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Host specificity of the Asian weevil, *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae), a potential biological control agent of mile-a-minute weed, *Polygonum perfoliatum* L. (Polygonales: Polygonaceae)

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Abstract

The annual vine *Polygonum perfoliatum* L. (mile-a-minute weed) is an invasive weed in natural areas and has been targeted for biological control in the United States. Host specificity of the Asian weevil *Rhinoncomimus latipes* Korotyaev, a potential biological control agent of mile-a-minute weed, was evaluated in China using qualitative laboratory choice and no-choice tests on 28 plant species in 18 families outside of the Polygonaceae and on 21 species within the Polygonaceae. An open-field choice-test was also conducted in China. In addition, quantitative assessments of adult and larval no-choice tests and adult-choice tests were conducted in a quarantine laboratory in the United States on 23 species of Polygonaceae (including five also tested in China) and five species in three other plant families. Adult weevils did not eat any plant species in families outside of the Polygonaceae in choice or no-choice tests. In no-choice tests with Polygonaceae, adults fed and survived for up to 30 days on a few species in the tribes Persicarieae, Polygoneae, and Rumiceae, but females did not oviposit on any plant except *P. perfoliatum*, and in choice tests adults almost exclusively ate *P. perfoliatum*. Neonate larvae placed on alternative hosts did not survive longer than 36 h, while 80% of neonates placed on *P. perfoliatum* survived and completed development at least to pupation. Of all plant species tested, only *P. perfoliatum* fell within the physiological host range of *R. latipes*. Our tests indicate that *R. latipes* is a host specialist with minimal potential for non-target effects if released in the United States.

Keywords: Weed biological control; *Rhinoncomimus latipes*; *Homorosoma chinensis*; Host range; Mile-a-minute weed; *Polygonum perfoliatum*; Weevil; Classical biological control

1. Introduction

Mile-a-minute weed, *Polygonum perfoliatum* L. (Polygonaceae), is an annual vine indigenous to temperate regions of Bhutan, China, India, Indonesia, Japan, Korea, Nepal, the Philippines, Russia, and Vietnam (Wu and Raven, 2003). It was introduced into a nursery in Stewartstown, Pennsylvania during the 1930s (Moul, 1948; Riefner, 1982) and has since spread to Delaware, the District of Columbia, Maryland, New Jersey, New York, Ohio, Virginia, West Virginia (Oliver, 1996), and Connecticut (Lamont and Fitzgerald, 2001). It has been listed as a noxious weed in several states (USDA-NRCS, 2002) because it forms dense, prickly thickets that displace native vegetation, interfere with forest regeneration, and reduce the recreational value of natural areas (McCormick and Hartwig, 1995; Mountain, 1989; Oliver, 1996).

The rapid spread and detrimental effects of *P. perfoliatum* in North America prompted the USDA Forest

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Service to initiate a classical biological control program in 1996. After 3 years of field surveys and laboratory testing in China, a geometrid moth, *Timandra griseata* Peterson (Lepidoptera: Geometridae), a bug, *Cletus schmidti* Kiritschulsko (Hemiptera: Coreidae), and a weevil, *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae), were identified as the most promising candidate biological control agents against *P. perfoliatum* (Jianqing et al., 2000). The geometrid was subsequently rejected for importation as insufficiently host specific (Price et al., 2003), while the coreid has not yet been completely evaluated.

The weevil was initially identified as *Homorosoma chinensis* (Wagner) (synonym: *Homorosoma chinense* Wagner) by Zhang Runzhi of the Zoological Institute of the Chinese Academy of Sciences. Specimens from a laboratory colony maintained in quarantine in Newark, DE, were later sent to Boris A. Korotyaev of the Zoological Institute of the Russian Academy of Sciences, a recognized world expert for the subfamily Ceutorhynchinae, who identified them as *R. latipes* Korotyaev (Korotyaev, 1997). Voucher specimens of *R. latipes* have been deposited in the University of Delaware Department of Entomology and Wildlife Ecology Insect Reference Collection.

Weevils in the subfamily Ceutorhynchinae are often, though not always, specialists that develop on a narrow range of plants within a single family, genus, or species (Anderson, 1993; Korotyaev, 1992; Morris, 1991). Although the holotype of *R. latipes* was reportedly collected from *Polygonum thunbergii* Seibold and Zuccarini in the Russian Far East (Korotyaev, 1997), *R. latipes* were observed only on *P. perfoliatum* during field surveys in China (Jianqing et al., 2000). Price et al. (2003) studied the biology of *R. latipes* (then referred to as *H. chinensis* [Wagner]) in quarantine in the United States. Adult *R. latipes* are about 2 mm long, eat young leaves of *P. perfoliatum*, and lay eggs on leaves and stems. After hatching, larvae bore into the stem where they complete development, then exit the stem and drop to the soil for pupation. Damage to the plant occurs primarily from larval feeding, which kills the stem from the exit hole to the stem terminal.

In no-choice tests in quarantine, *R. latipes* did not oviposit on buckwheat, *Fagopyrum esculentum* Moench, or rhubarb, *Rheum rhabarbarum* L., and newly hatched larvae placed on these hosts all died within 24 h (Price et al., 2003). Adult survival after 8 weeks was lower on both crop species than on the target weed (about 60% on *F. esculentum* and *R. rhabarbarum* vs 88% on *P. perfoliatum*), and the amount of foliage consumed from both crop species was about half the amount consumed from *P. perfoliatum*. When adult *R. latipes* were given a choice of *P. perfoliatum*, *F. esculentum*, or *R. rhabarbarum*, they did not consume any *R. rhabarbarum* and it consumed only trace amounts of *F. esculentum* (less than 3% of the amount of *P. perfoliatum* foliage consumed; Price et al., 2003).

Further testing of *R. latipes* on ornamental and native North American Polygonaceae was required before petitioning for release of this species in the United States. The results of the host specificity tests conducted on *R. latipes* in China and in quarantine in the United States are reported here.

2. Materials and methods

2.1. Tests conducted in China

Experiments were conducted at the Biological Control Institute, Chinese Academy of Agricultural Sciences, Beijing, China using a colony of *R. latipes* that originated from adults collected from *P. perfoliatum* in Hunan and Henan provinces of China in 1996 and infused annually with adults from one of these provinces. The colony was maintained and laboratory tests were conducted at 25–30 °C, 50–80% RH, with a 16-h photophase.

Test plants were selected that were closely related to the target weed or that were economically important plants used as human or livestock food, traditional medicines, or ornamentals. Taxonomy, common name, habitat, and uses of test plants are summarized in Table 1. Representative species from each of the three tribes of Polygonaceae and six sections of *Polygonum* L. sensu lato that are present in China (Wu and Raven, 2003) were included. Plants were grown from seed in a field adjacent to the laboratory. They were watered regularly but no fertilizer or artificial lights were used during propagation. Young plants were dug from the field and placed into pots for use in laboratory host-range tests.

For larval no-choice tests with non-Polygonaceae in China, eggs were removed from *P. perfoliatum* and placed in a petri dish containing moist filter paper. Soon after eclosion, five naïve neonates were transferred to test dishes using a small paintbrush. Each test dish contained moist filter paper and a leaf from the test plant. Three replicates of each potential host were used. Potential hosts were tested in batches and the target weed was included as a control with each batch. After all larvae had died in each dish, leaves were scored positive or negative for feeding, indicated by scraping of leaf epidermal cells and larval frass.

For larval no-choice tests with plants in the Polygonaceae, three naïve neonates were placed near the apex of a potted test plant, with three replicates per plant species. Each plant was checked every 3 h with a hand lens on the day of release and every 12 h thereafter. The maximum number of days that any neonate survived was recorded. When all larvae had died or pupated, plants were scored positive or negative for feeding.

Table 1
Plants used in China to evaluate the host specificity of *Rhinoncomimus latipes*

Taxonomic grouping[a]	Common name[b]	Habitat and uses[a]
Family Amaranthaceae (Vol. 5)		
Amarathus retroflexus L.	Redroot pigweed	Waste places; cultivated medicine
Family Asclepiadaceae (Vol. 16)		
Telosma cordata (Burm f.) Merr.	Tongkin flower	Open woods; cultivated medicine
Family Asteraceae (Vol. 20)		
Dendranthema grandiflorum (Ramatuelle) Kitamura	Florist's daisy	Cultivated ornamental
Family Brassicaceae (Vol. 8)		
Brassica rapa var. *glabra* Regel	Bok choy	Cultivated food
Brassica rapa var. *chinensis* (L.) Kitamura	Oilseed rape	Cultivated food (oil)
Family Convolvulaceae (Vol. 16)		
Ipomoea nil (L.) Roth	Japanese morning glory	Mountain slopes and fields; cultivated medicine and ornamental
Family Cucurbitaceae (Vol. 20)		
Cucumins sativus L.	Cucumber	Cultivated food
Luffa cylindrica (L.) M. Roemer	Sponge gourd	Cultivated food
Family Fabaceae (Vol. 10)		
Glycine max (L.) Merr.	Soybean	Cultivated food
Lablab purpureus (L.) Sweet	Hyacinth bean	Cultivated food and ornamental
Phaseolus coccineus L.	Scarlet runner	Cultivated food and ornamental
Family Juglandaceae (Vol. 4)		
Juglans regia L.	English walnut	Cultivated food and fiber
Family Lythraceae (Vol. 13)		
Lawsonia inermis L.	Henna	Cultivated medicine and dye
Family Liliaceae (Vol. 24)		
Allium fistulosum L.	Welsh onion	Cultivated food
Chlorophytum comosum (Thunberg) Jacq.	Spider plant	Cultivated ornamental
Family Meliaceae (Vol. 11)		
Aglaia odorata Lour.	Mock lime	Cultivated medicine and ornamental
Toona sinensis (Juss.) Roemer	Chinese cedar	Cultivated ornamental
Family Nyctaginaceae (Vol. 5)		
Mirabilis jalapa L.	Common four o'clock	Cultivated medicine and ornamental
Family Oleaceae (Vol. 15)		
Jasminum sambac (L.) Aiton	Arabian jasmine	Cultivated medicine and flavoring
Family Poaceae (Vol. 22)		
Zea mays L.	Corn	Cultivated food
Family Polygonaceae (Vol. 5)		
[Subfamily Polygonoideae]		
Tribe Persicarieae		
Fagopyrum dibotrys (D. Don) H. Hara	Perennial buckwheat	Moist valleys and grassy slopes; medicinal
Fagopyrum esculentum Moench	Common buckwheat	Cultivated food
Fagopyrum tataricum (L.) Gaertner	Japanese buckwheat	Mountainous regions; cultivated food
Polygonum sec. Aconogon		
Polygonum alpinum Allioni	Alpine knotweed	Forest margins and grassy slopes; cultivated food and medicine
Polygonum sec. Bistorta		
Polygonum bistorta L.	Meadow bistort	Grassy meadows; cultivated medicine
Polygonum sec. Cephalophilon		
Polygonum nepalense Meissner	Nepalese smartweed	Mountain slopes and moist valleys
Polygonum sec. Echinocaulon		
Polygonum bungeanum Turczaninow	Prickly smartweed	Wetlands, fields and roadsides
Polygonum perfoliatum L.	Mile-a-minute weed	Wetlands, fields and roadside
Polygonum sieboldii Meissner	Japanese fleece-flower	Wetlands
Polygonum thunbergii Sieb. and Zucc.	Ji ye liao	Wetlands

Table 1 (*continued*)

Taxonomic grouping[a]	Common name[b]	Habitat and uses[a]
Polygonum sec. Persicaria		
Polygonum hydropiper L.	Marshpepper smartweed	Wetlands
Polygonum posumbu Buchanan-Hamilton ex D. Don	Cong zhi liao	Mixed forests and moist valleys
Polygonum lapathifolium var. *lanatum* (Roxburgh) Steward	Pale smartweed	Waste places
Polygonum lapathifolium L.	Pale smartweed	Waste places
Polygonum orientale L.	Princes-feather	Waste places and lawns; cultivated ornamental
Tribe Polygoneae		
Polygonum sec. Polygonum		
Polygonum aviculare L.	Prostrate knotweed	Waste places; medicinal
Fallopia multiflora (Thunberg ex Murray) Haraldson	He shou wu	Mountain slopes and valleys
Reynoutria japonica Houttuyn	Japanese knotweed	Mountain slopes and valleys
Tribe Rumiceae		
Rheum altaicum Losinskaja	A er tai da huang	Forest valleys
Rumex japonicus Houttuyn	Curly dock	Wetlands and waste places; medicinal
Rumex obtusifolius L.	Broadleaf dock	Field edges and moist valleys
Family Pontederiaceae (Vol. 24)		
Eichhornia crassipes (Marti.) Solms-Laub.	Water-hyacinth	Pools, ditches, and rice fields; livestock feed
Family Portulacaceae (Vol. 5)		
Portulaca grandiflora Hooker	Pink purslane	Cultivated medicine and ornamental
Family Rosaceae (Vol. 9)		
Rosa chinensis Jacq.	China rose	Cultivated ornamental
Family Solanaceae (Vol. 17)		
Capsicum annuum L.	Cayenne pepper	Cultivated food and ornamental
Lycopersicon esculentum Miller	Tomato	Cultivated food
Nicotiana tabacum L.	Tobacco	Cultivated medicine
Solanum melongena L.	Eggplant	Cultivated food
Solanum nigrum L.	Black nightshade	Medicinal

[a] Taxonomy, habitat, and uses from Wu and Raven (2003, volume # indicated for each family).
[b] Common names from the Weed Science Society of America for weedy species, USDA-ITIS (2002) for species that are native or introduced into the United States, and Wu and Raven (2003, multiple volumes) for Chinese species not present in the United States.

Larval choice tests with non-Polygonaceae were conducted by placing 10 neonates in a petri dish containing moist filter paper and leaves or sections of leaves from *P. perfoliatum* and two or more other plant species. The same methods were used for larval choice tests with Polygonaceae, except that five neonates were used per petri dish. Leaves were scored positive or negative for feeding after all larvae had died. Larval choice tests were not replicated.

For adult no-choice tests, five adult weevils were placed in plastic cages ($80 \times 50 \times 50$ cm) with a potted test plant. Three cages were used as replicates for each plant species. Cages were monitored daily, and the maximum number of days that any weevil survived was recorded for each species of plant tested. After all weevils had died, plants were scored positive or negative for feeding, indicated by characteristic shot holes and scraping of leaf epidermal cells.

Multiple-choice tests with adult weevils were conducted with all plant species, and paired-choice tests were conducted with plants from the family Polygonaceae. For each choice test, 10 weevils were released in a plastic cage ($80 \times 100 \times 100$ cm) with a potted *P. perfoliatum* plant and one or more other test plants. Each plant was scored positive or negative for feeding after 7–10 days. Four replicates were included for each test.

A field-choice test was conducted to compare the acceptability of four potential host plant species in an outdoor open arena. In early May of 2002, a 10×6 m plot was planted with alternating rows of *P. perfoliatum* and one of three other test species, *F. esculentum*, *F. tartaricum* (L.) Gaertner, and *P. thunbergii*. A total of 150 *R. latipes* adults collected from Changsha, Hunan province were released in the plot individually or in pairs on all plant species except *P. perfoliatum* on 30 May. Each plant in the plot was examined for the presence of *R. latipes* weevils or their characteristic feeding damage every 2–3 days for 1 month.

Additional laboratory choice and no-choice tests with *P. thunbergii* were conducted in China in September 2003. No-choice and paired-choice tests were conducted with both cut shoots in small cylindrical cages and with potted plants in larger screen cages. Each cage had two male and two female *R. latipes* adults in each of 10 replicates. Tests were run for a minimum of 10 days.

2.2. Tests conducted in the United States

Experiments were conducted in a quarantine facility at the USDA-ARS Beneficial Insects Introduction Research Unit in Newark, DE, with a colony of *R. latipes* originating from adults collected in Changsha, Hunan Province in 2000 and reared on *P. perfoliatum* using the methods of Price et al. (2003). The colony was infused annually with 50–100 new adults collected from the same region of China. The quarantine room was maintained at 23 ± 1 °C, 60–65% RH, and with a 16-h photophase.

Test plants selected were closely related to the target weed, were recommended by the Technical Advisory Group for Biological Control Agents of Weeds in response to Petition 99-03 (R. Reardon and D. Price, 1999, unpublished report), or recorded as a potential host in the primary literature. Taxonomy, common name, status (native or introduced, and threatened or endangered in specific states where relevant), habitat, and uses of test plants are summarized in Table 2. Representative species from both subfamilies (Polygonoideae and Erigonoideae) and from five of the six tribes of Polygonaceae and all six sections of *Polygonum* L. sensu lato that are native to North America (Brandbyge, 1993; Gleason and Cronquist, 1991) were included. Neither of the two North American species belonging to the tribe Pterostegieae (subfamily Erigonoideae) was available for testing. However, both of these species are ecologically restricted to southwestern North America (Brandbyge, 1993), an area where *P. perfoliatum* is unlikely to establish (Okay, 1997).

Potential risk to federally listed threatened and endangered species was addressed by testing related species as surrogates. Of the 18 federally listed species of Polygonaceae (http://endangered.fws.gov, October 2003), 15 are in the subfamily Erigonoideae, two are in the genus *Polygonella*, and one is in the genus *Polygonum* (*P. hickmanii* H. Hinds and R. Morgan, Scott's Valley Polygonum, which occurs in dry, shallow soils in a restricted region of California). One *Polygonella* sp., one *Chorizanthe* sp., and four *Erigonum* spp. were tested as surrogates for federally listed species and also for native buckwheats in the genus *Erigonum*, a number of which have been reported as rare or threatened by Natural Heritage Programs in various states.

A few representatives from the families Plumbaginaceae and Caryophyllaceae were included because some botanists believe that these families have affinities with the Polygonaceae (Brandbyge, 1993; Cronquist, 1988). The family Cannabaceae was included because Morimoto and Lee (1992) indicated that an adult *H. chinense* was collected on Japanese hops, *Humulus japonicus* Siebold and Zuccarini, in Korea. European hops, *Humulus lupus* L., was included as an economically important plant that is closely related to Japanese hops.

Locally collected or commercially available seeds of each plant species were germinated on moist blotter paper sealed in plastic zip-lock bags and then planted in potting soil in plastic pots. Plants were grown in a greenhouse with supplementary lighting, drip-irrigated or watered as needed, and fertilized every other week.

Larval no-choice tests were conducted with naïve neonate larvae. Eggs were removed from *P. perfoliatum* using the tip of a syringe and placed in a petri dish containing moist filter paper. Eggs typically hatched on the morning of the fifth day after harvest, and neonates were transferred from the petri dish to a test plant soon after eclosion using a sable hair paintbrush. Ten neonates were placed near the apex of a potted test plant, which had a coating of petroleum jelly around the base of its stem to prevent the larvae from crawling away. Three plants were used as replicates for each species. All plants were checked once a day with a hand lens for dead larvae, or later for emerged adults. After 26 days, which is the time required for *R. latipes* to develop from egg to adult under quarantine conditions (Price et al., 2003), test plants were dissected under a microscope if all larvae had not been recovered dead or collected as adults. Where plants had larval tunneling and an emergence hole but no adult was recovered, it was assumed that the insect had developed at least to the pupal stage. Three replicates of *P. perfoliatum* were included each of the 3 months that alternative hosts were tested.

For adult no-choice tests, for each host plant, 10 newly emerged male and 10 newly emerged female *R. latipes* were isolated individually in clear plastic cylinders (10 cm in diameter and 30 cm high) with removable screen covers. The weevils were naïve, i.e., they had not had contact with any previous host plant, including *P. perfoliatum*. This was accomplished by removing *P. perfoliatum* stems from rearing containers on day 20 following oviposition, when the majority of *R. latipes* larvae had left the stems to pupate in moist vermiculite at the bottom of the rearing containers, but none had yet emerged as adults. The weevils were sexed by assessing the shape of the metasternum, which is more convex in the female than in the male (Price et al., 2003). Females were marked with Wite-out (Bic Corporation USA, Milford, CT) on their elytra, and cylinders were labeled (male, 1–10 and female, 1–10) to simplify sorting and mating.

Each cylinder cage was supplied with a freshly cut stem from a test plant in a 125-ml Erlenmeyer flask filled with water and stopped with cotton. Plant material was replaced with fresh stems every three days for 30 days.

Table 2
Plants used in the United States to evaluate the host specificity of *Rhinoncomimus latipes*

Taxonomic grouping[a]	Common name[b]	Status[c]	Habitat and uses[d]
Family Cannabaceae			
Humulus japonicus Siebold and Zuccarini	Japanese hops	Introduced	Wetlands and waste places
Humulus lupulus L.	European hops	Native and introduced	Cultivated flavoring
Family Caryophyllaceae			
Dianthus caryophyllus L.	Florist's carnation	Introduced	Cultivated ornamental
Family Plumbaginaceae			
Armeria maritima (Miller) Willdenow	Thrift seapink	Native	Cultivated ornamental
Limonium leptostachyum (Boisser) Kuntze	Statice	Introduced	Cultivated ornamental
Family Polygonaceae			
[Subfamily Polygonoideae]			
Tribe Coccolobeae			
Brunnichia ovata (Walter) Shinners	Redvine	Native	Wetlands
Coccoloba uvifera L.	Seagrape	Native	Cultivated ornamental
Tribe Persicarieae			
Polygonum sec. Bistorta			
Polygonum bistorta L.	Meadow bistort	Native and introduced	Cultivated ornamental
Polygonum sec. Echinocaulon			
Polygonum arifolium L.	Halberdleaf tearthumb	Native; endangered IL and threatened TN	Wetlands
Polygonum perfoliatum L.	Mile-a-minute weed	Introduced	Wetlands and waste places
Polygonum sagittatum L.	Arrowleaf tearthumb	Native	Wetlands
Polygonum sec. Persicaria			
Polygonum caespitosum Blume	Tufted knotweed	Native and introduced	Wetlands and waste places
Polygonum hydropiperoides Michaux	Mild smartweed	Native; threatened IN and NY	Wetlands and waste places
Polygonum lapathifolium L.	Pale smartweed	Native	Wetlands and waste places
Polygonum pensylvanicum L.	Pennsylvania smartweed	Native	Wetlands and waste places
Polygonum punctatum Elliot	Dotted smartweed	Native	Wetlands and waste places
Polygonum sec. Tovara			
Polygonum virginianum L.	Virginia smartweed	Native	Moist woods
Tribe Polygoneae			
Atraphaxis buxifolia Jaub and Spach	Atraphaxis	Introduced	Cultivated ornamental
Polygonella articulata (L.) Meissner	Coastal jointweed	Native; endangered IA and PA	Dry sandy soils
Polygonum sec. Polygonum			
Polygonum aviculare L.	Prostrate knotweed	Introduced	Lawns and waste places
Polygonum sec. Tiniaria			
Polygonum scandens L.	Hedge smartweed	Native	Wetlands and waste places
Tribe Rumiceae			
Rumex acetosa L.	Green sorrel	Introduced	Cultivated food
Rumex altissimus Wood	Pale dock	Native	Wetlands and waste places
Rumex obtusifolius L.	Broadleaf dock	Introduced	Fields and waste places
[Subfamily Eriogonoideae]			
Tribe Eriogoneae			
Chorizanthe staticoides Bentham	Turkish rugging	Native	Cultivated ornamental
Eriogonum fasiculatum Bentham	Mojave buckwheat	Native	Cultivated ornamental
Eriogonum giganteum S. Watson	Island buckwheat	Native	Cultivated ornamental
Eriogonum parvifolium Smith	Seacliff buckwheat	Native	Cultivated ornamental

[a] Suprageneric classification from Kubitzki et al. (1993). Sectional treatment of *Polygonum* L. from Ronse Decraene and Akeroyd (1988). Species names accepted by USDA-ITIS (2002).
[b] Common names from the Weed Science Society of America for weedy species and USDA-ITIS (2002) for non-weedy species.
[c] Status from USDA-NRCS (2002).
[d] Habitat and uses from Gleason and Cronquist (1991) and USDA-NRCS (2002).

Cuttings were taken from a single plant for all 20 cylinders when they were replaced, and cuttings contained inflorescences for all plants tested except those in the genera *Atraphaxis*, *Brunnichia*, *Coccoloba*, *Humulus*, *Rumex*, and *Polygonella*, which are dioecious or functionally unisexual (Kubitzki et al., 1993). Flowering plants were used whenever possible because female *R. latipes* have been shown to preferentially eat and oviposit on the developing seed heads of *P. perfoliatum* (Colpetzer et al., unpublished). During replacement, the weevils were allowed to mate in petri dishes. A sequential mating system that involved a regular rotation of mates was used to keep track of each individual. In trials where more males died than females, which frequently occurred, surviving males were sequentially mated to multiple females. *R. latipes* mated promptly, typically within 1 min of pairing a male and a female. Although copulation generally lasted less than a minute, the weevils were left together for approximately 2 h while stems were replaced.

Foliage consumed in the adult no-choice tests was quantified by pressing damaged plant parts, photocopying them onto transparencies, and processing the transparencies through a portable leaf area meter (LI-3000A, LI-COR, Lincoln, NE) twice before and twice after filling in the area eaten with a permanent marker. Eggs were counted on each plant stem under a dissecting microscope. *P. perfoliatum* was included as a test plant each month during the 6 months that alternative hosts were tested.

Adult-choice tests were conducted with host plants on which *R. latipes* adults fed and survived in no-choice tests. Three newly emerged naïve males and three newly emerged naïve females were placed in the center of a $28 \times 28 \times 28$ cm plastic cage with a potted *P. perfoliatum* plant and one or two other potted hosts. Three cages were used as replicates for each test. The amount of foliage consumed from each plant in each cage was quantified as described above, weekly for 2 weeks.

Survival on the different test plants during adult no-choice tests was analyzed using the non-parametric PROC LIFETEST with survival distribution functions calculated by the product limit (Kaplan–Meier) method (SAS Institute, 1999). Separation of the survival distribution functions was accomplished in nominated posterior comparisons between each novel host and the appropriate *P. perfoliatum* control. The conservative Wilcoxon χ^2 test statistic was used to assess significance in the overall and nominated posterior comparisons.

Total amount of foliage consumed in 30 days from each test plant by each weevil during adult no-choice tests was analyzed using PROC RANK and PROC GLM (SAS Institute, 1999). Combined, these procedures produced a test that approximated the non-parametric Kruskal–Wallis test and allowed the performance of posterior multiple comparison tests (SAS Institute, 2003). The least significant difference (LSD) multiple comparison test was employed for mean rank separation (Sprent and Smeeton, 2001). Total amount of foliage consumed per weevil per week during choice tests was analyzed in the same way.

3. Results

3.1. Tests conducted in China

In larval choice and no-choice tests, neonates did not eat detached leaves from any plant outside of the family Polygonaceae (see Table 1 for species tested). Within the family Polygonaceae, neonates ate leaves of *P. perfoliatum*, *Polygonum bistorta*, and *Rheum altaicum* in no-choice tests using potted plants, but they did not survive longer than 36 h on any plant other than *P. perfoliatum* (Table 3). Larvae ate only *P. perfoliatum* when confined to petri dishes containing leaves from several different plant species (Table 3).

Adult *R. latipes* ate *P. bistorta*, *Polygonum lapathifolium*, *P. perfoliatum*, *R. altaicum*, and *Rumex japonicus* in no-choice tests with potted plants in China, but females did not oviposit on any plant except *P. perfoliatum*. Adults survived for up to 69 days on *P. perfoliatum*, but no longer than 25 days on any other plant species tested (Table 3). About half the species supported adult survival for longer than expected with water alone (8 days in US quarantine studies; Price et al., 2003). When given a choice of *P. perfoliatum* and other species, adults only ate *P. perfoliatum* (Tables 1 and 3).

In the open field-test, 2 days after release of 150 adult *R. latipes* on *F. esculentum*, *F. tartaricum*, and *P. thunbergii* plants, 65 weevils were counted on *P. perfoliatum* plants, while only two weevils were found on *F. esculentum* and none on the other plant species. In subsequent counts, the numbers of weevils recorded from *P. perfoliatum* plants was 31 after 6 days, 20 after 12 days, and 10 after 22 days. An additional six weevils were counted on *F. esculentum* after 12 days; otherwise no *R. latipes* were observed on plants other than *P. perfoliatum.* Feeding damage occurred only on *P. perfoliatum*.

In additional laboratory tests with adult weevils, no feeding or oviposition was observed on *P. thunbergii* in either choice or no-choice tests, while substantial feeding occurred on *P. perfoliatum* in paired-choice tests. Most of the weevils kept with *P. thunbergii* in no-choice tests had died after 10 days (80% in cages with plants and 98% in cylinders with cut shoots).

3.2. Tests conducted in the United States

Rhinocomimus latipes neonates did not bore into stems or survive longer than 24 h when placed on any plant host other than *P. perfoliatum* in no-choice tests in quarantine, while 80% of the neonates transferred to *P. perfoliatum* survived at least to the pupal stage and 36% survived to adulthood (Table 4). Larvae placed on

Table 3
Host specificity of *Rhinoncomimus latipes* as indicated by choice and no-choice tests conducted in China on plants in the family Polygonaceae

Taxonomic grouping	Adult[a]						Larval[b]			
	No-choice			Multiple-choice		Paired-choice	No-choice			Multiple-choice
	F	O	S (days)	F	O	F	F	C	S (hours)	F
[Subfamily Polygonoideae]										
<u>Tribe Persicarieae</u>										
Fagopyrum bibotrys	–	–	4	–	–	–	–	–	12	–
Polygonum sec. Aconogon										
Polygonum alpinum	–	–	18	–	–	–	–	–	18	–
Polygonum sec. Bistorta										
Polygonum bistorta	+	–	18	–	–	–	+	–	24	–
Polygonum sec. Cephalophilon										
Polygonum nepalense	–	–	4	–	–	–	–	–	12	–
Polygonum sec. Echinocaulon										
Polygonum bungeanum	–	–	13	–	–	–	–	–	6	–
Polygonum perfoliatum	+	+	69	+	+	+	+	+	216	+
Polygonum sieboldii	–	–	3	–	–	–	–	–	12	–
Polygonum sec. Persicaria										
Polygonum hydropiper	–	–	4	–	–	–	–	–	6	–
Polygonum posumbu	–	–	5	–	–	–	–	–	12	–
Polygonum lapathifolium	+	–	25	–	–	–	–	–	12	–
Polygonum lapathifolium var.*lanatum*	+	–	25	–	–	–	–	–	12	–
Polygonum orientale	–	–	18	–	–	–	–	–	18	–
<u>Tribe Polygoneae</u>										
Fallopia multiflora	–	–	4	–	–	–	–	–	12	–
Reynoutria japonica	–	–	4	–	–	–	–	–	6	–
Polygonum sec. Polygonum										
Polygonum aviculare	–	–	3	–	–	–	–	–	12	–
<u>Tribe Rumiceae</u>										
Rheum altaicum	+	–	24	–	–	–	+	–	36	–
Rumex japonicus	+	–	14	–	–	–	–	–	6	–
Rumex obtusifolius	–	–	10	–	–	–	–	–	12	–

F, feeding; O, oviposition; S, survival time; and C, completed life cycle. Results scored as either "+" for positive or "–" for negative. Survival times refer to the maximum number of days or hours that any individual adult or larvae survived.

[a] No-choice tests conducted with five adult *R. latipes* in a cage with a potted plant. Multiple- and paired-choice tests conducted with 10 adult *R. latipes* in a cage with a potted *P. perfoliatum* plant and one or more other potted plants.

[b] No-choice tests conducted with three neonate *R. latipes* on a potted plant. Multiple-choice tests conducted with five neonate *R. latipes* and detached leaves in petri dishes.

plants other than *P. perfoliatum* typically crawled to the junction of the petiole and the stem and remained there until death, while those placed on *P. perfoliatum* fed briefly on the leaf and then bored into the petiole or stem.

Survival of adult *R. latipes* after 30 days in no-choice tests averaged 86.7 ± 3.3% (mean ± SEM) on *P. perfoliatum* and ranged from 0 to 80% on other plant species (Table 4). Overall survival distribution functions were significantly different on the test plants ($n = 20$, $df = 32$, $x^2 = 288.9818$, $P < 0.0001$). Nominated posterior comparisons between each novel host and the appropriate *P. perfoliatum* control indicated that survival distribution functions were significantly different than *P. perfoliatum* for all other hosts ($n = 20$, $df = 1$, $x^2 = 5.5406–49.9794$, $P < 0.0001–0.0186$) except *P. lapathifolium*, *P. punctatum* Elliot and *P. virginianum* L. ($x^2 = 2.0170$, $P = 0.1555$; $x^2 = 0.5181$, $P = 0.4716$; and $x^2 = 0.2115$, $P = 0.6456$, respectively).

Adult *R. latipes* consumed on average 3.06 ± 0.18 cm^2 (mean ± SEM) of *P. perfoliatum* in 30 days, while consumption of novel hosts ranged from 0 to 2.33 ± 0.40 cm^2 (Table 4). Overall foliage consumption was significantly different on the test plants ($n = 20$, $df = 32$, $F = 50.40$, $P < 0.0001$), and *R. latipes* consumed significantly more *P. perfoliatum* than any of the other test plants except *Rumex acetosa* L. (Table 4; LSD, $P > 0.05$). Female *R. latipes* deposited on average 48.7 ± 3.3 eggs (mean ± SEM) on *P. perfoliatum* in 30 days, while no eggs were deposited on any of the other hosts in no-choice tests (Table 4).

In choice tests, adult *R. latipes* strongly preferred *P. perfoliatum* to any of the alternative hosts that it was capable of eating or surviving on in no-choice tests

Table 4
Host specificity of *Rhinoncomimus latipes* as indicated by no-choice tests conducted in the United States (means ± SEMs are shown)

Taxonomic grouping	Adult no-choice tests[a]			Larval no-choice tests[b]	
	% Surviving for 30 days	Foliage consumed (cm^2/weevil/30 days)	Oviposition (eggs/female/30 days)	% Surviving to Pupation	% Surviving to Adulthood
Family Cannabaceae					
Humulus japonicus	0 b	0 g	0	0	0
Humulus lupulus	0 b	0 g	0	0	0
Family Caryophyllaceae					
Dianthus caryophyllus	0 b	0 g	0	0	0
Family Plumbaginaceae					
Armeria maritima	0 b	0 g	0	0	0
Limonium leptostachyum	0 b	0 g	0	0	0
Family Polygonaceae					
[Subfamily Polygonoideae]					
Tribe Coccolobeae					
Brunnichia ovata	0 b	0.01 ± 0.01 efg	0	0	0
Coccoloba uvifera	0 b	0 g	0	0	0
Tribe Persicarieae					
Polygonum sec. Bistorta					
Polygonum bistorta	0 b	0.04 ± 0.02 de	0	0	0
Polygonum sec. Echinocaulon					
Polygonum arifolium	20 b	0.02 ± 0.01 efg	0	0	0
Polygonum perfoliatum	86.7 ± 3.3 a	3.06 ± 0.18 a	48.7 ± 3.3	80 ± 1	36 ± 2
Polygonum sagittatum	0 b	0 g	0	0	0
Polygonum sec. Persicaria					
Polygonum caespitosum	10 b	0.09 ± 0.04 def	0	0	0
Polygonum hydropiperoides	20 b	0.07 ± 0.04 def	0	0	0
Polygonum lapathifolium	70 a	0.21 ± 0.08 cd	0	0	
Polygonum pensylvanicum	50 b	0.80 ± 0.17 b	0	0	0
Polygonum punctatum	80 a	0.08 ± 0.04 cd	0	0	0
Polygonum sec. Tovara					
Polygonum virginianum	80 a	1.17 ± 0.16 b	0	0	0
Tribe Polygoneae					
Atraphaxis buxifolia	0 b	0 g	0	0	0
Polygonella articulata	0 b	0 g	0	0	0
Polygonum sec. Polygonum					
Polygonum aviculare	50 b	0.53 ± 0.14 c	0	0	0
Polygonum sec. Tiniaria					
Polygonum scandens	60 b	1.18 ± 0.25 b	0	0	0
Tribe Rumiceae					
Rumex acetosa	60 b	2.33 ± 0.40 ab	0	0	0
Rumex altissimus	20 b	0.61 ± 0.15 c	0	0	0
Rumex obtusifolius	0 b	0 g	0	0	0
[Subfamily Eriogonoideae]					
Tribe Eriogoneae					
Chorizanthe staticoides	0 b	0.01 ± 0.01 efg	0	0	0
Eriogonum fasiculatum	0 b	0 g	0	0	0
Eriogonum giganteum	0 b	0 g	0	0	0
Eriogonum parvifolium	0 b	0 g	0	0	0

[a] Tests conducted with 10 newly emerged male and 10 newly emerged female *R. latipes* isolated individually in plastic cylinders and provided with a fresh cut stem from the test plant of interest (repeated six times for *P. perfoliatum* over the period that other plants were tested). Means sharing the same letters are not significantly different (PROC LIFETEST for survival and ANOVA-LSD for foliage consumption).

[b] Tests conducted with three replicate potted plants for each species and 10 neonate *R. latipes* on each replicate (repeated three times for *P. perfoliatum* over the period that other plants were tested).

(Table 5). The weevils consumed significantly more *P. perfoliatum* than any alternative host in all choice tests ($n = 6$, $df = 16$, $F = 27.79$, $P < 0.0001$), and in each test the amount of alternative host foliage consumed was less than 3% of the amount of *P. perfoliatum* foliage consumed.

Table 5
Foliage consumed (means ± SEMs) by *Rhinoncomimus latipes* naïve adults in six different choice tests conducted in the United States

Alternative host	Foliage consumed from alternative host (cm^2/weevil/week)	Foliage consumed from *P. perfoliatum* (cm^2/weevil/week)
Polygonum arifolium	0 b	0.61 ± 0.07 a
Polygonum lapathifolium	0.01 ± 0.01 b	
Polygonum caespitosum	0 b	0.54 ± 0.04 a
Polygonum punctatum	0 b	
Rumex acetosa	0.01 ± 0.01 b	0.67 ± 0.12 a
Polygonum pensylvanicum	0.01 ± 0.01 b	
Polygonum hydropiperoides	0.01 ± 0.01 b	0.81 ± 0.13 a
Polygonum virginianum	0.02 ± 0.02 b	
Polygonum aviculare	0 b	0.57 ± 0.05 a
Polygonum scandens	0.01 ± 0.01 b	
Rumex altissimus	0.01 ± 0.01 b	0.54 ± 0.04 a

Means sharing the same letters are not significantly different (ANOVA, LSD). Three replicate cages each contained three newly emerged male and three newly emerged female *R. latipes*, a potted *P. perfoliatum* plant, and one or two other potted plants.

4. Discussion

In host-range studies conducted in China and the United States, adult *R. latipes* ate and survived for up to 30 days on a few plant species in the tribes Persicarieae, Polygoneae, and Rumiceae of the family Polygonaceae, but female *R. latipes* oviposited and larvae bored into stems and completed their development only on *P. perfoliatum*. Based on these results, along with those of Price et al. (2003), release of *R. latipes* into North America should have no impact on native flora or economically important plants. Adult weevils that found themselves without *P. perfoliatum* in the field might consume a small amount of foliage of a few other species of Polygonaceae. However, because none of these other species are acceptable for egg-laying or support larval survival or development even if eggs were laid on them in error, it seems likely that the weevils would quickly disperse in search of *P. perfoliatum*.

Recent studies have highlighted the ecological risks associated with classical biological control (e.g., Louda et al., 2003a,b), but where damage to non-target plant species has occurred, it has resulted from imported insects that have adapted to eat physiologically acceptable but less preferred and less suitable hosts, in situations where the ‘‘preferred’’ host is not present (Louda et al., 2003b). Laboratory host-range testing has repeatedly been shown to predict an insect's physiological host range accurately, even though such tests may not always predict its ecological host range under field conditions (Louda et al., 2003a,b; Pemberton, 2000). In our studies, none of the plant species tested other than the target host fell within the physiological host range of *R. latipes.* Even when naïve neonates were placed on other plant species (necessary for testing because eggs were never laid on these species and eggs could not be transferred without desiccating) the larvae failed to bore into the stems and all died within 1–3 days.

Polygonum sagittatum and *P. arifolium*, the two closest native North American relatives to *P. perfoliatum*, experienced less than 1% of the herbivory observed on *P. perfoliatum* in adult no-choice tests. The flavonoid profiles of all three of these species are distinct (Park, 1987), which may account for the differential response by *R. latipes* adults to them. A number of flavonoids have been isolated that deter insect feeding or have insecticidal properties (e.g., Fang and Casida, 1999; Ho et al., 2003) and thus some of the distinctive flavonoids present in plant species related to *P. perfoliatum* may deter *R. latipes* feeding and oviposition.

Polygonum perfoliatum contains a number of neoflavonoids that have not been isolated from any other member of the family Polygonaceae (Sun and Sneden, 1999), and these may act as feeding and oviposition stimulants for *R. latipes*. Toxic secondary plant compounds have been shown to act as feeding stimulants for two monophagous weevil species in the same subfamily as *R. latipes* (Larsen et al., 1992; Nielsen et al., 1989; Peterson et al., 1998). The presence of unique neoflavonoids in *P. perfoliatum* may make this plant a good candidate for biological control because these compounds have a limited distribution in isolated plant species (Donnelly and Sheridan, 1988). Furthermore, the flavonoids found in the Polygonaceae are similar to the furanocoumarins found in the Apiaceae (Umbelliferae) in that both contain a coumarin nucleus (benzo-2-pyrone) and exhibit varying degrees of structural complexity. Berenbaum (1981) showed that the number of herbivorous insects specializing on plants in the Apiaceae increased with increasing complexity of furanocoumarins in the plants. If this pattern holds for herbivorous insects specializing on plants in the

Polygonaceae, then specialists on *P. perfoliatum* may be relatively common because neoflavonoids are unusual and more complex than flavones or flavonols.

Impacts of released biological control agents on the target weed are difficult to predict even if experiments are conducted in the native range, because of differences in biotic and abiotic factors affecting the plants and the insects in the introduced range. However, simulated damage studies suggest that larval feeding by *R. latipes* has the potential to kill small mile-a-minute plants and stunt and reduce seed production by larger plants (Colpetzer et al., unpublished). Thus, this weevil could have a substantial impact on *P. perfoliatum* if released into the weed's introduced range in North America. The research reported here suggests that *R. latipes* has a narrow host range and poses minimal potential risks to non-target plants.

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References

Anderson, R.S., 1993. Weevils and plants: phylogenetic versus ecological mediation of evolution of host plant associations in Curculioninae (Coleoptera: Curculionidae). Mem. Entomol. Soc. Can. 165, 197–232.

Berenbaum, M., 1981. Patterns of furanocoumarin distribution and insect herbivory in the Umbelliferae: plant chemistry and community structure. Ecology 62, 1254–1266.

Brandbyge, J., 1993. Polygonaceae. In: Kubitzki, K., Rohwer, J., Bittrich, V. (Eds.), The Families and Genera of Vascular Plants: Vol. II. Flowering Plants: Dicotyledons: Magnoliid, Hamamelid and Caryophyllid Families. Springer-Verlag, New York, pp. 531–544.

Cronquist, A., 1988. The Evolution and Classification of Flowering Plants, second ed. New York Botanical Garden, Bronx.

Donnelly, D.M.X., Sheridan, M.H., 1988. Neoflavonoids. In: Harbourne, J.B. (Ed.), The Flavonoids: Advances in Research Since 1980. Chapman and Hall, New York, pp. 211–232.

Fang, N., Casida, J.E., 1999. New bioactive flavonoids and stilbenes in cube resin insecticide. J. Nat. Prod. 62, 205–210.

Gleason, H., Cronquist, A., 1991. Manual of Vascular Plants of Northeastern United States and Adjacent Canada, second ed. New York Botanical Garden, Bronx.

Ho, S.H., Wang, J., Sim, K.Y., Gwendoline, C.L.E., Imiyabir, Z., Yap, K.F., Shaari, K., Goh, S.H., 2003. Meliternatin: a feeding deterrent and larvicidal polyoxygenated flavone from *Melicope subunifoliolata*. Phytochemistry 62, 1121–1124.

Jianqing, D., Weidong, F., Wu, Y., Reardon, R., 2000. Insects associated with mile-a-minute weed (*Polygonum perfoliatum* L.) in China: a three-year-survey report. In: Spencer, N.R. (Ed.), Proceedings of the X International Symposium on Biological Control of Weeds, 4–14 July 1999. Montana State University, Bozeman, pp. 225–231.

Korotyaev, B., 1992. On trophic specialization of palearctic weevils of the subfamily Ceutorhynchinae (Coleoptera: Curculionidae). In: Zombori, L., Peregovits, L. (Eds.), Proceedings of the Fourth European Congress of Entomology and the XIII Internationale Symposium für die Entomofaunistik Mitteleuropas, 1–6 September 1991. Gödöllîo, Hungary, pp. 510–512.

Korotyaev, B., 1997. New and little known species of weevils from East Asia (Coleoptera: Apionidae, Curculionidae). Zoosystematica Rossica 5, 285–288.

Kubitzki, K., Rohwer, J., Bittrich, V., 1993. The Families and Genera of Vascular Plants: Vol. II. Flowering Plants: Dicotyledons: Magnoliid, Hamamelid and Caryophyllid Families. Springer-Verlag, New York.

Lamont, E.E., Fitzgerald, J.M., 2001. Noteworthy plants reported from the Torrey Range—2000. J. Torrey Bot. Soc. 128, 409–414.

Larsen, L.M., Nielsen, J.K., Sorensen, H., 1992. Host plant recognition in monophagous weevils: specialization of *Ceutoryhncus inaffectatus* to glucosinolates from its host plant *Hesperis matronalis*. Entomol. Exp. Appl. 64, 49–55.

Louda, S.M., Pemberton, R.W., Johnson, M.T., Follett, P.A., 2003a. Nontarget effects—the Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. Annu. Rev. Entomol. 48, 365–396.

Louda, S.M., Arnett, A.E., Rand, T.A., Russell, F.L., 2003b. Invasiveness of some biological control insects and adequacy of their ecological risk assessment and regulation. Conserv. Biol. 17, 73–82.

McCormick, L., Hartwig, N., 1995. Control of the noxious weed mile-a-minute (*Polygonum perfoliatum*) in reforestation. North. J. Appl. For. 12, 127–132.

Morimoto, K., Lee, C.E., 1992. Curculionidae from Cheju Island, Korea, with descriptions of three species (Insecta, Coleoptera). Esakia 32, 1–18.

Morris, M.G., 1991. A taxonomic check list of the British Ceutorhynchinae, with notes, particularly on host plant relationships (Coleoptera: Curculionidae). Entomol. Gaz. 42, 255–265.

Moul, E., 1948. A dangerous weedy *Polygonum* in Pennsylvania. Rhodora 50, 64–66.

Mountain, W.L., 1989. Mile-a-minute (*Polygonum perfoliatum* L.): update—distribution, biology, and control suggestions. Regul. Hort. 15, 21–24.

Nielsen, J.K., Kirkeby-Thomsen, A.H., Petersen, M.K., 1989. Host plant recognition in monophagous weevils: specificity in feeding responses of *Ceutorhyncus constrictus* and the variable effect of sinigrin. Entomol. Exp. Appl. 53, 157–166.

Okay, J.A., 1997. How far will mile-a-minute go. In: Wu, Y., Fredericks, J., (Eds.), Mile-a-minute Info Link, Issue 3. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV, p. 2.

Oliver, J.D., 1996. Mile-a-minute weed (*Polygonum perfoliatum* L.), an invasive vine in natural and disturbed sites. Castanea 61, 244–251.

Park, C., 1987. Flavonoid chemistry of *Polygonum* sect. Echinocaulon: a systematic survey. Syst. Bot. 12, 167–179.

Pemberton, R.W., 2000. Predictable risk to native plants in weed biological control. Oecologia 125, 489–494.
Peterson, C.J., Tsao, R., Coats, J.R., 1998. Glucosinolate aglucones and analogues: insecticidal properties and a QSAR. Pestic. Sci. 54, 35–42.
Price, D.L., Hough-Goldstein, J., Smith, M.T., 2003. Biology, rearing and preliminary evaluation of host range of two potential biological control agents of mile-a-minute weed, *Polygonum perfoliatum* L. Environ. Entomol. 32, 229–236.
Riefner, R., 1982. Studies of the Maryland flora VIII: range extension of *Polygonum perfoliatum* L., with notes on introduction and dispersal in North America. Phytologia 50, 152–159.
Ronse Decraene, L.P., Akeroyd, J., 1988. Generic limits in *Polygonum* and related genera (Polygonaceae) on the basis of floral characters. Bot. J. Linn. Soc. 98, 321–371.
SAS Institute, 1999. SAS/STAT User's Manual, Version 8.0. SAS Institute, Cary, NC.
SAS Institute, 2003. SAS Customer Support Center. Available from http://support.sas.com.
Sprent, P., Smeeton, N.C., 2001. Nonparametric multiple comparison tests. In: Sprent, P., Smeeton, N.C. (Eds.), Applied Nonparametric Statistical Methods, third ed. Chapman and Hall/CRC Press, Boca Raton, FL, pp. 220–223.
Sun, X., Sneden, A.T., 1999. Neoflavonoids from *Polygonum perfoliatum*. Planta Med. 65, 671–673.
USDA-ITIS, 2002. Integrated Taxonomic Information System. Available from http://www.itis.usda.gov/.
USDA-NRCS, 2002. The Plants Database, Version 3.5. Baton Rouge, LA. Available from http://plants.usda.gov/.
Wu, Z., Raven, P.H., 2003. Flora of China. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis. Multiple volumes, some in draft form. Available from http://flora.huh.harvard.edu/china/.