



Field host-specificity of the mile-a-minute weevil, *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae)

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ABSTRACT

The safe practice of biological control relies, in part, on an accurate evaluation of a potential agent's host-specificity via testing through a "filter of safety". The results of laboratory tests may differ from those obtained in open field host-specificity tests, where agents are able to use their full range of host-selection behaviors. It was hypothesized that *Rhinoncomimus latipes* (Coleoptera: Curculionidae), the biological control agent released against mile-a-minute weed, *Persicaria perfoliata* (Polygonaceae), would not feed or oviposit on nontarget plants in a two-phase, open field setting. Ten weevils were placed at the base of each of 13 test plant species in a randomized complete block design with six replicates. Weevils placed at the base of mile-a-minute weed were marked with yellow fluorescent dust, and yellow weevils were subsequently found only on mile-a-minute. Weevils placed at the base of nontarget plants (marked with red fluorescent dust) rapidly colonized mile-a-minute weed. Three hours after release, the number of *R. latipes* found on mile-a-minute weed was significantly higher than predicted by a random distribution of weevils on all test plants. The likelihood of finding more weevils on mile-a-minute compared to nontarget plant species was 31.0% at 3 h and increased to 96.5% at 44 h after release. Whereas prerelease studies showed feeding at low levels on 9 of the 13 plant species tested here, under open field conditions *R. latipes* did not feed on any nontarget plant species and dispersed from these plants. In an open field setting, where the weevil was able to use its full range of host-selection behaviors, there was no observed risk of nontarget effects for any species tested.

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1. Introduction

The intentional release of insect agents for biological control of weeds is predicated on the assumption that host-specificity tests can accurately predict risk to nontarget plants under natural conditions in the introduced range (Louda et al., 2005). However, there is justified skepticism that the artificial conditions inherent in laboratory tests reflect the natural world (Blossey, 1995; Briese, 2005; Louda et al., 2005). Restrictions on insect behavior, which are common in confined cage studies (Turanli and Schaffner, 2004), and the use of potted plants grown under ideal conditions (Willis et al., 2003) raise concerns about whether results obtained in quarantine host-specificity trials accurately translate to the field.

In spite of concerns about the current testing paradigm, biological control as a practice continues to be an effective technique for the suppression of difficult to manage invasive weeds, especially when no other control options are available (Van Driesche et al., 2008). The current success of classical biological control can be

partially explained by its reliance on a "filter of safety" (Fig. 1), a series of tests used to accumulate information on the biology and host-specificity of natural enemies. Each "sieve" in the filter is an opportunity to sift and remove unsafe organisms capable of causing nontarget damage. While these tests currently lack the ability to predict indirect nontarget effects such as food web alterations (Louda and Arnett, 2000; Pearson et al., 2000; Pearson and Callaway, 2003; Müller-Schärer and Schaffner, 2008), field and laboratory tests conducted in the native and introduced ranges have been very successful in limiting direct nontarget effects of introduced organisms. The biological control program targeting mile-a-minute weed, *Persicaria perfoliata* (L.) H. Gross (formerly *Polygonum perfoliatum* L.), is described to identify key features of the filter of safety, and to highlight recent improvements to the process. New research on the field host-specificity of the mile-a-minute weevil, *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae), is presented as part of the validation phase of a biological control program to demonstrate the ability of prerelease risk assessment to accurately predict natural enemy host range.

The first sieve used to select a host-specific agent for weed biological control refines the total number of insect and pathogen species found on the plant in its native range to a few species of interest. This is accomplished by obtaining detailed information

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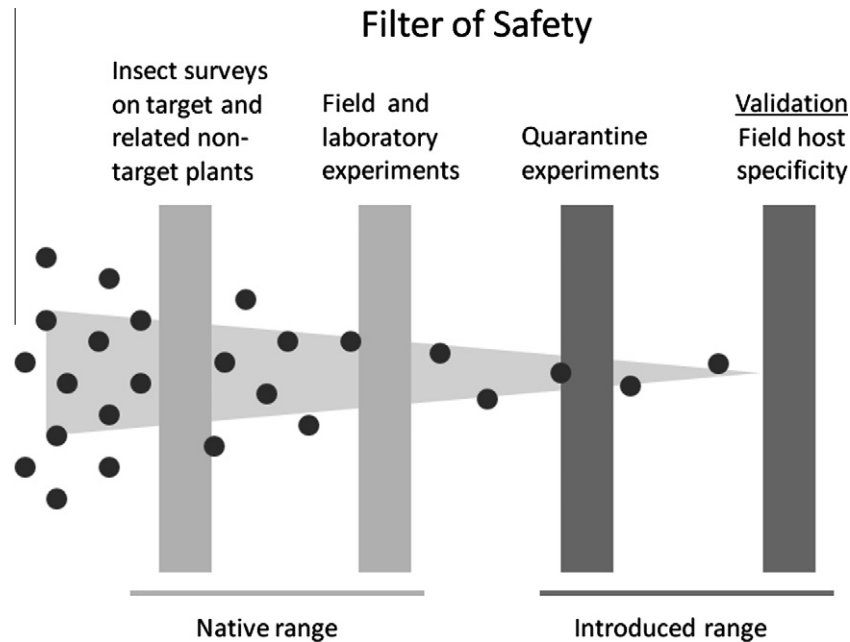


Fig. 1. Filter of safety used in modern biological control programs. From left to right, the first two “sieves” estimate the natural enemies’ ecological host range in the country of origin, and the third sieve determines the physiological host range by conducting starvation/no-choice experiments in a quarantine facility in the area of introduction. Dots represent insect species that pass through or are rejected as potentially unsafe at each stage. Following introduction, field experiments are used to validate results from laboratory experiments and determine the ecological host range in the area of introduction.

on the distribution, density, and life history of specific natural enemies, including the type and relative amount of damage imposed (Van Driesche et al., 2008). Surveys on closely related, nontarget species in the native range may provide early insight regarding host-specificity, and help to prioritize which agents should be considered for further study (Balciunas et al., 1994, 1996). Surveys and life history data collection over a six year period in China were used to refine the number of potential biological control agents for mile-a-minute weed from 111 species found on the plant to 11 species of interest (Ding et al., 2004). These surveys indicated that *R. latipes* relied on mile-a-minute weed for reproduction and development, making it a suitable insect for further study (Ding et al., 2004). Field surveys conducted on mile-a-minute weed in Japan confirmed these results (Miura et al., 2008).

Manipulative outdoor experiments in the native range allow potential biological control agents to engage in their full complement of host-finding behaviors when exposed to nontarget species of interest under natural conditions. From these experiments, conclusions can be drawn about how insects will respond to environmental and plant cues if released in the introduced range of the target weed (Clement and Cristofaro, 1995; Sheppard et al., 2005). Native range, open field host-specificity experiments are therefore used to provide either information supporting the release of potential agents (Turanli and Schaffner, 2004; Briese and Walker, 2008), or evidence to reject agents that are not host-specific (Dunn and Campobasso, 1993). Advancements in the design and interpretation of experiments, such as the use of two-phase open field tests (Briese et al., 2002), have improved the efficacy of these trials. When released in an open field with alternating rows of mile-a-minute weed and three nontarget plant species in China, *R. latipes* was observed on mile-a-minute weed and one other nontarget species. However, feeding damage was restricted to mile-a-minute weed (Colpetzer et al., 2004).

Laboratory experiments in the country of origin explore the physiological (fundamental) host range of a potential biological control agent using no-choice tests that confine insects with a single plant species (Schaffner, 2001; Sheppard et al., 2005). Often,

these tests are conducted with plants that are closely related to the target weed and available in the native range. For studies of *R. latipes*, 49 plant species representing 19 plant families were tested in China, including plants in three tribes and six sections within the Polygonaceae (Colpetzer et al., 2004). Choice and no-choice feeding trials for both adults and larvae demonstrated that mile-a-minute was the only acceptable host for larval development and adult oviposition (Colpetzer et al., 2004).

In the introduced range, quarantine studies evaluate nontarget native plant species that are closely related to the target weed, those recommended by the Technical Advisory Group for Biological Control Agents of Weeds, or those recorded as hosts in the primary literature (Van Driesche et al., 2008). The goal of this step is to fully determine the direct risk posed by a potential biological control agent to plants in the introduced range. *Rhinoncomimus latipes* larvae and adults were tested using choice and no-choice experiments on 28 plant species at a quarantine facility located near the area of introduction of mile-a-minute weed (Colpetzer et al., 2004). Incidental feeding was recorded on 15 test species (all within the Polygonaceae); however, there was no oviposition or larval survival to pupation and adulthood on any test plant species except mile-a-minute weed (Colpetzer et al., 2004). The combined results from studies conducted in China and the United States presented strong evidence that *R. latipes* would not cause damage to nontarget plants if released in the US. A petition for release was approved, and weevils have been released in ten Mid-Atlantic and Northeast states since 2004 (Hough-Goldstein et al., 2009; J.H.G., unpublished data).

Retrospective analysis (Alyokhin et al., 2001; Louda et al., 2005) and field validation of results obtained during laboratory studies are considered the final stage in documenting host-specificity of a biological control agent. While this information does not contribute to preventing nontarget effects in the introduced range, results can yield important lessons about the host-specificity testing paradigm (Taylor et al., 2007). For example, in some cases postrelease evaluations have documented a broader feeding range than predicted by quarantine studies. This has occurred when biological

control agents shipped directly from the native range were not synchronized with host plant phenology (Paynter et al., 2008; Withers et al., 2008), and when untested populations included cryptic species or host races with a different host range than the originally tested population (Withers et al., 2008). Postrelease evaluations have also been used to support the results of quarantine studies (Alyokhin et al., 2001; Breiter and Seastedt, 2007; Taylor et al., 2007). Even for the controversial release of *Rhinocyllus conicus* Frölich (Coleoptera: Curculionidae), which has resulted in damage to native nontarget plants, Arnett and Louda (2002) have verified that tests conducted in quarantine accurately predicted this risk. Finally, in some cases postrelease studies have demonstrated a narrower feeding range than predicted by quarantine tests (Willis et al., 2003; Center et al., 2007; Pratt et al., 2009). Results from these experiments have successfully ameliorated public fear regarding the release and distribution of biological control agents by documenting safety for nontarget plants (Breiter and Seastedt, 2007).

To validate the prerelease screening results obtained for *R. latipes*, a multiple-choice, open field host-specificity test was conducted that exposed weevils to a range of plant species closely related to mile-a-minute weed, or species that showed some evidence of host use by adult weevils in quarantine studies. A two-phase design was used, whereby removing the target weed simulated local extinction of the preferred host and forced insects to choose another host plant, disperse, or die (Briese et al., 2002). By later reintroducing the target weed, it was possible to determine if insects remained in the study area when the preferred host was absent. This study addressed the hypotheses that, when given a choice of related plants in the field (1) weevil populations would rapidly shift from nontarget plant species to mile-a-minute weed and (2) that weevils would disperse away from the study area when mile-a-minute weed was absent.

2. Materials and methods

2.1. Study species

The taxonomic placement of plants in the Polygonaceae was presented recent difficulty. Relationships within the family (Sanchez et al., 2009), and specifically the genus *Persicaria* (Kim and Donoghue, 2008) are under major revision and the generic placement of several species is tenuous. The nomenclature used in the current study comes from the Germplasm Resources Information Network (USDA ARS, 2010) and the Flora of North America (eFloras, 2010).

Mile-a-minute weed is an aggressive, annual vine native to Asia. The plant established in the US in Stewartstown, York County, Pennsylvania after arriving in the 1930s as a seed contaminant with Japanese holly (Moul, 1948). The expanding invasive range of the plant now includes 12 Mid-Atlantic and Northeast states (Hough-Goldstein et al., 2008a; EDDMapS, 2010). The weed forms dense monocultures in a variety of habitats, particularly in disturbed areas, where it can displace native vegetation and interfere with forest regeneration (Oliver, 1996). Mile-a-minute weed has characteristic backward projecting thorns on stems, leaf petioles and veins, which contribute to the plant's climbing habit. Furthermore, the plant is a prolific seed producer (Hough-Goldstein et al., 2008b) and forms a seed bank that can persist for at least six years (Hough-Goldstein et al., 2008a).

Nontarget species selected for the current study were either (1) locally available native plants closely related to the target weed, (2) plants that received some feeding damage during no-choice host-specificity tests, or (3) species on which some adult weevils survived for 30 days during those tests (Price et al., 2003; Colpetzer

et al., 2004). Mile-a-minute achenes were collected from field sites in Chester County, Pennsylvania in the fall 2008, allowed to air dry at room temperature for several months, and then submerged in water, with the water changed each week, and stored at 4 ± 1 °C for about 8 weeks before being planted in the greenhouse. This treatment has been shown to break both coat-imposed and chemical-based achene dormancy found in *P. perfoliata* (Colpetzer and Hough-Goldstein, 2004). Individual *P. perfoliata* plants were grown in round pots (30.5 cm diameter) containing Pro-mix (Premier Horticulture, Quebec, Canada). Buckwheat, *Fagopyrum esculentum* Moench (Johnny's Selected Seeds; Winslow, ME) plants were also grown from seed, with three or four small plants per pot. Ten additional nontarget plant species (Table 1) were collected locally the week of 13 July 2009 and transplanted to the study site within 12 h of collection. Plants were identified using flower structures (Gleason and Cronquist, 1991), and with the help of a botanist. Supplemental plant material was collected at the same time as test plants and kept in a greenhouse in pots to replace plants that did not survive initial transplant. One each of *Persicaria virginiana* and *Polygonum aviculare*, and two *Persicaria sagittata* were replaced before weevils were released in the plots. Plastic English ivy (*Hedera helix* L.) plants were purchased at a local craft supply store, cut to ~41 cm lengths, and "leaves" were cut to mimic the triangular-shaped foliage of *P. perfoliata*. This was done to test *R. latipes'* response to visual cues associated with the plant (Stenberg and Ericson, 2007) and to provide an object that offered structure but no possibility of nourishment. *Rhinoncomimus latipes* is in the subfamily Ceutorhynchinae (Korotyaev, 2006), which consists mostly of specialized feeders on higher plants, especially pioneer and ruderal species (Korotyaev, 1992). Adult *R. latipes* feed on mile-a-minute foliage; the larvae are stem borers (Price et al., 2003). The weevil has been reared since 2004 on potted *P. perfoliata* plants at the Philip Alampi Beneficial Insect Laboratory (Trenton, NJ) from a colony of weevils that were originally collected from two sites in Hunan Province, China (Hough-Goldstein et al., 2009). Insects have been reared, released, and distributed by the laboratory as part of the implementation phase of the biological control program. Under natural field conditions in the Mid-Atlantic US, adult *R. latipes* emerge from overwintering sites in the early spring and can complete at least three to four generations before entering a reproductive diapause between late August and late September (Lake, 2007). Weevil numbers in the field decline in late summer to early fall as insects begin to overwinter and mile-a-minute weed is killed by frost (Lake, 2007). Adult weevils overwinter in the leaf litter or soil (Fu Weidong, Chinese Academy of Agricultural Sciences, Beijing, personal communication). For the current experiment, a shipment of 1000 weevils was received on 23 July 2009 and kept in a 61 × 61 × 61 cm cage with three potted *P. perfoliata* plants for 5 days before the experiment was initiated.

2.2. Study site

An 18 by 40 m area on the University of Delaware Farm (Newark, DE) was tilled and treated with glyphosate one month prior to the start of the experiment to reduce competition from agricultural weeds. Six blocks were arranged in a two by three design, and each block contained 13 one by one meter plots arranged in three columns and four rows, with one additional plot in a fifth row. The distance between plants ranged from 1 to 4 m within blocks, and adjacent blocks were 9 m apart. Test plant species were randomly assigned within each block using the PLAN procedure in SAS (SAS Institute, 2008), and plants were transplanted into the center of plots. Plants of similar size were used to ensure that weevil selection was due to host affinity and not plant biomass (Willis et al., 2003). To facilitate plant establishment, compost was placed in dug holes, and plants were watered as needed. Plots were hand

Table 1

List of plant species used in field host-specificity trial.

Former name ^a	Status ^b	Common name ^b	Revised name ^c
<i>Polygonum perfoliatum</i> L.	Introduced	Mile-a-minute weed	<i>Persicaria perfoliata</i> (L.) H. Gross
<i>Polygonum arifolium</i> L.	Native	Halberdleaf tearthumb	<i>Persicaria arifolia</i> (L.) Harolds
<i>Polygonum sagittatum</i> L.	Native	Arrowleaf tearthumb	<i>Persicaria sagittata</i> (L.) H. Gross
<i>Polygonum virginianum</i> L.	Native	Virginia smartweed	<i>Persicaria virginiana</i> (L.) Gaertn.
<i>Polygonum lapathifolium</i> L.	Native	Pale smartweed	<i>Persicaria lapathifolia</i> (L.) Delarbre
<i>Polygonum pensylvanicum</i> L.	Native	Pennsylvania smartweed	<i>Persicaria pensylvanica</i> (L.) M. Gómez
<i>Polygonum caespitosum</i> Blume	Native & introduced	Tufted knotweed	<i>Persicaria longiseta</i> (Bruijn) Kitag. & <i>Persicaria posumbu</i> (Buch.-Ham. Ex D. Don) H. Gross
<i>Polygonum punctatum</i> Elliot	Native	Dotted smartweed	<i>Persicaria punctata</i> (Elliott) Small
<i>Polygonum hydropiper</i> L.	Introduced	Marshpepper knotweed	<i>Persicaria hydropiper</i> (L.) Delarbre
<i>Polygonum hydropiperoides</i> Michaux	Native (threatened NY & IN)	Swamp smartweed	<i>Persicaria hydropiperoides</i> (Michx.) Small
<i>Fagopyrum esculentum</i> Moench	Introduced	Common buckwheat	<i>Fagopyrum esculentum</i> Moench
<i>Polygonum aviculare</i> L.	Introduced	Prostrate knotweed	<i>Polygonum aviculare</i> L.

^a Scientific names reported in earlier literature on this system.^b Status and common name from USDA NRCS (2010).^c Revised name from USDA ARS (2010).

weeded regularly to remove competing vegetation, leaving only test plant species. An area encompassing a 0.8 km (0.5 mi) radius surrounding the test area was intensively searched for *P. perfoliata*, and none was found. Based on the large area surveyed, all weevils found in this short-term experiment were most likely the individuals released.

2.3. Field host-specificity trial

Once plants were established, 10 adult *R. latipes* were placed at the base of each plant, but not directly on any plant, to permit insect choice (Briese et al., 2002). To distinguish the starting plant of insects, weevils placed at the base of *P. perfoliata* were coated with yellow fluorescent dust (BioQuip Products Inc., Rancho Dominguez, CA), and red fluorescent dust was used to mark weevils placed at the base of the 12 nontarget test plants. A pilot study demonstrated that weevils marked with both yellow and red dust could oviposit, and that weevils coated with yellow and red dust were equally able to disperse from a central release plant to trap plants located 2.5 m ($\chi^2 = 0.4211$, $P = 0.5164$, $N = 38$) and 5 m away ($\chi^2 = 1.2857$, $P = 0.2568$, $N = 7$). Based on this dispersal ability and the block size used, dusted weevils could have accessed all plants within a block.

On 28 July 2009 at 12 pm, a thorough inspection for weevils, eggs, and feeding damage was completed for all plants in the test area. At 1 pm, weevils were released at the base of plants. Plants were inspected for weevils and feeding damage at 4 pm (3 h after release) and again at 10 pm (9 h after release, using a black light to reflect fluorescent dust). On day 2, searches for weevils and feeding damage were conducted at 8 am and 12 pm (19 and 23 h after release). On day 3, plants were examined at 9 am (44 h after release), and *P. perfoliata* plants were killed by clipping at the base of the stem 2 h later. The stems were subsequently secured to the ground using flexible metal wire. A search of all plants at 2 pm the same day (49 h after release) revealed that *P. perfoliata* plants had wilted and leaves curled so that accurate weevil counts on *P. perfoliata* were no longer possible. Therefore, surveys for weevil numbers, color, and damage were completed only for the 12 nontarget plants twice on day 4 (68 and 73 h after release), once on day 5 (98 h after release), and once on day 7 (145 h after release). Foliage was searched for eggs at each sample time on mile-a-minute, and periodically for nontarget plants.

On day 8 (4 August 2009), after 5 days of very low weevil counts on the 12 nontarget plants, the wilted *P. perfoliata* foliage was collected, sealed individually in plastic bags, brought to the laboratory and thoroughly inspected for weevils. Potted, greenhouse grown *P. perfoliata* trap plants were placed in the plots that originally con-

tained *P. perfoliata* at 9 am (164 h after release) and all plants were searched for weevils and feeding damage 5 h later (169 h after release), then once per day for the next 3 days. Plants were searched again the following week, on 11 August 2009 (337 h after release), and a thorough search of all plants for weevils, feeding damage, and eggs was conducted on 17 August 2009 (477 h after release). Weevils observed from 4 August until 17 August 2009 were collected individually in plastic vials, labeled with the date, host plant and block on which they were collected, and stored in a freezer. These weevils were later inspected in a dark room with a black light to detect traces of fluorescent powder.

2.4. Data analysis

Logistic regression was used to determine whether weevils were randomly distributed within the test area or actively choosing *P. perfoliata* (Agresti, 2007). Computations were performed using SAS (SAS Institute, 2008). A Wald Linear Hypothesis Test was used and the ratio of weevils on mile-a-minute weed to the total number of weevils on all plant species was compared to a reference value that represented a purely random distribution of weevils among the 13 plant species in each block. Firth's penalized likelihood method was used to correct for a lack of variability because of low numbers of weevils recovered on some plant species (Firth, 1993). The predicted probability, or likelihood, of finding more weevils on *P. perfoliata* than on nontarget plants was calculated by applying the inverse link of logistic regression to the intercept values. Individual logistic regressions were performed for each time period; a repeated measures analysis was not appropriate for this experiment because weevils were not marked individually and followed over time.

3. Results

Of the 780 weevils placed at the base of plants, between 149 and 158 individuals were recorded on foliage during the first five sample periods (Table 2). There was no apparent relationship between plant phylogeny and the number of weevils observed on plants, and four weevils were observed on the plastic plants during the first sample 3 h after release (Table 2). The average number of yellow weevils (started at base of *P. perfoliata*) observed on *P. perfoliata* remained fairly constant, while the number of red weevils (started at base of nontarget plant species) found on *P. perfoliata* increased during the first five sample times (Fig. 2). No yellow weevils were ever observed on a nontarget plant.

The host choice by weevils differed significantly from a random distribution at 3, 9, 19, 23, and 44 h after release (Table 3). The like-

Table 2
Total number of weevils counted on foliage of different plant species in all six blocks. *P. perfoliata* plants were killed 46 h after release, and potted *P. perfoliata* trap plants were added 164 h after release. Weevils observed between 169 and 477 h were collected and returned to the laboratory.

	Hours after release															
	3	9	19	23	44	49	68	73	98	145	169	193	217	241	337	477
Tribe Persicariae ^a																
<i>Persicaria</i> sec. Echinocaulon																
<i>Persicaria perfoliata</i> (L.) H. Gross	47	107	111	134	154	0	0	0	0	0	4	5	1	2	11	4
<i>Persicaria arifolia</i> (L.) Harold	11	5	1	2	0	0	0	2	0	0	0	0	0	0	0	0
<i>Persicaria sagittata</i> (L.) H. Gross	13	8	4	5	0	0	0	0	0	0	0	0	0	0	0	0
<i>Persicaria</i> sec. Tovar																
<i>Persicaria virginiana</i> (L.) Gaertn.	8	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Persicaria</i> sec. Eupersicaria																
<i>Persicaria lapathifolia</i> (L.) Delarbre	13	6	5	3	1	0	0	0	0	0	0	0	0	0	0	0
<i>Persicaria pennsylvanica</i> (L.) M. Gómez	14	7	6	1	0	0	0	0	1	0	0	0	0	0	0	0
<i>Persicaria longiseta</i> (Bruijn) Kitag.	5	2	3	1	0	0	1	0	0	0	0	0	0	0	0	0
<i>Persicaria punctata</i> (Elliott) Small	8	4	6	1	1	1	1	0	0	0	0	0	0	0	0	0
<i>Persicaria hydropiper</i> (L.) Delarbre	7	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Persicaria hydropiperoides</i> (Michx.) Small	3	4	3	1	2	2	0	0	0	0	0	0	0	0	0	0
<i>Fagopyrum esculentum</i> Moench	5	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Tribe Polygoneae																
<i>Polygonum aviculare</i> L.	12	5	5	0	0	0	1	0	0	0	0	0	0	0	0	0
Plastic plant	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	150	154	152	149	158	3	3	2	1	0	4	5	1	2	11	4

^a Taxonomic groupings are based on Kim and Donoghue (2008). Plant species are listed in order of their phylogenetic relationship to the target plant (*P. perfoliata*).

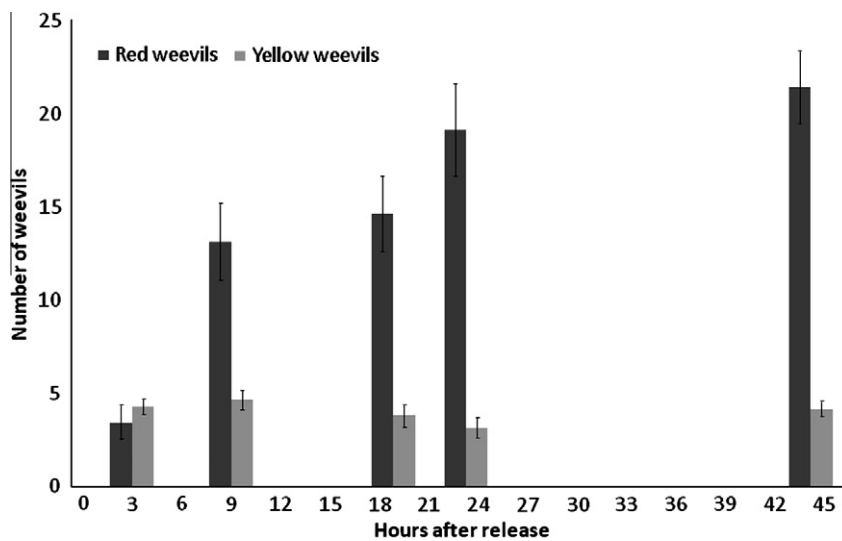


Fig. 2. Average number (\pm SEM) and color of weevils observed on *P. perfoliata* during the first 44 h of the experiment, before host-plant removal. Ten yellow weevils were initially placed at the base of each *P. perfoliata* plant and 10 red weevils were placed at the base of each of the 12 nontarget plant species.

likelihood of finding more weevils on *P. perfoliata* compared to nontarget plant species was 31.0% at 3 h and increased to 96.5% at 44 h after release (Table 3).

The number of weevils observed in the plots decreased to nearly zero after *P. perfoliata* plants were killed at 46 h (Table 2). Zero to three weevils were recorded for the entire study site at sample times between 49 h and 145 h after release, and no weevils were found on dead *P. perfoliata* plants inspected in the laboratory. After potted *P. perfoliata* trap plants were placed in the field at 164 h, four weevils were collected from these plants at a sample period 5 h later (169 h, Table 2). For the next 13 days, all weevils collected in the study site were found on *P. perfoliata* (Table 2). Of the 27 weevils collected, 14 had red fluorescent dust when examined and 13 had no apparent fluorescent dust. Twenty-three of the 27 collected weevils were found on *P. perfoliata* plants in three blocks located nearest to dense vegetative cover outside the study area.

No feeding damage or eggs were recorded for any plant except *P. perfoliata*. Eggs were observed on *P. perfoliata* trap plants in one of the blocks 15 days after release, and a total of 19 eggs were found on *P. perfoliata* plants at the end of the study (21 days after release).

4. Discussion

This field host-specificity test of the mile-a-minute weevil was conducted in three phases. First, a mixture of nontarget plants plus mile-a-minute was presented; next, mile-a-minute was removed; and finally, mile-a-minute weed was returned to the plots. Upon release, weevils rapidly dispersed from nontarget plants to mile-a-minute weed. By 44 h after release, 98% of weevils observed on foliage were found on mile-a-minute weed. After the mile-a-min-

Table 3Host choice by *R. latipes* under field conditions at different times after release.

Sample time	Intercept estimate	Wald's linear hypothesis test	df	P-value ^a	Predicted probability ^b
3 h after release	−0.8019	82.1564	1	<0.0001	31.0
9 h after release	0.8052	321.1203	1	<0.0001	69.1
19 h after release	1.1469	265.7599	1	<0.0001	75.9
23 h after release	2.3176	183.1783	1	<0.0001	91.0
44 h after release	3.3104	146.1158	1	<0.0001	96.5

^a Tests the null hypothesis that the ratio of weevils on mile-a-minute weed to weevils on nontarget plant species is equivalent to a purely random distribution.^b The predicted probability of finding more weevils on mile-a-minute weed than nontarget plants as calculated by applying the inverse link of logistic regression to the intercept values.

ute plants were killed, weevil numbers in the plots plummeted to nearly zero. Some weevils must have remained in the general area, however, because potted mile-a-minute trap plants placed in the plots 5 days later attracted weevils within 5 h. Of the weevils collected on trap plants, 85% were found on the side of the study area near dense vegetative cover.

Prior to release of the mile-a-minute weevil in 2004, laboratory adult no-choice tests documented feeding, though at low levels, by *R. latipes* on 9 of the 13 plant species used in this experiment (Price et al., 2003; Colpetzer et al., 2004). Furthermore, percent survival of adult weevils over 30 days on three species tested here (*Persicaria lapathifolia*, *Persicaria punctata*, and *P. virginiana*) was not significantly different from percent survival of adult insects on mile-a-minute weed (Colpetzer et al., 2004). However, under open field conditions, *R. latipes* dispersed from, and did not feed on any nontarget plant species, even when the preferred host plant was killed or absent. These results are in line with predictions from Colpetzer et al. (2004) about the risk of attack based on native range open field experiments and quarantine studies.

Previous work has shown that host-plant selection by insects occurs in two phases: searching, and contact testing (Finch and Collier, 2000; Schoonhoven et al., 2005). During the searching process, an appropriate landing structure is identified using volatile chemicals and visual stimuli (Finch and Collier, 2000). After landing, nonvolatile plant chemicals provide important cues for host-plant acceptance (Finch and Collier, 2000). Contact testing, or the process of repeatedly touching the plant surface with the antennae, ovipositor, tarsi or mouthparts, allows assessment of plant secondary compounds that are used by specialist herbivores to identify their host (Schoonhoven et al., 2005). Contact testing may be followed by a small test bite, during which food is kept in the preoral cavity and the consumed material is evaluated by the insect. If a plant is accepted, a longer bout of feeding may commence (Schoonhoven et al., 2005).

In prerelease risk assessment of biological control agents, an unavoidable limitation to quarantine host-specificity tests is that experiments conducted in cages can eliminate the cues an insect needs to select an appropriate host plant (Heard, 2000). This could lead to discrepancies between results obtained from laboratory and field studies, with the potentially damaging result of underestimating the fundamental host range of an insect natural enemy prior to release. In the current experiment conducted under open field conditions with all cues available to herbivores, there was no evidence of feeding damage to nontarget plants. Thus, when weevils utilize their full range of host-seeking behaviors and assess multiple cues, both the plastic plant and nontarget plant species are perceived merely as structure, explaining why weevils were found on these plants in the absence of feeding damage.

The role of secondary plant substances in stimulating specialist insect species to accept their host is well documented in the ecological literature (Fraenkel, 1959; Berenbaum and Zangerl, 2008), and is central to the safety and practice of biological control of weeds (Briese and Walker, 2008). Mile-a-minute weed has a un-

ique neoflavonoid profile containing several compounds that have not been isolated from any other members of the Polygonaceae (Sun and Sneden, 1999). The two closely related, native congeneric species tested in this study, *Persicaria arifolia* and *P. sagittata*, exhibit neoflavonoid profiles that are distinctly different from mile-a-minute weed (Park, 1987). Thus, the lack of feeding or oviposition on *P. arifolia* and *P. sagittata* supports the hypothesis that the unique chemistry of mile-a-minute weed is used as a cue for host-finding and acceptance (Colpetzer et al., 2004), and may be necessary for insect development or survival. In the field, these closely related species grow interspersed with mile-a-minute weed, and are not subject to feeding damage even when high weevil populations are present (E.L., personal observation).

The results of this field host-specificity study are in line with others that support the findings of prerelease evaluations conducted in quarantine (Willis et al., 2003; Breiter and Seastedt, 2007; Center et al., 2007; Taylor et al., 2007; Pratt et al., 2009). Whereas prerelease studies with *R. latipes* documented minor feeding on nontarget plant species (Price et al., 2003; Colpetzer et al., 2004), no damage to nontarget species was observed in this field study, even when the preferred host plant was absent. Because only plant species subject to the greatest risk of nontarget effects were selected for study, the results presented here are particularly compelling. We conclude that the filter of safety used to identify, test, and evaluate the host-specificity of the mile-a-minute weevil has been successful, and based on these studies *R. latipes* does not pose a threat to nontarget species.

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