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

Environmental Assessment, September 2012

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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 (PPB) is proposing to issue permits for release of the insect *Aphelinus glycinis* (Hymenoptera: Aphelinidae). The agent would be used by the applicant for biological control of soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), in the continental United States. Before permits are issued for release of *A. glycinis,* the APHIS–PPQ PPB needs to analyze the potential impacts of the release of this organism into the continental United States.

This environmental assessment[1] (EA) has been prepared, 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 *A. glycinis* to control soybean aphid in the continental United States. This EA considers a "no action" alternative and the potential effects of the proposed action.

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

Existing soybean aphid management options (discussed below) are expensive, temporary, or have non-target impacts. For these reasons, the applicant has a need to identify an effective, host-specific biological control organism and release it into the environment of the continental United States for the control of soybean aphid.

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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).

II. Alternatives

This section will explain the two alternatives available to the APHIS–PPQ PPB—no action (no issuance of permits) and issuance of permits for environmental release of *A. glycinis* in the continental United States. Although APHIS' alternatives are limited to a decision of whether to issue permits for release of *A. glycinis*, other methods available for control of soybean aphid in the United States are also described. These control methods are not decisions to be made by APHIS, and are likely to continue whether or not permits are issued for environmental release of *A. glycinis*. These are methods presently being used to control soybean aphid by growers in the United States.

A third alternative was considered, but will not be analyzed further. Under this third alternative, the APHIS–PPQ PPB would have issued permits for the field release of *A. glycinis*; however, the permits would contain special provisions or requirements concerning release procedures or mitigating measures, such as limited release of *A. glycinis* in the United States. No issues have been raised which would indicate that special provisions or requirements are necessary.

A. No Action

Under the no action alternative, the APHIS–PPQ PPB would not issue permits for the field release of *A. glycinis* for the control of soybean aphid—the release of this biological control agent would not take place. The following methods are presently being used to control soybean aphid in the United States; these methods will continue under the "no action" alternative and are likely to continue even if permits are issued for release of *A. glycinis*. 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 currently 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 can also be used (Pedersen and Lang, 2006; Magalhaes et al., 2009).

2. Host Plant Resistance

Aphid-resistant soybean plants have been developed to reduce 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. A parasitoid is an insect whose immature stages (larvae and pupae) live as parasites that eventually kill their hosts (typically other insects). *Aphelinus certus* has a broad host range, but is commonly found in soybean aphids in Asia, and has been found attacking soybean aphid in parts of North America (Heimpel et al., 2010; Frewin et al., 2010). It appears to have been accidentally introduced into the United States, arriving around the time when soybean aphid invaded, and it may have been introduced with soybean aphid. High levels of parasitism of soybean aphid by *A. certus* have been observed in Quebec and Ontario, but the parasitoid is not as common elsewhere.

A previously introduced strain of the European parasitoid *Aphelinus atriplicis* was released against soybean aphid in 2002. 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) and was found to attack the soybean aphid as well (Wu et al., 2004). Although this strain of *Aphelinus atriplicis* was released in nine Minnesota sites in 2002 (Heimpel et al., 2004), it has not been recovered since then. Another Asian parasitoid, *Binodoxys communis* (Hymenoptera: Braconidae), was released in the United States beginning in 2007; unfortunately, it has not established (Ragsdale et al., 2011).

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

Under this alternative, the APHIS–PPQ PPB would issue permits upon request and after evaluation of each application for the field release of *A. glycinis* 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 glycinis* Taxonomic Information

Insect Taxonomy
Order: Hymenoptera
Family: Aphelinidae
Genus: *Aphelinus*
Species: *glycinis* Hopper and Woolley
Common name none

A paper describing *A. glycinis* has been accepted for publication in the Journal of Hymenoptera Research. Voucher specimens of *A. glycinis* are held at the Beneficial Insect Introductions Research Unit, Agricultural Research Service (ARS), United States Department of Agriculture, Newark, Delaware; Texas A & M University, Department of Entomology; and the Smithsonian in the Systematic Entomology Laboratory, USDA-

ARS.

2. Description and Biology of *Aphelinis glycinis*

Aphelinus species are tiny (about 1 millimeter (mm) in length), stingless wasps. Like all parasitic wasps, the immature stages develop as 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 glycinis 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^{o}C, the wasps develop from egg to pupa in about 14 days; third instar wasps kill their hosts but leave the aphid host external skeleton (exoskeleton) intact, causing it to harden and turn black, a process called mummification. Adult *A. glycinis* wasps emerge from the mummified aphid about one week after pupation, 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 2 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.

Aphelinus species are weak fliers, searching primarily by walking (Fauvergue et al., 1995; Mason and Hopper, 1997). Another parasitoid in this genus, *Aphelinus asychis,* dispersed an average of 13 feet in one generation and 26 feet in three generations (Fauvergue and Hopper, 2009). However, these low distances were in fields with abundant hosts; they would have to disperse much further at the end of the growing season to track their hosts.

3. Geographic Range of *Aphelinis glycinis*

Aphelinus glycinis was collected from parasitized soybean aphids on soybean in 2007 near Xiyuan, Liaoning Province, Peoples Republic of China, and is not known to occur elsewhere. However, this province has a climate that closely matches the climate in the northern midwest United States where the majority of soybean is grown and where soybean aphid has become a major pest. It is expected that *A. glycinis* will be limited to this region and perhaps the Canadian provinces of Ontario and Quebec. In this region, it is expected that it will be found in soybean fields infested with soybean aphid during the growing season and on *Rhamnus* species (buckthorns that are woody perennial shrubs/trees in forest understories and crop borders) where soybean aphid overwinters.

4. Impact of *Aphelinis glycinis* on Soybean Aphid

In the laboratory, an individual female *A. glycinis* wasp can parasitize or kill by host feeding over 150 aphids during a lifetime of three weeks, but such long lifetimes are unlikely in the field. However, if females lived one week in the field they could parasitize and host feed on 40 to 50 aphids, if aphids are sufficiently abundant.

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 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), which explains 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.

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). It is also known from Kenya, and 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. glycinis* in the United States

1. Insects Related to Soybean Aphid

Information regarding insects taxonomically related to soybean aphid is included because insect species which are closely related to it have the greatest potential to be attacked by *A. glycinis*.

Of the 497 species of *Aphis* whose distributions are given 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. However, 86 *Aphis* species were described from collections in North America and thus are likely to be native species. Fortunately, most native *Aphis* species are not closely related to soybean aphid (*Aphis glycines*), and *A. glycinis* does not parasitize *Aphis* species that are distantly related to soybean aphid. 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 thought to be native to Asia and is a pest of potatoes in North America.

2. Insects Related to *Aphelinus glycinis*

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 are likely 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 have been 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 midwest. Thus, the native parasitoid species are unlikely to switch to attack the soybean aphid, and such 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*, *A. siphonophorae*) are in the *mali* complex to which *A. glycinis* belongs and thus are more closely related to *A. glycinis* than other native *Aphelinus* species. However, their hosts are very different from those parasitized by *A. glycinis*. Therefore, *A. glycinis* is not expected to compete with native species of *Aphelinus*.

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 are also able to transmit plant viruses such as alfalfa mosaic, soybean mosaic, and bean yellow mosaic, that can cause reduction in soybean yields.

For soybean growers in the north central United States, 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). Because of the soybean aphid invasion, pest management practices of soybean producers have changed dramatically. In 2000, less than 0.1 percent of soybeans in the north central states were treated with insecticides; by 2006, over 13 percent of the soybeans in these states were treated with insecticides, indicating that soybean aphid has been 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 in the United States. This is a major disruption in the 70 million acres where soybean is grown.

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. glycinis.*

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 also pose a threat to bees and aphid natural enemies such as lady beetle larvae and predatory bugs.

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 can result in a financial loss to growers if aphid populations would not have been high enough to cause economic damage.

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 have previously been released against soybean aphid. However, neither *A. atriplicis* nor *Binodoxys communis* have established in the environment and are therefore not likely to control soybean aphid.

These environmental consequences may occur even with the implementation of the biological control alternative, depending on the efficacy of *A. glycinis* to reduce soybean aphid infestations in the continental United States.

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

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

a. Scientific Literature

Aphelinus glycinis is new to science so there is no literature on its host range. Other species in the *mali* complex tend to have narrow host ranges, although a few, including *A. mali* itself, are reported to have broad host ranges. However, broad host ranges in the literature for species in the genus *Aphelinus* are suspect because similar looking species have frequently been confused with one another, resulting in false host reports (Heraty et al., 2007).

b. Host Specificity Testing

Host specificity testing of *A. glycinis* was conducted in a quarantine facility (Hopper, 2010, appendix A). Parasitism of 13 aphid species from five different genera on nine different plant species from eight families were tested (Table 1). These aphids span many of the aphids reported as hosts of the *A. mali* complex, of which *A. glycinis* is a member. Based on the aphid distributions in Blackman and Eastop (2006), many of these aphid species occur within the native geographic range of *A. glycinis*.

Table 1. Aphid and host plant species used to test *Aphelinus glycinis* host specificity (Hopper, 2010).

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

In the laboratory experiments in quarantine, oviposition and host feeding by *A. glycinis* were restricted almost completely to species in the genus *Aphis* that are closely related to soybean aphid (see appendix A for more detailed study information). Furthermore, parasitoid stinging that did not lead to mummification or host feeding did not increase mortality of any aphid species. Handling times were long so that ant-tended native aphids, such as *A. monardae* and *A. oestlundi*, are not expected to be at risk in the field because ants would have time to chase away *A. glycinis* if it were to attempt to oviposit or host feed on these aphid species.

2. Uncertainties Regarding the Environmental Release of *A. glycinis.*

Once a biological control agent such as *A. glycinis* 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. glycinis*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *A. glycinis* 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. glycinis* 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 action alternative, depending on the efficacy of *A. glycinis* 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).

No other soybean aphid biological control organisms released in the United States have successfully established. Therefore, no competitive interactions would occur between *A. glycinis* and other biological control organisms. In addition, as discussed previously, *A. glycinis* is not expected to compete with native species of *Aphelinus*. However, the non-native parasitoid *Aphelinus certus* has established in North America accidentally (Heimpel et al., 2010, Frewin et al., 2010). Because of its broad host range, *A. certus* was not considered for purposeful introduction against soybean aphid. The abundance of *A. certus* in the United States is unlikely to be affected by introduction of *A. glycinis* because *A. glycinis* does not attack many of the same host species used by *A. certus*. *Aphelinus certus* might interfere with establishment of *A. glycinis*, but given that these parasitoid species coexist in Asia, such interference is likely to be transitory.

Release of *A. glycinis* 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, and 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. glycinis,* there will be no effect on any listed insect species or designated critical habitat in the continental United States. In host specificity testing, *A. glycinis* attacked only *Aphis* species in the *Aphis gossypii/glycines* complex. No federally listed threatened or endangered insects belong to the aphid family, Aphididae (USFWS, 2012). 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. glycinis* 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. No circumstances that would trigger the need for special environmental reviews are involved in implementing the preferred alternative. Therefore, no disproportionate effects on children are anticipated as a consequence of the field release of *A. glycinis*.

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 Illinois, Indiana, Michigan, Minnesota, Ohio, Iowa, North Dakota, and South Dakota. 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. The addresses of participating APHIS units, cooperators, and consultants (as applicable) follow.

U.S. Department of Agriculture
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Animal and Plant Health Inspection Service
Plant Protection and Quarantine
Registrations, Identification, Permits, and Plant Safeguarding
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U.S. Department of Agriculture
Agricultural Research Service
Beneficial Insect Introductions Research Unit
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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 *Aphelinis glycinis* (Hopper, 2010).

In laboratory experiments in quarantine, attack by *A. glycinis* on the soybean aphid and non-target aphid species was evaluated, as well as the mechanisms underlying differences in parasitism among aphid species. Parasitoids in the genus *Aphelinus* could kill aphids in three ways: oviposition which leads to aphid mummification, stinging (with or without oviposition) that leads to aphid death but no mummification, and host feeding. These experiments tested for each type of mortality. Parasitism of 13 aphid species in 5 genera in 2 tribes on 9 plant species in 9 families was measured. These aphids span most of the phylogeny of aphids reported as hosts of the *Aphelinus mali* complex, of which *A. glycinis* is a member. Furthermore, these aphid-plant combinations provide contrasts of aphids in the same versus different genera on the same versus different plants. Based on the aphid distributions in Blackman and Eastop (2006), many of these aphid species occur within the native geographic range of *A. glycinis*, so these aphids and this parasitoid are likely to have been in contact for at least 10,000 years. The exceptions are *D. noxia*, which is native to central to western Asia and Europe, and *Aphis oestlundi* and *A. monardae*, both native to North America. The goal was to explore the phylogenetic and host plant limits on parasitism as has been recommended for host specificity testing of entomphagous insects (Kuhlmann et al., 2005). Three rounds of testing were done: round one involved species in five aphid genera distributed across the phylogeny of known hosts of the *A. mali* complex; round two narrowed the testing to species in the genus *Aphis* but involved more species in this genus and included species native to North America; round three used aphid species from rounds one and two, but involved detailed direct observations of parasitoid and aphid behavior, as well as measurement of aphid suitability.

Parasitism in 24 hour exposure of aphids on plant. Individual female parasitoids were exposed to one of 13 aphid species. One to five-day old females that had been with males and aphids since emergence were used 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 ensure that the females had a full egg load. Each female parasitoid was placed in a cage (10 centimeters (cm) in 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 was delayed until the larval parasitoids had killed and mummified their hosts. Therefore, a combination of acceptance of hosts for oviposition and suitability of hosts for parasitoid development was measured.

Female parasitoids had the choice of whether to oviposit or not in a particular host species. This is frequently the choice parasitoids make in the field. The idea 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 was recorded. Seven to 14 females on each of 13 aphid species were tested.

After 24 hours exposure, the number of aphids mummified by *A. glycinis* varied with aphid species ($F = 5.7$; df = 12, 123; $P < 0.0001$; Fig. 1). 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*, such as *A. gossypii*, *A. monardae*, and *A. oestlundi*, but not on aphids that are more phylogenetically distant, such as *A. asclepiadis*, *A. nerii*, and *A. rumicis*. The low numbers of mummified aphids for *A. asclepiadis* and *A. nerii* may have resulted from their host plant, *Asclepias syriaca*, which is known to produce toxic secondary chemicals which aphids could sequester for their defense (Mooney et al., 2008; Warashina and Noro, 2000). Although the number of mummified aphids varied greatly among aphid species, the proportion of mummies from which adults emerged was high (0.93±0.02) and did not vary significantly among species that were parasitized. The proportion of females among parasitoid progeny varied with species ($F = 2.89$; df = 4, 42; $P = 0.03$), but only because *A. gossypii* produced a low proportion of females (0.11±0.06) compared to other species of *Aphis* (e.g. 0.46±0.10 for *A. glycines*)

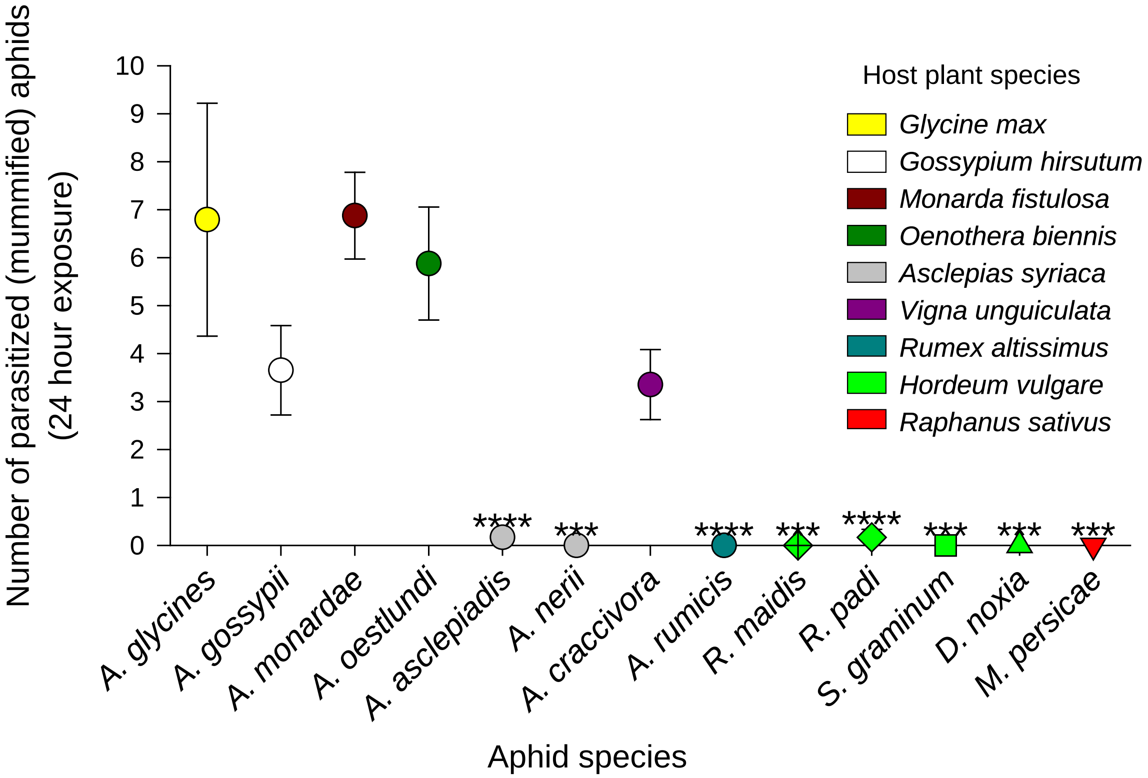

Fig. 1. Number of aphids parasitized (mummified) of various aphid species exposed to *Aphelinus glycines* for 24 hours. Aphid species are ordered by phylogenetic distance from *Aphis glycines*. Asterisks indicate level of difference from *Aphis glycines*: ***P<0.001; ****P<0.0001. Error bars are standard errors of means.

Direct observation of parasitoid and aphid behavior. Behavior of parasitoids and aphids were directly observed to determine the factors affecting host use patterns from the experiment above, observing 18 to 21 female parasitoids exposed to each of 12 aphid species. A randomized complete-block design was used with each block done on a single day. Each female parasitoid was exposed to 10 second and third instar aphids on an excised leaf of the appropriate plant species in small arenas (10 millimeters (mm) in diameter by 4 mm in height). The arenas were formed using two microscope slides with vinyl foam weather-stripping glued to them; on one slide, the weather-stripping had a 10 mm diameter hole bored into it, exposing the slide surface through which observations were made. A freshly cut leaf from a plant was clamped between the slides (weather-stripping against the leaf) and the leaf petiole placed in 1 percent agar in a small vial to maintain turgor. Aphids were placed in the arenas about 0.5 hour before beginning observations to allow them to settle and begin feeding on the leaves. One-day old females were exposed to 3 second and third instar *A. glycines* for about 0.5 hour before being observed.

The behaviors of each female parasitoid and the aphids with which she was enclosed were observed continuously for 25 minutes under a zoom binocular stereoscope at 10-30X magnification and these behaviors were recorded using an attached video camera and digital video tape recorder. Locations and sequences of behaviors were noted on maps of the arenas, and these locations and sequences were verified by reviewing the tape recordings.

On encountering a potential host, *A. glycinis* 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). They turn 180^o to face away from the aphid, extend their ovipositor, and insert it into the aphid (a sting). Females sometimes failed to contact the aphid with their ovipositor (a miss) and made several attempts to approach and sting the aphid. Besides ovipositing in an aphid, *A. glycinis* females may also paralyze them, make a wound with their ovipositor, and drink hemolymph from the wound (host-feeding). Female parasitoids paralyze and kill the aphids on which they feed and thus do not oviposit in them.

The following data was recorded for aphids: whether an aphid was approached; if approached, the outcome of each encounter (stung, missed, host-fed); the duration of the longest sting, the duration of the encounter with longest sting from approach until the parasitoid leaves the aphid (handling time), and the presence of aphid defense behaviors (kicking, bucking, rotating around while retaining mouth parts in the leaf, walking away, and cornicular secretion). From these data, the number of aphids approached, the numbers of aphids stung, and the numbers of aphids fed upon were tabulated. Aphid defense was calculated as the number of aphids defending themselves weighted by the frequencies of defensive behaviors (kicking, bucking, rotating, walking away, and cornicular secretion) when approached by female parasitoids. For aphids that were not approached, whether they changed location from the beginning to the end of the observation period was recorded. Female parasitoids were removed after the observation period, their ovaries were dissected in Ringer’s solution and mixed with neutral red stain, and the number of mature eggs was counted under 40-100X magnification (neutral red does not pass the chorion of mature eggs and thus they remain unstained).

If a female parasitoid stung one or more aphids, either all the aphids were dissected from a given observation period to determine whether eggs were laid, or all aphids were transferred from a given observation period to the appropriate host plant species and were reared to determine whether parasitoid progeny survived to mummification and whether exposure to parasitoids otherwise affected aphid survival. Aphids were dissected in batches of five on glass microscope slides. Each batch was placed in a small drop of Ringer's solution and covered with a separate cover slip. Parasitoid eggs from the dissected aphids were counted at 40-100X magnification. Aphids were dissected from half the replicates and aphids were reared from the other half of the replicates, yielding 10 replicates of dissected aphids and 10 replicates of reared aphids per aphid species that a female parasitoid stung. For aphid species that were not stung, these aphids were neither dissected nor reared after observation.

Because sampling for eggs in aphids was destructive, the number of aphids with parasitoid eggs and the number that mummified could not be directly compared. Instead, Spearman's rank correlation coefficient (ρ) was used to test the relationship between the mean number of mummified aphids and the mean number of aphids in which parasitoids oviposited.

In these short, direct, behavioral observations in small arenas, the behavior of female *A. glycinis* varied with aphid species, and the differences among species increased while the range of species involved in specific behaviors narrowed through the steps in parasitism (approach, sting, oviposition or host feeding, mummification, adult emergence). In particular, *A. glycinis* was limited to some, but not all species in the genus *Aphis*.

The number of aphids approached varied with species ($F = 13.9$; df = 11, 222; $P < 0.0001$; Fig. 2), primarily because three species, *A. rumicis*, *D. noxia*, and *M. persicae*, were rarely approached, whereas in the genus *Aphis* (excluding *A. rumicis*), about half the available aphids (5.3±0.5 to 6.5±0.6) were approached.

The number of aphids stung varied with species ($F = 13.2$; df = 11, 221; $P < 0.0001$; Fig. 2). Aphids in the genus *Aphis* were stung 2-4 times more often than those outside this genus, with the exceptions of *A. rumicis* which was rarely stung and *S. graminum* which was stung almost as often as *Aphis* species. Aphid survival rate was not correlated with stings per aphid (Spearman's $\rho = -0.11$; $P = 0.28$; n =102), indicating that parasitoid stings (other than those that resulted in mummified or host-fed aphids) did not cause aphid mortality.

The number of aphids in which parasitoids oviposited varied strongly with species ($F = 2.9$; df = 11, 129; $P < 0.002$; Fig. 2). Parasitoids rarely laid eggs in aphids outside the genus *Aphis*, and in the genus *Aphis*, parasitoids oviposited in 0.7±0.3 to 1.9±0.4 aphids, again with the exception of *A. rumicis* which received no eggs. Egg load for the 1-2 day-old *A. glycinis* females used in this experiment was 11.2±0.3. Although 1 percent of the parasitoids carried 1−3 eggs, 95 percent carried more than five eggs (and 5 percent carried more than 16 eggs) so these females could have laid eggs in all the aphids they approached. However, the ratio of oviposition to approaches was 0.27±0.07 for *A. glycines* and varied from 0.12±0.05 for *A. nerii* to 0.37±0.07 for *A. oestlundi* among species in the genus *Aphis* (excluding *A. rumicis*). This low ratio of ovipositions to approaches can be explained in part by host feeding. Females often host fed during observations, despite having been exposed to three *A. glycines* nymphs for 0.5 hour before observations. The number of aphids on which parasitoids host fed varied with species (*F*

= 5.1; df = 11, 221; *P* < 0.0001; Fig. 2). Parasitoids rarely fed on aphids outside the genus *Aphis*, and in the genus *Aphis*, parasitoids fed on 0.2±0.1 to 1.2±0.2 aphids. Both *A. nerii* and *A. rumicis* were fed on less often than *A. glycines*. Contrary to previous speculation that feeding host range should be broader than oviposition host range, the choice of aphids on which to host feed was essentially the same as the choice of aphids in which to oviposit (Fig. 3).

For aphid species in which parasitoids oviposited, handling time varied among aphid species for encounters that might have involved oviposition (*F* = 2.2; df = 11, 550; *P* = 0.01). This handling time varied from 1.0±0.4 minutes for *A. nerii* to 2.4±0.3 minutes for *A. glycines*. For aphids on which parasitoids host fed, handling time varied from 8.1±0.7 minutes for *A. gossypii* to 10.9±1.2 minutes for *A. glycines*, but no differences among aphid species were significant. Given these long handling times, it is unlikely that *A. glycinis* would be able to parasitize ant-defended aphids, like *A. monardae* and *A. oestlundi* in the field (Wyckhuys et al., 2007).

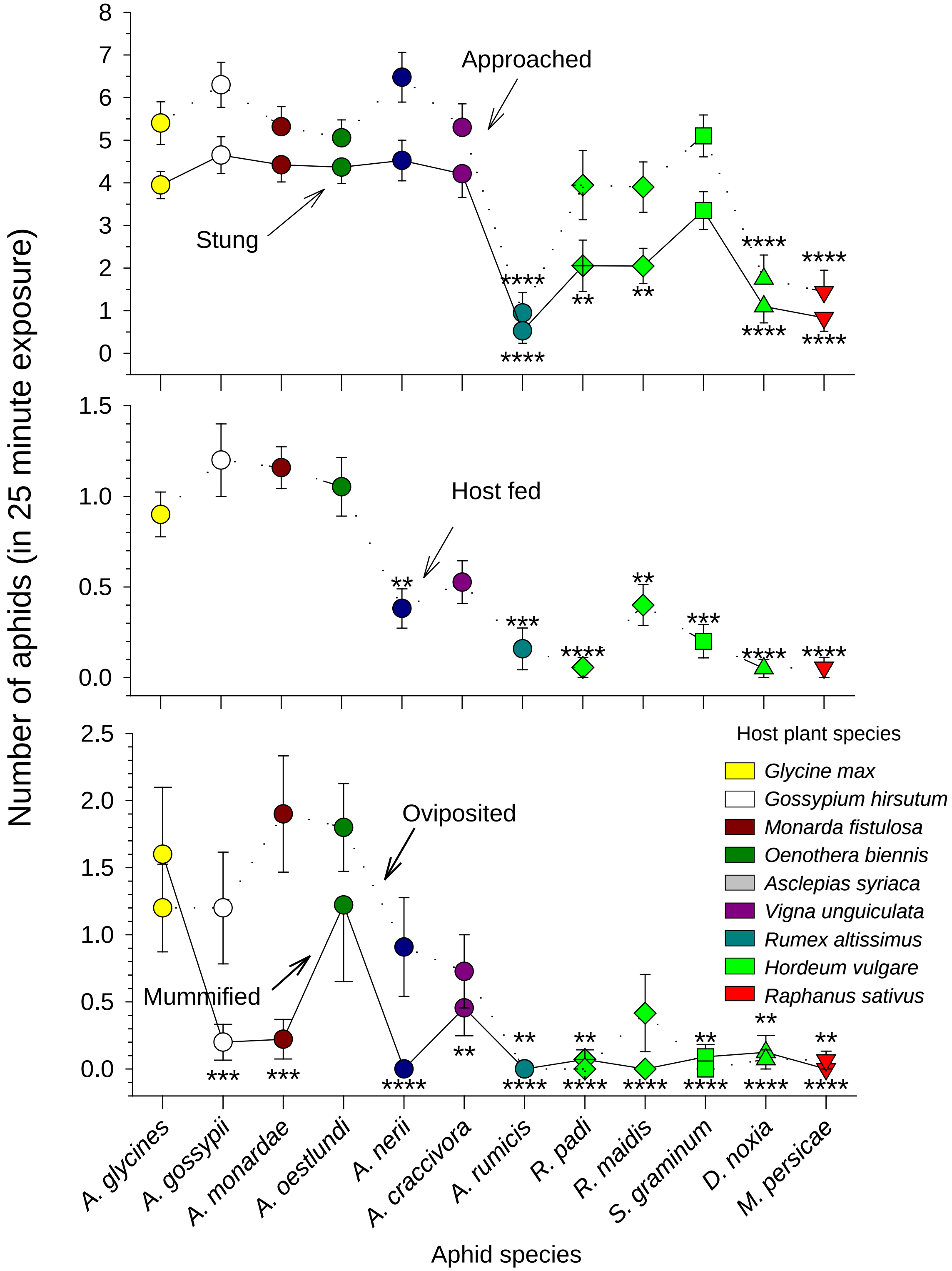

Fig. 2. Number of aphids approached, stung, host-fed, oviposited, and mummified by *Aphelinus glycines* in 25 minute observations. Asterisks above or below means indicate difference from *Aphis glycines*: ** *P*<0.01; ****P*<0.001: *****P*<0.0001. Error bars are standard errors of means.

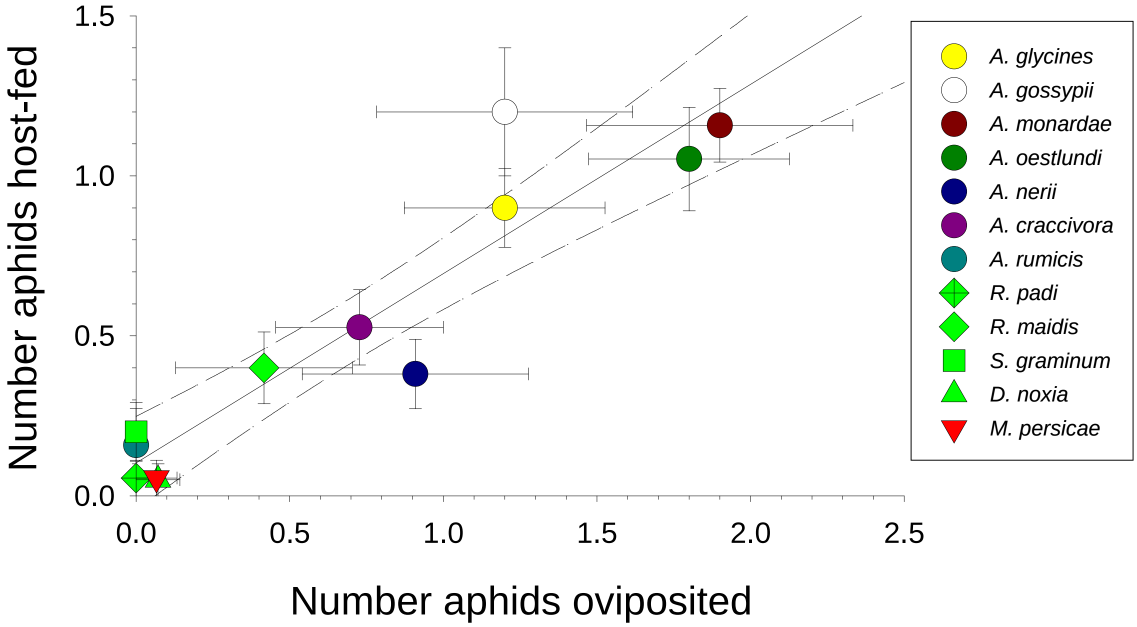

Fig. 3. Host-feeding versus oviposition in aphids by *Aphelinus glycines*. Error bars are standard errors of means. Solid line is least-squared regression; dotted lines are 95% confidence intervals for regression.

The number of mummified aphids varied with species (*F* = 3.3; df = 11, 131; *P* = 0.0006; Fig. 2). *Aphelinus glycinis* produced more mummies on *A. glycines* (1.6±0.5) than on all other species except *A. oestlundi*. Few or no mummies were produced on species in genera other than *Aphis* or on *A. rumicis*, which is not surprising, given the low rate of oviposition in these species. Furthermore, few mummies were produced on *A. gossypii*, *A. monardae,* and *A. nerii* as well, although female parasitoids oviposited as often in these species as in *A. glycines*. The proportion of adults which emerged from mummified aphids was high (0.95±0.05) and did not vary with species.

Aphid behavioral defense varied with species (*F* = 28.0; df = 11, 182; *P* < 0.0001; Fig. 4). *Aphis craccivora* and *S. graminum* defended themselves more than *A. glycines*, but *A. oestlundi* defended themselves less than *A. glycines*. In the genus *Aphis*, the proportion of aphids that parasitoids stung that either received an egg or were host-fed varied from 0.62±0.11 for *A. glycines* through 0.24±0.08 for *A. craccivora* to 0.03± 0.03 for *A. rumicis*, and this proportion was not correlated with the level of aphid behavioral defense. Handling time increased with amount of aphid behavioral defense (*F* = 8.83; df =1, 559; *P* = 0.003), and the *Aphis* species that defended itself most, *A. craccivora*, did receive relatively low numbers of eggs. Nonetheless, oviposition was determined primarily by detection and acceptance of aphids by parasitoids and was little affected by aphid behavioral defenses. Thus, while aphid defensive behavior may mediate host specificity of parasitoids, parasitoids are able to circumvent these defenses in the species that are hosts. The effectiveness of defensive behavior varies with aphid instar (Gerling et al. 1990; Wyckhuys et al. 2008) and thus the high suitability aphid species may be inaccessible

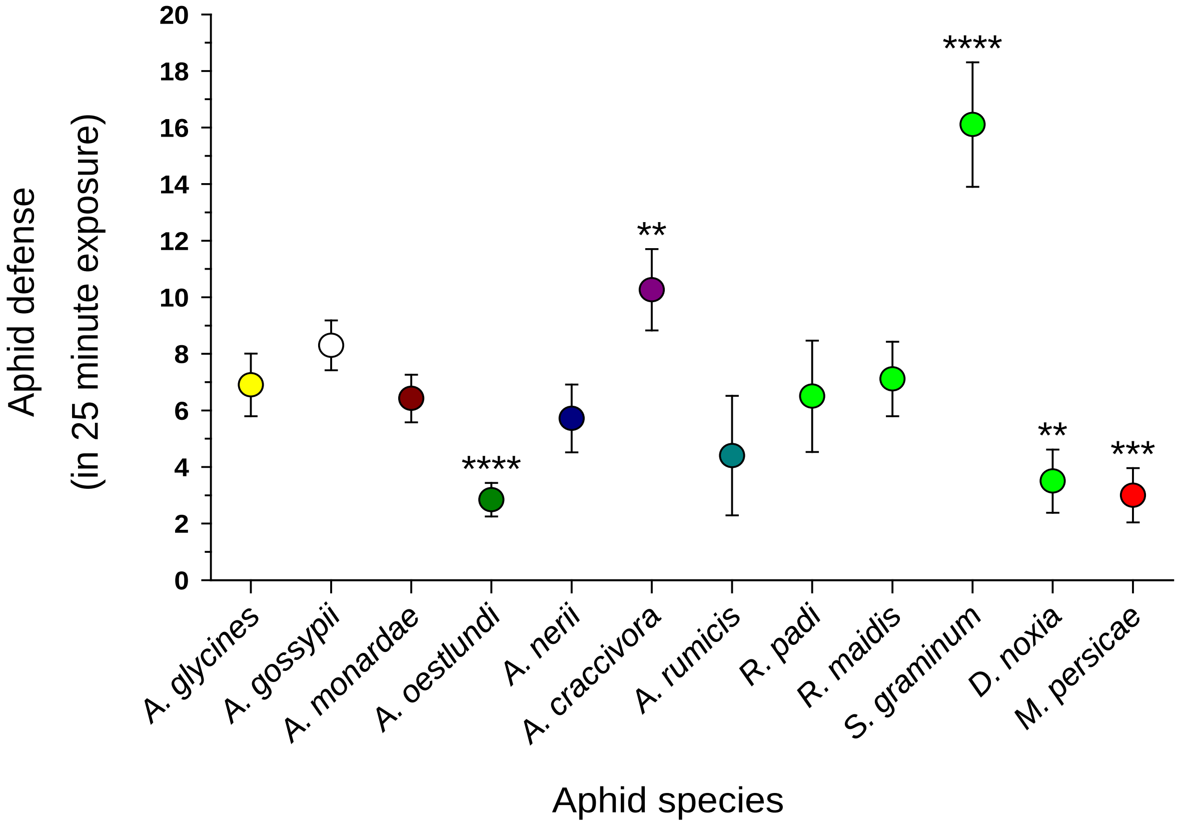

Fig. 4. Defense by aphids approached by female *Aphelinus glycines*. Defense was measured as the number of aphids defending themselves weighted by the frequencies of defensive behaviors (kicking, bucking, rotating, walking away, and cornicular secretion) when approached by female parasitoids in 25 minute observations. Error bars are standard errors of means. Asterisks indicate difference in aphid defense from *Aphis glycines*: $P<0.05$; ** $P<0.01$; ***$P<0.001$; ****$P<0.0001$.

in later instars. Aphid parasitoids may have behaviors adapted to efficiently approach some aphid species but not others, leading to behavioral specialization on these species. Oviposition by *A. glycinis* in an aphid species was correlated with progeny survival in that species (Spearman's $\rho = 0.59$; $P = 0.04$; n =12; Fig. 5) , so our results were consistent with the preference-performance hypothesis (Jaenike, 1978) and results from other parasitoid species (Ode et al., 2005; van Alphen and Vet, 1986; Driessen et al., 1991; Kraaijeveld et al., 1995; Chau and Mackauer, 2001; Bilu et al., 2006). However, the correlation is weak because several aphid species in which parasitoids oviposited were poor hosts for parasitoid development. Host-plant chemistry explains some of this lack of suitability. Aphids are known to sequester secondary metabolites when feeding on toxic plants (Mooney et al., 2008) and these compounds can be detrimental to aphid natural enemies (Helms et al., 2004). *Aphelinus glycinis* mummified few aphids of species on milkweed, which suggests that milkweed toxins limited the suitability of aphids on milkweed. Milkweed synthesizes cardenolides which are sequestered by aphids (Mooney et al., 2008), and these cardenolides affect the fitness of various aphid natural enemies

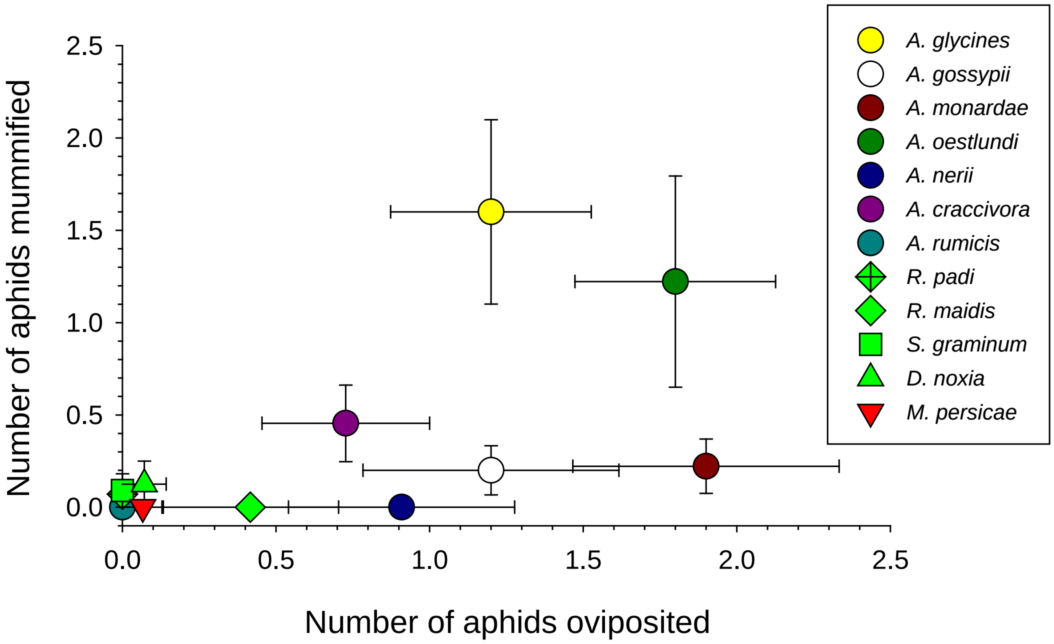

Fig. 5. Aphid species preference (oviposition) versus performance (aphid mummification = survival through third instar) in *Aphelinus glycines*. Error bars are standard errors of means.

(Pasteels, 1978; Malcolm, 1989). In particular, the low suitability of *A. nerii* may have resulted from its ability to sequester toxic host-plant allelochemicals (Rothschild et al., 1970; Malcolm, 1989; Malcolm, 1989). Low suitability on milkweed-feeding aphids may also result from other chemical defenses such as the steroidal pregnane glycosides (Warashina and Noro, 2000). *Aphelinus glycinis* also mummified few *Aphis gossypii* on *Gossypium hirsutum* and *A. monardae* on *Monarda fistula*, and both of these plants produce defensive chemicals (Elsebae et al., 1980; Johnson et al., 1998) that these aphids could sequester. However, little is known about whether these aphids sequester these chemicals or whether the chemicals affect parasitoids.
Another potential source of resistance to immature parasitoids in aphids are bacterial endosymbionts like *Hamiltonella defensa*, which interferes with successful development of *Aphidius ervi* in the pea aphid *Acyrthosiphon pisum* (Oliver et al., 2003). *Hamiltonella defensa* has been found in *A. craccivora* (Oliver et al., 2005), and was present at the start of our experiments on *A. glycinis*. However, the *A. craccivora* culture lost its *H. defensa* without change in the suitability, indicating that *H. defensa* did not defend against *A. glycinis*.

In the absence of effects of endosymbionts or plant secondary metabolites, mortality of parasitoids in some aphid species (*A. craccivora* and *R. maidis*) may have resulted from low nutritional quality for parasitoid development. Parasitoid larvae have nutritional needs that are strongly stage-specific, as a result of the complex pathways of nutritional physiology associated with the parasitic lifestyle (Godfray, 1994; Jervis et al., 2008).

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

September 2012

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 glycinis* (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. glycinis*, 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/plant_health/ea/index.shtml

The EA analyzed the following two alternatives in response to a request for permits authorizing environmental release of *A. glycinis*: (1) no action, and (2) issue permits for the release of *A. glycinis* 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. glycinis*. Legal notice of the EA was made available in the Federal Register on August 3, 2012 for a 30-day public comment period. One anonymous comment was received on the EA, in opposition to the proposed release of *A. glycinis*. 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. glycinis*. 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. glycinis* poses no threat to the health of humans.

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

- 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. glycinis* 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. glycinis*) and, therefore, no Environmental Impact Statement needs to be prepared.

Jeff Grode ______________ Date 9/6/12 ______________

Jeff Grode
Associate Executive Director
Plant Health Programs
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture