the model. Adult *T. planipennisi* are only present in the summer; for adults, the model was run only for June through September, and because adults are free living outside of the tree, relative humidity was included as a factor.

For larvae, the CMI for Lansing MI, which is representative of all the locations in the upper Midwest, was 0.66 when comparisons were made with Changchun in Jilin Province, China. CMI values were similar throughout the central part of the United States (Figure 3).

Figure 3. Composite Match Index generated by CLIMEX showing the similarity in yearly climate between Changchun, Jilin Province, China and North America for *T. planipennisi* larvae.



Source of the culture/agent in nature (name of collector, name of identifier). EAB larvae parasitized by *T. planipennisi* were collected near Changchun City (43°54'N, 125°12'E), Jilin Province, China by Dr. Tonghai Zhao, Chinese Academy of Forestry, Beijing and shipped to Dr. Leah Bauer's quarantine laboratory in E. Lansing, Michigan. Mature parasitoid larvae can be held in chill (4ºC) for up to 4 months; *T.*

planipennisi pupate and emerge as adults after approximately two weeks at 25ºC.

EAB/ *T. planipennisi* interaction (e.g., parasitoid, pathogen, parasite, competitor, antagonist, etc.) No entomopathogens or hyperparasitoids have been discovered infecting or attacking *T. planipennisi* in shipments received from China. The interactions between *T. planipennisi* and EAB are close and host-specific because this type of parasitoid is a koinobiont[2], and evolved the means to overcome physiological resistance mechanisms while it develops within its host's body cavity. Interactions between *T. planipennisi* and EAB parasitoids in North America will be minimal since few natural enemies attack EAB here. In Michigan during 2003 and 2004, research on EAB natural enemies demonstrated 0.7% EAB larval parasitism. The larval parasitoids reared from almost 3,000 EAB larvae included three native parasitoids: *Phasgonophora sulcata* (Hymenoptera: Chalcididae), *Spathius floridanus* (Hymenoptera: Braconidae), *Atanycolus* sp. (Hymenoptera: Braconidae), and one exotic ectoparasitoid, *Balcha indica* (Hymenoptera: Eupelmidae). This parasitism rate for EAB in the United States is low when compared to those reported in the literature for native *Agrilus* spp. and for EAB in China (Liu et al., 2003; Yang et al., 2005). Should *T. planipennisi* become introduced and established in North American EAB populations, its impact on these incidental parasitoids will be insignificant because their primary hosts attack different host trees (Bauer et al., 2004; 2005).

Life history (including dispersal capability and damage inflicted on EAB). *T. planipennisi* is a gregarious larval endoparasitoid; host larvae continue feeding after parasitoid oviposition until external symptoms of parasitism appear. Symptoms include bulges on the surface of the host integument (outer covering of the body), causing the host cadaver to look like a small braided rope. The larval parasitoids are then mature and ready to exit the host cadaver during their wandering phase just prior to pupation. Parasitized host larvae produce a range of 5 to 122 parasitoids, with an average of 35.2 parasitoid larvae per host. *T. planipennisi* attack both third- and fourth-instar EAB larvae, and overwinters as mature larvae in EAB galleries (Liu et al., in press). In the spring, the parasitoid larvae form naked pupae, emerge as adults, and chew a 1-mm diameter hole through the bark from which the wasp brood exits the host gallery and tree.

In the laboratory, the egg-larval period for *T. planipennisi* is about 11 days and its pupal period is ca. 10 days (Table 2). Adult longevity of females is around 24 days, almost twice that of males, and one generation requires approximately three weeks. Their sex ratio is 3.5:1 (female:male), and

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average fecundity is 42 progeny/female. Adult parasitoids are maintained with a streak of honey and moist cotton ball during exposure to host larvae. Female parasitoids will not oviposit unless host larvae are inserted in ash branches.

Table 2. The biology of *T. planipennisi* reared in the laboratory at 25ºC (Bauer, 2007b).

Stage	Egg-larval	Pupal		Adult	
		Female	Male	Female	Male
Duration (d)	11.3 ± 4.2	10.3 ± 1.5	10.2 ± 2.3	23.6 ± 19.2	14.0 ± 10.3
Sex ratio	3.5 : 1 (female : male)				
Fecundity	42.4 ± 4.5				

The biological attributes of *T. planipennisi*, including female-biased sex ratio, short generation time, and high rates of parasitism and reproduction, are characteristics of successful biocontrol agents (Kimberling, 2004). No data are available on the dispersal capability of *T. planipennisi,* but despite their small size adult females live more than three weeks; both sexes are active jumpers and fliers in the laboratory. Dispersal, however, may be facilitated by selecting release sites with connecting corridors of ash, such as along riparian areas.

Known host organisms based on valid literature records, host data from museum specimens, and unpublished records. This larval endoparasitoid was discovered in China in 2003. Specimens were submitted to Drs. Zhang and Huang, Chinese Academy of Science, Beijing, China for taxonomic description. Drs. Yang and Wang, Chinese Academy of Forestry, Beijing, China, eventually described this EAB parasitoid as *T. planipennisi* (Yang et al., 2006).

History of past use of *T. planipennisi*. *Tetrastichus planipennisi* has never been used as a biocontrol agent.

Pathogens, parasites, hyperparasitoids of agent and how to eliminate them from a culture of the agent. No pathogens, parasitoids, or hyperparasitoids have been observed attacking *T. planipennisi*, either in observations in China or in specimens shipped to the United States. Insects used to establish colonies or to release in the field will be allowed to emerge as adults in quarantine and only healthy, unparasitized adults will be used.

Other closely related genera, sibling species or closely-similar species

in North America. *Tetrastichus* is large and diverse genus awaiting further taxonomic clarification. Several species in this genus have been introduced as biocontrol agents of other invasive species including *Tetrastichus asparagi* for the common asparagus beetle, *Crioceris asparagi* (Coleoptera: Chrysomelidae) (Capinera and Lilly, 1975).

IV. Affected Environment

A. North American *Agrilus* species

The "Nomina Insecta Nearctica; a check list of the insects of North America" (Poole, 1997) lists 164 species of *Agrilus* in North America (see appendix 1). Most are not considered pests; however, Solomon (1995) considers 24 *Agrilus* species to cause injury to trees and shrubs under certain circumstances. Most species of *Agrilus* are unable to colonize healthy trees; in fact, EAB is not considered a pest throughout its native range. *Agrilus* species typically attack trees stressed by factors such as drought, damage from other insects, or poor silvicultural practices. Species such as *A. anxius* (bronze birch borer) and *A. bilineatus* (Weber) (two-lined chestnut borer) are often considered major pest species in forest and landscape situations, but they are typically acting as secondary pests on already stressed trees or trees not native to the United States. *Agrilus* species in North America could potentially be at risk from attack by the three parasitoids proposed for environmental release.

B. Ash Resources of North America

Hosts of EAB: The known hosts of EAB in the United States are ash trees (*Fraxinus* spp.) including *F. americana* (white ash), *F. nigra* (black ash), *F. pennsylvanica* (green ash), and some varieties of horticultural ash. Twenty-two species of ash grow in the United States, of which sixteen are native (USDA, NRCS, 2006). See appendix 2 for distribution maps of native *Fraxinus* species in the United States. There is increasing evidence that EAB will attack all *Fraxinus* spp., although innate susceptibility varies by species and variety (Liu et al., 2003; Wie et al., 2004; Rebek et al., 2006; Liu et al., in press). The FS estimates that 7,553,000,000 ash trees in United States timberlands are potentially susceptible to EAB (USDA, FS, 2004).

Ash trees are present as ornamentals, street trees, or timber trees in all of the lower 48 states. Each *Fraxinus* sp. is adapted to slightly different habitats within forest ecosystems. Several species are tolerant of poorly-drained sites and wet soils, protecting environmentally-sensitive riparian areas; e.g. pure stands of black ash grow in bogs and swamps in northern areas where they provide browse, thermal cover, and protection for wildlife such as deer and moose. In agricultural and shelterbelt areas, ash provides vital shelter for livestock; e.g. ca. 25% of all trees in North Dakota are *Fraxinus* spp. Bark of young ash trees is a favored food of mammals including beaver, rabbit, and porcupines; older trees provide habitat for cavity-nesting birds such as wood ducks, woodpeckers, chickadees, and nuthatches; seeds are consumed by ducks, song and game birds, small mammals, and insects.

Ash timber is valued for applications requiring strong, hardwood, but with less rigidity than maple. In the Eastern United States, a net volume of 114 billion board feet of ash sawtimber is harvested, comprising 7.5% of the volume of all hardwood species (FR, 2003). The impact varies by state, but in Michigan alone an estimated 7.7 billion board feet of ash timber is harvested annually. In 2001, ash accounted for over 149 million board feet of timber products produced in the United States. White ash is the primary commercial hardwood used in production of tool handles, baseball bats, furniture, flooring, containers, railroad cars and ties, canoe paddles, snowshoes, boats, doors, and cabinets; green ash is used for both solid wood applications such as crating, boxes, handles, and for fiber in the manufacture of high grade paper; black ash is typically used for interior furniture, cabinets (FR, 2003), and Northeastern Native Americans require ash for the art of basketry.

Beyond manufacturing, ash trees play an important role in the urban landscape due to their historical resistance to pests and tolerance of adverse growing conditions, such as soil compaction and drought. Many of the ash trees that now serve as street, shade, and landscape trees were planted to replace elm trees destroyed by Dutch elm disease; ash trees now comprise 5-20% of all street trees throughout North America. In the United States, urban areas cover about 3.5% of the total land area, contain more than 75% of the population, and support about 3.8 billion trees. The City of Chicago has approximately 603,000 ash trees that provide 14.4% of leaf area (FR, 2003). Trees are considered vital to the health of cities because they sequester gaseous air pollutants and particulate matter, help people conserve energy through the shade they provide, assist in the dispersal of storm water, provide shelter belts for urban fauna, and contribute aesthetic pleasure to the lives of city-dwellers and tourists. Ash is a vital component of the urban forest.

V. Environmental Consequences

A. No Action Alternative

EAB is an invasive wood-boring beetle that is spreading rapidly and poses a serious threat to ash trees in the United States if not controlled. Despite state and federal quarantines designed to contain EAB, the lack of effective methods to detect EAB-infested trees and the large size of the infestation has resulted in a shift by regulatory agencies from a strategy of area-wide eradication to one focused on eradication in outlying areas and containment in the core infestation area (GAO, 2006). In the United States, EAB eradication efforts involved the removal of all ash trees within a circle of specified radius around known infestations (typically ½ mile). By the time an infestation was discovered and treated, however, EAB had usually already dispersed outside the eradication zone. The bronze birch borer, *A. anxius* Gory, a native species closely related to EAB, is known to spread at a rate of 10 to 20 miles per year, and this has been proposed as an estimate for EAB's natural dispersal rate (FR, 2003). Besides natural dispersal, the spread of EAB has been accelerated through human-assisted movement of infested ash firewood, timber, solid-wood packing materials, and nursery stock. This resulted in the spread of EAB from Michigan to Maryland and Virginia in 2003. As EAB spreads throughout North America, regulatory agencies, land managers, and the public are seeking sustainable management tools to reduce EAB population densities and to slow its spread (Cappaert et al., 2005; GAO, 2006; Poland and McCullough, 2006). Since its discovery, EAB has killed more than 20 million ash trees in Michigan, Ohio and Indiana (MSU, 2007).

The potential path of expansion of the EAB infestation is through Ohio and Indiana into the hardwood forests of the Northeast through Pennsylvania and New York and into the Appalachian Mountain States through Kentucky. In addition, spread of the pest through the Upper Peninsula of Michigan to Wisconsin and through Ontario to New York is possible. The economic impact would be severe if EAB spread from currently infested areas into the forests of the northeastern United States where nursery, landscaping, timber, recreation, and tourism industries are economically critical.

Michigan implemented a moratorium on importing and selling ash nursery stock in the Lower Peninsula of the State, impacting at least 9,519 nurseries (McPartlan et al., 2006). The State's 1,847 logging companies and sawmills were also affected by their inability to receive ash logs from the quarantined area (McPartlan et al., 2006). Additionally, more than 2,500 private campgrounds in the tri-state area have been impacted; many are losing business when campers are told that they cannot bring firewood from quarantined areas (McPartlan et al., 2006). The area also supports industries that utilize ash for tool handles, rail road ties and pallet production.

The continued spread of this pest would threaten these resources and permanently alter the Midwest's forest ecosystem that in some areas is made up of 20 to 40 percent ash (McPartlan et al., 2006). In addition to its value in forest ecosystems and for the timber industry, ash has become an extremely popular urban/suburban landscape tree because of its tolerance of less than ideal planting conditions and resistance to gypsy moth and other pests. It is currently the most commonly planted tree in new residential and commercial developments. Ash was planted widely in Midwestern States to replace elms lost to Dutch elm disease, and it is common in parks, public spaces, and neighborhoods across the United States. The potential national impact of EAB on the urban environment alone is 0.5 to 2 percent loss of total leaf area, or 30-90 million trees with a loss of $20-60 billion dollars (McPartlan et al., 2006).

The spread of EAB infestations could potentially have an enormous impact on the U.S. nursery industry, municipal governments, and individual home owners. As many as 300 million landscape ash trees have been planted in Michigan alone. Removal and replacement costs would be staggering. In an initial economic analysis of EAB, the USDA, FS estimated that EAB, if not contained and eradicated, could cause approximately $7 billion in additional costs to state and local governments and landowners to remove and replace dead and dying ash trees in urban and suburban areas over the next 25 years (McPartlan et al., 2006). One city in Michigan reported substantially higher water and electrical use because of the loss of ash as shade trees (Victor Mastro, personal communication).

B. Biological control alternative (preferred alternative)

1. Environmental and Economic Impacts of the Proposed Release of *S. agrili*

Known impact on vertebrates including humans: *Spathius agrili* is an obligate parasitoid of wood boring larvae, specifically the EAB. As such, it will rarely come into contact with humans or other vertebrates, and if it does, it is incapable of stinging or biting. Braconid wasps have no known adverse impacts on humans or other vertebrates.

Direct impact of *S. agrili* (e.g. intended effects on EAB, direct effects on non-targets). Percentage parasitism by *S. agrili* in China can reach 30-50%, and in a few stands 85-90% of the EAB are parasitized (Yang et al., 2005). Because *S. agrili* has three generations per year compared with just one for EAB, generational percentage parasitism will be greater than that measured during a single collection. *Spathius agrili* clearly has the potential to cause considerable mortality to EAB populations. In China, it is probable that EAB is not typically a pest because of the interaction between host plant resistance and natural enemies. While it is clear that American ash species are not resistant to EAB per se, they can withstand some attack as evidenced by callusing of EAB galleries (Gould, 2007). In China, where *F. pennsylvanica* (native to North America) was attacked by EAB, parasitism by *O. agrili* and *T. planipennisi* reduced EAB populations by 74% and the trees continued to produce fruit. *Spathius agrili* will not have to cause 100% mortality of EAB to impact the health of ash stands, because ash can successfully withstand some attack (Gould, 2007).

To evaluate the effects of *S. agrili* on non-target insect species, no-choice host specificity tests were conducted in China and the United States to determine the physiological host range of *S. agrili* and possible direct effects on non-target species. In 2003-2005, potential host larvae were collected in the ash forest where *S. agrili* was attacking EAB in Tianjin, China to determine whether *S. agrili* would attack other species in the same forest habitat. All species tested were boring insects, including some that were closely related to EAB and others that were not.

Spathius agrili finds hosts to parasitize by hearing sounds or feeling vibrations produced by feeding larvae. Studies have shown that they do not attack prepupae or molting larvae that are not feeding. All test larvae, therefore, had to be feeding inside their natural hosts during testing. To accomplish this, 1-1.5 cm diameter twigs were split longitudinally and a 3 cm long groove was cut through to the bark on one side. The test larvae were placed in these chambers, and the twigs were reassembled and secured with rubber bands. The ends of the twigs were sealed with paraffin wax to prevent desiccation. The twigs were placed into 11.5 by 2.8 cm diameter glass vials that contained a newly eclosed mated *S. agrili* female. The parasitoids were fed honey streaked on the side of the vial. In tests in the United States, the larvae were checked weekly for the presence of *S. agrili* eggs or larvae until the parasitoid females died. In China the twigs were only checked after the female died. All parasitized larvae were held to determine if *S. agrili* could develop to the adult stage.

In the early host specificity tests, *S. agrili* did not parasitize wood boring Lepidoptera, a longhorned beetle (Cerambycidae), or the one *Agrilus* species tested (Table 3). Of the three species whose larvae attack ash, only EAB was parasitized. Further testing was, therefore, confined to members of the genus *Agrilus*, which might be at risk because they are closely related to EAB. In the United States, the two-lined chestnut borer, *A. bilineatus,* in oak, and the bronze birch borer, *A. anxius*, in birch were tested. These species were chosen because oak and birch can be found in close proximity to ash, they could be collected in reasonable numbers for testing, and they are two *Agrilus* species that sometimes occur in outbreak situations.

Table 3. No-choice host specificity testing from 2003-2005 in China on boring larvae collected in the same ash plantation forest as *S. agrili*. There was no attack on non-target species (Gould, 2007).

Species	Order and Family	Host Plant used in testing	N	Percentage Parasitism	Outcome
Agrilus planipennis	Coleoptera: Buprestidae	*Fraxinus velutina*	30	33%	Adults produced
Agrilus auriventris	Coleoptera: Buprestidae	*Citrus reticulata*	30	0%	No attack
Ostrinia orientalis	Lepidopera: Pyralidae	*Xanthium sibiricum*	30	0%	No attack
Chilo luteellus	Lepidoptera: Pyralidae	*Phragmites communis*	30	0%	No attack
Holcocerus insularis	Lepidoptera: Cossidae	*Fraxinus velutina*	30	0%	No attack
Sylepta derogata	Lepidoptera: Pyralidae	*Gossypiμm herbaceμm*	30	0%	No attack
Carposina niponensis	Lepidoptera: Carposinidae	*Zizyphus jujuba*	30	0%	No attack
Thyestilla gebleri	Coleoptera: Cerambycidae	*Abutilon theophrasti*	30	0%	No attack
Pyralid larva	Lepidoptera: Pyralidae	*Fraxinus velutina*	30	0%	No attack

In China, *S. agrili* attacked *A. zanthoxylumi, A. mali,* and *A. inamoenus*, but at rates that were lower than attack on EAB (Table 4). Adult *Spathius* were produced on *A. mali*, and *S. agrili* developed to the pupal stage on *A. inamoenus*, but mites attacked and killed the pupae before adults could emerge. *Spathius agrili* developed through the pupal stage on *A. zanthoxylumi,* but they seem to have entered diapause and may emerge as adults in the spring. No attack occurred on other *Agrilus* species, *Sphenoptera* sp. or *Eucryptorrhynchus chinensis*.

Spathius agrili attacked some species of *Agrilus* other than EAB in the no-choice tests, but attack rates were significantly lower than for its primary host, the EAB (Table 4). Two out of thirty *A. bilineatus* were attacked and both attacks resulted in cocoons but only two adult males were reared from one egg cluster, and the other egg cluster produced cocoons, but no adults emerged. However, this indicates that *A. bilineatus* could serve as a potential host of *S. agrili.* No *S. agrili* larvae survived on the single parasitized *A. anxius*.

Table 4. Host specificity testing in 2006 in China and the United States. All species except *Eucryptorrhynchus* (weevil) are buprestid beetles. Attack rate on all non-target species was significantly less than the control (EAB) (Fisher's Exact Test) (Gould, 2007).

Species	Host Plant used in testing	N	Percentage Parasitism	Outcome
Agrilus planipennis United States	*Fraxinus americana*	30	27%	Adults produced
Agrilus bilineatus	*Quercus alba*	30	7%	Adult males only
Agrilus anxius	*Betula papyrifera*	30	3%	No larval survival
Agrilus planipennis China	*Fraxinus velutina*	23	65%	Adults produced
Agrilus zanthoxylumi	*Zanthoxylum bungeanum*	31	32%	Cocoons produced – too early for adults
Agrilus mali	*Malus micromalus*	30	27%	Adults produced
Agrilus inamoenus	*Citrus reticulata*	15	7%	Cocoons produced but attacked by mites
Agrilus sorocinus	*Albizzia julibris*	15	0%	No attack
Agrilus lewisiellus	*Juglans regia*	26	0%	No attack
Sphenoptera sp.	*Artemisia ordosica*	30	0%	No attack
Eucryptorrhynchus chinensis	*Ailanthus altissima*	5	0%	No attack

No-choice tests determine the physiological host range of a parasitoid by giving them no other option but to oviposit on a non-target host. They are not allowed to accept or reject the host plant inhabited by potential non-target hosts. To determine the ecological host range of *S. agrili*, olfactometer tests were conducted in China to investigate whether *S. agrili* is attracted to plant species harboring the larvae tested in no-choice tests. Naïve mated *S. agrili* females were placed in vertical y-tube olfactometers and given a choice of leaves and twigs of various host plants or clean air. *Spathius agrili* was only attracted to *F. pennsylvanica*, *F. velutina*, and *Salix babylonica* (willow) (Table 5). The two *Fraxinus* species are native to the United States. Even though some attack occurred on larvae found in *Citrus reticulata, Malus micromalus*, and *Zanthoxylum bungeanum* in no-choice tests, *S. agrili* females were not attracted to these tree species. In nature, if parasitoids are not attracted to the host tree they are unlikely to encounter and parasitize the non-target larvae. *Spathius agrili* was attracted to willow leaves, and at least three *Agrilus* species attack willow in the United States: *Agrilus pratensis pratensis* (adults 4-6 mm long), *A. politus* (adults 5.0-8.5 mm long) and *A. quadriguttatus* (size could not be found in literature). These insects are quite small compared with adult EAB, which are 8.5-13.5 mm long. *Spathius agrili* only attacks large EAB larvae, and *A. pratensis* and *A. politus* undoubtedly are too small to be at risk of attack. Even if *S. agrili* is attracted to willow in the United States, it is unlikely to encounter suitable non-target hosts.

That some attack by *S. agrili* on non-target species occurred is not totally surprising given that the native *S. floridanus* does attack EAB in the United States. However, parasitism of EAB by this native *Spathius* is extremely low, well below 0.5%, despite an abundance of EAB. This low level of parasitism is certainly not sufficient to affect EAB population growth or dynamics and it is not expected that *S. agrili* will significantly impact populations of native *Agrilus*.

A fourth piece of evidence concerning host specificity was gathered in China by collecting larvae of six *Agrilus* species and rearing them to determine their parasitoid fauna. A total of 2,074 *Agrilus* larvae of six species were collected and not a single *S. agrili* was recovered (Table 6).

Given the combination of evidence from no-choice tests (lower parasitism rates or no attack on non-target species), olfactometer tests (only attracted to ash and willow), the lack of *S. agrili* reared from other *Agrilus spp.* in China, and the fact that a native *Spathius* species rarely attacks EAB, the release of *S. agrili* is not expected to have adverse direct effects on non-target species.

Table 5. Response of *S. agrili* females to twigs and leaves of trees that are hosts of *Agrilus* species tested in China. * indicates that *S. agrili* was either significantly attracted to or repelled by the test stimulus (Gould, 2007).

Tree species	Wasps towards stimulus	Wasps away from stimulus	Potential insect host	p value
F. pennsylvanica	29	9	*A. planipennis*	0.0008*
F. velutina	27	8	*A. planipennis*	0.0003*
Salix babylonica	23	12	*A. rotundicollis*, *A. viridis*, *Meliboeus cerskyi*	0.0205*
Citrus reticulata	15	16	*A. auriventris, A. inamoenus*	0.5000
Malus micromalus	19	17	*A. mali*	0.6911
Zanthoxylum bungeanum	23	15	*A. zanthoxylumi*	0.1279
Juglans regia	14	22	*A. lewisiellus*	0.1215
Albizzia julibrissin	18	18	*A. sorocinus*	0.4340
Pyrus bretschneideri	15	15	*Lampra limbata*	0.5722
Prunus persica	18	20	*Ptosima chinensis*	
Populus deltoides	18	17	*Melanophila picta, Poecilonota variolosa*	0.6321
Crataegus pinnatifida	14	18	*Caraebus* sp.	0.2983
Euonymus japonica	17	18	*A. nakanei*	0.5000
Ailanthus altissima	10	23	*Eucryptorrhynchus brandti*	0.0175*

Table 6. Parasitoids emerging from *Agrilus* species collected in China. No *S. agrili* were reared (Gould, 2007).

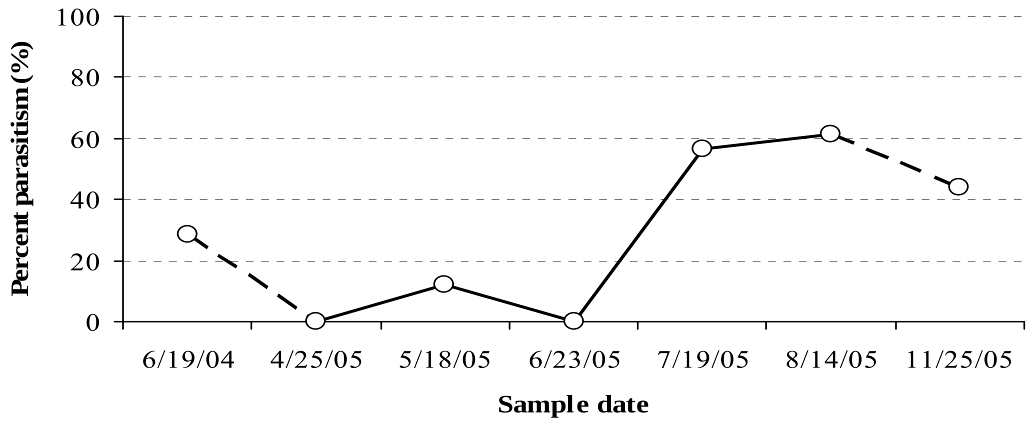
Agrilus species	Collection Province	Number larvae collected	Parasitoids recovered
A. mali	Shaanxi	427	*Tetrastichus* sp and *Doryctes* sp.
	Xinjiang	502	*Atanycolus* sp. and *Eupelmus* sp
A. lewisiellus	Shaanxi	227	*Tetrastichus* sp.
A. zanthoxylumi	Shaanxi	515	*Tetrastichus* sp. and Braconidae
A. sorocinus	Tianjin	176	*Tetrastichus* sp.
A. auriventris	Zhejiang	87	*Spathius* sp. not *agrili*
A. inamoenus	Hunan	140	No parasitoids

2. Environmental and Economic Impacts of the Proposed Release of *O. agrili*

Known impact on vertebrates including humans: *Oobius agrili* is a very small (ca. 0.9 mm) obligate egg parasitoid of a wood boring insect, specifically the emerald ash borer. As such, it will rarely come into contact with humans or other vertebrates, and if it does, it is incapable of stinging or biting. Encyrtid wasps have no known adverse impacts on humans or other vertebrates.

Direct impact of *Oobius agrili* (e.g. intended effects on targets, direct effects on non-targets). After discovery of *O. agrili* in China in 2004, its effects on EAB populations were quantified by sampling EAB eggs monthly from April through August in an infested ash plantation in Jilin Province; samples in November 2005 were taken to determine its overwintering stage (Liu et al., in press). Based on the number of *O. agrili* adults successfully emerging from EAB eggs after arrival in the FS quarantine laboratory in Michigan, parasitism rates reached 56.3% parasitism in July and 61.5% in August (Figure 4). The number of parasitoids reared from November collections vs. numbers reared from spring collections suggests that overwintering mortality does occur. Year-to-year variations in parasitism rates can be observed by comparing parasitism rates from samples taken in June 2004 and those taken June 2005. This variation may be caused by differences in the overwintering survival of *O. agrili,* developmental rates EAB populations, and other biotic/abiotic factors.

Figure 4. Parasitism (%) by *O. agrili* of field-collected EAB eggs from Jilin Province, China in June 2004 (time of discovery) and during seasonal sampling in 2005 (Bauer, 2007a).



To evaluate the direct effects of *O. agrili* on non-target insect species, no-choice assays were performed in the laboratory using eggs of six *Agrilus* spp., two cerambycids, and four lepidopterans (Table 7). These insects were selected based on taxonomic similarity to EAB; overlap in habitat and/or niche with EAB; risk to beneficial, threatened, or endangered insects; feasibility of acquiring or rearing enough eggs to perform and replicate reliable assays (Badendreier et al., 2005).

Table 7. Oviposition by *O. agrili* females during no-choice assays after exposure to 0- to 6-day old eggs of each species (Bauer, 2007a).

Order/Family	Species	Host	Egg size (mm)	Oviposit?
Coleoptera				
Buprestidae	*Agrilus planipennis* (control)	Ash	1.4 x 1.0	Yes
	A. anxius	Birch	1.3 x 0.7	Yes
	A. bilineatus	Oak	1.2 x 0.8	Yes
	A. ruficollis	Raspberry	1.1 x 0.7	Yes
	A. cyanescens	Oak	1.0 x 0.6	No
	A. egenus	Black locust	0.6 x 0.6	No
	A. subcinctus	Ash	0.6 x 0.4	No
Cerambycidae	*Neoclytus acuminatus*	Ash	1.2 x 0.5	No
	Megacyllene robiniae	Locust	2.4 x 1.0	No
Lepidoptera				
Tortricidae	*Choristoneura rosaceana*	Apple	0.5 x 0.5	No
Pieridae	*Pieris rapae*	Cabbage	1.0 x 0.5	No
Bombycidae	*Bombyx mori*	Mulberry	1.2 x 1.2	No
Sphingidae	*Manduca sexta*	Tobacco	1.4 x 1.4	No

In no-choice assays, *O. agrili* did not oviposit in eggs of the cerambycids and lepidopterans (Table 7). *O. agrili* may oviposit and develop in *Agrilus* eggs from different species, but only those with eggs similar in size to EAB eggs (Table 7). These included *A. anxius* (bronze birch borer), *A. bilineatis* (two-lined chestnut borer), and *A. ruficollis* (red-necked cane borer), which are considered aggressive pests of birch, oak, and raspberry, respectively. Although many native *Agrilus* spp. are considered destructive pests of forest and shade trees, most prefer stressed, weakened, or dying trees.

Paired no-choice and choice assays were performed (Badendreier et al., 2005) for two of the *Agrilus* spp. accepted by *O. agrili* during the no-choice assays. The results of the choice assays demonstrate that *O. agrili* shows a clear preference for EAB (*A. planipennis*) eggs over those of *A. anxius* (Figure 5) and *A. ruficollis* (Figure 6).

Figure 5. Paired no-choice and choice assays to compare oviposition (%±SD) rates of *O. agrili* on *A. anxius* eggs (Bauer, 2007a).

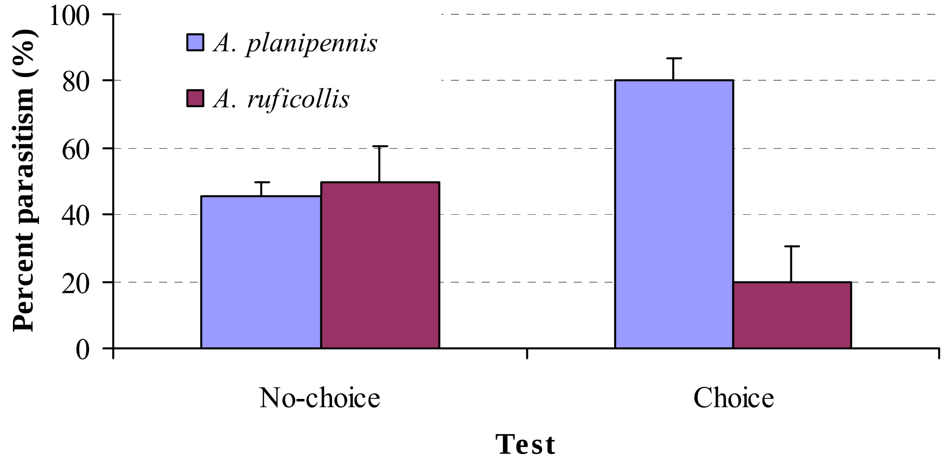
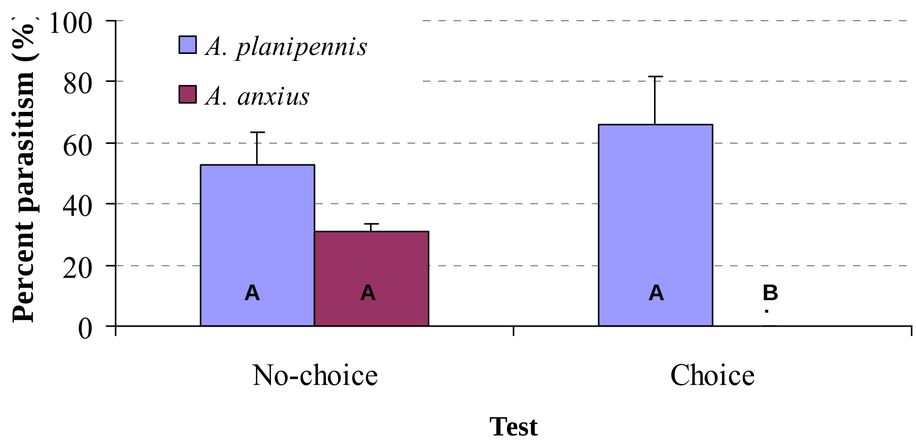


Figure 6. No-choice and choice assays to compare oviposition (%±SD) rates of *O. agrili* on *A. ruficollis* eggs (Bauer 2007a).

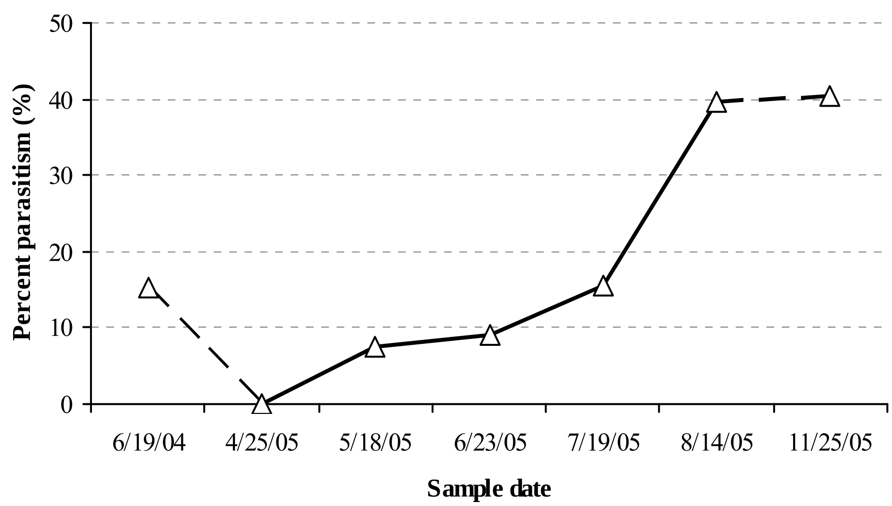


3. Environmental and Economic Impacts of the Proposed Release of *T. planipennisi.*

Known impact on vertebrates including humans: *Tetrastichus planipennisi* is small (females 4-5 mm; males 2-3 mm) and gregarious larval endoparasitoid of EAB. As such, it will rarely come into contact with humans or other vertebrates, and if it does, it is incapable of stinging or biting. Eulophid wasps have no known adverse impacts on humans or other vertebrates.

Direct impact of *T. planipennisi* (e.g. intended effects on EAB, direct effects on non-targets). After discovery of *T. planipennisi* in China in 2003 (Liu et al., 2003), effects on EAB populations were quantified by sampling EAB larvae monthly from April through August in an infested ash plantation in Jilin Province; samples in November 2005 were taken to determine its overwintering stage (Liu et al., in press). *T. planipennisi* completes four generations per year. In 2005, parasitism averaged 22.4% and peaked at approximately 40% in August (Figure 7). Year-to-year variations in parasitism rates can be observed by comparing parasitism rates from samples taken in June 2004 and those taken in June 2005. This variation may be caused by differences in the overwintering survival of *T. planipennisi,* developmental rates of EAB populations, and other environmental factors.

Figure 7. Seasonal abundance of *T. planipennisi* parasitizing *A. planipennis* (EAB) larvae in Jilin Province, 2004-2005 (Bauer, 2007b; Liu et al., in press).



It was also found that third- and fourth-instar EAB larvae are available as hosts throughout spring, summer, and fall for emerging *T. planipennisi* adults, allowing for a rapid population build-up during the summer (Liu et al., in press). Similar distributions in EAB larval stages are observed in Michigan as populations increase throughout an area (Cappaert et al., 2005).

To evaluate the direct effects of *T. planipennisi* on potential non-target insect species, no-choice assays were performed in the laboratory with larvae of EAB and eight species of buprestid (five *Agrilus* and three *Chrysobothris*), five cerambycids, two lepidopterans, and one hymenopteran (Table 8). These insects were selected based on taxonomic similarity to EAB; overlap in habitat and/or niche with EAB; risk to beneficial, threatened, or endangered insects; feasibility of acquiring or rearing enough larvae to perform and replicate reliable assays (Badendreier et al., 2005).

Table 8. Oviposition by *T. planipennisi* during no-choice assays after exposure to last-instar larvae of the following species (Bauer, 2007b).

Order/Family	Species	Host	Accepted?
Coleoptera			
Buprestidae	*Agrilus planipennis*	Ash	Control
	A. subcinctus	Ash	No
	A. anxius	Birch	No
	A. bilineatus	Oak	No
	A. ruficollis	Raspberry	No
	A. putillus	Maple	No
	Chrysobothris femorata	Apple	No
	C. floricola	Pine	No
	C. sexsignata	Pine	No
Cerambycidae	*Neoclytus acuminatus*	Ash	No
	Megacyllene robiniae	Locust	No
	Astylopsis sexguttata	Pine	No
	Monochamus scutellatus	Pine	No
	Unknown	Red Maple	No
Tenebrionidae	*Tenebrio molitor*	Grains	No
Lepidoptera			
Pyralidae	*Galleria mellonella*	Beeswax	No
Sphingidae	*Manduca sexta*	Solanaceae	No
Hymenoptera			
Cephidae	*Janus abbreviatus*	Willow	No

The results of the no-choice assays of *T. planipennisi* indicate that this parasitoid is highly specific to EAB (Table 8), thus choice assays were not performed.

4. Effects on the Physical Environment and Indirect Effects of the Release of the Three Parasitoids

Effects on physical environment (e.g. water, soil and air resources): Trees in the genus *Fraxinus* are important components of many forested ecosystems throughout North America and are planted extensively as urban and shelterbelt trees. USDA Forest Service Forest Inventory and Analysis estimates that establishment of EAB throughout the United States could result loss of approximately 2.6% of trees in our timberlands, or 8 billion trees (USDA, FS, 2004). White, blue, and Oregon ash grow on fertile uplands and river terraces; green, black, Carolina, and pumpkin ash are wetland species; and velvet and single-leaf ash grow in semi-deserts and canyons. If EAB populations are not managed, ash resources throughout North America could be devastated (MacFarlane and Meyer, 2005). The loss of ash over large geographical areas will adversely affect water, soil, and air resources. Ash is an important riparian tree and is often found along river banks. Removing ash from stream banks will likely affect bank soil retention and stream processes. The successful deployment of EAB biocontrol agents such as *S. agrili, O. agrili,* and *T. planipennisi* will have a positive impact on the physical environment by moderating EAB population increase, thus limiting tree damage.

Indirect effects (e.g. potential impacts on organisms that depend on EAB or non-target species including potential competition with resident biological control agents). Successful management of EAB using biological control agents, including *S. agrili, O. agrili,* and *T. planipennisi,* will result in positive, indirect effects on U.S. municipalities, land owners, wood industries, Native American basketry, forest biodiversity, wildlife, riparian areas, and organisms dependent on *Fraxinus* spp. (e.g. the cerambycid: red-headed ash borer, *Neoclytus acuminatus;* the sphingid: great ash sphinx, *Sphinx cheri*; the sesiids: ash clearwing, *Podesia syringae* and banded ash clearwing, *P. aurecincta*; the scolytids: eastern, westren, and northern ash beetles, *Hylesinus aculeatus, H. californicus,* and *H. criddlei*).

Indirect effects to ongoing biological control projects that utilize *Agrilus* species to control weeds should also be considered. *Agrilus hyperici* was released against Klamath weed in the western United States with mixed results. In northern Idaho it is beneficial in assisting two Klamath weed beetles, *Chrysolina* sp., in controlling the target weed (Campbell and McCaffery, 1991), but in California it has been displaced by *C. quadrigemina* (McCaffrey et al., 1995). *Agrilus hyperici* is a root feeder acting on a rangeland weed. Another exotic buprestid, *Sphenoptera jugoslavica*, also a root feeder, was released against spotted, diffuse, and squarrose knapweeds. Based on the results of research in the field in China and laboratory host specificity studies, *S. agrili, O. agrili,* and *T. planipennisi* are host specific, and are not expected to attack other buprestid species.

5. Uncertainties Regarding the Environmental Release of *S. agrili, O. agrili,* and *T. planipennisi.*

Once biological control agents such as *S. agrili, O. agrili,* and *T. planipennisi* are released into the environment and become established, there is a slight possibility they could move from the target insect (EAB) to attack nontarget insects, such as native *Agrilus* species. Native species that are closely related to the target species are the most likely to be attacked (Louda *et al.*, 2003). If other insect species were to be attacked by *S. agrili, O. agrili,* or *T. planipennisi*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *S. agrili, O. agrili,* and *T. planipennisi* generally spread without intervention by man. In principle, therefore, release of these parasitoids at even one site should be considered equivalent to release over the entire area in which potential hosts occur and in which the climate is suitable for reproduction and survival. Post-release evaluations of *S. agrili, O. agrili,* and *T. planipennisi* populations and their effects on EAB and other non-target species will be conducted by APHIS and FS researchers.

In addition, these agents may not be successful in reducing EAB populations in the continental United States. Approximately 12% of all parasitoid introductions have led to significant sustained control of the target pests, but the majority of introductions have failed to provide control of the pest (Greathead and Greathead, 1992) either because introduction did not lead to establishment or establishment did not lead to control (Lane et al., 1999). Actual impacts on EAB populations by *S. agrili, O. agrili,* and *T. planipennisi* will not be known until after release occurs and post-release monitoring has been conducted. The environmental consequences discussed under the "no action" alternative may occur even with the implementation of the biological control alternative, depending on the efficacy of those agents to reduce EAB populations in the continental United States.

6. Cumulative Impacts

"Cumulative impacts are defined as the impact on the environment which results from the incremental impact of the action when added to other past, present and reasonably foreseeable future actions regardless of what agencies or person undertakes such other actions" (40 CFR 1508.7).

Currently, no other biological control agents have been released against EAB in the continental United States, although hundreds, if not thousands of parasitoid species have been released in the United States to attack various insect pests such as mealybugs, aphids, whiteflies, and agriculturally important lepidopteran pests.

APHIS has put quarantines in place to prevent the movement of EAB out of EAB-infested areas of the United States (7 CFR Subpart 301.53). This area may expand as new infestations are discovered. Quarantines are put in place to prevent the artificial spread of EAB through movement of infested firewood or other infested wood materials.

APHIS, in cooperation with the appropriate State Departments of Agriculture, conducts an EAB management program that includes survey for EAB to determine new areas of infestation, and eradication of EAB by cutting infested ash trees in outlying areas.

Release of the proposed parasitoids will have no negative cumulative impacts in the continental United States because of the host specificity of the parasitoids to EAB. Effective biocontrol of EAB will have beneficial effects to current EAB management activities, and may result in protection of ash resources and reduction in removals of infested trees.

7. Endangered Species Act

Section 7 of the Endangered Species Act (ESA) and ESA's implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of federally listed threatened endangered species or result in the destruction or adverse modification of critical habitat.

A direct effect of the release of these parasitoids would be the potential attack of eggs or larvae of federally listed insects, resulting in additional declines of these species. In particular, the valley elderberry longhorned borer (*Desmocerus californicus dimorphus* Fisher) (Family Cerambycidae), a longhorned borer that mines the interior wood of its host plant (Paine et al., 2004) could be perceived to be at risk by these parasitoids.

During host specificity testing, *S. agrili* did not attack any non-target species other than some *Agrilus* species. The larvae of the valley elderberry longhorned beetle would not be accessible to the relatively short ovipositor of *S. agrili*. In addition, this is not closely related to EAB and would not likely be accepted as a host by *S. agrili*. *Spathius agrili* must attack its hosts, even EAB, as they are feeding inside of wood, so it is unlikely to attack any other threatened or endangered insects, which are all external feeders.

During laboratory host-specificity testing, *O. agrili* rejected the eggs of cerambycids tested: red-headed ash borer (*Neoclytus acuminatus*) eggs on ash and locust borer (*Megacyllene robiniae*) on black locust. The eggs of the valley elderberry longhorned borer are about 2.5-3.0 mm long and are shaped like a football with longitudinal ridges, whereas EAB eggs are ca. 1 mm in diameter and flat, oppressed between bark layers or in bark crevices. Moreover, a closely related encyrtid, *Avetienella longoi*, was released against the eucalyptus longhorned borer, *Phoracantha semipunctata*, in California where no impact on the valley elderberry longhorned beetles has been found. In addition, *O. agrili* did not oviposit in eggs of Lepidoptera and Coleoptera, other than *Agrilus* eggs of similar size to EAB.

During laboratory host-specificity testing, *T. planipennisi* rejected larvae of five cerambycids tested: *Astylopsis sexguttata, Neoclytus acuminatus*, *Megacyllene robiniae, Monochamus scutellatus,* and an unknown species. Moreover, *T. planipennisi* did not oviposit in larvae of any Lepidoptera, Coleoptera (other than EAB), or Hymenopteran tested.

Thus, due to the host specificity of the three parasitoids, their release will have no effect on the wood boring Valley elderberry longhorned beetle or its designated critical habitat.

There would be no effect on cave-dwelling or subterranean species (Kretschmarr Cave mold beetle, Coffin Cave mold beetle, Comal Springs dryopid beetle, Tooth Cave ground beetle, *Rhadine exilis, Rhadine infernalis,* Helotes mold beetle); the American burying beetle where eggs and larvae occur in soil and carcasses; aquatic species (Comal Springs riffle beetle, Hungerford's crawling water beetle, Hine's emerald dragonfly, Ash meadows naucorid); species that lay eggs in the sand or soil (Northeastern beach tiger beetle, Delta green ground beetle, Mount Hermon June beetle, Delhi Sands flower-loving fly, Zayante band-winged grasshopper, Ohlone tiger beetle, Puritan tiger beetle, Salt Creek tiger beetle). The eggs and larvae of these species occur in habitats where these parasitoids would not occur. These parasitoids would not be attracted to eggs and larvae in these habitats.

There would be no effect on butterfly, skipper, and moth species (Karner blue butterfly, Bay checkerspot butterfly, Behren's silverspot butterfly, Callippe silverspot butterfly, El Segundo blue butterfly, Fender's blue butterfly, Lange's metalmark butterfly, Lotis blue butterfly, Mission blue butterfly, Mitchell's satyr butterfly, Myrtle's silverspot butterfly, Oregon silverspot butterfly, Palos Verdes blue butterfly, Quino checkerspot butterfly, Saint Francis' satyr butterfly, San Bruno elfin butterfly, Schaus swallowtail butterfly, Smith's blue butterfly, Uncompahgre fritillary butterfly, Kern primrose sphinx moth, Carson wandering skipper, Laguna

Mountains skipper, Pawnee montane skipper). From host specificity testing, no lepidopteran species were accepted as hosts. In addition, these parasitoids are adapted to attacking larvae and eggs of wood-boring species and would not be attracted to eggs and larvae of non wood-boring species.

VI. Other Issues

Consistent with Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-income Populations," APHIS considered the potential for disproportionately high and adverse human health or environmental effects on any minority populations and low-income populations. The environmental and human health effects from the proposed applications are minimal and are not expected to have disproportionate adverse effects to any minority or low-income populations.

Consistent with EO 13045, "Protection of Children From Environmental Health Risks and Safety Risks," APHIS considered the potential for disproportionately high and adverse environmental health and safety risks to children. No circumstances that would trigger the need for special environmental reviews are involved in implementing the preferred alternative. Therefore, it is expected that no disproportionate effects on children are anticipated as a consequence of implementing the preferred alternative.

VII. Agencies, Organizations, and Individuals Consulted

This EA was prepared and reviewed by APHIS and FS. The addresses of participating APHIS units, cooperators, and consultants (as applicable) follow.

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine
Otis Pest Survey, Detection, and Exclusion Laboratory
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Otis ANGB, MA 02542-5008

U.S. Department of Agriculture
Animal and Plant Health Inspection Service

Policy and Program Development
Environmental Services
4700 River Road, Unit 149
Riverdale, MD 20737–1238

U.S. Department of Agriculture
U. S. Forest Service
Northern Research Station
1407 S. Harrison Rd.
East Lansing, MI 48823

U.S. Department of the Interior
Fish and Wildlife Service
4401 N. Fairfax Dr.
Arlington, VA 22203

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Appendix 1. *Agrilus* species in North America (from Nearctica Insecta Nomina (Poole, 1997) http://www.nearctica.com/nomina/beetle/colb-c.htm#anchor8802 *last accessed* March 15, 2007)

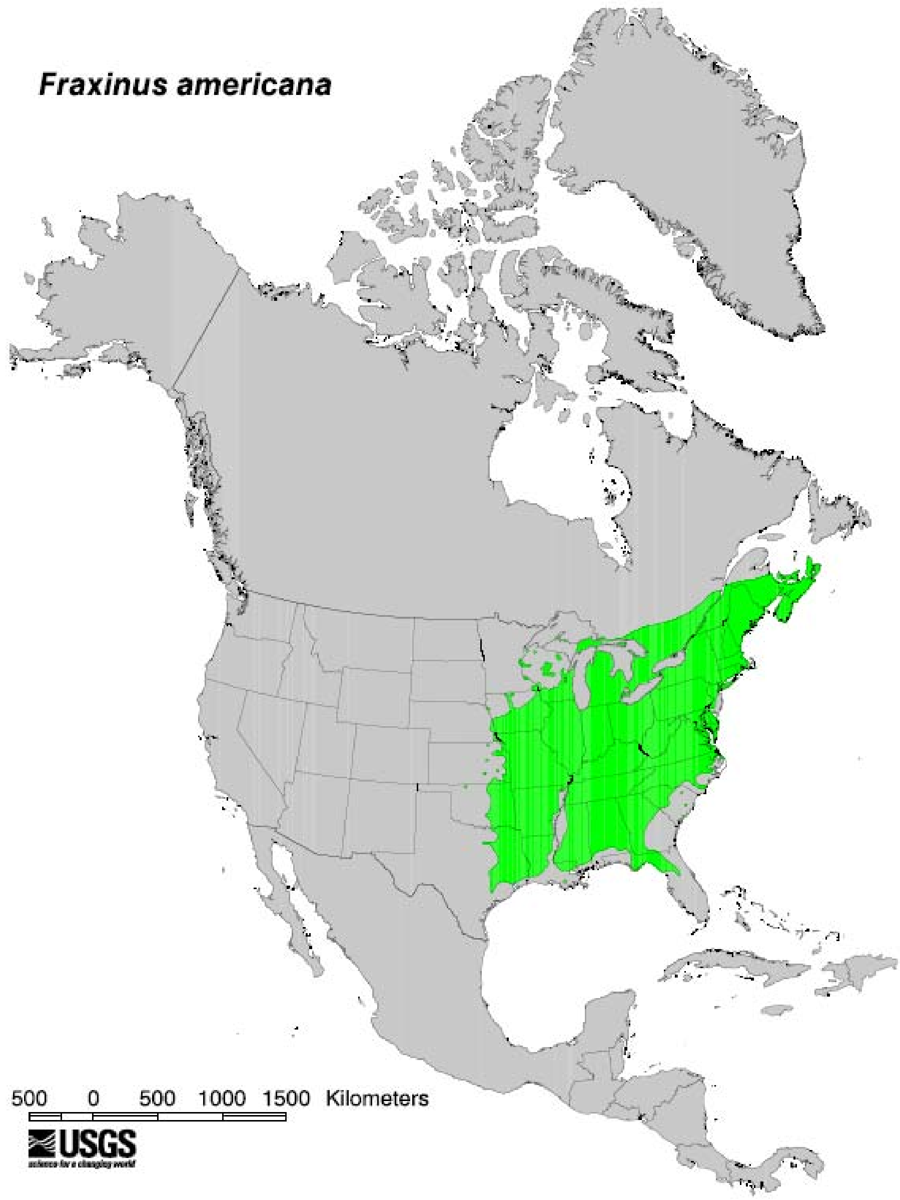
Agrilus Curtis 1825

Agrilus abditus Horn 1891 (Agrilus)
Agrilus abductus Horn 1891 (Agrilus)
Agrilus abjectus Horn 1891 (Agrilus)
Agrilus abstersus Horn 1891 (Agrilus)
Agrilus acaciae Fisher 1928 (Agrilus)
Agrilus acutipennis Mannerheim 1837(Agrilus)
Agrilus addendus Crotch 1873 (Agrilus)
Agrilus aeneocephalus Fisher 1928 (Agrilus)
Agrilus albocomus Fisher 1928 (Agrilus)
Agrilus amelanchieri Knull 1944 (Agrilus)
Agrilus angelicus Horn 1891 (Agrilus)
Agrilus anxius Gory 1841 (Agrilus)
Agrilus apachei Knull 1938 (Agrilus)
Agrilus arbuti Fisher 1928 (Agrilus)
Agrilus arcuata Say 1825 (Buprestis)
Agrilus arizonicus Obenberger 1936 (Agrilus)
Agrilus arizonus Knull 1934 (Agrilus)
Agrilus atricornis Fisher 1928 (Agrilus)
Agrilus audax Horn 1891 (Agrilus)
Agrilus aureus Chevrolat 1837 (Agrilus)
Agrilus aurichalceus Redtenbacher 1849 (Agrilus)
Agrilus auroguttatus Schaeffer 1905 (Agrilus)
Agrilus baboquivariae Fisher 1928 (Agrilus)
Agrilus barberi Fisher 1928 (Agrilus)
Agrilus benjamini Fisher 1928 (Agrilus)
Agrilus bentseni Knull 1954 (Agrilus)
Agrilus bilineata Weber 1801 (Buprestis)
Agrilus blandus Horn 1891 (Agrilus)
Agrilus burkei Fisher 1917 (Agrilus)
Agrilus catalinae Knull 1940 (Agrilus)
Agrilus cavatus Chevrolat 1835 (Agrilus)
Agrilus cavifrons Waterhouse 1889 (Agrilus)
Agrilus celti Knull 1920 (Agrilus)
Agrilus cephalicus LeConte 1860 (Agrilus)
Agrilus cercidii Knull 1937 (Agrilus)
Agrilus champlaini Frost 1912 (Agrilus)
Agrilus chiricahuae Fisher 1928 (Agrilus)
Agrilus cladrastis Knull 1945 (Agrilus)
Agrilus cliftoni Knull 1941 (Agrilus)
Agrilus cochisei Knull 1948 (Agrilus)
Agrilus concinnus Horn 1891 (Agrilus)
Agrilus crataegi Frost 1912 (Agrilus)
Agrilus criddlei Frost 1920 (Agrilus)
Agrilus crinicornis Horn 1891 (Agrilus)
Agrilus cupreomaculatus Duges 1891 (Agrilus)
Agrilus cupreonitens Fisher 1928 (Agrilus)
Agrilus cyanescens Ratzeburg 1838 (Buprestis)
Agrilus davisi Knull 1941 (Agrilus)
Agrilus defectus LeConte 1860 (Agrilus)
Agrilus derasofasciatus Boisduval and LeConte 1835 (Agrilus)
Agrilus difficilis Gory 1841 (Agrilus)
Agrilus diospyroides Knull 1942 (Agrilus)
Agrilus dolli Schaeffer 1904 (Agrilus)
Agrilus dozieri Fisher 1918 (Agrilus)
Agrilus duncani Knull 1929 (Agrilus)
Agrilus egeniformis Champlain and Knull 1923 (Agrilus)
Agrilus egenus Gory 1841 (Agrilus)
Agrilus eleanorae Fisher 1928 (Agrilus)
Agrilus esperanzae Knull 1935 (Agrilus)
Agrilus exhuachucae Knull 1937 (Agrilus)
Agrilus exiguellus Fisher 1928 (Agrilus)
Agrilus exsapindi Vogt 1949 (Agrilus)
Agrilus fallax Say 1839 (Agrilus)
Agrilus falli Fisher 1928 (Agrilus)
Agrilus felix Horn 1891 (Agrilus)
Agrilus ferrisi Dury 1908 (Agrilus)
Agrilus fisherellus Obenberger 1936 (Agrilus)
Agrilus fisheriana Knull 1930 (Agrilus)
Agrilus floridanus Crotch 1873 (Agrilus)
Agrilus frisoni Fisher 1943 (Agrilus)
Agrilus frosti Knull 1920 (Agrilus)
Agrilus fulminans Fisher 1928 (Agrilus)
Agrilus fuscipennis Gory 1841 (Agrilus)
Agrilus geminata Say 1823 (Buprestis)
Agrilus geronimoi Knull 1950 (Agrilus)
Agrilus gibbicollis Fall 1901 (Agrilus)
Agrilus gillespiensis Knull 1947 (Agrilus)
Agrilus granulata Say 1823 (Buprestis)
Agrilus hazardi Knull 1966 (Agrilus)
Agrilus heterothecae Knull 1972 (Agrilus)
Agrilus horni Kerremans 1900 (Agrilus)
Agrilus howdeni Knull 1957 (Agrilus)
Agrilus huachucae Schaeffer 1905 (Agrilus)
Agrilus hualpaii Knull 1939 (Agrilus)
Agrilus imbellis Crotch 1873 (Agrilus)
Agrilus impexus Horn 1891 (Agrilus)
Agrilus inhabilis Kerremans 1900 (Agrilus)
Agrilus jacobinus Horn 1891 (Agrilus)
Agrilus juglandis Knull 1920 (Agrilus)
Agrilus lacustris LeConte 1860 (Agrilus)
Agrilus lautuellus Fisher 1928 (Agrilus)
Agrilus lecontei Saunders 1871 (Agrilus)
Agrilus limpiae Knull 1941 (Agrilus)

Agrilus liragus Barter and Brown 1950 (Agrilus)
Agrilus macer LeConte 1858 (Agrilus)
Agrilus malvastri Fisher 1928 (Agrilus)
Agrilus masculinus Horn 1891 (Agrilus)
Agrilus mimosae Fisher 1928 (Agrilus)
Agrilus muticus LeConte 1858 (Agrilus)
Agrilus neabditus Knull 1935 (Agrilus)
Agrilus neoprosopidus Knull 1938 (Agrilus)
Agrilus nevadensis Horn 1891 (Agrilus)
Agrilus nigricans Gory 1841 (Agrilus)
Agrilus niveiventris Horn 1891 (Agrilus)
Agrilus oblongus Fisher 1928 (Agrilus)
Agrilus obolinus LeConte 1860 (Agrilus)
Agrilus obscurilineatus Vogt 1949 (Agrilus)
Agrilus obsoletoguttatus Gory 1841 (Agrilus)
Agrilus obtusus Horn 1891 (Agrilus)
Agrilus ohioensis Knull 1951 (Agrilus)
Agrilus olentangyi Champlain and Knull 1925 (Agrilus)
Agrilus olivaceoniger Fisher 1928 (Agrilus)
Agrilus ometauhtli Fisher 1938 (Agrilus)
Agrilus ornatulus Horn 1891 (Agrilus)
Agrilus osburni Knull 1937 (Agrilus)
Agrilus otiosus Say 1839 (Agrilus)
Agrilus palmacollis Horn 1891 (Agrilus)
Agrilus palmerleei Knull 1944 (Agrilus)
Agrilus parabductus Knull 1954 (Agrilus)
Agrilus paracelti Knull 1972 (Agrilus)
Agrilus paramasculinus Champlain and Knull 1923 (Agrilus)
Agrilus parapubescens Knull 1934 (Agrilus)
Agrilus parkeri Knull 1935 (Agrilus)
Agrilus parvus Saunders 1871 (Agrilus)
Agrilus pensus Horn 1891 (Agrilus)
Agrilus pilosicollis Fisher 1928 (Agrilus)
Agrilus politus Say 1825 (Buprestis)
Agrilus prosopidis Fisher 1928 (Agrilus)
Agrilus pseudocoryli Fisher 1928 (Agrilus)
Agrilus pseudofallax Frost 1923 (Agrilus)
Agrilus pubescens Fisher 1928 (Agrilus)
Agrilus pubifrons Fisher 1928 (Agrilus)
Agrilus pulchellus Bland 1865 (Agrilus)
Agrilus puncticeps LeConte 1860 (Agrilus)
Agrilus putillus Say 1839 (Agrilus)
Agrilus quadriguttatus Gory 1841 (Agrilus)
Agrilus quadriimpressus Ziegler 1845 (Agrilus)
Agrilus quercicola Fisher 1928 (Agrilus)
Agrilus quercus Schaeffer 1905 (Agrilus)
Agrilus restrictus Waterhouse 1889 (Agrilus)
Agrilus ruficollis Fabricius 1787 (Buprestis)
Agrilus salviaphilos Manley 1979 (Agrilus)
Agrilus santaritae Knull 1937 (Agrilus)
Agrilus sapindi Knull 1938 (Agrilus)
Agrilus sapindicola Vogt 1949 (Agrilus)
Agrilus sayi Saunders 1871 (Agrilus)
Agrilus scitulus Horn 1891 (Agrilus)
Agrilus shoemakeri Knull 1938 (Agrilus)
Agrilus sierrae Van Dyke 1923 (Agrilus)
Agrilus sinuatus Olivier 1790 (Buprestis)
Agrilus snowi Fall 1905 (Agrilus)
Agrilus subcinctus Gory 1841 (Agrilus)
Agrilus subtropicus Schaeffer 1905 (Agrilus)
Agrilus townsendi Fall 1907 (Agrilus)
Agrilus toxotes Obenberger 1935 (Agrilus)
Agrilus transimpressus Fall 1925 (Agrilus)
Agrilus utahensis Westcott 1991 (Agrilus)
Agrilus ventralis Horn 1891 (Agrilus)
Agrilus viridescens Knull 1935 (Agrilus)
Agrilus viridis Linnaeus 1758 (Buprestis)
Agrilus vittaticollis Randall 1838 (Buprestis)
Agrilus walsinghami Crotch 1873 (Agrilus)
Agrilus waltersi Nelson 1985 (Agrilus)
Agrilus wenzeli Knull 1934 (Agrilus)

Appendix 2. Distribution maps of *Fraxinus* species in the United States.

(Maps from USGS, Earth Surface Processes, Digital Representations of Tree Species Range Maps from "Atlas of United States Trees" by Elbert L. Little and Other Publications < http://esp.cr.usgs.gov/data/atlas/little/> *last accessed* March 21, 2007.)




Fraxinus anomala
500 0 500 1000 1500 Kilometers
USGS
science for a changing world


Fraxinus berlandieriana
500 0 500 1000 1500 Kilometers
USGS
science for a changing world


Fraxinus caroliniana
500 0 500 1000 1500 Kilometers
USGS
science for a changing world

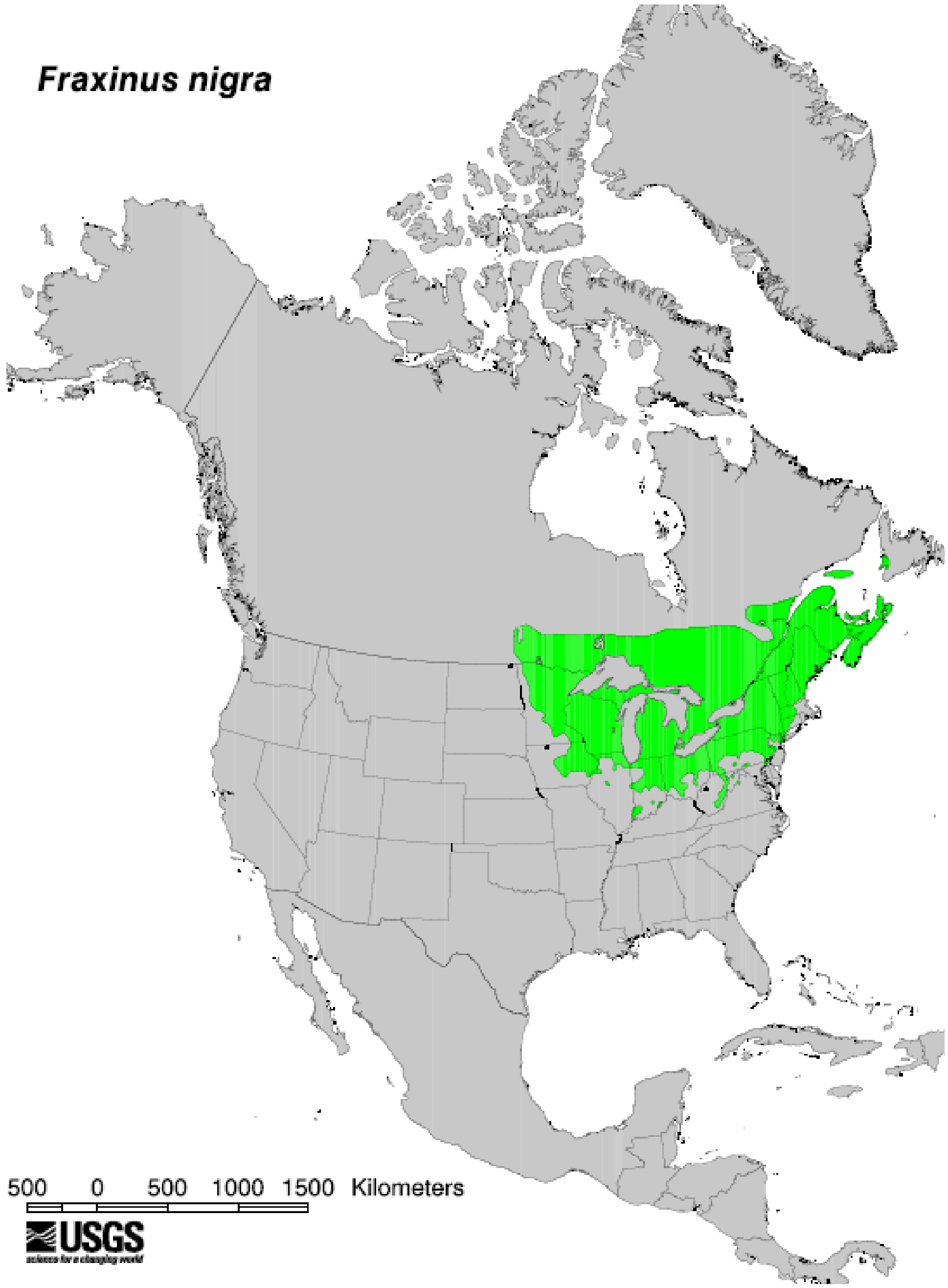

Fraxinus cuspidata
500 0 500 1000 1500 Kilometers
USGS


Fraxinus dipetala
500 0 500 1000 1500 Kilometers
USGS


Fraxinus gooddingii
500 0 500 1000 1500 Kilometers
USGS
science for a changing world


Fraxinus greggii
500 0 500 1000 1500 Kilometers
USGS
science for a changing world


Fraxinus latifolia
500 0 500 1000 1500 Kilometers
USGS
science for a changing world


Fraxinus nigra
500 0 500 1000 1500 Kilometers
USGS
science for a changing world


Fraxinus papillosa
500 0 500 1000 1500 Kilometers
USGS
science for a changing world


Fraxinus pennsylvanica
500 0 500 1000 1500 Kilometers
USGS


Fraxinus profunda
500 0 500 1000 1500 Kilometers
USGS
science for a changing world

Fraxinus quadrangulata
500 0 500 1000 1500 Kilometers
USGS
science for a changing world


Fraxinus texensis
500 0 500 1000 1500 Kilometers
USGS


Fraxinus velutina
500 0 500 1000 1500 Kilometers
USGS
science for a changing world