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Field Release of *Aphelinus rhamni* (Hymenoptera: Aphelinidae) for Biological Control of the Soybean Aphid, *Aphis glycines* (Hemiptera: Aphididae), in the Continental United States

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I. Purpose and Need for the Proposed Action

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Pest Permitting Branch is proposing to issue permits for release of the insect *Aphelinus rhamni* (Hymenoptera: Aphelinidae). This organism would be used by the applicant for biological control of soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), in the continental United States. Before issuing permits for release of *Aphelinus rhamni*, APHIS–PPQ needs to analyze the potential impacts of the release of this organism into the continental United States.

This environmental assessment[1] (EA) was prepared to be consistent with USDA–APHIS' National Environmental Policy Act of 1969 (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 the parasitoid wasp, *A. rhamni*, to control soybean aphid in the continental United States. A parasitoid is an insect whose immature stages (larvae and pupae) live as parasites that eventually kill their hosts (typically other insects). This EA considers a “no action” alternative and the potential effects of the proposed action.

The applicant’s purpose for releasing *A. rhamni* is to reduce the severity of damage to soybean (*Glycine max*) from infestations of soybean aphid in the continental United States. In 2000, the soybean aphid (native to Asia), was found in North America, and is now a major soybean pest infesting 42 million acres in 2003 which resulted in decreased soybean yields and greatly increased control costs (Ragsdale et al., 2011; Landis et al., 2008; Song and Swinton, 2009). The soybean aphid invaded most soybean production regions in North America, including 21 states and three Canadian provinces since originally reported in North America in 2000 (Ragsdale et al., 2004).

The applicant identified several purposes for releasing this particular insect species for biological control of soybean aphid. First, impacts to non-target species are expected to be low. Second, this parasitic wasp may exhibit utility as a biological control agent on the soybean aphid’s alternate buckthorn host, *Rhamnus* species. Lastly, the distribution of *A. rhamni* in the United States may differ from the expected distribution of *A. glycinis*, a related biological control agent of soybean aphid recently

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[1] 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).

approved for release in the United States. Deployment of both insect species has the potential to provide enhanced control of soybean aphid over a broader area in the United States than *A. glycinis* alone. These parasitic wasps are insects whose immature stages (larvae and pupae) live as parasites that eventually kill their hosts (typically other insects).

Most of the existing soybean aphid management options (discussed below) are expensive, temporary, and/or include non-target impacts. For these reasons, there is a need to identify and release a complement of effective, host-specific biological control organisms against soybean aphid in the continental United States.

II. Alternatives

This section will explain the two alternatives available to APHIS–PPQ: no action (no issuance of permits) and issuance of permits for environmental release of *A. rhamni* in the continental United States. Although APHIS' alternatives are limited to a decision of whether to issue permits for release of *A. rhamni*, we describe other methods currently used to control soybean aphid by soybean producers in the United States. Use of these control methods is not an APHIS decision, and their use is likely to continue whether or not APHIS-PPQ issued permits for environmental release of *A. rhamni*.

APHIS–PPQ considered a third alternative but will not analyze it further. Under this third alternative, APHIS–PPQ would issue permits for the field release of *A. rhamni*. The permits, however, would contain special provisions or requirements concerning release procedures or mitigating measures, such as limited releases of *A. rhamni* in the United States. There are no issues raised indicating that special provisions or requirements are necessary.

A. No Action

Under the no action alternative, APHIS–PPQ would not issue permits for the field release of *A. rhamni* for the control of soybean aphid — the release of this biological control agent would not occur, and current methods to control soybean aphid in the United States will continue. Use of these methods is likely to continue even if APHIS–PPQ issues permits for release of *A. rhamni*. Presently, control of soybean aphid in the United States is limited to chemical control, plant resistance, and biological control methods.

1. Chemical Control

Insecticide treatments are the most effective method to manage soybean aphids in North America. Foliar sprays of insecticides provide temporary suppression (7 to 14 days) of soybean aphid populations. Insecticides including esfenvalerate, dimethoate, chlorpyrifos, zeta-cypermethrin, methyl parathion, permethrin, and lambda cyhalothrin are or have been labeled for foliar application against soybean aphid (Ostlie, 2002). Soybean seed treatments using the neonicotinoid insecticides imidacloprid and thiamethoxam for systemic control of soybean aphids also can be used (Pedersen and Lang, 2006; Magalhaes et al., 2009).

2. Host Plant Resistance

Aphid-resistant soybean plants were bred to support reduced aphid populations. Host plant resistance works by inhibiting aphid growth and development and increasing plant tolerance to aphid feeding.

3. Biological Control

Aphelinus certus is a parasitoid native to Asia, accidentally introduced into the United States when the soybean aphid invaded (Heimpel et al., 2010). *A. certus* has a broad host range (Heimpel et al., 2010; Frewin et al., 2010), and was not considered for purposeful introduction against soybean aphid. In the long term, introduction of another parasitoid is unlikely to affect the abundance of *A. certus* because these insects coexist in Asia.

Another Asian parasitoid, *Binodoxys communis* (Hymenoptera: Braconidae), was released in the United States beginning in 2007; unfortunately, it did not establish in the environment (Ragsdale et al., 2011). In 2002, a previously introduced strain of the European parasitoid *Aphelinus atriplicis* was released against soybean aphid. This strain was first released in the western United States against the Russian wheat aphid (Hopper et al., 1998; Prokrym, 1998; Heraty et al., 2007), but it also attacks the soybean aphid (Wu et al., 2004). Although this strain of *Aphelinus atriplicis* was released in nine Minnesota sites in 2002 (Heimpel et al., 2004), it was not recovered since that time.

Most recently, APHIS–PPQ approved the parasitoid *Aphelinus glycinis* for environmental release into the United States (Hopper, 2010; USDA-APHIS, 2012). This related species originated from Xiyuan, Liaoning Province, Peoples Republic of China. The climate in this area closely matches the climate in the northern Midwest United States where soybean aphid is a major pest. It is expected that *A. glycinis* will become limited to this region, but may extend its range into Ontario and Quebec provinces in Canada. Establishment is expected to occur primarily in soybean fields infested with soybean aphid during the growing season.

B. Issue Permits for Environmental Release of *A. rhamni.*

Under this alternative, APHIS–PPQ would issue permits upon request and after evaluation of each application for the field release of *A. rhamni* for the control of soybean aphid in the continental United States. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

1. *Aphelinus rhamni* Taxonomic Information

Insect Taxonomy

Order:	Hymenoptera
Family:	Aphelinidae
Genus:	*Aphelinus*
Species:	*rhamni* Woolley and Hopper
Common name:	none

The species description of *A. rhamni* was published in 2012 (Hopper et al., 2012). Voucher specimens of *A. rhamni* are held at three locations. These include (1) the Beneficial Insect Introductions Research Unit, Agricultural Research Service (ARS), United States Department of Agriculture, Newark, Delaware, (2) Texas A&M University, Department of Entomology, and (3) the Smithsonian in the Systematic Entomology Laboratory, USDA−ARS.

2. Description and Biology of *Aphelinis rhamni*

Aphelinus species are tiny (about 1 millimeter (mm) in length), stingless parasitic wasps. The immature stages develop as internal parasitoids of arthropods where, in this case, feeding of the wasp larva inside the host aphid eventually kills the host aphid. Many species of *Aphelinus* have demonstrated their importance in the biological control of aphids (Hemiptera: Aphididae).

Aphelinus rhamni females prefer second to fourth instar (an immature developmental stage) aphid hosts for oviposition (egg laying), but will lay eggs in all stages, including winged adults. Females use an egg laying organ called an ovipositor to insert an egg into the aphid. The inserted egg hatches and the wasp larva feeds on the internal organs of the aphid host. At 20°C, the wasps develop from egg to pupa in about 14 days; third instar wasps kill their hosts leaving intact the aphid host external skeleton (exoskeleton) hardened and black, in a process called mummification. Adult *A. rhamni* wasps emerge from the mummified aphid about one week after pupation by chewing a hole through the host exoskeleton to escape. Adult wasps eat plant nectar and honeydew (a sugary liquid secreted by aphids and other sap-feeding insects). Adult females also feed on aphids to obtain nutrients for egg production by piercing or “stinging” the aphids with their ovipositor and drinking the hemolymph (insect blood) from the wound, killing about two aphids per day by such feeding. Females emerge as adults with five to 15 mature eggs, but produce more eggs throughout their lives, maturing about 10 eggs per day. In the laboratory, adult wasps live for two to three weeks, but they are unlikely to survive this long in the field (Hopper, 2011).

Aphelinus species are weak fliers, searching primarily by walking (Fauvergue et al., 1995; Mason and Hopper, 1997). Another related parasitoid, *Aphelinus asychis,* dispersed an average of 13 feet in one generation and 26 feet in three generations in fields with abundant hosts

(Fauvergue and Hopper, 2009). Insects would have to disperse much further at the end of the growing season to track their hosts.

3. Geographic Range of *Aphelinis rhamni*

Aphelinus rhamni was collected from parasitized soybean aphids on *Rhamnus* species in 2005 near Daxing (Beijing, Hebei Province) Peoples Republic of China, and is not known to occur elsewhere (Hopper et al., 2012). The present distribution of soybean aphid in the United States matched fairly well with a climate-based expected distribution of *A. rhamni* (Dymex Simulator Application software, version 2.01.025, (c) CSIRO, 2004). It is expected that *A. rhamni* may not establish in the full southern range of the soybean aphid because this pest is found further south than the climate match for Beijing. *Aphelinus rhamni* is expected to establish primarily in the northern half of the United States east of the Rocky Mountains (Hopper, 2011).

4. Impact of *Aphelinus rhamni* on Soybean Aphid

In the laboratory, an individual female *A. rhamni* wasp parasitizes or kills by host feeding over 200 aphids during a lifetime of three weeks, but long lifetimes in the field are unlikely. If females lived one week in the field, each is expected to parasitize and host feed on 70 aphids, if aphids are sufficiently abundant (Hopper, 2011).

III. Affected Environment

A. Soybean Aphid

The soybean aphid is a host alternating species, which means it must disperse between two different types of host plants between summer and winter. Females reproduce asexually on soybean from June to September, producing winged and wingless forms. In September, winged males and parthenogenetic (a form of reproduction in which an unfertilized egg develops into a new individual) females migrate to their primary host plants, *Rhamnus* species, and the migratory females bear sexual, flightless females, which mate with the migratory males and lay overwintering eggs. In the spring, parthenogenetic females hatch from these eggs, and several generations of females reproduce asexually on *Rhamnus* species before winged aphids move to soybean plants. Although soybean aphids are weak fliers, the rapid rate of spread of soybean aphid indicates a high rate of windborn dispersal. In the laboratory, soybean aphid has a high rate of population increase at 25^{o}C, with a steep decline to a negative rate when temperatures are higher than 30^{o}C (McCornack et al., 2004). This can explain why it has not moved further south in the United States where temperatures are warmer. At 25^{o}C, females can produce 73 offspring during their lifetime, with a generation time of 10 days. The combination of high dispersal rate and high reproductive rate mean that soybean aphid can rapidly reach damaging levels over wide regions, if not properly managed (Hopper, 2011).

B. Areas Affected by Soybean Aphid

1. Native and Worldwide Distribution

The soybean aphid is native to East Asia, found in eastern Russia, China, Japan, and Southeast Asia (Vietnam, Thailand, Indonesia, Malaysia, and the Philippines). There are reports in Kenya, and it invaded Australia in 2000.

2. Present Distribution in North America

In North America, it is currently known from Ontario and Quebec in Canada, and from the following 29 U.S. states: Arkansas, Connecticut, Delaware, Georgia, Indiana, Iowa, Illinois, Kansas, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New Jersey, New York, North Dakota, Ohio, Oklahoma, Pennsylvania, South Dakota, Tennessee, Virginia, West Virginia, and Wisconsin (Ragsdale et al., 2011).

3. Soybean Aphid Hosts

Soybean (*Glycine max*) is the summer host of soybean aphid while various buckthorn species (*Rhamnus* species) are the winter hosts. Several native and a few introduced species of *Rhamnus* occur in the United States.

C. Insects Related to Soybean Aphid and *A. rhamni* in the United States

1. Insects Related to Soybean Aphid

Information regarding insects taxonomically related to soybean aphid is included because closely related insect species have the greatest potential for attack by *A. rhamni*.

Of the 497 species of *Aphis* with distributions listed in Blackman and Eastop (2006), 119 are reported from North America. Of these species found in North America, 33 are widely distributed throughout the world, and many of these are invasive exotic pests. Only 86 species of *Aphis* were described from collections in North America, and consequently may be considered native species. Fortunately, most native *Aphis* species are not closely related to soybean aphid (*Aphis glycines*). The soybean aphid is closely related to the cotton aphid, *Aphis gossypii*, which is also native to Asia and a major agricultural pest, and to the buckthorn-potato aphid, *Aphis nasturtii*, which shares its overwintering host (*Rhamnus* species). *Aphis nasturtii* is a pest of potatoes in North America, and believed to be native to Asia (Hopper, 2011).

2. Insects Related to *Aphelinus rhamni*

Thirty-one species of *Aphelinus* are reported from North America, however, only about half of these species (14) were described from North America (listed in Appendix A with their authors and species complex membership, as well as reported distributions and hosts). These 14 *Aphelinus* species are reported exclusively or almost exclusively from North America, and likely are native.

The remaining *Aphelinus* species reported from North America were described elsewhere, are reported from one to several other continents, and therefore are unlikely to be native to North America. Several have been introduced for biological control of pest aphids (Hopper et al., 1998; Jackson et al., 1971; van den Bosch et al., 1959). Three native *Aphelinus* species were reported from *Aphis* species, *A. marlatti*, *A. nigritis*, and *A. semiflavus*, but only *A. semiflavus* parasitizes a species closely related to the soybean aphid, being reported from the cotton aphid in the Midwestern United States. The native parasitoid species appear unlikely to switch to attack the soybean aphid, because these types of switches have not been observed (Lin and Ives, 2003; Rutledge et al., 2004; Kaiser et al., 2007; Costamagna and Landis, 2007; Noma and Brewer, 2008; Noma et al., 2010). Five native *Aphelinus* species (*A. lapisligni*, *A. mali*, *A. prociphili*, *A. sanborniae*, and *A. siphonophorae*) are in the *mali* complex to which *A. rhamni* belongs. These species are more closely related to *A. rhamni* than other native *Aphelinus* species, yet their hosts are different from those parasitized by *A. rhamni* (see Appendix A). Therefore, *A. rhamni* is not expected to compete with native species of *Aphelinus*.

Climate projections suggest both *A. rhamni* and *A. glycinis* are likely to establish in the northern Midwest of the United States (Hopper, 2010; Hopper, 2011; Hopper, 2012). Establishment of *A. glycinis* may preferentially occur on the soybean host, while *A. rhamni* is expected to establish on the buckthorn hosts.

IV. Environmental Consequences

A. No Action

a. Effect of soybean aphid on host plants

1. Impact of Soybean Aphid on the Environment

Soybean aphids suck sap from plants. When aphid infestations are large, infested leaves are wilted or curled. Direct feeding damage from aphids may include plant stunting, reduced pod and seed counts, and yellowing of leaves. Soybean aphids also transmit plant viruses such as alfalfa mosaic, soybean mosaic, and bean yellow mosaic; these diseases cause yield reduction in soybeans.

For soybean growers in the north central United States, the soybean aphid is the first insect pest to consistently cause important losses over wide areas (Ragsdale et al., 2004), with yield decreases of up to 40 percent (Ragsdale et al., 2007). Invasion of the soybean aphid led to dramatic changes in the pest management practices of soybean producers. In 2000, less than one-tenth of one percent of soybeans in the north central states were treated with insecticides. By 2006, over 13 percent of soybeans in these states were treated with insecticides. This indicates soybean aphid was responsible for a 130-fold increase in the use of insecticides (Ragsdale et al., 2011). Control of the soybean aphid currently relies on insecticide use, and millions of acres of soybeans have been sprayed since the invasion of this pest. This is a major disruption in the 70 million acres of soybean production within the country.

2. Impact from the Use of Other Control Methods

The continued use of chemical control, plant resistance, and biological control at current levels would result if the “no action” alternative is chosen, and may continue even if permits are issued for environmental release of *A. rhamni.*

a. Chemical Control

Foliar applications may not sufficiently control soybean aphids and prevent yield loss, especially if large quantities of soybean aphids are surviving on lower leaves. Foliar sprays may pose a threat to bees and aphid natural enemies, such as lady beetle larvae and predatory bugs. In a screening assay, both λ-cyhalothrin and dimethoate were harmful to *A. certus* (Frewin et al., 2012). This suggests chemical pest controls may limit the effectiveness of various parasitoid wasps released as potential biocontrol organisms.

Seed treatments of systemic insecticides such as imidacloprid do not last the entire growing season. In addition, seed treatment insecticides are applied at the beginning of the growing season before it is known if treatment for soybean aphid is even necessary. This creates a financial loss to growers if aphid populations would not have been high enough to trigger economic damage requiring pest treatments.

b. Plant Resistance

Although plant resistance inhibits aphid growth and development and increases plant tolerance to aphid feeding, plant resistance in certain cases has been overcome by soybean aphids.

c. Biological Control

Two biological control organisms, *Aphelinus atriplicis* and *Binodoxys communis* were released against soybean aphid, however, neither organism established in the environment and provides control of the soybean aphid. The related *A. glycinis* recently was approved for environmental release in the continental United States (Hopper, 2010; USDA-APHIS, 2012). This organism is expected to establish primarily on the soybean host.

These impacts from the use of other control methods may have environmental consequences even with the implementation of the biological control alternative, depending on the efficacy of *A. rhamni* to reduce soybean aphid infestations in the continental United States.

B. Issue Permits for Environmental Release of *A. rhamni*

1. Impact of *A. rhamni* on Non-target Insects

a. Scientific Literature

Published literature on the host range of *A. rhamni* includes only the information in the species description. In the field, *Aphis glycines* is the only known host, but in laboratory experiments, *Aphelinus rhamni* parasitizes *Aphis glycines*, closely related species in this genus, and rarely *Rhopalosiphum padi* L. and *Schizaphis graminum* (Hopper et al., 2012). Other species in the *mali* complex tend to have narrow host ranges; a notable exception is *A. mali* itself which reportedly has a broad host range. On the other hand, literature reports of broad host ranges may reflect confounding effects from cryptic species within this genus (Heraty et al., 2007).

b. Host Specificity Testing

Host specificity testing of *A. rhamni* in containment examined three ways that aphids were killed: oviposition leading to mummification, stinging the aphid to death without mummification, and host feeding (Hopper, 2011).

Parasitism of 13 aphid species in five genera in two tribes on 10 plant species in nine families was measured (Table 1). These aphids covered a range of closely related aphids reported as hosts of the *A. mali* complex (see Appendix A), of which *A. rhamni* is a member. Furthermore, the aphid-plant combinations provided contrasts of aphids in the same versus different genera, on the same versus different plants (see Appendix B). Based on the aphid distributions in Blackman and Eastop (2006), most of these aphid species occur within the native geographic range of *A. rhamni,* so these aphids and this parasitoid are likely to have been in contact for at least 10,000 years. The exceptions are *Diuraphis noxia*, which is native to central to western Asia and Europe, and *Aphis oestlundi* and *Aphis monardae*, which are native to North America.

Table 1. Aphid and host plant species used to test *Aphelinus rhamni* host specificity.

Aphid species	Plant species	Plant family
Aphis glycines	*Glycine max*	Fabaceae
Aphis gossypii	*Gossypium hirsutum*	Malvaceae
Aphis gossypii	*Asclepias syriaca*	Apocynaceae
Aphis monardae	*Monarda fistulosa*	Laminacea
Aphis oestlundi	*Oenothera biennis*	Onagraceae
Aphis nerii	*Asclepias syriaca*	Apocynaceae
Aphis asclepiadis	*Asclepias syriaca*	Apocynaceae
Aphis craccivora	*Vigna unguiculata*	Fabaceae
Aphis rumicis	*Rumex altissimus*	Polygonaceae
Rhopalosiphum maidis	*Hordeum vulgare*	Poaceae
Rhopalosiphum padi	*Hordeum vulgare*	Poaceae
Schizaphis graminum	*Hordeum vulgare*	Poaceae
Diuraphis noxia	*Hordeum vulgare*	Poaceae
Myzus persicae	*Raphanus sativus*	Brassicaceae

Tests of parasitism in 24-hour exposures of aphids to *A. rhamni* consisted of *A. rhamni* females being placed in cages with 100 aphids of a single species on plant foliage. In this study, *A. rhamni* rarely or never parasitized aphids outside of the genus *Aphis.* Within the genus *Aphis, A. rhamni* produced mummified aphids on species closely related to the target aphid, *A. glycines* (e.g., *A. gossypii, A. monardae,* and *A. oestlundi*), but not on more distantly related *Aphis* species.

Direct observation of the behavior of *A. rhamni* and aphids determined host use patterns. Continuous microscopic observation of each female parasitoid (and the aphids to which she was exposed) for 25 minutes under magnification showed *A. glycines* is the most suitable species for *A. rhamni* development, by producing an adult parasitoid per oviposition. In most other species in the genus *Aphis*, there were less than half as many *A. rhamni* adults per oviposition produced.

In summary, laboratory experiments showed oviposition and host feeding by *A. rhamni* was restricted almost completely to species in the genus *Aphis* closely related to the target. Furthermore, parasitoid stinging that did not lead to mummification or host feeding did not increase mortality of any aphid species. Handling times of aphids by *A. rhamni* were long, so ant-fended native aphids, such as *A. monardae* and *A. oestlundi*, should not be at risk in the field. The target pest, soybean aphid, was the most suitable aphid species for *A. rhamni* development (Hopper, 2011). See Appendix B for details on the host specificity study.

Additional studies determined if *A. rhamni* might be likely to shift to non-target aphid hosts under certain conditions. In these studies, it was found that stresses decreasing the length of life of *A. rhamni* did not increase its acceptance of low quality aphid host species. Specificity appears to be stable, and low-quality, non-target species should not have increased risk of attack when *A. rhamni* is stressed from starvation or age.

2. Uncertainties Regarding the Environmental Release of *Aphelinus rhamni.*

Once a biological control agent such as *A. rhamni* is released into the environment and becomes established, there is a possibility it could move from the target insect (soybean aphid) to attack nontarget insects, such as native aphid species. Native species that are closely related to the target species are the most likely to be attacked (Louda et al., 2003). If other aphid species were to be attacked by *A. rhamni*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *A. rhamni* 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.

In addition, these agents may not be successful in reducing soybean aphid populations in the continental United States. Approximately 12 percent 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 soybean aphid populations by *A. rhamni* will not be known until after release and establishment occurs. Actual impacts on the establishment of *A. glycinis*, including potential competitive interactions will not be known until after release and establishment of *A. rhamni* occurs. The environmental consequences discussed under the no action alternative may occur even with the implementation of the action alternative, depending on the efficacy of *A. rhamni* to reduce soybean aphid in the continental United States.

3. Cumulative Impacts

“Cumulative impacts are defined as the impacts 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).

To date, other soybean aphid biological control organisms released in the United States have not successfully established. Therefore, competitive interactions would not occur among any organisms previously released. The release of *A. glycinis* is not expected to produce competitive interactions with *A. rhamni* because habitat partitioning of the two parasitoids between soybean aphids on the two alternate plant hosts (soybean and *Rhamnus* species) is anticipated (K. Hopper, pers. comm.).

The two species also have different predicted geographical distributions based on climate matching, with the distribution of *A. glycinis* extending further north and *A. rhamni* further south (Hopper, 2010). As discussed previously, *A. rhamni* is not expected to compete with native species of *Aphelinus*.

The non-native parasitoid *Aphelinus certus* Yasnosh accidentally established in North America (Heimpel et al., 2010, Frewin et al., 2010). It was found over a wide geographic range in the eastern United States and Canada since 2005. In laboratory experiments, it has a host range of at least four genera in two subfamilies of aphids, and on at least four host plant species (Heimpel et al., 2010). The abundance of *A. certus* in the United States is unlikely to be affected by introduction of *A. rhamni* because this parasitoid's host range is narrower than for *A. certus*. *Aphelinus certus* might interfere with establishment of *A. rhamni*, but the coexistence of these parasitoid species in Asia suggests any interference is likely to be short-lived.

Release of *A. rhamni* is not expected to have any negative cumulative impacts in the continental United States because of its host specificity to soybean aphids. Effective biological control of soybean aphid will have beneficial effects for soybean growers in the United States, may result in a long-term, non-damaging method to assist in the control of soybean aphid, and may reduce the use of insecticides on soybean.

4. 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 and endangered species, or result in the destruction or adverse modification of critical habitat.

APHIS has determined that, based on the host specificity of *A. rhamni*, there will be no effect on any listed insect species or designated critical habitat in the continental United States. In host specificity testing, *A. rhamni* only attacked species of aphids. There are no federally listed threatened or endangered insects belong to the aphid family, Aphididae (USFWS, 2012). There are no federally listed species are known to depend on or utilize soybean aphid.

V. 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. There are no adverse environmental or human health effects anticipated from the field release of

A. rhamni and its release will not 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. There are no circumstances that would trigger the need for special environmental reviews involved in implementing the preferred alternative. Therefore, there are no disproportionate effects on children anticipated because of the field release of *A. rhamni*.

EO 13175, “Consultation and Coordination with Indian Tribal Governments”, was issued to ensure that there would be “meaningful consultation and collaboration with tribal officials in the development of Federal policies that have tribal implications….” Consistent with EO 13175, APHIS sent letters of notification and requests for comment and consultation on the proposed action to tribes in areas where the soybean aphid occurs. APHIS will continue to consult and collaborate with Indian tribal officials to ensure that they are well-informed and represented in policy and program decisions that may impact their agricultural interests, in accordance with EO 13175.

VI. Agencies, Organizations, and Individuals Consulted

This EA was prepared and reviewed by APHIS and ARS. The addresses of participating APHIS units and any applicable cooperators are provided.

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Policy and Program Development
Environmental and Risk Analysis Services
4700 River Road, Unit 149
Riverdale, MD 20737

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine
Registrations, Identification, Permits, and Plant Safeguarding
4700 River Road, Unit 133
Riverdale, MD 20737

U.S. Department of Agriculture
Agricultural Research Service
Beneficial Insect Introductions Research Unit
501 South Chapel Street

Newark, DE 19713

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Appendix A. *Aphelinus* species described from North America and their species complexes, distributions, and reported hosts

Species	**Author**	**Species complex**	**Reported distribution**	**Reported hosts**
howardii	Dalla Torre, 1898	*abdominalis*	California (Howard, 1895)	*Acyrthosiphon pisum* (Herting, 1972); *Macrosiphum euphorbiae* (Sullivan and van den Bosch, 1971); *Illinoia liriodendri* (Zuparko and Dahlsten, 1993)
jucundus	Gahan, 1924	*abdominalis*	California (Gahan, 1924); New York (Griswold, 1926)	*Acyrthosiphon malvae* (Griswold, 1926); *Acyrthosiphon pseudodirhodum* (Griswold, 1927); *Aulacorthum solani* (Herting, 1972); *Macrosiphum euphorbiae* (Gahan, 1924); *Myzus persicae* (Griswold, 1927)
semiflavus	Howard, 1908	*asychis*	Colorado (Howard, 1908); Indiana (Hartley, 1922); Kansas, Oklahoma (Kelly, 1917); Minnesota, New Mexico (Webster and Phillips, 1912); New York (Griswold, 1927); Ohio (Girault, 1917)	*Acyrthosiphon pisum, Aphis gossypii, Cerusaphis viburnicola, Rhopalosiphum maidis, Macrosiphoniella sanborni, Macrosiphum avenae* (Hartley, 1922); *Aphis rumicis, Brevicoryne brassicae, Lipaphis pseudobrassicae, Sitobion avenae* (Peck, 1963);*Chaitophorus viminalis, Schizaphis graminum* (Webster and Phillips, 1912); *Myzus persicae* (Howard, 1908); *Myzaphis rosarum* (Peck, 1963)
marlatti	Ashmead, 1888	*asychis*	Kansas (Ashmead, 1888b); Ontario; Quebec (Peck, 1963)	*Aphis* sp. (Ashmead, 1888b); *Myzus persicae* (Herting, 1972)
lapisligni	Howard, 1917	*mali*	Oregon (Howard, 1917); Idaho (Smith, 1923); Washington (Smith, 1923)	*Brachycaudus helichrysi* (Peck, 1963); *Nearctaphis bakeri* (Howard, 1917)
mali	Haldeman, 1851	*mali*	Arkansas (Becker, 1918); Colorado (Gillette and Taylor, 1908); District of Columbia, Illinois, Missouri (Howard, 1881); Kansas (Lohrenz, 1911); Nebraska (Bruner, 1894); Pennsylvania (Cresson, 1862)	*Eriosoma americanum* (Girault, 1909); *Eriosoma crataegi* (Howard, 1881); *Eriosoma lanigerum* (Haldeman, 1859)
prociphili	Carver, 1980	*mali*	Iowa (Carver, 1980)	*Prociphilus fraxinifolii* (Carver, 1980)
sanborniae	Gahan, 1924	*mali*	Pennsylvania (Gahan, 1924); Ohio (Delong and Jones, 1926)	*Sanbornia juniperi* (Gahan, 1924); *Nasonovia houghtonensis* (Delong and Jones, 1926)
siphonophorae	Ashmead, 1888	*mali*	Florida (Ashmead, 1888a); New Jersey (Peck, 1963)	*Macrosiphum sp.* (Ashmead, 1888a)
nigritus	Howard, 1908	*varipes*	South Carolina (Howard, 1908); Kansas, Minnesota (Webster and Phillips, 1912); New Mexico (Webster, 1909); Oklahoma (Jackson et al., 1970); Texas (Archer et al., 1974)	*Schizaphis graminum* (Howard, 1908); *Hysteroneura setariae* (Webster and Phillips, 1912); *Aphis helianthi* (Rogers et al., 1972); *Rhopalosiphum maidi* (Jackson et al., 1970)
aureus	Gahan, 1924	*subgenus Mesidia*	California (Gahan, 1924)	*Chaitophorus nigrae* (Gahan, 1924)
automatus	Girault, 1911	*subgenus Mesidia*	Illinois (Girault, 1911); Virginia (Girault, 1916); California (Zuparko and Dahlsten, 1995)	*Chaitophorus sp.* (Girault, 1911); *Hysteroneura setariae* (Girault, 1916); *Eucallipterus tiliae* (Zuparko and Dahlsten, 1995)

gillettei	Howard, 1914	*subgenus Mesidia*	Colorado (Howard, 1914)	*Diuraphis tritici* (Howard, 1914)
perpallidus	Gahan, 1924	*subgenus Mesidia*	Iowa (Gahan, 1924); Alabama (Edelson and Estes, 1987); California (Peck, 1963); Florida (Mizell and Schiffhauer, 1990); Texas (Watterson and Stone, 1982)	*Chromaphis juglandicola* (Peck, 1963); *Melanocallis fumipennellus, Monellia costalis* (Peck, 1963); *Monellia caryella* (Bueno and Stone, 1983); *Monelliopsis pecanis* (Edelson and Estes, 1987)

Appendix B. Host specificity tests for *Aphelinus rhamni* (Hopper 2011; 2012).

Parasitism in 24-hour exposure of aphids on plant. Individual female parasitoids were exposed to one of 12 aphid species. One to five day old females were used that had been with males and aphids since emergence and thus had the opportunity to mate, host-feed, and oviposit. Females were isolated from males and aphids for 24 hours before using them in experiments to insure that the females had a full egg load. Each female parasitoid was placed in a cage (10 centimeters (cm) diameter by 22 cm tall) enclosing the foliage of a potted plant of the appropriate species with 100 aphids of mixed instars of a single species. Female parasitoids were removed after 24 hours and were used only once.

Because these parasitoids can parasitize a maximum of 20 aphids in 24 hours, this abundance of aphids and period of exposure allowed parasitoids to use their full egg complement. Furthermore, the density of aphids, amount of plant material, and cage size meant that parasitoids were not limited by search rate. Parasitism scoring occurred after larval parasitoids killed and mummified their hosts. Therefore, a combination of acceptance of hosts for oviposition and suitability of hosts for parasitoid development were measured.

Female parasitoids had the choice to oviposit or not to oviposit in a particular host species. This is frequently the choice parasitoids make in the field. The purpose of this study was to measure parasitism in an environment that appears to harbor only one aphid species on only one plant species and where female parasitoids re-encounter this combination repeatedly starting with a full egg complement.

Ten days after exposure of aphids to parasitoids, any mummified aphids were collected and held for adult parasitoid emergence. After the adults emerged, the number of mummified aphids and the number and sex of adult parasitoids were recorded. Eight to 32 females on each of 12 aphid species were tested.

After 24 hours exposure, the number of aphids mummified by *A. rhamni* varied with aphid species (F = 13.1; df = 12, 213; P < 0.0001; Fig. 3).Female parasitoids rarely or never parasitized aphids outside the genus *Aphis*; within the genus *Aphis*, female parasitoids produced mummified aphids on species phylogenetically close to *A. glycines*, e.g. *A. gossypii*, *A. monardae*, and *A. oestlundi*, but not on aphids that are more phylogenetically distant, e.g. *A. nerii* and *A. rumicis*. The low numbers of mummified aphids for *A. nerii* may have resulted from their host plant, *A. syriaca,* which is known to produce toxic secondary chemicals which aphids could sequester for their defense (Mooney et al., 2008, Warashina and Noro, 2000). This effect is supported by many fewer mummies for *A. gossypii* on *A. syriaca* than on *G. hirsutum* (Fig. 3).

The proportion of mummies from which parasitoid adults emerged was generally high (0.93±0.01) but varied among species (F = 4.6; df = 8, 67; P = 0.0002) because *A. oestlundi* had a lower adult emergence rate (0.64±0.01) than the other aphids. Sex ratio of parasitoid progeny did not differ from equality (0.52±0.02) and did not differ among aphid species.

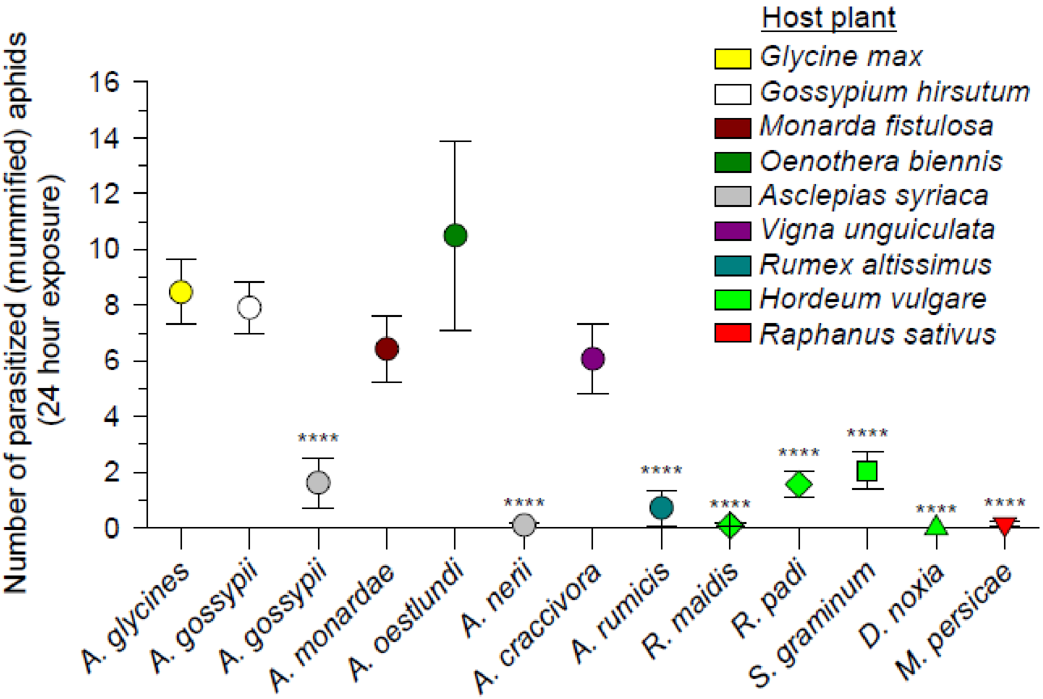

Fig. 3. Number of aphids parasitized (mummified) of various aphid species exposed to *Aphelinus rhamni* for 24 hours. Aphid species are ordered by phylogenetic distance from *Aphis glycines*. Asterisks indicate level of difference from *Aphis glycines*: ****P<0.0001. Error bars are standard errors of means.

Direct observations of behavior in experiments on the stresses of starvation and age, egg load, and experience affecting acceptance of low versus high quality host species for oviposition by *Aphelinus rhamni* found female parasitoids may behave differently after encountering low versus high quality hosts for longer periods. Starved *Aphelinus rhamni* females persisted in ovipositing more in high-quality aphid species than in low quality aphid species. Females oviposited about twice as often in *Aphis glycines* than in *A. craccivora* or *R. padi* (F = 5.8; df = 2,128; P = 0.004). Starved and fed females laid about the same numbers of eggs (1.0 ± 0.2 versus 1.1 ± 0.2, respectively), and there was no interaction between nutrition and aphid species in their effect on oviposition. Aphid species and egg load did interact in their effect on oviposition (F = 5.9; df = 3,124; P = 0.0009).Honey-fed parasitoids exposed to *A. glycines* oviposited more when they carried more eggs, but oviposition in the other aphid species did not increase with egg load, and increased only slightly in water-fed females exposed to *A. glycines*. This pattern is the opposite of what one would expect if female parasitoids became choosier as eggs became more limiting. The higher oviposition in *A. glycines* did not result from stinging this species more often. Indeed, female parasitoids stung *A. craccivora* more often than *A. glycines*, which was stung at the about the same rate as *R. padi* (6.3±0.4, 4.1±0.3, and 3.7±0.3 aphids, respectively; *F*

= 17.3; df = 2,156; P < 0.0001), although *A. glycines* was oviposited in twice as often as the other aphid species. The lack of correlation between stinging and oviposition, probably results because *Aphelinus* females usually do not directly contact aphids before ovipositor insertion, and thus are may not assess host quality until aphids are stung. On encountering a potential host, *Aphelinus rhamni* females stop at about half their body length away from the aphid. Without touching the aphid, females sway from side to side several times with antennae extended (an approach). Stinging did not vary with nutrition, egg load, nor did these factors interact with aphid species in its effect on numbers of aphids stung.

Effect of age on oviposition in low versus high quality hosts. Ten-day old *Aphelinus rhamni* females persisted in ovipositing more in a high quality aphid species than in a low quality aphid species. Females oviposited over twice as often in *A. glycines* than in *R. padi* (F = 13.0; df = 1,76; P = 0.0006). Young and old females laid about the same number of eggs (1.1 ± 0.2 versus 0.9 ± 0.2 eggs, respectively), and there was no interaction between parasitoid age and aphid species.

As in the nutrition experiment, there was a strong interaction between aphid species and egg load in their effect on oviposition (F = 6.6; df = 3,125; P = 0.0002). Young parasitoids exposed to *A. glycines* oviposited more when they carried more eggs, but oviposition did not increase significantly with egg load in parasitoids exposed to *R. padi* or in older parasitoids exposed to *A. glycines*. As in the nutrition experiment, the preference for high quality hosts persisted in the face of low versus high egg load. At the start of observations, young females carried about 3 more eggs than older females (9.5 ± 0.9 versus 6.2 ± 0.6, respectively; F = 453.3; df = 2,77; P < 0.0001), and by the end of observations, both young and old females still had eggs they had not oviposited (8.0 ± 0.8 and 5.3±0.6, respectively).

The higher oviposition in *A. glycines* was partly a result of stinging this aphid species more often. Unlike the starvation experiment, where *A. glycines* and *R. padi* where stung equally often, female parasitoids in this experiment stung *A. glycines* slightly more often than *R. padi*.

Effect of age on oviposition in low versus high quality hosts. Ten-day old *Aphelinus rhamni* females persisted in ovipositing more in a high quality aphid species than in a low quality aphid species. Females oviposited over more than twice as often in *A. glycines* than in *R. padi* (F= 13.0; df = 1,76; P = 0.0006). Young and old females laid about the same number of eggs (1.1± 0.2 versus 0.9 ± 0.2 eggs, respectively), and there was no interaction between parasitoid age and aphid species.

As in the nutrition experiment, there was a strong interaction between aphid species and egg load in their effect on oviposition (F = 6.6; df = 3,125; P = 0.0002). Young parasitoids exposed to *A. glycines* oviposited more when they carried more eggs, but oviposition did not increase significantly with egg load in parasitoids exposed to *R. padi* or in older parasitoids exposed to *A. glycines*. As in the nutrition experiment, the preference for high quality hosts persisted in the face of low versus high egg load. At the start of observations, young females carried about 3 more eggs than older females (9.5 ± 0.9 versus 6.2 ± 0.6, respectively; F = 453.3; df = 2,77; P < 0.0001), and by the end of observations, both young and old females still

had eggs they had not oviposited (8.0 ± 0.8 and 5.3±0.6, respectively). The higher oviposition in *A. glycines* was partly a result of stinging this aphid species more often.

Unlike the starvation experiment, where *A. glycines* and *R. padi* where stung equally often, female parasitoids in this experiment stung *A. glycines* slightly more often than *R. padi* (5.6±0.4 versus 4.4±0.3, respectively; $F = 5.3$; df = 1,77; $P = 0.02$).However, this difference was only 1.3 fold more stings in *A. glycines* than *R. padi*, whereas oviposition was 2.5 fold greater in *A. glycines* than in *R. padi*. Stinging did not vary with age, egg load, nor did these factors interact with aphid species in its effect on numbers of aphids stung. The frequency of host feeding did not vary with age, but parasitoids fed on *A. glycines* more frequently than on *R. padi* (32 percent versus 6 percent of parasitoids fed on each species; likelihood ratio $\chi_2 = 8.2$; n = 74; $P = 0.003$), and this difference was similar to that for honey-fed parasitoids in the nutrition experiment of the same age. Host feeding did not vary with egg load for either aphid species, age, or their combinations.

Differences in aphid defense cannot explain differences in oviposition between species because *A. glycines* defended itself as much as *R. padi* (2.3 ± 0.3 versus 2.1±0.3 defensive behaviors per approach), but received over twice as many eggs. Amount of aphid defense behavior did not vary with aphid species, age, or their interaction.

Both young and old *Aphelinus rhamni* females produced more progeny on high quality host species than on low quality host species when exposed to the aphids for 24 hours.

Conclusions. Stresses that decreased life expectancy did not increase acceptance of low quality host species by *Aphelinus rhamni*. Egg load was also lower in stressed females, which resorbed eggs in response to starvation and aging, so changes in life expectancy and egg load may have counterbalanced one another. However, the decrease in egg load was small relative to the decrease in life expectancy, particularly for starved females, which carried about three-quarters of the egg load of honey-fed females, but would die the day following the assays.

Furthermore, although oviposition increased with egg load for females exposed to *A. glycines*, oviposition did not vary with egg load for females exposed to low quality aphid species. The outcome of this pattern was that females became less choosy as egg load decreased, rather than becoming choosier, as theory predicts. Besides having lower life expectancy, older females were kept without aphids as they aged so they experienced a more aphid-poor environment than did young parasitoids. It was expected this would make parasitism of low quality aphids by older females more frequent, yet it did not. These results demonstrate that *A. rhamni* specificity is behaviorally stable rather than dynamic, and low-quality non-target species should not have increased risk of attack when parasitoids are stressed from starvation or age or when they accumulate eggs.

Decision and Finding of No Significant Impact for Field Release of *Aphelinus rhamni* (Hymenoptera: Aphelinidae) for Biological Control of the Soybean Aphid, *Aphis glycines* (Hemiptera: Aphididae), in the Continental United States

June 2014

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) Pest Permitting Branch (PPB), is proposing to issue permits for release of an insect, *Aphelinus rhamni* (Hymenoptera: Aphelinidae), in the continental United States. The agent would be used by the applicant for the biological control of soybean aphid, *Aphis glycines* (Hemiptera: Aphididae). Before permits are issued for release of *A. rhamni*, APHIS must analyze the potential impacts of the release of this organism into the continental United States in accordance with USDA APHIS National Environmental Policy Act implementing regulations (7 Code of Federal Regulations Part 372). APHIS has prepared an environmental assessment (EA) that analyzes the potential environmental consequences of this action. The EA is available from:

U.S. Department of Agriculture
Animal and Plant Health inspection Service
Plant Protection and Quarantine
Registrations, Identification, Permits, and Plant Safeguarding
4700 River Road, Unit 133
Riverdale, MD 20737
http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth?1dmy&urile=wcm%3apath%3a%2FAPHIS_Content_Library%2FSA_Our_Focus%2FSA_Plant_Health%2FSA_Domestic_Pests_And_Diseases%2FSA_Environmental_Assessments%2F

The EA analyzed the following two alternatives in response to a request for permits authorizing environmental release of *A. rhamni*: (1) no action, and (2) issue permits for the release of *A. rhamni* for biological control of soybean aphid. A third alternative, to issue permits with special provisions or requirements concerning release procedures or mitigating measures, was considered. However, this alternative was dismissed because no issues were raised that indicated that special provisions or requirements were necessary. The No Action alternative, as described in the EA, would likely result in the continued use at the current level of chemical, host plant resistance, and biological control methods of soybean aphid. These control methods described are not alternatives for decisions to be made by the PPB, but are presently being used to control soybean aphid in the United States and may continue regardless of permit issuance for field release of *A. rhamni*. Legal notice of the EA was made available in the Federal Register on May 2, 2014 for a 30-day public comment period. One anonymous comment was received on the EA, in opposition to the proposed release of *A. rhamni*. However, no substantive information was presented in the comment.

I have decided to authorize the PPB to issue permits for the environmental release of *A. rhamni*. The reasons for my decision are:

- This biological control agent is sufficiently host specific and poses little, if any, threat to the biological resources, including non-target insect species of the United States.

- The release will have no effect on federally listed threatened and endangered species or their habitats in the United States.

- *A. rhamni* poses no threat to the health of humans.

- No negative cumulative impacts are expected from release of *A. rhamni.*

- There are no disproportionate adverse effects to minorities, low-income populations, or children in accordance with Executive Order 12898 "Federal Actions to Address Environmental Justice in Minority Populations and Low-income Populations" and Executive Order 13045, "Protection of Children from Environmental Health Risks and Safety Risks."

- While there is not total assurance that the release of *A. rhamni* into the environment will be reversible, there is no evidence that this organism will cause any adverse environmental effects.

I have determined that there would be no significant impact to the human environment from the implementation of the preferred alternative (issuance of permits for the release of *A. rhamni*) and, therefore, no Environmental Impact Statement needs to be prepared.

Emily Pullins
Director
Regulations, Permits, and Manuals
Plant Health Programs
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

Date July 21, 2014