



Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Field Release of *Aphelinus hordei* (Hymenoptera: Aphelinidae) for Biological Control of the Russian Wheat Aphid, *Diuraphis noxia* (Hemiptera: Aphididae), in the Contiguous United States

**Final Environmental Assessment,
July 2021**

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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), Pests, Pathogens, and Biocontrol Permits (PPBP) is proposing to issue permits for release of the insect *Aphelinus hordei* (Hymenoptera: Aphelinidae). This organism would be used by the permit applicant for biological control of Russian wheat aphid (RWA), *Diuraphis noxia* (Hemiptera: Aphididae), in the contiguous United States.

APHIS has the authority to regulate biological control organisms under the Plant Protection Act of 2000 (Title IV of Pub. L. 106–224). Applicants who wish to study and release biological control organisms into the United States must receive PPQ Form 526 permits for such activities.

This environmental assessment (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. hordei*, to control RWA in the contiguous 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. Notice of this EA was made available in the Federal Register on May 5, 2020 for a 30-day public comment period. Three comments were received on the EA by the close of the comment period. Responses to these comments are included in appendix D of this EA.

The applicant’s purpose for releasing *A. hordei* is to reduce the severity of damage to wheat and barley from infestations of RWA in the western United States. RWA has become a major pest of wheat and barley since it was detected in the western United States in 1986. Wheat varieties resistant to RWA began to be used in 1996, and research has revealed multiple genes in wheat (Fazel-Najafabadi et al., 2015) and barley (Dahleen et al., 2015) that can provide resistance to RWA. However, aphid genotypes able to overcome one or more of these resistance genes began appearing by 2003 (Haley et al., 2004), and four resistant genotypes of RWA have been discovered, some of which have become widespread (Puterka et al., 2015; Randolph et al., 2009). Biological control agents specific to RWA may not only provide direct control of this aphid, but also may slow the spread of virulent genotypes of the aphid.

Most of the existing RWA management options (discussed below) are expensive, temporary, have not been effective, and/or include non-target impacts. For these reasons, there is a need to identify and release an effective, host-specific biological control organism against RWA in the contiguous United States.

II. Alternatives

This section will explain the two alternatives available to PPBP: no action (no issuance of permits) and issuance of permits for environmental release of *A. hordei* in the contiguous United

States. Although APHIS' alternatives are limited to a decision of whether to issue permits for release of *A. hordei*, we describe other methods currently used to control RWA by wheat and barley 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 PPBP issues permits for environmental release of *A. hordei*.

The PPBP considered a third alternative but will not analyze it further. Under this third alternative, PPBP would issue permits for the field release of *A. hordei*. The permits, however, would contain special provisions or requirements concerning release procedures or mitigating measures, such as limited releases of *A. hordei* 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, the PPBP would not issue permits for the field release of *A. hordei* for the control of RWA — the release of this biological control agent would not occur, and current methods to control RWA in the United States will continue. Use of these methods is likely to continue even if PPBP issues permits for release of *A. hordei*. Presently, control of RWA in the United States is limited to chemical control, host plant resistance, cultural, and biological control methods.

1. Chemical Control

Insecticide treatments are the most effective method to manage RWA in North America. Insecticides including dimethoate, malathion, and chlorpyrifos have been labeled for foliar application against RWA. Imidacloprid is effective as a seed treatment for dryland wheat (Pike et al., 1993; van der Westhuizen et al., 1994)

2. Host Plant Resistance

Aphid-resistant plants are available to support reduced aphid populations. Research has revealed at least 11 genes providing resistance to RWA in wheat and barley (Dahleen et al., 2015; Randolph et al., 2009). Host plant resistance works by inhibiting aphid growth and development and increasing plant tolerance to aphid feeding.

3. Cultural control

Destroying or removing volunteer grain plants can help reduce or delay the buildup of RWA populations. Also, maintaining adequate soil moisture and fertilization can reduce damage because plants stressed for water or nutrients are more susceptible to damage from RWA.

4. Biological Control

Among the most important of natural enemies of RWA are parasitoids in the genus *Aphelinus* (Hopper et al., 1998). *Aphelinus atriplicis* was introduced and established against RWA in 1996 (Hopper et al., 1998). This parasitoid has become the most frequently encountered natural enemy

of RWA (Brewer et al., 2005), being found on 13–16 percent of sentinel pots of RWA-infested wheat in Colorado, Nebraska, and Wyoming (Noma et al., 2005). Other natural enemies that attack RWA in the United States include the parasitoids *Aphelinus asychis*, *Aphelinus varipes* (Hymenoptera: Aphelinidae), *Aphidius avenaphis*, *Aphidius ervi*, *Aphidius matricariae*, *Diaretiella rapae*, *Lysiphlebus testaceipes*, *Praon yakimanum*, *Praon unicum*, *Praon occidentale* (Hymenoptera: Braconidae), and the predators *Leucopis gaimarii* (Diptera: Chamaemyiidae) and *Eupeodes volucris* (Diptera: Syrphidae).

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

Under this alternative, PPBP would issue permits for the field release of *A. hordei* for the control of RWA in the contiguous United States. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

1. *Aphelinus hordei* taxonomic information

Insect Taxonomy

Order:	Hymenoptera
Family:	Aphelinidae
Genus:	<i>Aphelinus</i>
Species:	<i>hordei</i> Kurdjumov
Common name:	none

Ferrière (1965) considered *A. hordei* and *A. varipes* to be the same species (synonymy), and Graham (1976) supported this synonymy, but it is incorrect because the two species are reproductively incompatible and phylogenetically distinct (Heraty et al., 2007). Furthermore, they differ in wing hairs (Nikol'skaya and Yasnosh 1966; Prinsloo and Nesar, 1994).

2. Description and biology of *Aphelinus hordei*

The genus *Aphelinus* is comprised of more than 90 species (Hopper et al., 2012; Noyes, 2015). *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 hordei 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 (Rohne, 2002). 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 (Christiansen-Weniger, 1994). Adults emerge about one week after pupation by chewing a hole through the host exoskeleton.

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.

Egg load, the numbers of aphids mummified per day, adult emergence rate from mummified aphids, and sex ratio of *A. hordei* attacking various aphid species were measured in the laboratory. The number of aphids mummified per day: mean 12 (range 9–16), proportion of adult emergence of *A. hordei* from mummified aphids: mean 0.89 (range 0.83–0.95), sex ratio (proportion males): mean 0.43 (range 0.29–0.56), egg load: 12 (11–14) eggs to 17 (15–19) eggs depending on whether they have four or six ovarioles. An ovariole is one of the tubes of which the ovaries are composed.

Field dispersal of *A. hordei* was not evaluated by the researcher, and there is no information on its dispersal in the literature. *Aphelinus* adults are small and are weak fliers; searching for hosts and mates primarily while walking (Fauvergue and Hopper, 2009; Fauvergue et al., 1995). 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 dispersal distances were in a field with abundant aphids; the parasitoids would have to disperse much further at the end of the growing season to track their hosts. Intentionally and accidentally introduced species of *Aphelinus* have spread rapidly (Heimpel et al., 2010; Noma et al., 2005; Prinsloo et al., 2002), suggesting that they can be carried long distances rapidly by winds. Parasitism of winged aphids also may aid *A. hordei* in finding aphid colonies when the aphids have dispersed (Zhang et al., 2009; Zhang et al., 2012).

3. Geographic range of *Aphelinus hordei*

Aphelinus hordei has been reported from France (Heraty et al., 2007), the Ukraine (Kurdjumov, 1913), and the Republic of Georgia (Yasnosh, 2002), and these countries appear to delimit the extremes of its natural geographical range. Extensive exploration for natural enemies of RWA in Eurasia did not recover it beyond this range (Heraty et al., 2007; Yasnosh, 2002). *Aphelinus hordei* has been introduced into South Africa for control of RWA (Prinsloo et al., 2002; Prinsloo, 1998; Prinsloo and Naser, 1994). Using the climate extremes of the known distribution of *A. hordei* in Europe, its potential distribution in North America was projected based on climatic match. This projection was done with a Composite Match Index calculated from similarity in maximum and minimum temperature, total rainfall, and rainfall pattern, using the Match Climates procedure in the Dymex Simulator Application software (version 2.01.025, copyright CSIRO, 2004). Based on climate match and preferred host distribution *A. hordei* will likely be limited to the western United States, southwestern Canada, and perhaps central Mexico (Hopper, 2017).

4. Impact of *Aphelinus hordei* on Russian wheat aphid

In the laboratory, an individual *A. hordei* female can parasitize or kill by host feeding over 100 aphids during a lifetime of two to 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 40–50 aphids, if aphids were sufficiently abundant (Hopper, 2017).

III. Affected Environment

A. Russian Wheat Aphid

The RWA is wingless, pale yellow-green or gray-green insect lightly dusted with white wax powder. It is 1.4-2.3 mm in length. In North America, RWA is similar to other *Diuraphis* species including *Diuraphis mexicana*, a native species that occurs primarily on *Bromus* species and does not attack wheat or barley; *Diuraphis frequens* which occurs in Eurasia as well as North America and has rarely been found on wheat; and, *Diuraphis tritici*, which may have invaded North America from Asia and has occasionally done damage to wheat.

RWA feeds and develops on grass and cereal species (Blackman and Eastop, 2006). In North America, RWA does best on wheat and barley (Armstrong et al., 1991). RWA females produce female progeny parthenogenetically (a form of reproduction in which an unfertilized egg develops into a new individual), and they overwinter as adults. However, a few populations undergo sexual reproduction in the fall, and in this case, overwinter as eggs (Puterka et al., 2012). RWA females produce winged and wingless forms during April to September, but they can reproduce at temperatures from 1 to 40°C (Merrill et al., 2009). In the laboratory, RWA can produce 55 progeny per lifetime of a female at 18°C. At that temperature, the time from hatching to reproduction is about 10 days (Merrill et al., 2009). Although the winged adults are weak fliers, the rapid rate of spread of RWA after it was first detected in the United States suggests a high rate of windborne dispersal. The combination of high dispersal rate and high reproductive rate means that the RWA can rapidly reach damaging levels over wide regions if not properly managed.

B. Areas Affected by Russian Wheat Aphid

1. Native and worldwide distribution

RWA is native to Central Asia, the Middle East, Southern Europe, and North Africa, being reported from six Asian countries, 19 European countries, and five African countries (CABI, 2016). It has recently spread to Australia, western China, Kenya, Zimbabwe, South Africa, and North and South America. RWA is seldom a pest in Eurasia, its area of origin, although short-lived outbreaks have been reported (Fernandez et al., 1992; Grossheim, 1914; Tuatay and Remaudière, 1964).

2. Present distribution in North America

In North America, RWA is currently known from Mexico, the three western Canadian provinces (Alberta, British Columbia, and Saskatchewan), and from the following 18 states in the western United States: Arizona, California, Colorado, Idaho, Kansas, Montana, Nebraska, Nevada, New Mexico, North Dakota, Oklahoma, Oregon, South Dakota, Texas, Utah, Washington, Wisconsin, and Wyoming (CABI, 2016). RWA has not spread to the eastern United States, and is limited to the western parts of Kansas, Nebraska, North Dakota, and South Dakota.

3. Russian wheat aphid hosts

RWA feeds and develops only on certain grasses (C3), and in North America, it does best on wheat and barley (Armstrong et al., 1991).

RWA is a major pest of wheat and barley in the western United States, causing over \$1 billion in damage and control costs since 1986 (Morrison and Peairs, 1998).

C. Insects Related to Russian Wheat Aphid and *A. hordei* in the United States

1. Insects related to Russian wheat aphid

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

Of the ten species of *Diuraphis* whose distributions are given in Blackman and Eastop (2006), three besides RWA (*D. noxia*) are reported from North America: *D. frequens*, *D. mexicana*, and *D. tritici*. Both *D. frequens* and *D. tritici* are found in Europe and Asia, may have invaded North America from there, and are occasional pests of wheat. Only *D. mexicana* appears to be native to North America, where it is found on *Bromus* species (Miller et al., 2005).

2. Insects related to *Aphelinus hordei*

Thirty-one species of *Aphelinus* are reported from North America; however, only about half of these species (15) were described from North America (listed in appendix A with their authors and species complex membership, as well as reported distributions and hosts). These 15 *Aphelinus* species are reported exclusively or almost exclusively from North America, and thus are probably native. The remaining *Aphelinus* species reported from North America were described elsewhere, are reported from one to several other continents, and thus 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). Prior to the introductions of parasitoids to control RWA, there was only one report of an *Aphelinus* species parasitizing any of the four species of *Diuraphis* found in North America. Lajeunesse and Johnson (1991) found *Aphelinus* sp. nr. *varipes* (Foerster) parasitizing both RWA and *Diuraphis tritici* on wild grasses in Montana. In surveys since the introduction of *Aphelinus* species against RWA, only *A. asychis* and *A. atriplicis* and have been reported parasitizing RWA, and *A. asychis* parasitism levels have been very low (Brewer et al., 2005; Noma et al., 2005), so native *Aphelinus* species, other than *Aphelinus* sp. nr. *varipes*, have not switched to attack RWA.

Hybridization with native species is a risk in biological control introductions (Hopper et al., 2006), but *A. hordei* is reproductively isolated from the other species of *Aphelinus* that have been tested, including closely related species in the *A. varipes* complex (Heraty et al., 2007). This is in part because *A. hordei* males show very different courtship behavior than males of closely related species (Rhoades, 2015). Furthermore, the genome size of *A. hordei* is quite different from that of other species in the *A. varipes* complex and its chromosomes are different than that

of species in the *A. mali complex*, making viable hybrids unlikely (Gokhman et al., 2017).

IV. Environmental Consequences

A. No Action

a. Effect of Russian wheat aphid on host plants

1. Impact of Russian wheat aphid on the environment

RWA sucks sap from plants. While feeding, it injects a toxin into the plant that causes many of the damage symptoms. Damage often appears as white, longitudinal streaks on the leaves and sometimes the stem. Heavily infested plants are stunted and distorted. Infested leaves curl up and remain in a rigid upright position rather than drooping as normal.

RWA develops on barley (*Hordeum vulgare*, *H. murinum*, *H. pusillum*), wheat (*Triticum aestivum*, *T. cylindricum*, *T. dicoccum*, *T. dicoccoides*, *T. durum*, *T. monococcum*, *T. tauschii*, *T. timopheevi*, *T. turgidum*), rye (*Secale cereale*), oats (*Avena sativa*), rice (*Oryza sativa*), triticale (*Triticum aestivum* x *Secale cereale*), canarygrass (*Phalaris canariensis*), timothy (*Phleum pratense*), and on grasses of the genera *Bromus*, *Elymus*, and *Agropyron* (Stoetzel, 1987; Hughes, 1988, Kindler and Springer, 1989; Armstrong et al., 1991, as cited in Kaplin and Sharapova, 2017). Wheat and barley are the primary hosts of RWA (Armstrong et al., 1991). RWA also transmits plant viruses such as barley yellow dwarf virus and barley stripe mosaic virus.

After RWA was detected in the western United States in 1986 (Stoetzel, 1987), it rapidly became a major pest of wheat and barley crops (Brooks et al., 1994), causing over \$1 billion in damage and control costs in the United States during the first decade after being detected (Morrison and Peairs, 1998). In the 18 U.S. states where RWA has invaded, a total of 41 million acres of wheat and barley were harvested in 2016, producing grain worth \$8 billion (National Agricultural Statistics Service, 2016). In the three Canadian provinces where RWA has invaded, a total of 24 million acres of wheat and barley were harvested in 2015, producing grain worth about \$6 billion (Statistics Canada, 2015). In Mexico, a total of 2 million acres of wheat and barley were harvested in 2012 producing grain worth \$1 billion (Foreign Agricultural Service, 2017), but data on the distribution of RWA in Mexico are not readily available so it is not clear how much of the Mexican wheat and barley acreage is at risk.

RWA outbreaks have become more rare and magnitude and geographical extent have decreased since the widespread use of resistant wheat and barley varieties. However, RWA strains able to overcome one or more of the resistance genes in wheat and barley began appearing by 2003 (Haley et al., 2004). Four genotypes of RWA have been discovered that can overcome the resistance genes in wheat and barley, some of which have become widespread (Puterka et al., 2015; Randolph et al., 2009).

2. Impact from the use of other control methods

The continued use of chemical control, plant resistance, cultural control, 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. hordei*.

a. Chemical Control

Variation in RWA susceptibility to insecticides suggests the possibility that *D. noxia* may develop resistance to them (Brewer and Kaltenbach, 1995). Neonicotinoid seed-treatments could provide an alternative to previously resistant wheat and barley varieties. However, there is growing concern that neonicotinoids have significant non-target impacts and are contributing to declines in pollinators, especially honey bees (Di Prisco et al., 2013; Sanchez-Bayo and Goka, 2014), as well as predatory and parasitic insects, aquatic insects, and to fish, frogs, birds, and bats that feed on insects (Hallmann et al., 2014; Sanchez-Bayo, 2014).

b. Plant Resistance

RWA genotypes able to overcome plant resistance genes began appearing by 2003 (Haley et al., 2004). At least four virulence genotypes have been identified in RWA that can overcome one or more of these resistance genes, and some of these genotypes have become widespread (Puterka et al., 2015; Randolph et al., 2009).

c. Cultural Control

Cultural control can be useful in reducing RWA damage, but alone is not effective in eliminating RWA.

d. Biological Control

Aphelinus atriplicis Kurdjumov (Hymenoptera: Aphelinidae) was introduced and established against RWA by 1996 (Hopper et al., 1998) and has become the most frequently encountered natural enemy of RWA (Brewer et al., 2005; Noma et al., 2005). Nonetheless, RWA remains a pest.

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. hordei* to reduce RWA infestations in the contiguous United States.

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

1. Impact of *A. hordei* on non-target insects

a. Scientific Literature

Aphelinus hordei was described from adults that emerged from RWA collected near Poltava, Ukraine, in 1911 (Kurdjumov, 1913), and *A. hordei* has since been reported from RWA in France (Heraty et al., 2007; Hopper et al., 2017), and in the Republic of Georgia (Yasnosh, 2002). During extensive exploration for natural enemies of RWA during 1988–1994 throughout the distribution of RWA in Eurasia, *A. hordei* was not found in any aphid species other than

RWA, although *Aphelinus* species were collected from all aphid species on barley, wheat, and occasionally wild grasses (Heraty et al., 2007; Hopper et al., 1998). *Acyrtosiphon pisum* has been reported as a host (Yasnosh, 2002), but this appears to have been a record for *A. varipes*, not *A. hordei*. The literature on the hosts of *Aphelinus* species suffers from confounding of cryptic parasitoid species (Heraty et al., 2007), which can increase estimates of host ranges when the parasitoid species have narrow and different host ranges. Host ranges may also be underestimated because of under-reporting of host aphids that are not pests (De Nardo and Hopper, 2004).

b. Host Specificity Testing

See appendix B for a complete description of host specificity testing methods and results.

Parasitism in 24-hour exposure of aphids on plants - Methods.

Parasitism was measured and behavior observed of *A. hordei* when exposed to 16 aphid species in six genera and two tribes on six host plant species in four families (table 1). These aphids and host plants were chosen to provide contrasts of aphid species in the same versus different genera and tribes on the same versus different host plant species (Hopper, 2017). Based on the aphid distributions in Blackman and Eastop (2006) and references cited therein, many of these aphid species occur within the native geographic range of RWA, so these aphids and this parasitoid are likely to have been in contact for at least 10,000 years. The exceptions are *Aphis glycines*, which is native to east Asia, and *Aphis helianthi*, *Aphis oestlundii*, *Aphis monardae*, and *D. mexicana* which are 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 entomophagous (insect feeding) insects (Kuhlmann et al., 2005).

Table 1. Aphids and host plants on which parasitism by *Aphelinus* species was measured.

Aphid		Host Plant			
Tribe	Species	Family	Species	Variety	Common Name
Macrosiphini	<i>Diuraphis noxia</i> (Kurdjumov)	Poaceae	<i>Hordeum vulgare</i> L.	Lacey	barley
	<i>Diuraphis tritici</i> (Gillette)	Poaceae	<i>Hordeum vulgare</i> L.	Lacey	barley
	<i>Diuraphis mexicana</i> (Baker)	Poaceae	<i>Bromus marginatus</i> Nees ex Steudel	Garnet	mountain brome
	<i>Myzus persicae</i> (Sulzer)	Brassicaceae	<i>Raphanus sativus</i> L.	Cherry Belle	radish
	<i>Acyrtosiphon pisum</i> Harris	Fabaceae	<i>Vicia faba</i> L.	Windsor	fava bean
Aphidini	<i>Aphis craccivora</i> Koch	Fabaceae	<i>Vicia faba</i> L.	Windsor	fava bean
	<i>Aphis glycines</i> Matsumura	Fabaceae	<i>Glycine max</i> (L.)	Pioneer 91Y70	soybean
	<i>Aphis gossypii</i> (Glover)	Malvaceae	<i>Gossypium hirsutum</i> L.	SG 105	cotton
	<i>Aphis helianthin</i> Monell	Asteraceae	<i>Helianthis annuus</i> L.	-	common sunflower
	<i>Aphis monardae</i> Oestlund	Laminaceae	<i>Monarda fistulosa</i> L.	-	wild bergamot
	<i>Aphis nerii</i> Boyer de Fonscolombe	Apocynaceae	<i>Asclepias syriaca</i> L.	-	common milkweed
	<i>Aphis oestlundi</i> Gillette	Onagraceae	<i>Oenothera biennis</i> L.	-	common evening-primrose
	<i>Aphis rumicis</i> L.	Polygonaceae	<i>Rumex latissimus</i> Wood	-	pale dock
	<i>Rhopalosiphun maidis</i> (Fitch)	Poaceae	<i>Hordeum vulgare</i> L.	Lacey	

Aphid		Host Plant			
Tribe	Species	Family	Species	Variety	Common Name
	<i>Rhopalosiphum padi</i> (L.)	Poaceae	<i>Hordeum vulgare</i> L.	Lacey	
	<i>Schizaphis graminum</i> (Rondani)	Poaceae	<i>Hordeum vulgare</i> L.	Lacey	

Table 2. Origins of *Aphelinus* species collected from grain aphids and tested for host specificity on *Diuraphis noxia*. All parasitized aphids were collected on wheat or barley with the exception of *R. maidis* which was collected on maize.

<i>Aphelinus</i>							
complex	species	Aphid host	Country	Region	Locations	Number	Year
<i>asychis</i>	<i>asychis</i> Walker	<i>D. noxia</i>	France	Herauld	Prades-le-Lez	>200	2000
	<i>asychis</i> Walker	<i>R. padi</i>	France	Herauld	Gedera	>200	2000
<i>varipes</i>	<i>albipodus</i> Hayat & Fatima	<i>R. padi</i>	Israel	Judea	Satchergesi	81	1999
	<i>atriplicis</i> Kurdjumov	<i>D. noxia</i>	Republic of Georgia	Tblisi		101	2000
	<i>atriplicis</i> Kurdjumov	<i>D. noxia</i>	USA	CO, NE, WY	Sterling, Scottsbluff, Cheyenne	584	2006
	<i>certus</i> Yasnosh	<i>R. maidis</i>	China	Hebei	Langfang	88	2005
	<i>hordei</i> Kurdjumov	<i>D. noxia</i>	France	Herauld	Prades-le-Lez	40	2000
	<i>hordei</i> Kurdjumov	<i>D. noxia</i>	France	Herauld	Montpelier	69	2011
	<i>kurdjumovi</i> (Kurdjumov)	<i>R. madi</i>	Republic of Georgia	Tbilisi	Satchergesi	80	2000
	<i>varipes</i> (Förster)	<i>R. madi</i>	France	Herauld	Prades-le-Lez	107	2000

In the first set of experiments, parasitism of seven aphid species was measured (RWA, *M. persicae*, *R. maidis*, *R. padi*, *S. graminum*, *A. glycines*, and *A. gossypii*) on four host plant species by the nine populations of *Aphelinus* in seven species (Table 2) to test their specificity among aphid species across much of the phylogeny of aphids and on several host plant species. When these experiments were conducted, the parasitoids had been in culture 12 to 52 generations. Because the first set of experiments showed that *A. hordei* had a narrow host range that included RWA, parasitism by *A. hordei* was measured in an experiment involving three species of *Diuraphis* (RWA, *D. tritici*, and *D. mexicana*), as well as well as 11 other aphid species (*M. persicae*, *A. pisum*, *A. craccivora*, *A. helianthi*, *A. monardae*, *A. nerii*, *A. oestlundii*, *A. rumicis*, *R. maidis*, *R. padi*, and *S. graminum*) that included seven aphid and six plant species not used in the first set of experiments. When this experiment was conducted, *A. hordei* had been in culture 15 to 55 generations.

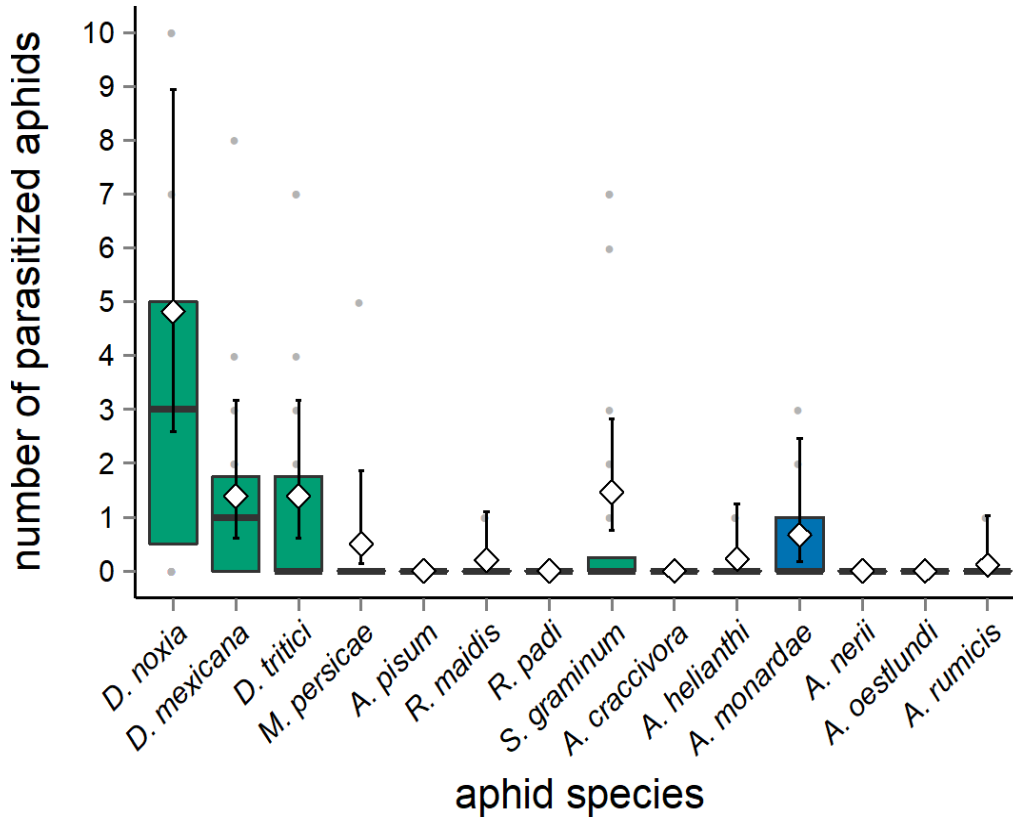
For these experiments, 1- to 5-day old females were used that had been with males and aphids since emergence, and thus, had the opportunity to mate, host-feed, and oviposit. Females were isolated from males and aphids for 24 hours before using them in experiments to ensure that the females had a full egg load. In each experimental unit, a single female parasitoid was put in a cage (10 centimeters (cm) in diameter by 22 cm in height) 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 about 30 aphids in 24 hours, this abundance of aphids and period of exposure allowed parasitoids to use their full egg load. Furthermore, the density of aphids, amount of plant material, and cage size meant that parasitoids were not limited by search rate. Parasitism was scored after the larval parasitoids killed and mummified their hosts. Therefore, a combination of acceptance of hosts for oviposition and suitability of hosts for parasitoid development was measured. This type of experiment is often called a no-choice experiment. However, female parasitoids had the choice to oviposit or not when they sequentially encountered individuals of a particular host species. This is frequently the choice aphid parasitoids have in the field because aphids are distributed in colonies often of a single aphid species. 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 load. 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.

Parasitism in 24-hour exposure of aphids on plants - Results.

Aphelinus hordei parasitized a very narrow range of aphid species primarily in the genus *Diuraphis*. This was the only species with a narrow host range. *Aphelinus hordei* was the only parasitoid that specialized on RWA attacking it significantly more often than even the more closely related *Diuraphis* spp. In the first experiment where seven species of *Aphelinus* were evaluated, *A. hordei* only attacked RWA.

Figure 1. Parasitism of 14 species of aphids exposed to *Aphelinus hordei*. Diamonds are means and vertical lines are asymptotic 95% confidence intervals of the means. Boxes indicate lower and upper quartiles and the horizontal line indicates the median.



For *A. hordei*, emergence rates were high, even for aphid species it rarely parasitized, except *M. persicae*, from which parasitoid adults rarely emerged. The sex ratios of *A. hordei* on RWA and *S. graminum* tended to be female-biased.

In the experiment on the host specificity of *A. hordei* alone, parasitism varied strongly among aphid species. *Aphelinus hordei* primarily parasitized species in the genus *Diuraphis*, particularly RWA, and little or no parasitism outside this genus (figure 1). Rates of adult emergence from mummified aphids were high for RWA (mean = 0.89 [0.77-1.00]), *D. mexicana* (0.84 [0.69-1.00]), and *S. graminum* (0.95 [0.75-1.00]), but lower for *D. tritici* (0.66 [0.46-0.85]) and *A. monardae* (0.56 [0.26-0.85]). However, the low number of replicates with parasitized aphids for species other than RWA meant that adult emergence rates were not quite significantly different among aphid species (model deviance = 0.6, df = 4; residual deviance = 3.0, df = 43; $P = 0.07$). Adult sex ratios did not differ from 50:50 and were not affected by aphid species (model

deviance = 0.4, df = 3; residual deviance = 5.7, df = 39; $P = 0.48$).

Direct observation of parasitoid and aphid behavior - Methods.

To determine the mechanisms affecting parasitism of aphid species by *A. hordei*, behavior of female parasitoids exposed to aphids was directly observed. Female parasitoids were observed with each of nine aphid species (*D. noxia*, *D. mexicana*, *D. tritici*, *M. persicae*, *R. maidis*, *S. graminum*, *A. helianthi*, *A. monardae*, and *A. rumicis*) that included species for which *A. hordei* did and did not produce parasitized aphids in the experiments described above. In each experimental unit, a day-old female parasitoid was exposed to ten second/third instar aphids, the preferred stages for parasitism, on an excised leaf of the appropriate plant species in small arenas (10 mm diameter by 4 mm 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 in 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 was placed in 1 percent agar in a small vial to keep it from drying out. Aphids were placed in the arenas about 30 minutes before beginning observations to allow them to settle and begin feeding on the leaves. Each parasitoid female was also exposed to three RWA in an arena prior to each observation bout and the number of aphids fed upon was counted. A randomized complete-block design was used, with each block of nine aphid species done on a single day. When this experiment was conducted, the parasitoids had been in culture 27–78 generations. The experimental unit in these analyses was an unmated, female parasitoid exposed to aphids of a single species on a leaf of the appropriate host plant. Ten females of *A. hordei* were observed for each of nine aphid species.

Each female parasitoid and the aphids with which she was enclosed were observed continuously for 25 minutes under a binocular stereoscope at 10–30X magnification. Behavior of the parasitoids and aphids were recorded with a video camera attached to the microscope and connected to a digital videotape recorder (for date/time stamping) and a DVD burner (for archiving). Identities of aphids and sequences of behaviors were also noted by hand on maps of the arenas, and these locations and sequences were verified by reviewing the DVD recordings.

On encountering a potential host, *A. hordei* 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 then turn 180° 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 at approaching and stinging the aphid. Besides ovipositing in an aphid, *Aphelinus* females may also paralyze them, make a wound with their ovipositor, and drink hemolymph from the wound (host-feeding). The aphid individuals paralyzed for feeding die, even if female parasitoids do not actually feed on them, and females do not oviposit in aphids paralyzed for feeding. When female parasitoids were exposed to aphid species that they did not approach or sting, they began to run and/or flit after a short period, suggesting that they would have left the aphid patch if they had not been constrained by the arena.

An aphid was recorded if approached, and if approached, the outcome of each encounter (stung, missed, host-fed). For the longest sting that did not lead to host feeding, the duration of the

encounter was measured from approach until the parasitoid left the aphid (handling time), and for the longest sting that led to host feeding, the duration of the encounter was measured from approach until the parasitoid left the aphid (host-feeding time). For aphids that were approached, the number of each type of defense behavior (kicking, bucking, rotating around while retaining mouth parts in the leaf, walking away, and cornicular secretion) was recorded. Patch-leaving time, defined as the time to flitting or running, was measured. From these data, the number of aphids approached, the numbers of aphids stung, and the numbers of aphids fed upon was tabulated. Aphid defense was calculated as the number of aphids that defended themselves times the frequency of each defense behavior. Female parasitoids were removed after the observation period, their ovaries were dissected in Ringer's solution mixed with neutral red stain, and the number of mature eggs were counted under 40–100X magnification.

If a female parasitoid stung one or more aphids, either all the aphids from a given observation period were dissected to determine whether eggs were laid, or all the aphids were transferred from a given observation period to the appropriate host plant species and were reared to determine whether parasitoid progeny survived to aphid mummification and whether exposure to parasitoids otherwise affected aphid survival. Aphids were dissected from half the replicates and aphids reared from the other half of the replicates, yielding five replicates of dissected aphids and five replicates of reared aphids per aphid species that female parasitoids stung. Aphids were dissected in batches of five on glass microscope slides, each batch 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. For replicates in which no aphids were stung, the aphids were not dissected nor reared after observation.

Direct observation of parasitoid and aphid behavior - Results.

Most of the host specificity of *A. hordei* can be explained by differences in the behavior of female parasitoids when they encountered different aphid species. When exposed to species in the genus *Diuraphis*, *A. hordei* females approached 5–8 aphids on average during the 25-minute observation periods. *Rhopalosiphum maidis* and *S. graminum* were the only non-*Diuraphis* species approached with even moderate frequency, and the other non-*Diuraphis* species were rarely, if ever, approached.

Within the genus *Diuraphis*, *A. hordei* females stung most of the aphids they approached, although there was a tendency to sting RWA more often per approach (96 percent stung per approach) compared to *D. mexicana* and *D. tritici* (90 percent and 84 percent, respectively). Outside the genus *Diuraphis*, *A. hordei* females stung both *R. maidis* and *S. graminum* 45 percent of the time they were approached, but the other non-*Diuraphis* species were never stung. Although *A. hordei* females approached and stung *D. mexicana* and *D. tritici* as often as RWA, the parasitoids oviposited more often in RWA than in the other *Diuraphis* species. This meant that the proportion of ovipositions per sting was higher for RWA (64 percent) than for *D. mexicana* (42 percent) or *D. tritici* (28 percent). *Aphelinus hordei* females occasionally oviposited in *S. graminum* but never in other non-*Diuraphis* species.

Handling time of aphids that were not fed upon varied among aphid species, from a mean of 69 seconds for RWA to a mean of 24 seconds for *R. maidis*. This variation probably arose from longer times that oviposition takes, compared to non-oviposition stings, together with fewer

ovipositions in species other than RWA and particularly in non-*Diuraphis* species. Survival of *A. hordei* progeny in RWA was higher (75 percent) than in *D. mexicana* (36 percent) or *D. tritici* (22 percent).

The numbers of aphids that were fed upon varied with aphid species, with female *A. hordei* feeding only on *Diuraphis* species. Female *A. hordei* consumed an average of 0.7 RWA, 0.5 *D. tritici*, and 0.4 *D. mexicana*, during the 25-minute observation period, but these differences were not significant. Handling times for aphids that were fed upon were about ten times longer than those for aphids that were not fed upon, being a mean 687 seconds for *D. mexicana*, 631 seconds for RWA, and 537 seconds for *D. tritici*. However, the differences among *Diuraphis* species were not significant.

The shorter handling times of *A. hordei* for species other than RWA may reflect female parasitoid rejection of poor host quality during stings, which would lead to less frequent oviposition and thus shorter handling times. Like other *Aphelinus* species, *A. hordei* has relatively large eggs that take a long time to pass through their long, thin ovipositors into host aphids. In support of this, the handling times for *A. hordei* are much longer than those reported for the other major group of aphid parasitoids, the aphidiine braconids (Wu et al., 2011) that carry much smaller eggs and have much shorter ovipositors with a large egg canal (Le Ralec et al., 1986). These long handling times probably mean that *A. hordei* is unlikely to attack ant-defended aphids (Wyckhuys et al., 2009).

Among aphids approached by *A. hordei*, defense behavior varied among aphid species with RWA defending itself least and *S. graminum* defending itself most. The time to patch leaving of *A. hordei* females was much longer for *Diuraphis* species than for other aphid species.

At the start of the observation periods, female *A. hordei* with four ovarioles carried an average of 12 eggs and females with six ovarioles carried an average of 17 eggs. Female *A. hordei* oviposited 8 percent of their eggs on average during the 25-minute observation periods. Although two females carried only two eggs and one carried three eggs, the rest of the females carried at least six eggs, and a maximum of four eggs were laid in aphids; thus, these females did not appear to be egg-limited. Furthermore, there was no correlation between the numbers of aphids in which female *A. hordei* laid eggs and their egg loads.

Summary of host specificity results.

Among the seven *Aphelinus* species tested, which included all species found on grain crops during exploration for parasitoids of RWA in Eurasia (Heraty et al., 2007; Hopper et al., 1998), only *A. hordei* specialized on *Diuraphis* species, and in particular, RWA. The specialization on *Diuraphis* species resulted from oviposition restricted almost exclusively to aphids in this genus. Female *A. hordei* very rarely approached aphids on host plants other than barley, and even with aphids on barley, walked over non-host aphid species completely ignoring them. Furthermore, *A. hordei* rarely stung non-*Diuraphis* species but did sting exuviae (the shed “skin” or exoskeleton of an insect) of *Diuraphis* species. Female *A. hordei* rarely touched host aphids or exuviae with their antennae but did orient headfirst towards them at a distance before stinging them. Within *Diuraphis*, female *A. hordei* oviposited more and their progeny survived better in RWA than in other *Diuraphis* species. Handling times were long so that ant-fended native aphids, like *A. monardae* and *A. oestlundii*, should not be at risk in the field.

2. Impact on human and animal health.

Aphelinus hordei is a tiny, stingless wasp. 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) and they pose no risk to humans, livestock, or wildlife.

3. Uncertainties regarding the environmental release of *Aphelinus hordei*

Once a biological control agent such as *A. hordei* is released into the environment and becomes established, there is a possibility it could move from the target insect (RWA) 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. hordei*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *A. hordei* generally spread without intervention by man. In principle, therefore, release of this parasitoid 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 RWA populations in the contiguous 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 RWA populations by *A. hordei* will not be known until after release and establishment occurs. Monitoring will be conducted by the permittee to determine the establishment of *A. hordei* (appendix C). 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. hordei* to reduce RWA in the contiguous United States.

4. 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).

Increase in hyperparasitoid abundance, resulting in increased parasitism of resident parasitoid species - This risk is low given the generally low levels of hyperparasitism of *Aphelinus* species. It would be at its greatest in the early stages of the introduction, when *A. hordei* abundances would be high because of initially high abundances of RWA itself. However, once biological control takes effect, *A. hordei* abundances would be expected to be low (as they are in Eurasia,

where RWA rarely reaches pest levels), and any contribution to hyperparasitoid abundances would be small.

Hybridization with native parasitoids species - Hybridization between introduced and native parasitoids might cause shifts in the behavior and ecology of both the introduced and native species, particularly changes in host specificity (Hopper et al., 2006). However, because of large differences in mating behavior between species complexes of *Aphelinus* (Rhoades, 2015), there is no risk of hybridization between *A. hordei* and species outside the *A. varipes* complex. Hybridization within *Aphelinus* species complexes is rare, and only occurs between very closely related species that are extremely difficult to distinguish morphologically (Heraty et al., 2007; Hopper et al., 2012; Kazmer et al., 1996; Shirley et al., 2017) and have similar genome sizes and chromosome number and appearance. *A. hordei* does not hybridize with any of the species in the *A. varipes* complex that have been tested (Heraty et al., 2007).

Competition with native parasitoids - *Aphelinus hordei* is restricted to species in the genus *Diuraphis*, and *Diuraphis* species are rarely parasitized by native parasitoids. *Aphelinus asychis* does parasitize RWA both in the laboratory (De Farias and Hopper, 1999; Hopper et al., 2017) and in the field (Noma et al., 2005), but *A. asychis* has been reported attacking aphids in multiple genera on a variety of host plants (Hopper et al., 2017; Mackauer and Finlayson, 1967; Rautapaa, 1972; Sanchez et al., 2011; Takada et al., 2011; Wilbert and Lauenste, 1974) and so is unlikely to be affected by competition from *A. hordei* on RWA alone.

Competition with resident biological control agents and other natural enemies - *Aphelinus atriplicis* was introduced and established against RWA (Hopper et al., 1998), and this parasitoid has become the most frequently encountered parasitoid of RWA (Brewer et al., 2005). However, *A. atriplicis* has a broad host range (Hopper et al., 2017) so that *A. hordei* will have an included niche and cannot outcompete *A. atriplicis* on the aphid species that *A. hordei* does not parasitize. Native and resident predators do prey on RWA (Lee et al., 2005; Mohamed et al., 2000; Noma et al., 2005), but these predator species have broad prey ranges and are superior competitors to *Aphelinus* species. Predation on parasitized aphids may present a barrier to establishment of *A. hordei*.

Decrease in honeydew from RWA, resulting in less food for natural enemies – This effect is an inevitable outcome of controlling RWA, no matter the method. Because RWA prevents leaves from unfurling and tends to remain inside the furled leaves, its honeydew is less accessible than honeydew produced by aphids that feed in the open. In any case, RWA honeydew was not available prior to its invasion, and loss of this honeydew should not be considered an environmental hazard.

Release of *A. hordei* is not expected to have any negative cumulative impacts in the contiguous United States because of its host specificity to RWA. Effective biological control from introduced *A. hordei* may not only provide safe, effective, and long-term control of RWA, but the parasitoid may also slow the spread of virulent genotypes that are able to overcome plant resistance to RWA. The potential benefits of introducing *A. hordei* are hard to estimate quantitatively because of uncertainties about the effects of releasing this parasitoid on the future frequencies and geographical distributions of virulent genotypes of RWA. Furthermore, it is hard to estimate the risks of non-target impacts of insecticides that might buffer against the impact of

virulent aphid genotypes on wheat and barley varieties previously resistant to RWA. However, even modest levels of direct control of RWA and reduction of the spread of virulent genotypes would provide hundreds of millions of dollars of benefits per year to small grain production in North America and these benefits could persist over decades.

5. 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. hordei*, there will be no effect on any listed insect species or designated critical habitat in the contiguous United States. In host specificity testing, *A. hordei* only attacked species of aphids. There are no federally listed threatened or endangered insects belong to the aphid family, Aphididae (USFWS, 2018). There are no federally listed species are known to depend on or utilize RWA.

V. Other Issues

In Executive Order (EO) 13985, Advancing Racial Equity and Support for Underserved Communities Through the Federal Government, each agency must assess whether, and to what extent, its programs and policies perpetuate systemic barriers to opportunities and benefits for people of color and other underserved groups. In EO 12898, Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations, Federal agencies must identify and address disproportionately high and adverse human health or environmental impacts of proposed activities. Consistent with these EOs, 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. hordei* 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. hordei*.

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 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 below.

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
Pests, Pathogens, and Biocontrol Permits
4700 River Road, Unit 133
Riverdale, MD 20737-1236

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

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CABI—see Centre for Agriculture and Biosciences International.

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

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

Species	Author	Species complex	Reported distribution	Reported hosts
<i>gillettei</i>	Howard, 1914	<i>subgenus Mesidia</i>	Colorado (Howard, 1914)	<i>Diuraphis tritici</i> (Howard, 1914)
<i>perpallidus</i>	Gahan, 1924	<i>subgenus Mesidia</i>	Iowa (Gahan, 1924); Alabama (Edelson and Estes, 1987); California (Peck, 1963); Florida (Mizell and Schiffhauer, 1990); Texas (Watterson and Stone, 1982)	<i>Chromaphis juglandicola</i> (Peck, 1963); <i>Melanocallis fumipennellus</i> , <i>Monellia costalis</i> (Peck, 1963); <i>Monellia caryella</i> (Bueno and Stone, 1983); <i>Monelliopsis pecanis</i> (Edelson and Estes, 1987)

Appendix B. Host Specificity Testing Methods and Results

(From: Hopper, 2017)

Host-Specificity Testing

B.1 Selection of non-target arthropods

Parasitism and behavior of *A. hordei* when exposed to 16 aphid species in six genera and two tribes on six host plant species in four families were measured (Table 1). These aphids and host plants were chosen to provide contrasts of aphid species in the same versus different genera and tribes on the same versus different host plant species. Based on the aphid distributions in Blackman and Eastop (2006) and references cited therein, many of these aphid species occur within the native geographic range of *D. noxia*, so these aphids and this parasitoid are likely to have been in contact for at least 10,000 years. The exceptions are *Aphis glycines*, which is native to east Asia, and *Aphis helianthi*, *Aphis oestlundii*, *Aphis monardae*, and *D. mexicana* which are 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 entomophagous insects (Kuhlmann et al., 2005).

B.2 Laboratory tests

In laboratory experiments in quarantine, parasitism of *D. noxia* and the non-target aphid species discussed above was measured. Experiments on the mechanisms underlying differences in parasitism among these aphid species were also conducted. Parasitoids in the genus *Aphelinus* could kill aphids by ovipositing in them and by host feeding on them, and our experiments on mechanisms tested for both types of mortality.

Table 1. Aphids and host plants on which parasitism by *Aphelinus* species was measured.

Aphid		Host Plant			
Tribe	Species	Family	Species	Variety	Common Name
Macrosiphini	<i>Diuraphis noxia</i> (Kurdjumov)	Poaceae	<i>Hordeum vulgare</i> L.	Lacey	barley
	<i>Diuraphis tritici</i> (Gillette)	Poaceae	<i>Hordeum vulgare</i> L.	Lacey	barley
	<i>Diuraphis mexicana</i> (Baker)	Poaceae	<i>Bromus marginatus</i> Nees ex Steudel	Garnet	mountain brome
	<i>Myzus persicae</i> (Sulzer)	Brassicaceae	<i>Raphanus sativus</i> L.	Cherry Belle	radish
	<i>Acyrtosiphon pisum</i> Harris	Fabaceae	<i>Vicia faba</i> L.	Windsor	fava bean
Aphidini	<i>Aphis craccivora</i> Koch	Fabaceae	<i>Vicia faba</i> L.	Windsor	fava bean
	<i>Aphis glycines</i> Matsumura	Fabaceae	<i>Glycine max</i> (L.)	Pioneer 91Y70	soybean
	<i>Aphis gossypii</i> (Glover)	Malvaceae	<i>Gossypium hirsutum</i> L.	SG 105	cotton
	<i>Aphis helianthin</i> Monell	Asteraceae	<i>Helianthus annuus</i> L.	-	common sunflower
	<i>Aphis monardae</i> Oestlund	Laminaceae	<i>Monarda fistulosa</i> L.	-	wild bergamot
	<i>Aphis nerii</i> Boyer de Fonscolombe	Apocynaceae	<i>Asclepias syriaca</i> L.	-	common milkweed
	<i>Aphis oestlundii</i> Gillette	Onagraceae	<i>Oenothera biennis</i> L.	-	common evening-primrose
	<i>Aphis rumicis</i> L.	Polygonaceae	<i>Rumex latissimus</i> Wood	-	pale dock
	<i>Rhopalosiphum maidis</i> (Fitch)	Poaceae	<i>Hordeum vulgare</i> L.	Lacey	
	<i>Rhopalosiphum padi</i> (L.)	Poaceae	<i>Hordeum vulgare</i> L.	Lacey	
	<i>Schizaphis graminum</i> (Rondani)	Poaceae	<i>Hordeum vulgare</i> L.	Lacey	

Table 2. Origins of *Aphelinus* species collected from grain aphids and tested for host specificity on *Diuraphis noxia*. All parasitized aphids were collected on wheat or barley with the exception of *R. maidis* which was collected on maize.

<i>Aphelinus</i>							
complex	species	Aphid host	Country	Region	Locations	Number	Year
<i>asychis</i>	<i>asychis</i> Walker	<i>D. noxia</i>	France	Herauld	Prades-le-Lez	>200	2000
	<i>asychis</i> Walker	<i>R. padi</i>	France	Herauld	Gedera	>200	2000
<i>varipes</i>	<i>albipodus</i> Hayat & Fatima	<i>R. padi</i>	Israel	Judea	Satchergesi	81	1999
	<i>atriplicis</i> Kurdjumov	<i>D. noxia</i>	Republic of Georgia	Tblisi		101	2000
	<i>atriplicis</i> Kurdjumov	<i>D. noxia</i>	USA	CO, NE, WY	Sterling, Scottsbluff, Cheyenne	584	2006
	<i>certus</i> Yasnosh	<i>R. maidis</i>	China	Hebei	Langfang	88	2005
	<i>hordei</i> Kurdjumov	<i>D. noxia</i>	France	Herauld	Prades-le-Lez	40	2000
	<i>hordei</i> Kurdjumov	<i>D. noxia</i>	France	Herauld	Montpelier	69	2011
	<i>kurdjumovi</i> (Kurdjumov)	<i>R. madi</i>	Republic of Georgia	Tbilisi	Satchergesi	80	2000
	<i>varipes</i> (Förster)	<i>R. madi</i>	France	Herauld	Prades-le-Lez	107	2000

Parasitism in 24 hour exposure of aphids on plants - Methods. Several experiments were carried out to measure differences in parasitism among aphid and plant species. In the first set of experiments, parasitism was measured of seven aphid species (*D. noxia*, *M. persicae*, *R. maidis*, *R. padi*, *S. graminum*, *A. glycines*, *A. gossypii*) on four host plant species by the nine populations of *Aphelinus* in seven species (Table 2) to test their specificity among aphid species across much of the phylogeny of aphids and on several host plant species. When these experiments were conducted, the parasitoids had been in culture 12–52 generations. Because the first set of experiments showed that *A. hordei* alone had a narrow host range than included *D. noxia*, parasitism by *A. hordei* was measured in an experiment involving three species of *Diuraphis* (*D. noxia*, *D. tritici*, *D. mexicana*), as well as well as 11 other aphid species (*M. persicae*, *A. pisum*, *A. craccivora*, *A. helianthi*, *A. monardae*, *A. nerii*, *A. oestlundii*, *A. rumicis*, *R. maidis*, *R. padi*, *S. graminum*) that included seven aphid and six plant species not used in the first set of experiments. When this experiment was conducted, *A. hordei* had been in culture 15–25 generations.

For these experiments, 1–5-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 ensure that the females had a full egg load. In each experimental unit, a single female parasitoid was put in a cage (10 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. Replicates in which females were not recovered or died before the end of the exposure period were not used in analyses. In the first set of experiments, missing or dead females constituted <8% (72/910) of those exposed with 0–4 missing for each combination of aphid species and parasitoid population. After these were removed, there was information for 6–29 females from each of the nine parasitoid populations on each of the seven aphid species for a total of 838 females. In the 24-hour experiment with *A. hordei*, missing or dead females constituted <8% (15/200) of those exposed with 0–2 missing for each aphid species. After these were removed, there was information for 9–28 females of *A. hordei* on 14 aphid species for a total of 185 females.

Because these parasitoids can parasitize a maximum of about 30 aphids in 24 hours, this abundance of aphids and period of exposure allowed parasitoids to use their full egg load. Furthermore, the density of aphids, amount of plant material, and cage size meant that parasitoids were not limited by search rate. The researchers waited until the larval parasitoids killed and mummified their hosts before scoring parasitism. Therefore, a combination of acceptance of hosts for oviposition and suitability of hosts for parasitoid development was measured. This type of experiment is often called a no-choice experiment. However, female parasitoids had the choice to oviposit or not when they sequentially encountered individuals of a particular host species. This is frequently the choice aphid parasitoids have in the field because aphids are distributed in colonies often of a single aphid species. 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 load. 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.

Three rounds of testing were done: round one involved species in five aphid genera distributed across the phylogeny of known hosts of the *A. varipes* complex; round two narrowed the testing to species in the genus *Diuraphis* 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 plants - Results. These *Aphelinus* species from grain aphids differed greatly in host range (Fig. 1). Four species (*A. albipodus*, *A. asychis*, *A. atriplicis*, and *A. certus*) parasitized all or all but one of the aphid species on all or nearly all the plant species tested. Two species (*A. kurdjumovi* and *A. varipes*) parasitized several, but not all, of the aphid species on several plant species. *Aphelinus hordei*, which parasitized one aphid species on one plant species, was the only species with a narrow host range. Within these categories, species showed different host specificities (Table 3). Parasitism by both populations of *A. asychis* did not differ among aphid species, nor was there an interaction between collection host and aphid species in their effects on parasitism. However, *A. asychis* originally collected from *R. padi* produced more parasitized aphids than those collected from *D. noxia*. Parasitism differed marginally among aphid species for *A. albipodus*, mostly because of low parasitism of *M. persicae*. Parasitism differed among aphid species for *A. atriplicis*, and collection country interacted with aphid species in their effects on parasitism. *Aphelinus atriplicis* from the Republic of Georgia parasitized few *M. persicae* and moderate numbers of *A. gossypii*, whereas *A. atriplicis* from the United States parasitized few *A. gossypii* and many *M. persicae*. *Aphelinus certus* readily parasitized all aphid species on all plant species, except for *D. noxia*, which it rarely parasitized. *Aphelinus hordei* was the only parasitoid that specialized on *D. noxia*.

Rates of adult emergences from mummified aphids were high (means 0.7–1.0 adults per mummified aphid) for all aphids but *M. persicae*, for which parasitoid species that showed low parasitism tended to show low emergence (Fig. 2). This caused significant effects of aphid species on rates of adult emergences for six of the seven *Aphelinus* species studied (Table 4). For *A. hordei*, emergence rates were high, even for aphid species it rarely parasitized, except *M. persicae*, from which parasitoid adults rarely emerged.

Adult sex ratios were highly variable within aphid species (Fig. 3), and for five out of the seven *Aphelinus* species, sex ratio did not differ among aphid species they parasitized (Table 5). *Aphelinus kurdjumovi* and *A. varipes* had male-biased sex ratios when they parasitized *Aphis* species, suggesting that these aphids may be poor quality hosts for these parasitoid species. For the other *Aphelinus* species, sex ratio did not significantly differ from 50:50, although the sex ratios of *A. hordei* on *D. noxia* and *S. graminum* tended to be female-biased.

In the experiment on the host specificity of *A. hordei* alone, parasitism varied strongly among aphid species (model deviance = 98.0, df = 13; residual deviance = 111.7, df = 171; $P < 0.00001$). *Aphelinus hordei* parasitized primarily species in the genus *Diuraphis*, particularly *D. noxia*, and little or no parasitism outside this genus (Fig. 4). Rates of adult emergences from mummified aphids were high for *D. noxia* (mean = 0.89 [0.77-1.00]), *D. mexicana* (0.84 [0.69-1.00]), and *S. graminum* (0.95 [0.75-1.00]), but lower for *D. tritici* (0.66 [0.46-0.85]) and *A. monardae* (0.56 [0.26-0.85]). However, the low number of replicates with parasitized aphids for

species other than *D. noxia* meant that adult emergence rates were not quite significantly different among aphid species (model deviance = 0.6, df = 4; residual deviance = 3.0, df = 43; $P = 0.07$). Adult sex ratios did not differ from 50:50 and were not affected by aphid species (model deviance = 0.4, df = 3; residual deviance = 5.7, df = 39; $P = 0.48$).

Direct observation of parasitoid and aphid behavior - Methods. To determine the mechanisms affecting parasitism of aphid species by *A. hordei*, behavior of female parasitoids exposed to aphids was directly observed. Female parasitoids were observed with each of nine aphid species (*D. noxia*, *D. mexicana*, *D. tritici*, *M. persicae*, *R. maidis*, *S. graminum*, *A. helianthi*, *A. monardae*, *A. rumicis*) that included species for which *A. hordei* did and did not produce parasitized aphids in the experiments described above. In each experimental unit, a day-old female parasitoid was exposed to ten second/third instar aphids, the preferred stages for parasitism, on an excised leaf of the appropriate plant species in small arenas (10 mm diameter by 4 mm 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 in it, exposing the slide surface through which observations were made. A leaf freshly cut from a plant was clamped between the slides (weather-stripping against the leaf) and the leaf petiole placed in 1% agar in a small vial to maintain turgor. Aphids were placed in the arenas about 30 minutes before beginning observations to allow them to settle and begin feeding on the leaves. Each parasitoid female was also exposed to three *D. noxia* in an arena prior to each observation bout and counted the number fed upon. A randomized complete-block design was used with each block of nine aphid species done on a single day. When this experiment was conducted, the parasitoids had been in culture 27–78 generations. The experimental unit in these analyses was an unmated, female parasitoid exposed to aphids of a single species on a leaf of the appropriate host plant. Ten females of *A. hordei* were observed for each of nine aphid species.

Each female parasitoid and the aphids with which she was enclosed was observed continuously for 25 minutes under a binocular stereoscope at 10–30X magnification. Behavior of the parasitoids and aphids was recorded with a video camera attached to the microscope and connected to a digital videotape recorder (for date/time stamping) and a DVD burner (for archiving). Identities of aphids and sequences of behaviors were also noted by hand on maps of the arenas, and these locations and sequences were verified by reviewing the DVD recordings. On encountering a potential host, *A. hordei* 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 (which was called an approach). They then turn 180° to face away from the aphid, extend their ovipositor, and insert it into the aphid (which was called a sting). Females sometimes failed to contact the aphid with their ovipositor (which was called a miss) and made several attempts at approaching and stinging the aphid. Besides ovipositing in an aphid, *Aphelinus* females may also paralyze them, make a wound with their ovipositor, and drink hemolymph from the wound (host-feeding). The aphid individuals paralyzed for feeding die, even if female parasitoids do not actually feed on them, and females do not oviposit in aphids paralyzed for feeding. When female parasitoids were exposed to aphid species that they did not approach or sting, they began to run and/or flit after a short period, suggesting that they would have left the aphid patch if they had not been constrained by the arena.

Whether an aphid was approached, and if approached, the outcome of each encounter (stung,

missed, host-fed) was recorded. For the longest sting that did not lead to host feeding, the duration of the encounter was measured from approach until the parasitoid left the aphid (handling time), and for the longest sting that lead to host feeding, the duration of the encounter was measured from approach until the parasitoid left the aphid (host-feeding time). For aphids that were approached, the number of each type of defense behavior (kicking, bucking, rotating around while retaining mouth parts in the leaf, walking away, and cornicular secretion) was recorded. Patch-leaving time, which was defined as the time to flitting or running, was also measured. From these data, the number of aphids approached, the numbers of aphids stung, and the numbers of aphids fed upon was tabulated. Aphid defense was calculated as the number of aphids that defended themselves times the frequency of each defense behavior. Female parasitoids were removed after the observation period, their ovaries were dissected in Ringer's solution mixed with neutral red stain, and the number of mature eggs were counted under 40–100X magnification (neutral red does not pass through the chorion of mature eggs and thus they remain unstained).

If a female parasitoid stung one or more aphids, either all the aphids from a given observation period were dissected to determine whether eggs were laid, or all the aphids from a given observation period were transferred to the appropriate host plant species and reared to determine whether parasitoid progeny survived to aphid mummification and whether exposure to parasitoids otherwise affected aphid survival. Aphids were dissected from half the replicates and aphids were reared from the other half of the replicates, yielding five replicates of dissected aphids and five replicates of reared aphids per aphid species that female parasitoids stung. Aphids were dissected in batches of five on glass microscope slides, each batch placed in a small drop of Ringer's solution and covered with a separate cover slip. Parasitoid eggs were counted from the dissected aphids at 40–100X magnification. For replicates in which no aphids were stung, the aphids were neither dissected nor reared after observation.

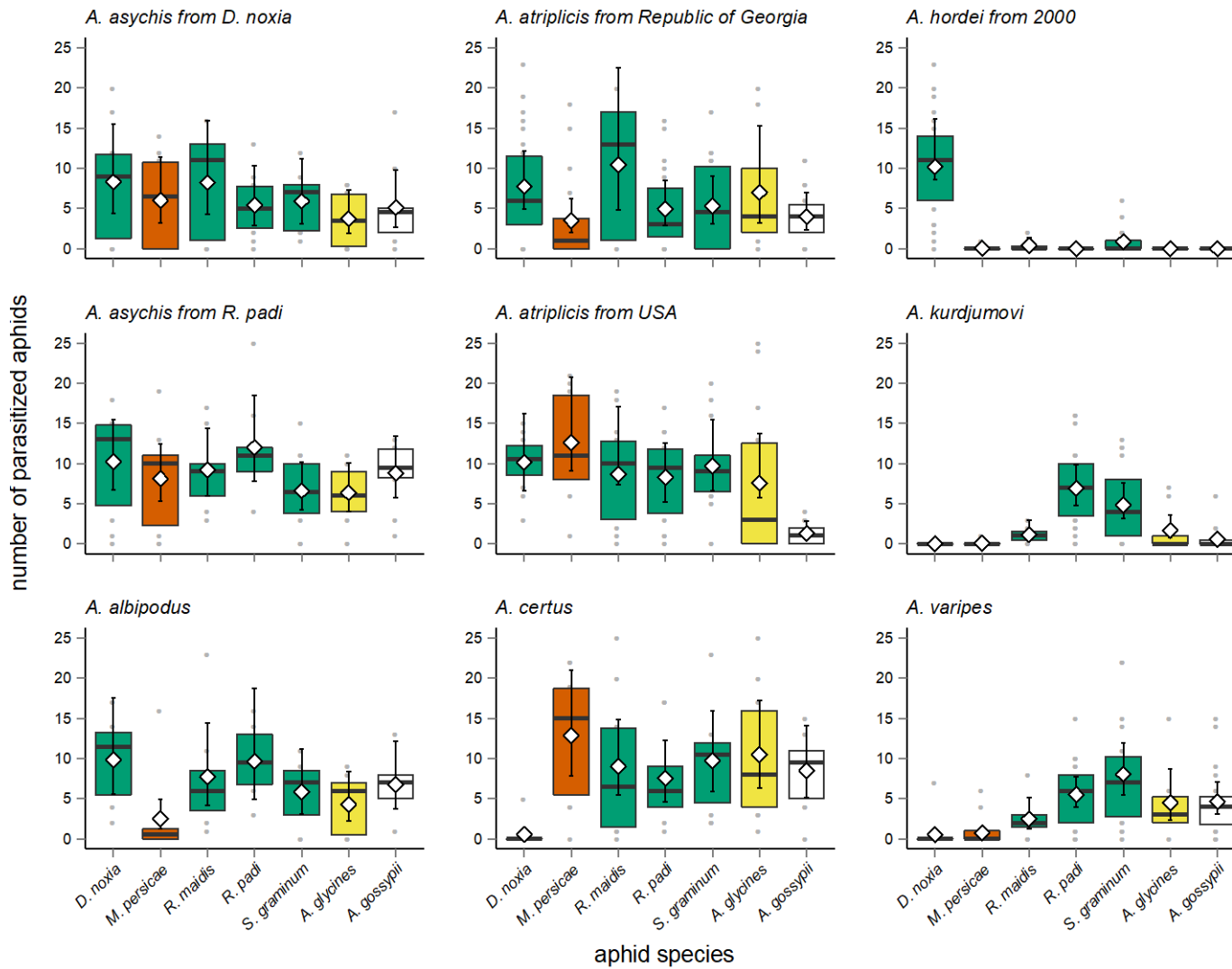
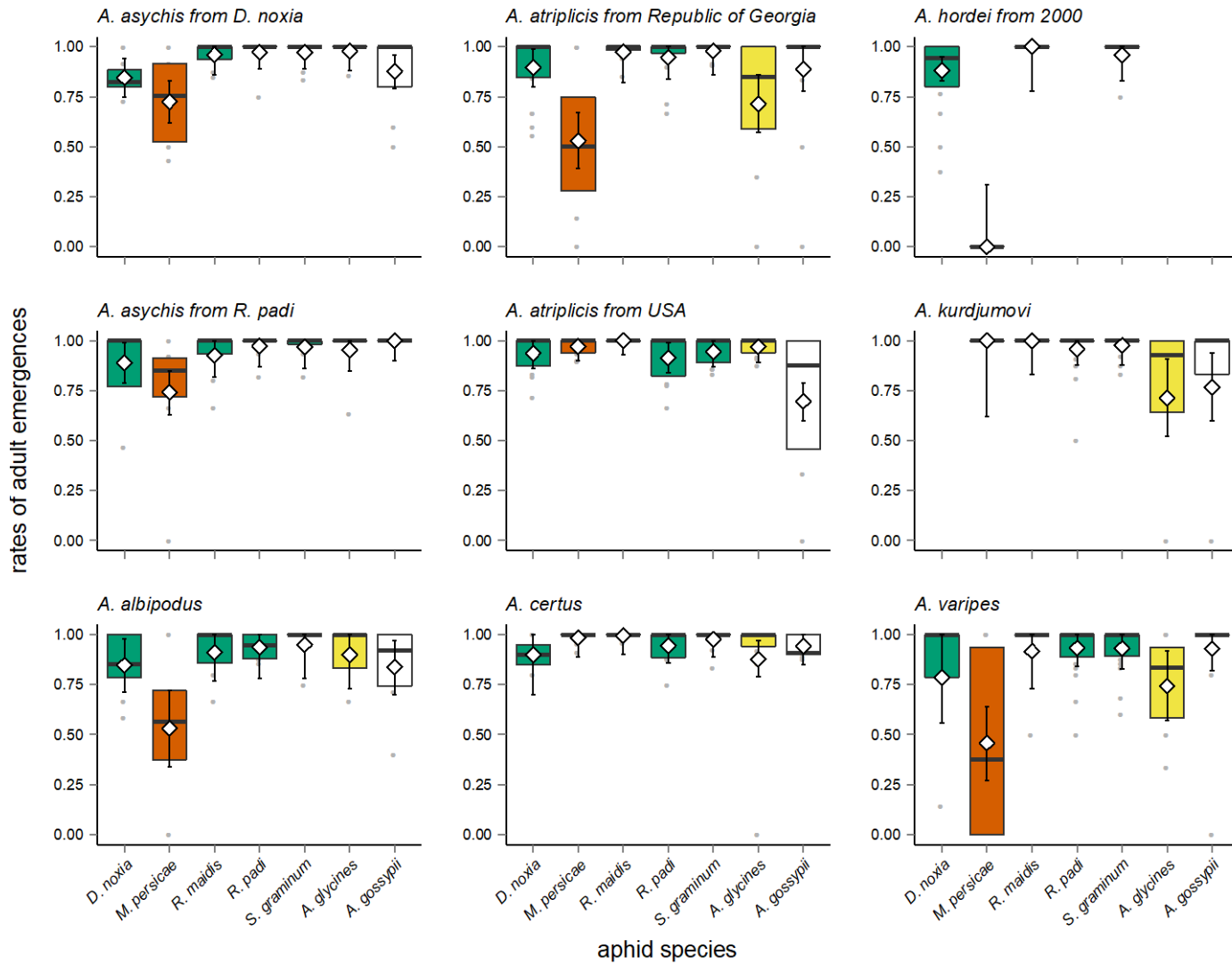


Figure 1. Parasitism of seven species of aphids exposed to species in the genus *Aphelelus* collected from wheat, barley, and maize. Diamonds are means and vertical lines are asymptotic 95% confidence intervals of the means. Upper and lower sides of the boxes indicate lower and upper quartiles and the horizontal line indicates the median. Grey dots are outliers beyond the quartiles. Host plants on which aphids were exposed are indicated by the fill colors of the boxes: green = barley; brown = radish; yellow = soybean; white = cotton.

Figure 2. Adult emergences from seven species of aphids parasitized by species in the genus *Aphelinus* collected from wheat, barley, and maize. Diamonds are means and vertical lines are asymptotic 95% confidence intervals of the means. Upper and lower sides of the boxes indicate lower and upper quartiles and the horizontal line indicates the median. Grey dots are outliers beyond the quartiles. Host plants on which aphids were exposed are indicated by the fill colors of the boxes: green = barley; brown = radish; yellow = soybean; white = cotton.



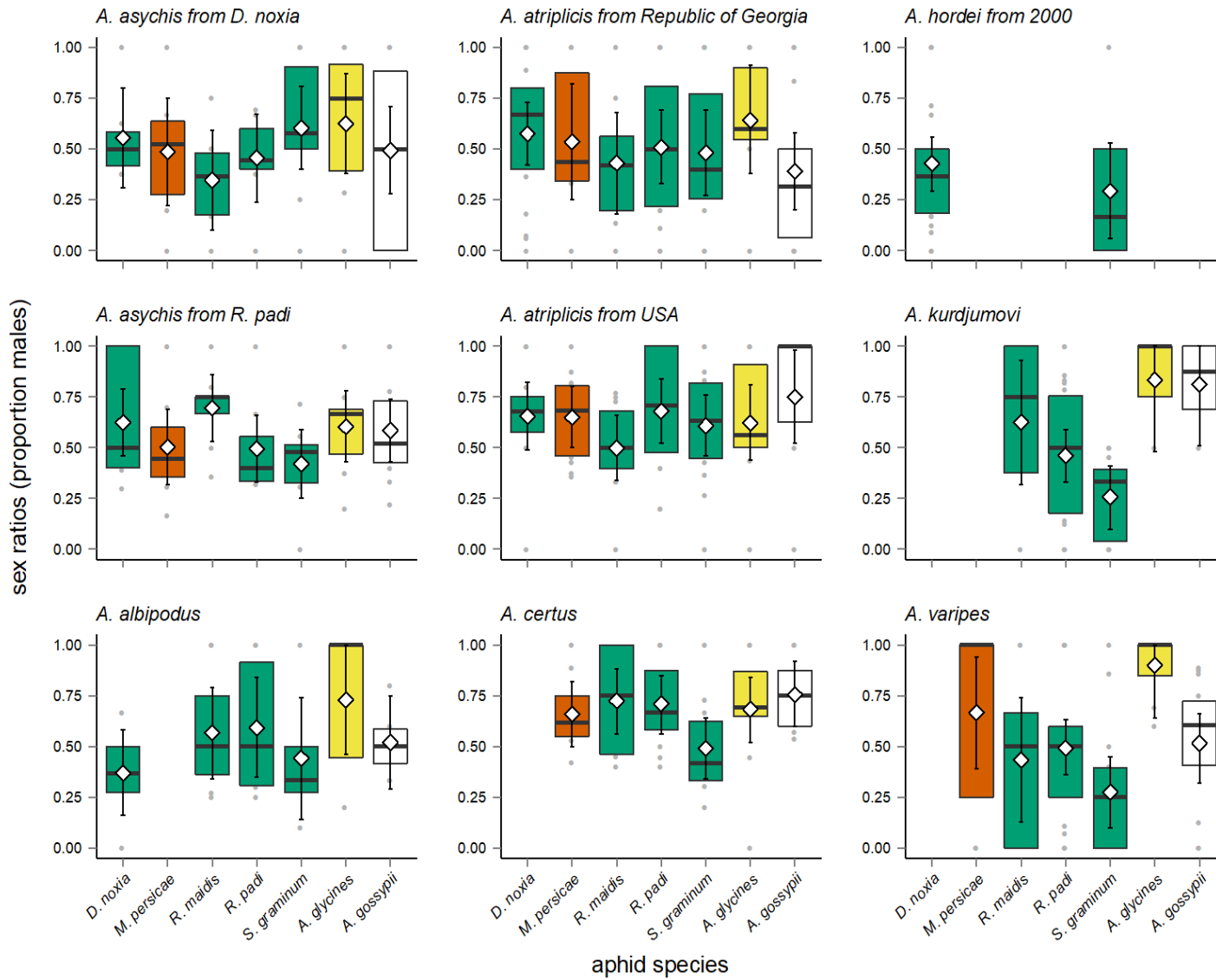


Figure 3. Sex ratios of adults emerging from seven species of aphids parasitized by species in the genus *Aphelinus* collected from wheat, barley, and maize. Diamonds are means and vertical lines are asymptotic 95% confidence intervals of the means. Upper and lower sides of the boxes indicate lower and upper quartiles and the horizontal line indicates the median. Grey dots are outliers beyond the quartiles. Host plants on which aphids were exposed are indicated by the fill colors of the boxes: green = barley; brown = radish; yellow = soybean; white = cotton.

Table 3. Analyses of deviance for effect of aphid species on parasitism (mummies formed after 24 hours of exposure) by parasitoids in the genus *Aphelinus* collected from aphids on wheat, barley, and maize.

Parasitoid species	Factor	model		residual		P
		Degrees of freedom (df)	Deviance	df	Deviance	
<i>asychis</i>	Collection host	1	6.3	134	179.1	0.01
	aphid species	6	7.4	128	171.7	0.28
	interaction	6	2.7	122	169.0	0.85
<i>albipodus</i>	aphid species	6	12.3	44	62.5	0.06
<i>altriplicis</i>	collection country	1	8.9	189	262.9	0.003
	aphid species	6	17.3	183	245.6	0.008
	interaction	6	15.7	177	229.9	0.02
<i>certus</i>	aphid species	6	48.0	65	82.6	<0.00001
<i>hordei</i>	aphid species	6	19.9	214	262.6	0.003
<i>kurdjumovi</i>	aphid species	6	161.3	110	96.1	<0.00001
<i>varipes</i>	aphid species	6	84.5	116	139.1	<0.00001

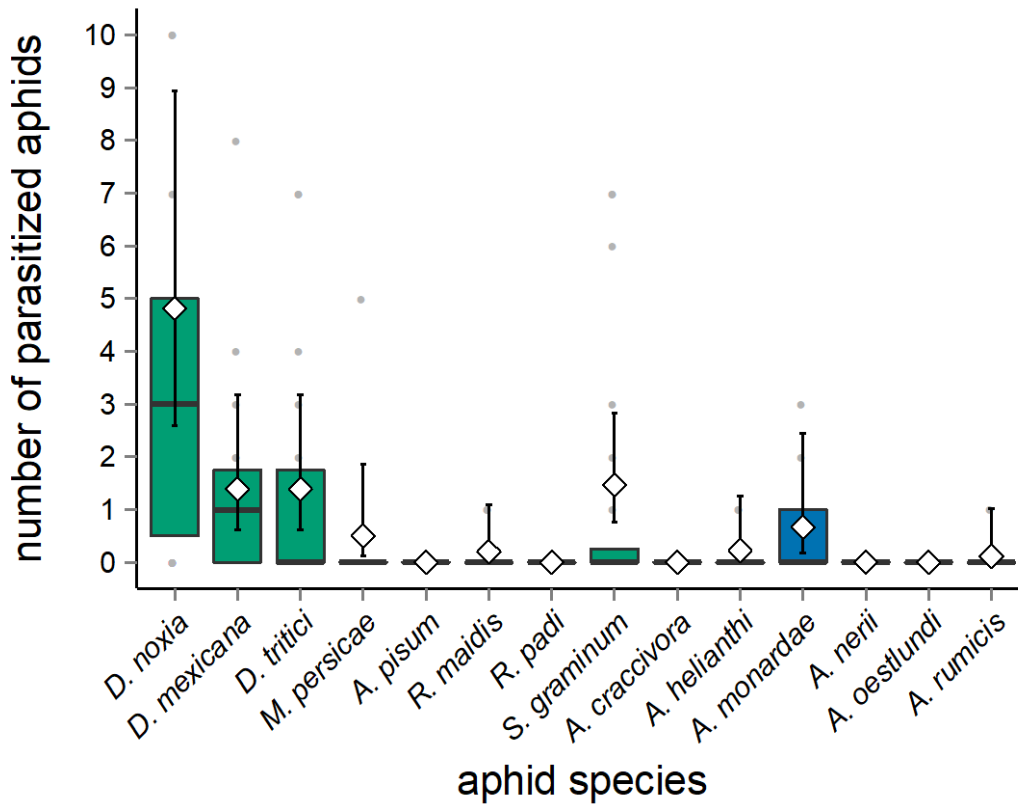
Table 4. Analyses of deviance for effect of aphid species on adult emergence rate of parasitoids in the genus *Aphelinus* collected from aphids on wheat, barley, and maize.

Parasitoid species	Factor	model		residual		P
		Degrees of freedom (df)	Deviance	df	Deviance	
<i>asychis</i>	Collection host	1	0.004	114	2.8	0.65
	aphid species	6	0.7	108	2.2	<0.00001
	interaction	6	0.07	102	2.1	0.72
<i>albipodus</i>	aphid species	6	0.5	36	1.3	0.02
<i>altriplicis</i>	collection country	1	0.2	172	7.2	0.02
	aphid species	6	0.7	166	6.6	0.002
	interaction	6	1.4	160	5.1	<0.00001
<i>certus</i>	aphid species	6	0.1	53	1.0	0.56
<i>hordei</i>	aphid species	3	0.8	31	0.7	<0.00001
<i>kurdjumovi</i>	aphid species	5	0.4	49	1.8	0.05
<i>varipes</i>	aphid species	6	1.4	77	4.0	0.0002

Table 5. Analyses of deviance for effect of aphid species on sex ratio of parasitoids in the genus *Aphelinus* collected from aphids on wheat, barley, and maize.

Parasitoid species	Factor	model		residual		P
		Degrees of freedom (df)	Deviance	df	Deviance	
<i>asychis</i>	Collection host	1	0.1	113	9.0	0.32
	aphid species	6	0.2	107	8.8	0.83
	interaction	6	0.6	101	8.2	0.26
<i>albipodus</i>	aphid species	5	0.5	31	2.7	0.36
<i>altriplicis</i>	collection country	1	0.6	159	15.6	0.02
	aphid species	6	0.5	153	15.2	0.60
	interaction	6	0.4	147	14.8	0.74
<i>certus</i>	aphid species	5	0.4	51	2.9	0.18
<i>hordei</i>	aphid species	1	0.1	31	3.3	0.31
<i>kurdjumovi</i>	aphid species	4	1.6	43	13.9	0.001
<i>varipes</i>	aphid species	5	2.1	68	8.0	0.004

Figure 4. Parasitism of 14 species of aphids exposed to *Aphelinus hordei*. Diamonds are means and vertical lines are asymptotic 95% confidence intervals of the means. Boxes indicate lower and upper quartiles and the horizontal line indicates the median.

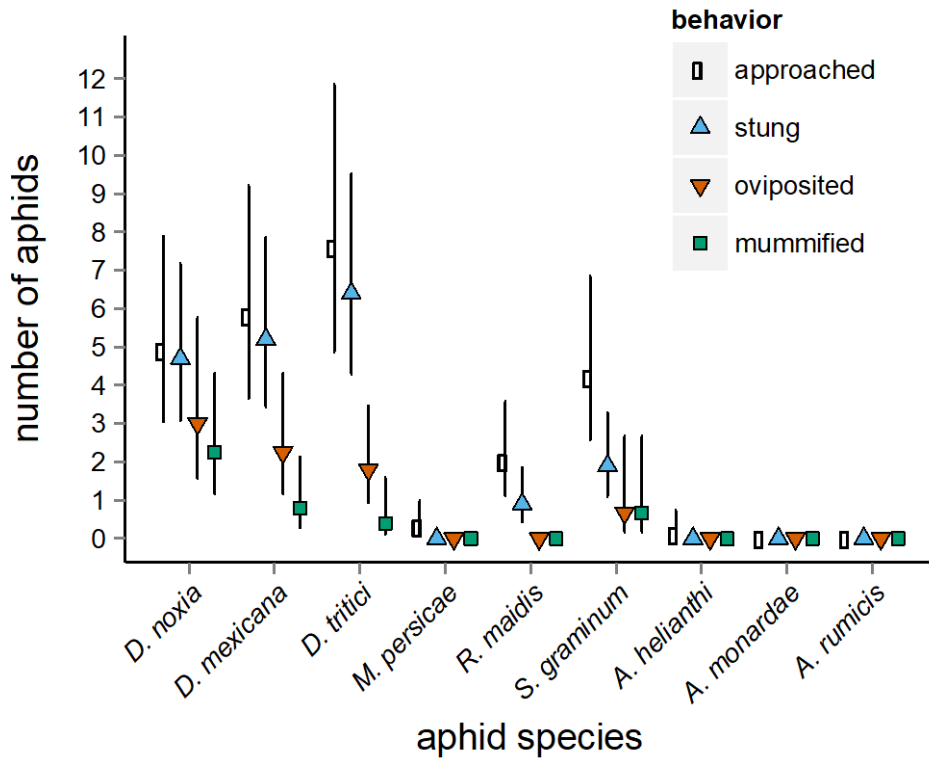


Direct observation of parasitoid and aphid behavior - Results. Most of the host specificity of *A. hordei* can be explained by differences in the behavior of female parasitoids when they encountered different aphid species (Fig. 5; Table 6). When exposed to species in the genus *Diuraphis*, *A. hordei* females approached 5–8 aphids on average during the 25-minute observation periods. *Rhopalosiphum maidis* and *S. graminum* were the only non-*Diuraphis* species approached with even moderate frequency, and the other non-*Diuraphis* species were rarely, if ever, approached.

Within the genus *Diuraphis*, *A. hordei* females stung most of the aphids they approached, although there was a tendency to sting *D. noxia* more often per approach (96% stung per approach) compared to *D. mexicana* and *D. tritici* (90% and 84%, respectively). Outside the genus *Diuraphis*, *A. hordei* females stung both *R. maidis* and *S. graminum* 45% of the time they were approached, but the other non-*Diuraphis* species were never stung. Although *A. hordei* females approached and stung *D. mexicana* and *D. tritici* as often as *D. noxia*, the parasitoids oviposited more often in *D. noxia* (3 [2–6] aphids with eggs) than in the other *Diuraphis* species (2 [1–4] aphids with eggs). This meant that the proportion of ovipositions per sting was higher for *D. noxia* (64%) than for *D. mexicana* (42%) or *D. tritici* (28%). *Aphelinus hordei* females occasionally oviposited in *S. graminum* but never in other non-*Diuraphis* species. Handling time of aphids that were not fed upon varied among aphid species (Table 6), from a mean of 69 [48–100] seconds for *D. noxia* to a mean of 24 [16–36] seconds for *R. maidis* (Fig. 6). This variation probably arose from longer times that oviposition takes, compared to non-oviposition stings, together with fewer ovipositions in species other than *D. noxia* and particularly in non-*Diuraphis* species. Survival of *A. hordei* progeny in *D. noxia* was higher (75%) than in *D. mexicana* (36%) or *D. tritici* (22%), which may explain part of the differences in realized parasitism among these aphid species in the experiments above.

The numbers of aphids that were fed upon varied with aphid species, with female *A. hordei* feeding only on *Diuraphis* species. Female *A. hordei* consumed 0.7 [0.3–1.5] *D. noxia*, 0.5 [0.2–1.2] *D. tritici*, and 0.4 [0.2–1.1] *D. mexicana*, during the 25 minute observation period, but these differences were not significant (model deviance = 0.9, df = 2; residual deviance = 24.8, df

Figure 5. Behavior of *Aphelinus hordei* when exposed to nine species of aphids. Symbols represent means and vertical lines are asymptotic 95% confidence intervals of the means.



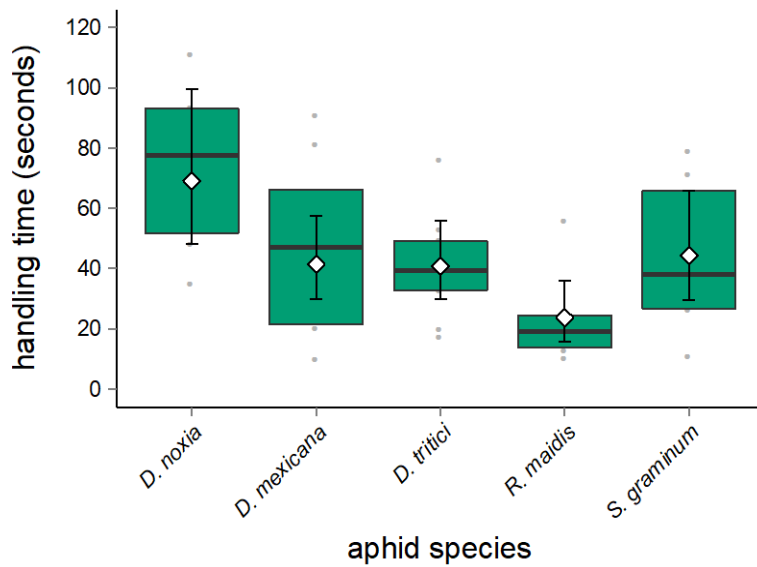


Figure 6. Handling times of *Aphelinus hordei* for species of aphids in which female parasitoids would oviposit. Diamonds are means and vertical lines are asymptotic 95% confidence intervals of the means. Upper and lower sides of the boxes indicate lower and upper quartiles and the horizontal line indicates the median. Gray dots are outliers beyond the quartiles.

Table 6. Analysis of deviance for effect of aphid species on behavior of *Aphelinus hordei* and aphids.

		Model		Residual		<i>P</i>
		df	deviance	df	deviance	
Number of aphids	approached	8	171.2	81	82.0	<0.00001
	stung	8	204.8	81	63.3	<0.00001
	oviposited	8	32.4	15	18.0	0.00008
	Host-fed	8	36.0	81	24.8	0.00002
Handling time	Not host-fed	4	14.5	33	39.4	0.006
	Host-fed	2	1.9	9	12.1	0.38
Aphid defense		4	10.9	46	57.8	0.03

Figure 7. Amount of defense behavior by aphid species in which female *Aphelinus hordei* would oviposit. Diamonds are means and vertical lines are asymptotic 95% confidence intervals of the means. Upper and lower sides of the boxes indicate lower and upper quartiles and the horizontal line indicates the median. Gray dots are outliers beyond the quartiles.

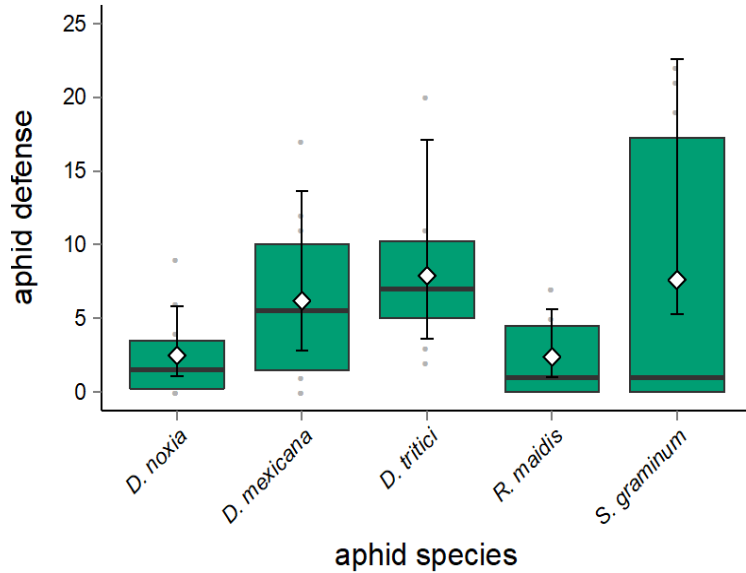
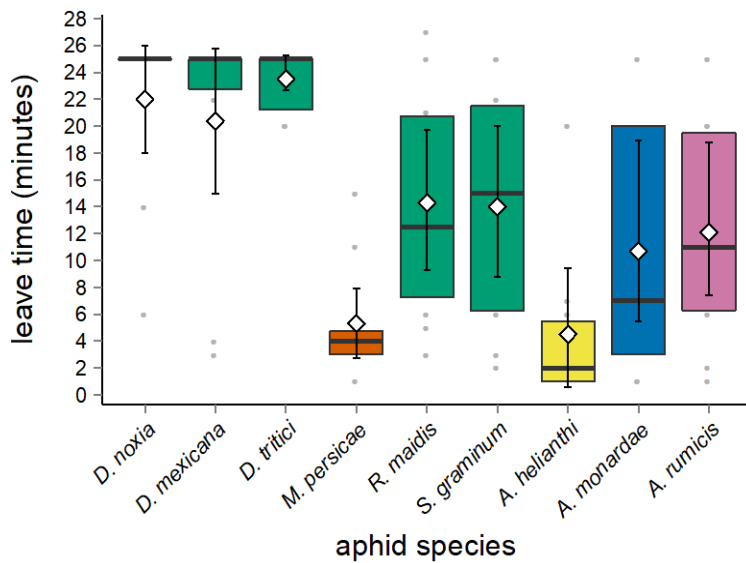


Figure 8. Leaving times *Aphelinus hordei* when exposed to nine species of aphids. Diamonds are means and vertical lines are asymptotic 95% confidence intervals of the means. Upper and lower sides of the boxes indicate lower and upper quartiles and the horizontal line indicates the median. Gray dots are outliers beyond the quartiles.



= 27; $P = 0.65$). Handling times for aphids that were fed upon were about ten times longer than those for aphids that were not fed upon, being 687 [494–956] seconds for *D. mexicana*, 631 [512–778] seconds for *D. noxia*, and 537 [435–662] seconds for *D. tritici*, and the differences among *Diuraphis* species were not significant (Table 6).

The shorter handling times of *A. hordei* for species other than *D. noxia* may reflect female parasitoid rejection of poor host quality during stings, which would lead to less frequent oviposition and thus shorter handling times. Like other *Aphelinus* species, *A. hordei* has relatively large, anhydronic eggs that take a long time to pass through their long, thin ovipositors into host aphids. In support of this, the handling times for *A. hordei* are much longer than those reported for the other major group of aphid parasitoids, the aphidiine braconids (Wu et al., 2011) that carry much smaller eggs and have much shorter ovipositors with a large egg canal (Le Ralec et al., 1986). These long handling times have implications for the impacts of intraguild predation on parasitoid foraging behavior (Martinou et al., 2010), and probably mean that *A. hordei* is unlikely to attack ant-defended aphids (Wyckhuys et al., 2009).

Among aphids approached by *A. hordei*, defense behavior varied among aphid species (Table 6), with *D. noxia* defending itself least and *S. graminum* defending itself most (Fig. 7). The time to patch leaving of *A. hordei* females were much longer for *Diuraphis* species than for other aphid species (Fig. 8; Cox proportional-hazards likelihood ratio = 39.5, $df = 8$, $P < 0.00001$).

At the start of the observation periods, female *A. hordei* with four ovarioles carried 12 [11–14] eggs and females with six ovarioles carried 17 [15–19] eggs. Female *A. hordei* oviposited 8% of their eggs on average during the 25-minute observation periods. Although two females carried only two eggs and one carried three eggs, the rest of the females carried at least six eggs, and a maximum of four eggs were laid in aphids, so these females did not appear to be egg-limited. Furthermore, there was no correlation between the numbers of aphids in which female *A. hordei* laid eggs and their egg loads (model deviance = 0.24; $df = 1$; residual deviance = 21.6, $df = 20$; $P = 0.63$).

Summary of host specificity results. Among the seven *Aphelinus* species tested, which included all species found on grain crops during exploration for parasitoids of *D. noxia* in Eurasia (Heraty et al., 2007; Hopper et al., 1998), only *A. hordei* specialized on *Diuraphis* species, and in particular, *D. noxia*. The specialization on *Diuraphis* species resulted from oviposition restricted almost exclusively to aphids in this genus. Female *A. hordei* very rarely approached aphids on host plants other than barley, and even with aphids on barley, walked over non-host aphid species completely ignoring them. Furthermore, *A. hordei* rarely stung non-*Diuraphis* species but did sting exuviae of *Diuraphis* species. Female *A. hordei* rarely touched host aphids or exuviae with their antennae but did orient headfirst towards them at a distance before stinging them. Within *Diuraphis*, female *A. hordei* oviposited more and their progeny survived better in *D. noxia* than in other *Diuraphis* species. Handling times were long so that ant-tended native aphids, like *A. monardae* and *A. oestlundii*, should not be at risk in the field.

B.3 Information on the biological control agent from the area of origin

Aphelinus hordei was described from adults that emerged from *D. noxia* collected near Poltava, Ukraine, in 1911 (Kurdjumov, 1913), and *A. hordei* has since been reported from *D. noxia* in France (Heraty et al., 2007; Hopper et al., 2017), and in the Republic of Georgia (Yasnosh, 2002). During extensive exploration for natural enemies of *D. noxia* during 1988–1994 throughout the distribution of *D. noxia* in Eurasia, *A. hordei* was not found on any aphid species other than *D. noxia*, although *Aphelinus* species were collected from all aphid species on barley, wheat, and occasionally wild grasses (Heraty et al., 2007; Hopper et al., 1998).

Host specificity of *Aphelinus* species obtained by rearing parasitoids from field-collected hosts suffers from two major problems. First, publications about host species of *Aphelinus* from field collections merely state that the parasitoid species was reared from a host species but do not estimate parasitism levels. In our experiments, parasitism of some aphid species was found at very low levels for some parasitoid species, and field records might include these aphids in the host range of the parasitoids even though they are rarely attacked. Second, closely related *Aphelinus* species can be very difficult to distinguish so host records from the literature may confound cryptic species of parasitoids. Ferrière (1965) synonymized *A. hordei* with *A. varipes* and the synonymy was supported by Graham (1976), but this synonymy is incorrect because the two species are morphologically, reproductively and phylogenetically distinct (Heraty et al., 2007; Prinsloo et al., 2002). Laboratory experiments show that *A. varipes* does not parasitize *D. noxia*, whereas *A. hordei* is a specialist on *Diuraphis* species, and especially *D. noxia*.

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Appendix C. *Aphelinus hordeii* release and post-release monitoring protocols.

Reference specimens

Voucher specimens of *A. hordei* are kept in molecular grade ethanol at -20°C at the Beneficial Insect Introductions Research Unit, Agricultural Research Service, United States Department of Agriculture, Newark, Delaware and at Texas A&M University, Department of Entomology. Specimens of *A. hordei* will also be submitted to the Systematic Entomology Laboratory, USDA-Agricultural Research Service (ARS), Washington, DC.

Procedures for handling *Aphelinus hordei* in containment

Aphelinus hordei was collected as mummified RWA in wheat and barley fields near Montpellier, France, in 2011, and shipped to the quarantine facility at the USDA-ARS, Beneficial Insect Introductions Research Unit, Newark, Delaware. This material was screened for hyperparasitoids and insect pathogens, and a parasitoid culture was established using 23 female and 18 male parasitoids. After one generation, the culture was split into 6 subcultures; each subculture has been maintained with an adult population size >200 and sex ratio of about 1:1 males:females. Parasitoids are reared on RWA on barley plants enclosed in cages (10 cm diameter by 22 cm tall) in pots (10 cm). The cultures are held at 20°C, 50–70 percent relative humidity, and 16:8 hours (Light:Dark) photoperiod in Percival plant growth chambers. About 200 adult parasitoids per cage are transferred to new cages at 3-week intervals.

Sufficiently large numbers of parasitoids will be reared and released to ensure establishment. Previous research indicates that releasing numbers in the thousands at a given site is more likely to establish populations than releasing numbers in the hundreds (Hopper and Roush, 1993) so about 40,000 parasitoids will be released in each field, at 10 locations, and these releases will be repeated at three-week intervals during the field season.

Location and timing of first releases

Parasitoids will be released in wheat and barley fields near Fort Collins, Colorado that RWA is found to infest. Releases will be made throughout the period in the wheat and barley growing seasons when RWA is present and abundant on these host plants (May–July). Field-releases may begin in the spring of 2019 and will continue, if necessary, through 2020 or 2021. The parasitoids will be released as mummified aphids from which adult parasitoids will emerge and disperse.

Monitoring

Wheat fields at the release sites and grasslands near the release sites will be monitored for a minimum of 3 years post-release to determine whether *A. hordei* has established. Once *A. hordei* has established near Fort Collins, sampling will be expanded to cover a greater geographical

area. However, if spread is rapid collaborators who are working on pests of wheat and barley will be relied on to provide information about detections of *A. hordei* in sampling efforts in other western states (Idaho, Kansas, Montana, Nebraska, North Dakota, South Dakota, Washington).

During the monitoring, data on per-plant RWA and parasitoid densities and impact of the parasitoid on the target will be collected using exclosure cages, quadrat samples, Berlese funnels, and emergence canisters. This will be done by following protocols that have been effective in previous research on aphid parasitoids, including those that parasitize RWA in Eurasia (Basky and Hopper, 2000; Chen and Hopper, 1997; Fauvergue and Hopper, 2009; Hopper et al., 1995; Lee et al., 2005; Liu et al., 2004; Miao et al., 2007).

Field exclosures will involve enclosing plants in cages to exclude parasitoids and measuring aphid population growth with and without parasitoid access. Aphids will be exposed to three levels of exclusion, uncaged and coarse-mesh cages and fine-mesh cages, with 40–80 replicates at each level in each field. Cages will be coarse or fine-mesh sacks supported by a metal frame. Natural enemies will have complete access to aphids on uncaged plants, access by parasitoids on coarse-mesh caged plants, and no access to aphids on fine-mesh caged plants. Sticky traps will be added to half of the caged plants to control for aphid emigration. If aphid numbers increase on caged plants because aphids could not emigrate, the plants in cages with sticky traps would show less increase than those without sticky traps because aphids attempting to emigrate would be trapped and not able to return to the plant. If necessary, plants will be artificially infested with aphids. A subset of plants will be destructively sampled from each site and the aphids counted at weekly or bi-weekly intervals for two months. To count the aphids, they will be extracted from plant material into 70 percent ethanol by putting the material in Berlese funnels for 48 hours. To count the aphids on sticky traps, the aphids will be removed from the traps by rinsing the traps with solvent, the aphids will be filtered from the solvent, and resuspended in 70 percent ethanol. Each sample will be spread in a gridded petri dish and aphids will be counted under a dissecting microscope. All grid cells will be counted for samples with less than 100 aphids but a randomly chosen subset will be counted for samples with more than 100 aphids. Aphid numbers per sample will be calculated by dividing the number of aphids counted by the proportion of the sample counted. Analysis of variance with generalized linear models with the appropriate data distributions will be used to test the effects of parasitoid exclusion, emigration, and sample date on aphid density.

For surveys, aphids and parasitoids will be sampled in quadrats randomly located along two transects diagonally across each field on each sample date with samples on each date displaced from samples on previous dates. For each quadrat, the plant growth stage and the numbers of aphid colonies found will be recorded. To estimate aphid density, a haphazard subsample of colonies in each quadrat will be collected. These colonies will be placed (along with plant tissue) in separate plastic bags for each quadrat and brought to the laboratory, where the number of healthy and mummified aphids will be counted. Mummified aphids will be kept in microtiter plates and the numbers of each parasitoid species that emerge will be counted. Healthy aphids will be pooled by field and reared to determine whether they were parasitized and by which species. Aphid density will be calculated for each quadrat by multiplying the mean number of aphids per colony by the number of colonies per quadrat. The effects of sample date and field on

aphid density and parasitism will be tested using analysis of variance with generalized linear models with the appropriate data distributions.

To determine the impact of *A. hordei* on non-target species, other aphid species near the release sites will be sampled. The aphids identified in laboratory experiments as hosts for *A. hordei*, and present in Colorado, will be included in this sampling. These include the following three species (with host plant to be sampled): *D. mexicana* (*Bromus* species), *D. frequens* (wheat, *Elymus* species), and *D. tritici* (wheat, *Bromus* species). In addition to these aphids, three other aphid species found on wheat will be sampled, *S. graminum*, *R. maidis* and *R. padi*, that *A. hordei* rarely or never parasitized in laboratory experiments (Hopper et al., 2017).

Data from the monitoring described above will be used to detect changes in the target pest in the presence versus absence of *A. hordei*. If *A. hordei* is found parasitizing the non-*Diuraphis* species, field surveys and enclosure experiments will be conducted to test the impact of *A. hordei* on the population dynamics of these non-target species.

Appendix D. Response to Comments

Notice of this environmental assessment was made available in the Federal Register on May 5, 2020 for a 30-day public comment period. Three comments were received on the EA by the close of the comment period. One commenter indicated a general distrust of APHIS and did not raise any comments specific to the proposed release of *Aphelinus hordei*. Comments received and responses are below.

1) I believe that whatever pesticides or herbicides are being applied to wheat and barley crops within the United States (i.e., whatever pesticides are labeled for these crops), they are weakening the natural ability of these grass plants to produce the natural chemicals necessary to discourage the RWA from attacking them.

Response: This comment is beyond the scope of this environmental assessment. There is no evidence that insecticides or herbicides have weakened wheat and barley crops so that their natural ability to resist RWA has been diminished. As discussed in the EA, host plant resistance is a method used to reduce RWA populations.

2) I am pretty certain that if you release RWA for biological control, this will create other serious issues down the road. And of course, by that time the decisions made by USDA will be irreversible. Please take time to do trials of this, not necessarily using FULL organic methods, but just without using any pesticides or herbicides.

Response: Researchers have conducted extensive host specificity testing of *Aphelinus hordei*, as described in this environmental assessment. *Aphelinus hordei* has been found to specialize on *Diuraphis* species, and in particular, RWA. Once approved for release, post release monitoring protocols as described in appendix C of this document will be used to evaluate potential impacts on non-target species.

3) From a functional review standpoint, all results should be presented in tables. As written, many of the results are not easily depicted and therefore cannot be adequately assessed. There is no explanation of the analysis performed on the host specificity data. This is atypical when evaluating a biological control agent. This data is extremely important for understanding the agent's inclination to not only utilize the target organism but non-targets as well.

Response: Typically, an environmental assessment does not include extensive technical information. This information was included in the petition submitted to APHIS, reviewed by the Biological Control Review Committee that recommended the release of *A. hordei* and subsequently evaluated by APHIS. However, the full host specificity testing protocols and results, including statistical analyses used and additional tables and figures, have now been included in appendix B of this document and are also found in Hopper et al., 2017.

4) The high parasitism rates are considered outliers in the parasitism graph, which is somewhat perplexing. Some of these non-target parasitism rates (including aphids in two non-*Diuraphis*

genera) approach levels seen in the target, Russian wheat aphid. Therefore, it appears that under some conditions, this wasp can parasitize multiple genera of aphids at high levels. This would consequently make this species a less than optimal biological control agent.

Response: This data was collected in no-choice tests. It is very common for insects to attempt to oviposit in a very wide range of potential hosts when given no choice. Similarly, most insects exhibit a wider physiological host range (where they can develop on a host) under no-choice conditions than what their realized or ecological host range would be under field conditions. The reference to “high parasitism rates” pertains to single data points that are statistical outliers as opposed to parasitism rates (averages). This relates to their statistical consideration and presentation; it is unusual for figures or tables to be included in EAs or even in journal articles that contain so much information, much less the individual data points of statistical outliers that exist in all data sets.

In the first study, *A. hordei* was compared to six other *Aphelinus* spp in its ability to attack seven aphid species. Demonstrating by far the most specificity of all the parasitoids, *A. hordei* parasitized an average of >10, a median of approximately 11, and in some cases nearly 25 RWA per replicate, fully an order of magnitude greater than the next most frequently attacked host, the pest *Schizaphis graminum*, where it parasitized an average of about 1 host and a median of zero hosts per replicate (although in a small number of cases it did attack three to six hosts) (figure 1 in appendix B). In a second study where *A. hordei* was evaluated against a wider range of hosts (including several additional species of *Diuraphis*), there was an average of five RWA attacked, about 1.5 individuals of *D. tritici*, *D. mexicana*, and *S. graminum*. For all non-*Diuraphis* spp., the median number of individuals parasitized per replicate was zero which indicates that in most replicates no aphids were attacked and *A. hordei* would be highly unlikely to reproduce sufficiently on these species to maintain a population (figure 4 in appendix B). Additionally, when those non-target aphids are approached by *A. hordei*, oviposition and mummification rates are near zero (figure 5 in appendix B). Statistical analyses for these studies are included in Table 3 and in the text in appendix B.

5) The assessment appears to contradict itself in at least one important place. Consider the following statements regarding the two non-target aphids *Rhopalosiphum maidis* and *Schizaphis graminum* on pages 17-19 of the draft EA: *R. maidis* and *S. graminum* are "approached with...moderate frequency" and are "stung...45 percent of the time they are approached." Yet "*A. hordei* rarely stung non-*Diuraphis* species." The issue here this is with qualifying terms "moderate" and "rarely". Providing numerical values is best, without the inclusion of qualifying terms that may be misleading or confusing.

Response: APHIS agrees that qualitative terms can make it more difficult to evaluate information. However, figures in support of this section were included in the petition and presented quantitative evidence in support of the qualitative terms; however they were not included in the EA because they were not considered critical to the evaluation of potential risks of releasing *A. hordei*. They are included here in Appendix B along with all statistical comparisons. Additionally, there was an errant statement by APHIS in the original Environmental Assessment that "*A. hordei* parasitized one species"; this statement should have

only applied to the first study examining seven *Aphelinus* species. Later studies with *A. hordei* alone demonstrated that it would attack other *Diuraphis* spp. as well as other non-target species in low numbers (figures 1, 4, and 5 in appendix B). However, the average number of offspring produced was three times higher in RWA than the other *Diuraphis* spp. and even greater in comparison to the non-*Diuraphis* spp. (figure 4 in appendix B). An important consideration here is that none of these non-targets are species of concern and most are considered plant pests. Even in the unlikely event of significant spillover parasitism, it is likely to be deemed beneficial. *Diuraphis mexicana* is unlikely to be encountered by *A. hordei* attacking RWA in crop fields because its hosts are found in significantly different habitats (e.g., on mountain brome)

Differences in reproduction by *A. hordei* in the non-target aphid species are primarily the result of differences in the behavioral response of *A. hordei* to the different potential hosts. *Aphelinus hordei* approached and oviposited less in non-target aphids than in RWA (figure 5 and table 6 in appendix B) and defense behaviors were higher in non-target species (figure 6 in appendix B).

6) The assessment notes that another *Aphelinus* species, *A. atriplicis*, was introduced to control Russian wheat aphid but has apparently not controlled it. Is there any particular reason why the species under current consideration (*A. hordei*) is likely to provide better control? There should be further discussion as to why the congeneric *A. hordei* is a preferred biological control agent when its sister species performed so poorly.

Response: The impact and efficacy of biological control agents can vary significantly between biotypes within a species, much less between species. The observation that a congener has not controlled RWA has little predictive power as to the effect that *A. hordei* may, or may not, have on RWA. *Aphelinus hordei* is demonstrated in these studies to be more specific to RWA than *A. atriplicis* (figure 1 in appendix B) and may therefore have greater impact on the target pest. However, even incremental additional parasitism may benefit grain producers, particularly in the western Great Plains where RWA is a significant pest.

Because potential efficacy cannot be evaluated *a priori*, APHIS focuses its analysis on potential risk and that is the basis of our decision making. The approval process that is undergone is to address risk and safety.