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Host Specificity of *Longitarsus quadriguttatus* Pont., a Below-Ground Herbivore for the Biological Control of Houndstongue

Mark Schwarzländer

CABI Bioscience Centre Switzerland, CH-2800 Délemont, Switzerland; and Department of Zoology, Christian-Albrechts-University, D-24098 Kiel, Germany

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***Cynoglossum officinale* is a Eurasian plant that has become an invasive weed of rangelands in the United States and Canada. It is a herbaceous, monocarpic, short-lived perennial. Since there are no satisfactory means of control of houndstongue, a biological control project was initiated in 1988. The first species introduced for the control of houndstongue is a root-mining weevil, *Mogulones cruciger*, which was first released in Canada in 1997. The flea beetle *Longitarsus quadriguttatus* is another below-ground herbivore species considered for the biological control of *C. officinale*. Previous host-specificity investigations with 49 European test-plant species demonstrated that larval development of the flea beetle is limited largely to plant species within the tribe Cynoglosseae in Boraginaceae. However, investigations neither included any native North American Boraginaceae species nor examined results of earlier tests for plant species that were susceptible to attack by the flea beetle. Therefore, additional host-range investigations were conducted between 1994 and 1996. Different no-choice and choice tests were conducted with 7 plant species that showed susceptibility to attack in previous tests and 5 native North American plant species. Experiments were carried out under laboratory and open-field conditions. *L. quadriguttatus* was host specific in these tests. Two European test-plant species outside the genus *Cynoglossum* and 2 native North American *Cynoglossum* species supported complete development of the flea beetle to some extent, but in all cases the test plants were significantly less suitable hosts than houndstongue. The remaining 5 European and 3 native North American test-plant species did not support development and/or survival of *L. quadriguttatus*. The introduction of *L. quadriguttatus* into Canada was approved in 1998.**

***Key Words: Longitarsus quadriguttatus*; *Cynoglossum officinale*; houndstongue; biological control; host specificity.**

INTRODUCTION

Houndstongue (*Cynoglossum officinale* L., Boraginaceae) is a herbaceous, hemicryptophytic, monocarpic, short-lived, perennial native to Europe and Asia Minor (Tutin *et al.*, 1972). Seeds germinate in spring, produce rosettes in the 1st year, and flower in the 2nd or subsequent years. Houndstongue is found in relatively low densities throughout Europe but occurs less frequently in the extreme north and south. Plants grow typically in ruderal habitats, along roadsides, in sand dunes, or in open woodlands (Tutin *et al.*, 1972; Hegi, 1975). A detailed review of the biology and ecology of *C. officinale* is provided by de Jong *et al.* (1990).

Houndstongue was introduced into North America probably in the middle of the 19th century as a contaminant of cereal (Brand, 1921; Knight *et al.*, 1984). First reported around Montreal (Macoun, 1884), houndstongue now occurs in all Canadian provinces except Prince Edward Island and Newfoundland. It is a problem mainly in British Columbia, in which it reaches high densities (Upadhyaya and Cranston, 1991). In the United States, houndstongue occurs in most mainland states. The plant is abundant in the northwestern states and is particularly prevalent in the western half of Montana, eastern Washington, eastern Oregon, central Idaho, and northern Wyoming (Forcella and Harvey, 1981; Rice, 1997). Houndstongue is a colonizer of disturbed areas. Heavy infestations are reported in forest sites that were cleared for cattle grazing, on abandoned cropland, or in sites along mining operations and road constructions (R. S. Cranston and J. L. Pethybridge, 1986, unpublished report). A detailed description of the problems caused by houndstongue in North America is given by Dickerson and Fay (1982) and Upadhyaya *et al.* (1988).

Houndstongue infestations are managed by means of chemical control with 2,4-D amine (1.12 kg a.i./ha), chlorsulfuron (0.14 kg a.i./ha), or dicamba (1.12 kg a.i./ha), when applied in spring or fall, and picloram (0.56–1.12 kg a.i./ha) (Upadhyaya and Cranston,

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1991). However, application of these herbicides is restricted to roadsides or waste areas due to potential damage to native plants, forage plants, and conifer seedlings. Generally, chemical control of houndstongue infestations is uneconomical because of the size of areas that need to be treated and the high cost of herbicides. Mechanical control can be effective when plants are cut 0–7 cm above ground during the flowering season (Dickerson and Fay, 1982). However, this control technique is feasible only where houndstongue infestations are small or easy to reach. Because of the topography of most areas in which houndstongue is a problem, mechanical management techniques are either impractical or ineffective.

In North America, houndstongue is not attacked to any noticeable degree by phytophagous insects (Upadhyaya *et al.*, 1988). Therefore, it has a competitive advantage over native rangeland species. The aim of the biological control program against houndstongue is to reduce its present competitive superiority through the introduction of host-specific insect herbivores from Europe. The first biocontrol agent studied, the most abundant herbivore species associated with houndstongue in Europe, was the root-mining weevil *Mogulones cruciger* Hbst. (Coleoptera, Curculionidae). Following life-history and host-range studies, the weevil was released in Canada in 1997 (Schwarzlaender, 1997, unpublished). It is expected that attack by *M. cruciger* will reduce the reproductive output, competitive ability, and survival of houndstongue (Prins *et al.*, 1992; Wesselingh, 1995). However, it was demonstrated that plants can survive and even reproduce at high *M. cruciger* attack rates (Schwarzlaender, 1998). Therefore, the biology and host specificity of another, below-ground herbivore, the flea beetle *Longitarsus quadriguttatus* Pont. (Coleoptera, Chrysomelidae), was investigated (Jordan, 1997).

L. quadriguttatus is reported to occur in southern and central Europe and is closely associated with its host plant *C. officinale* (Koch, 1992). Mohr (1966) stated that the flea beetle species should be found predominantly on *Nonea pulla* (L.) DC, but this host record could not be confirmed during previous host-range investigations (Jordan, 1997). Adult *L. quadriguttatus* feed on aerial plant parts of houndstongue, whereas larvae mine in roots. The beetles emerge between the end of May and the middle of June and can live until mid-October. Eggs are laid usually between the bases of petioles or at the root crown. *L. quadriguttatus* has three instars. Larvae develop during late summer and autumn. They mine in the cortex of the tap root and in secondary roots. They hibernate in the roots, complete development in early spring, and pupate in the soil in April (Jordan, 1997).

Previous host specificity investigations were composed of adult feeding, oviposition, and larval development tests with 49 European plant species. Adult feeding occurred to some extent on most of the 31 Boraginaceae species tested. Normal feeding, oviposition, and larval development were restricted mainly to plant species within the tribe Cynoglosseae Gürke (Jordan, 1997). However, 7 plant species that belonged to four genera within three different tribes, all different from Cynoglosseae, were susceptible to attack by *L. quadriguttatus* to some extent: *Lithospermum officinale* L. (tribe Lithospermae Gürke) was accepted during adult feeding and oviposition tests and supported complete development of *L. quadriguttatus* (Jordan, 1997). *Echium vulgare* L. and *E. italicum* L. (tribe Echieae Gürke) were accepted as host plants during adult feeding and oviposition tests. Both species supported larval development to some extent. In addition, adults could be reared from *E. vulgare* (Jordan, 1997). On both, *Anchusa officinalis* L. and *A. azurea* Mill. (tribe Anchuseae Gürke) adult feeding could be observed during host-range investigations. Larval development was partly supported by both species, and *A. azurea* was accepted for oviposition (Jordan, 1997). *Symphytum officinale* L. and *S. grandiflorum* Steven (tribe Anchuseae Gürke) were accepted during adult feeding tests. Moreover, both species supported larval development of *L. quadriguttatus* to a limited extent (Jordan, 1997). Thus, 4 plant species outside the genus *Cynoglossum* supported complete development of *L. quadriguttatus* but to a smaller extent than houndstongue.

Despite these results, the investigations on the host specificity of *L. quadriguttatus* were not continued with plant species that partly or completely supported development of the flea beetle nor were any native North American Boraginaceae species tested. Therefore, the present study was undertaken to provide complementary data on the host specificity of *L. quadriguttatus* for European plant species that were susceptible to attack and five native North American test-plant species.

MATERIALS AND METHODS

L. quadriguttatus adults used for studies were either obtained from a rearing maintained at the CABI Bioscience Centre in Delémont (Centre) or collected at a field site in Sollenau, Austria. The site (47°55'N, 16°15'E) is an old gravel pit, that was in use until 1991. Deer and rabbit activity regularly causes disturbance of the soil surface. The proportion of ground cover in the gravel pit varies considerably depending on precipitation and consists mainly of perennial grasses and annual and perennial herbaceous plant species. The field site was also used to conduct the open-field test described below.

In addition to seven susceptible European test-plant species, i.e., *L. officinale, E. vulgare, E. italicum, A. officinalis, A. azurea, S. officinale,* and *S. grandiflorum,* five native North American plant species were

included in the host-range investigations: *C. occidentale* Gray. and *C. grande* Dougl.; *Lithospermum ruderale* Dougl., a common species that occurs sympatrically with houndstongue in North America, and *Amsinckia tessalata* Gray. and *A. carinata* Nels. & Macbr., two representatives of a widespread genus (*Amsinckia* Lehm.), that is closely related to *Cynoglossum* but does not occur in Europe. The latter two species were also selected for investigating more thoroughly the acceptance of plants within the tribe Eritricheae (Gürke) by *L. quadriguttatus* because it is the most prevalent and diverse Boraginaceae tribe in North America.

No-Choice Oviposition and Longevity Tests with Susceptible European Test-Plant Species and Cynoglossum grande

No-choice oviposition, longevity, and adult feeding tests were conducted in 1994 with seven test-plant species that had been attacked in previous tests and with the native North American *C. grande.* Five pairs of *L. quadriguttatus* were kept in transparent plastic cylinders (160 mm in height, 100 mm in diameter) covered with a gauze lid, with four replicates for each test-plant species and the control (*C. officinale*). In addition, four cylinders containing no leaves (starvation control) were set up. Leaves of the test plants, inserted in small, wet horticulture sponge blocks, were continuously offered to the beetles. Feeding units (feeding holes of approximately 1 mm^2 in size) were counted every 3 to 4 days. The horticulture sponge blocks were dissected for eggs, and cylinders were checked for dead adults. Since most but not all beetles used for the test were field collected and thus had a feeding history, leaves of the control were offered to all beetles for 18 days prior to the experiment to standardize test conditions. During this period, dead beetles were replaced. Eggs laid and adults feeding during this period were not included in later data analysis. Because of the limited number of *C. grande* leaves available, tests with this plant species had to be stopped after 23 days. Thus, data for this period were summarized and compared with those for the control separately.

No-Choice Oviposition Tests with Two Native North American Amsinckia Species

These tests were conducted separately from other no-choice oviposition tests because of the availability of plant material of these native Boraginaceae species. In 1996, leaves of the control or one test-plant species were offered simultaneously to individual pairs of *L. quadriguttatus* for 22 days. The beetles were kept in transparent plastic cylinders covered with a gauze lid (160 mm in height, 100 mm in diameter). Five cylinders were set up for each test-plant species and the control. Leaves were offered in wet horticulture sponge blocks (30 × 30 × 30 mm) and changed every 4 days. The sponge blocks were dissected for eggs.

No-Choice Development Tests

No-choice oviposition and larval development tests were conducted in 1994 with two critical European test-plant species, i.e., *E. vulgare* and *L. officinale,* and the control. Five pairs of *L. quadriguttatus* were released on a gauze-covered, potted control or one test plant. Four replicates were set up for each plant species. In addition, 40 newly hatched larvae each were transferred using a fine paintbrush onto root crowns of six potted native North American *L. ruderale* and six potted control plants between 15 August and 20 September 1994. The plants were kept under a dry shelter in the Centre's garden during the winter. Emergence of beetles was checked regularly during May and June 1995.

No-Choice Larval Transfer Tests

From 21 July to 21 August 1995, 50 newly hatched larvae, obtained from a laboratory breeding colony, were transferred onto potted plants of four European test-plant species, the two native North American *Cynoglossum* species, and the control (*C. officinale*). Five replicates were used for each test-plant species. All plants were embedded in sand and kept under a dry shelter in the Centre's garden during the winter. Emergence of adults of the new beetle generation was checked regularly during June and July 1996.

Multiple-Choice Oviposition and Larval Development Tests under Field-Cage Conditions

On 21 August 1995, 10 pairs of *L. quadriguttatus* were released into a field cage (100 cm high, 130 cm long, 100 cm wide) in the Centre's garden. The cage contained five potted plants of each of six test-plant species and the Canadian biotype of *C. officinale.* Plant species used were *C. occidentale, C. grande, L. officinale, E. vulgare, S. grandiflorum,* and *S. officinale.* The plants were arranged within the cage in a completely randomized block design. The pots were embedded in sawdust and exposed to the beetles throughout autumn. Thereafter, plants were kept in the cage. At the beginning of June 1996, all plants were transferred into emergence traps consisting of a black, plastic, garbage bag (17 liter volume) with a tube and a transparent plastic cup (80 mm in height, 65 mm in diameter) attached to its top. Emergence of adults of the new generation was checked at regular intervals from 5 June 1996 onward.

Open-Field Test

Multiple-choice oviposition and larval development tests were conducted in summer 1994 within a 100 ×

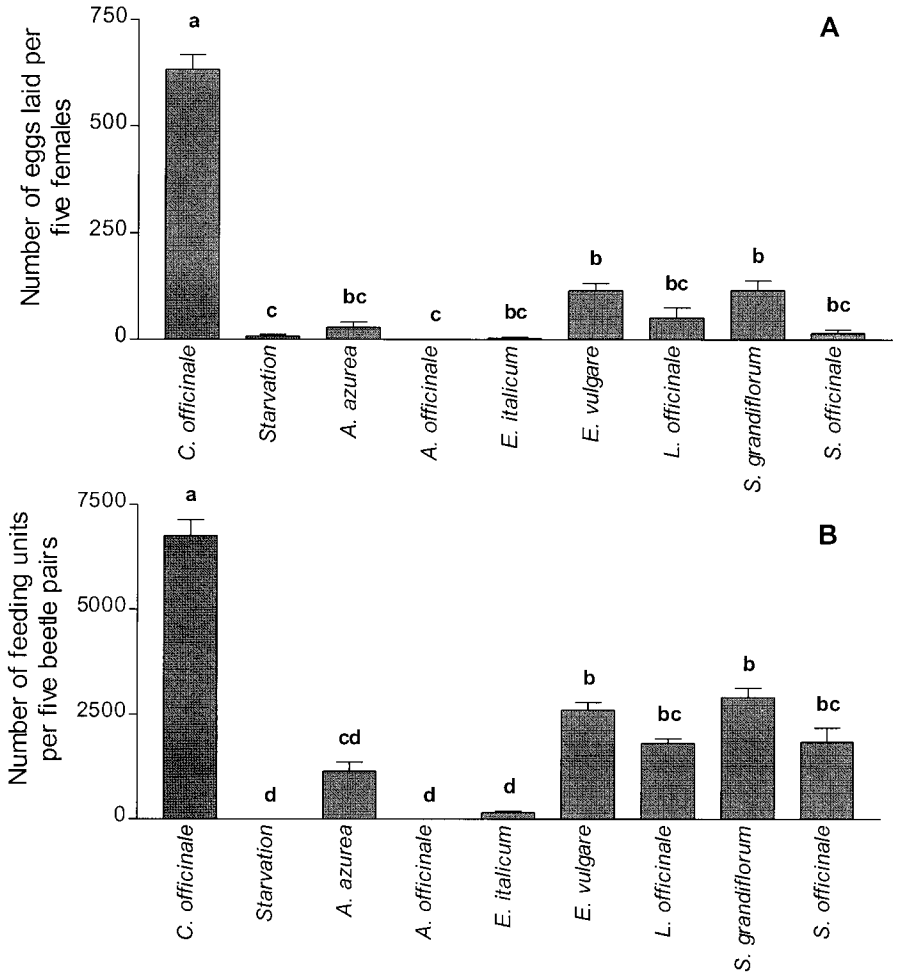

FIG. 1. Oviposition and adult feeding of *L. quadriguttatus* during no-choice tests with susceptible test-plant species. (A) Oviposition; (B) Adult feeding; vertical bars, means ± SE; $n = 4$. Bars denoted by the same letters are not significantly different, $P < 0.05$, by Scheffé's Multiple Comparison Test.

5 m area on a slope in the gravel pit in eastern Austria. Eight plots were set up, each consisting of four plants, i.e., two potted controls and two potted plants of one of the test-plant species (*C. grande, S. grandiflorum, E. vulgare,* and *L. officinale*). Each test-plant species/control plant plot was replicated twice. Plants were grown in a greenhouse at the Centre and transported to Austria on 19 July 1994, where they were carefully dug into the soil without destroying the surrounding vegetation. All plants were returned on 21 September and kept in the Centre's garden. Plants and soil were transferred into emergence traps in May 1995 to check for emergence of adult *L. quadriguttatus.*

RESULTS

No-Choice Oviposition and Longevity Tests with Susceptible Test-Plant Species

L. quadriguttatus showed a strong preference for its field host, *C. officinale,* in both oviposition and adult feeding (Fig. 1). However, when fed with *S. grandiflorum* and *E. vulgare,* females were able to continue limited egg production. Five females laid on average 115 eggs during their life span on each of these two species, i.e., approximately 23 eggs per female. In comparison, more than 600 eggs were laid by groups of five females on the control *C. officinale,* i.e., approximately 120 eggs per female (Fig. 1). Oviposition on the other plant species tested was negligible. The adults fed also on *S. officinale* and *L. officinale* (Fig. 1) but feeding and oviposition rates indicated that the food quality of these plant species was not optimal for egg development compared with that of the control. The results for longevity correspond with those for adult feeding (Figs. 1 and 2). Adults of *L. quadriguttatus* survived longer on houndstongue than on any other test species.

Significant differences were found after 23 days (test duration) for female survival rates and the number of eggs laid on the native North American *C. grande* (Table 1). No significant differences were found in male

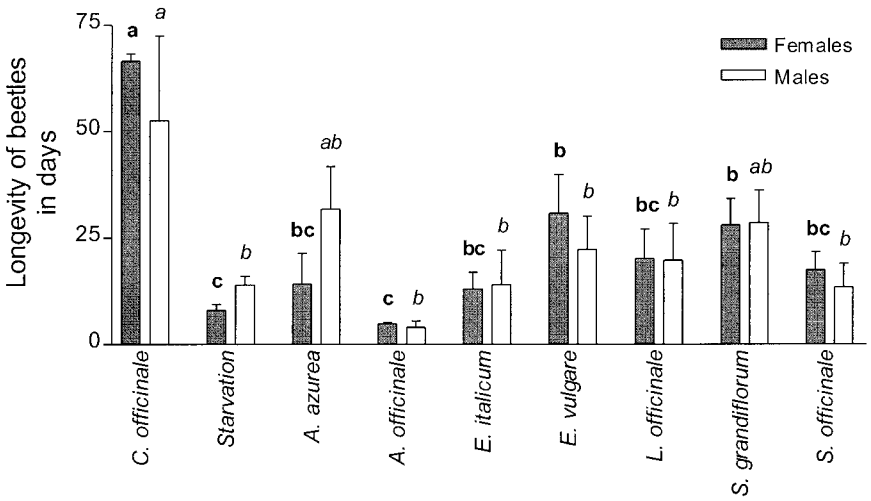

FIG. 2. Mean longevity (± SD) of female and male *L. quadriguttatus* during no-choice tests with susceptible plant species. Vertical bars, means ± SD; $n = 4$; within sexes, bars denoted by the same letters are not significantly different, $P < 0.05$, by Scheffé's Multiple Comparison Test.

survival rates and adult feeding. Similar to the results for *S. officinale* and *L. officinale,* females were able to continue egg development when fed with *C. grande.* The number of eggs laid by five females within the period of 23 days indicates that *C. grande* is probably a more suitable host plant for *L. quadriguttatus* than *E. vulgare* and *S. grandiflorum*.

No-Choice Oviposition Tests with Two Native North American Amsinckia Species

Neither *A. tessalata* nor *A. carinata* were accepted for oviposition by *L. quadriguttatus,* although adult feeding occurred on both species. In contrast, a mean number of 29 eggs per female was laid on the *C. officinale* plants (Table 2). Two of five males fed with *A. tessalata* and two of five males and three of five females fed with *A. carinata* died during the tests. In contrast, all adults fed with houndstongue leaves were still alive upon test termination.

No-Choice Development Tests

The number of beetles emerging from control plants in both tests was smaller than expected. In both tests, no adults could be reared from any of the test-plant species (Table 3).

No-Choice Larval Transfer Tests

Similar to the no-choice development tests, the survival rates of *L. quadriguttatus* larvae on control plants was not satisfactory (Table 4). The only plant species, other than the control, that supported complete development of *L. quadriguttatus* during larval development was *C. grande* (Table 4). The number of beetles emerging from *C. grande* was significantly smaller than that from the control plants ($P = 0.043$; $n = 5$; Mann–Whitney test).

TABLE 1

Comparison of Longevity, Oviposition, and Adult Feeding in No-Choice Tests with *Cynoglossum officinale* and *C. grande*

Plant species	Mean ± SE no. of beetles alive after test		Mean ± SE no. of eggs laid	Mean ± SE no. of feeding
	Females	Males		
C. officinale	5.0 ± 0.0[a]	2.3 ± 1.8	163.0 ± 10.9	2493.3 ± 209.8
C. grande	2.8 ± 1.5	2.0 ± 0.7	134.8 ± 4.9	2543.3 ± 394.7
*P**	<0.05	*NS*	<0.05	*NS*

[a] Means represent sums for cylinders, each containing five pairs of *L. quadriguttatus.*

* Mann–Whitney test; $n = 4$.

TABLE 2

Results of No-Choice Tests with Two North American *Amsinckia* Species

Plant species	No. replicates	Mean no. eggs laid ± SE	No. dead	
			Males	Females
C. officinale	5	29.0 ± 1.3	0	0
A. tessalata	5	0 ± 0	2	0
A. carinata	5	0 ± 0	2	3

TABLE 3

Results of No-Choice Development Tests with *Echium vulgare, Lithospermum officinale,* and the Native North American *L. ruderale*

Plant species	No. replicates	Total no. larvae transferred	Total no. beetles emerged	Mean % survival ± SE
C. officinale	4	—	12	—
E. vulgare	4	—	0	—
L. officinale	4	—	0	—
C. officinale	6	240	26	10.8 ± 3.3
L. ruderale	6	240	0	0.0 ± 0.0

Multiple-Choice Oviposition and Larval Development Tests under Field-Cage Conditions

Adults emerged from 10 June to 12 July 1996. The native North American *C. grande* was not attacked during this test, whereas one beetle emerged from *C. occidentale* (Table 5). Of the European test-plant species offered, one of the five *E. vulgare* plants was attacked. On average, 31 beetles emerged from each of the control plants offered. Attack rates of the control and test-plant species differed significantly (*C. occidentale* and *E. vulgare, P* = 0.0071, *n* = 5, Mann–Whitney test).

Open-Field Test

Because of the open-field conditions, with naturally growing houndstongue plants surrounding the experimental area, the relatively low attack rate of control plants was expected (Table 6). *S. grandiflorum* was the only test species attacked in the experiment. Two beetles emerged from one of the two *S. grandiflorum* replicates. In contrast, 33 beetles emerged from the corresponding control plants (Table 6).

TABLE 4

Results of No-Choice Larval Transfer Tests

Plant species	No. replicates	Total no. beetles emerged	Mean % survival
C. officinale	5	38	15.2 ± 2.8
C. occidentale	5	0	0 ± 0
C. grande	5	12	4.8 ± 2.7
L. officinale	5	0	0 ± 0
E. vulgare	5	0	0 ± 0
S. grandiflorum	5	0	0 ± 0
S. officinale	5	0	0 ± 0

TABLE 5

Results of Multiple-Choice Oviposition and Larval Development Tests under Field-Cage Conditions

Plant species	No. plants		Mean ± SE no. of beetles emerged
	Offered	Attacked	
C. officinale	5	5	31.4 ± 12.8
C. grande	5	0	0 ± 0
C. occidentale	5	1	0.2 ± 0.2
L. officinale	5	0	0 ± 0
E. vulgare	5	1	0.2 ± 0.2
S. grandiflorum	5	0	0 ± 0
S. officinale	5	0	0 ± 0

DISCUSSION

Comparison of Previous and Present Host Specificity Results

A few European plant species outside the genus *Cynoglossum* are able to support complete development of the flea beetle species. In previous investigations, these plant species were *Rindera umbellata* (Waldst. & Kitt), *E. vulgare, L. officinale,* and a few of the European *Cynoglossum* species (Jordan, 1997). In the present study, adults could be reared from *E. vulgare* and the native North American *C. occidentale* and *C. grande.* In contrast to previous tests, during which 14 specimens of *L. quadriguttatus* emerged from five *L. officinale* plants exposed in a multiple-choice field-cage test (Jordan, 1997), this plant species was not attacked in any of the tests performed during this study. Differences in the plant quality and phenotypic or genotypic differences of the flea beetles used in the two tests might be the causes of the differential susceptibility of *L. officinale* to *L. quadriguttatus* attack (Schmid, 1992; Bernays and Chapman, 1994 and references therein).

Host Specificity with Regard to Environmental Test Conditions

The performance of similar host-specificity tests under varying environmental conditions is an important tool for obtaining better information concerning both the realized or ecological host range and the potential or physiological host range of an insect species (Cullen, 1989; Marohasy, 1998). In addition to investigations under laboratory conditions, open-field tests (Clement and Cristofaro, 1995), field-cage tests in cases in which open-field tests are not possible, or ideally both types of tests should be conducted. The latter was done with selected test-plant species in this study (Tables 3–6). Jordan (1997) demonstrated for several Boraginaceae species that were previously accepted by *L. quadriguttatus* that the same plant species remained free from

TABLE 6

Results of Oviposition and Larval Development Tests under Open-Field Conditions

Plant species	Replicate	Total no. of beetles emerged		Mean ± SE no. of beetles emerged from	
		Control	Test	Control	Test
C. grande	1	5	0	2.0 ± 0.7	0 ± 0
	2	4	0		
S. grandiflorum	1	33	2	9.8 ± 4.4	0.5 ± 0.5
	2	6	0		
E. vulgare	1	6	0	2.3 ± 0.9	0 ± 0
	2	3	0		
L. officinale	1	11	0	3.5 ± 2.3	0 ± 0
	2	3	0		

attack when tests were performed under more natural conditions. In the present study, the native North American *C. grande* supported complete development of *L. quadriguttatus* under no-choice conditions. However, when beetles were given a choice and tests were carried out in field cages or in an open-field situation, the plant species remained free from attack (Tables 4–6). This host-acceptance pattern occurs frequently during host specificity investigations of potential biocontrol agents (e.g., Cullen, 1989; Whapshere, 1989; Olckers *et al.*, 1995; Balciunas *et al.*, 1996 and references therein). The differences in results with regard to environmental conditions of host specificity tests are usually explained by the ability of insect females to express their complete host-choice behavior when tests are conducted under more natural conditions (Cullen, 1989; Harris and McEvoy, 1995; Briese *et al.*, 1995; Clement and Cristofaro, 1995; Marohasy, 1998; McFadyen, 1998).

However, the results for the other three test-plant species that supported complete development of *L. quadriguttatus* during the course of this study deviate from this explanation. *C. occidentale, E. vulgare,* and *S. grandiflorum* were not accepted as host plants under no-choice conditions but were accepted under more natural test conditions, i.e., during the multiple-choice field-cage test and the open-field test. Variation in plant phenotype and/or genotype cannot be used as an argument for explaining the differences of results between no-choice and choice tests because plants used for these tests originated from the same population and were kept under the same conditions prior to and after performance of the tests. In addition, it was observed that plants exposed in the field cage and at the field site grew better than those kept in the greenhouse for the no-choice tests. However, it seems unlikely that the growth differences of plants due to differing growing conditions at the time of exposure to beetles might have influenced the susceptibility of plants to attack by the root herbivore, but the possibility cannot be excluded.

Risk of Potential Nontarget Effects

Both native North American *Cynoglossum* species used for host-range investigations, i.e., *C. occidentale* and *C. grande,* supported development of *L. quadriguttatus* to some extent. The North American distribution of *C. grande* extends from California to British Columbia along the far western mountain ranges at elevations below 1500 m (Hitchkock *et al.*, 1971). Occurrence records in Canada could so far not be confirmed (Scoggan, 1979). In the United States, the plant species is found only on the west side of the Cascade range and does not overlap much with the distribution of *C. officinale,* which is more common east of the Cascade–Sierra Nevada–Coast ranges (Hitchkock *et al.*, 1971). *C. occidentale* is also restricted to the far western mountain ranges of California, Oregon, and Washington but occurs at higher elevations (900–2100 m) in Ponderosa pine woods (Hitchkock *et al.*, 1979). A third *Cynoglossum* species native to North America that could not be obtained for testing, *C. boreale* Fern., has a more eastern distribution. In areas in western North America in which the plant species occurs, its southern distribution borders at latitudes in Northern Canada (Scoggan, 1979). Thus, the distribution of *C. boreale* does not overlap with that of houndstongue.

Neither geographical distribution or habitat differences that separate native North American *Cynoglossum* species from sympatric occurrence with houndstongue nor the small degree of acceptance and the strong preference of *L. quadriguttatus* for *C. officinale* during host specificity tests can guarantee that native *Cynoglossum* species will not be attacked by the flea beetle. However, for the same reasons, the probability of encounter and the risk of *L. quadriguttatus* establishment on these plant species in North America are very small. A detailed benefit–risk assessment for the introduction of the flea beetle species into North America was not part of this study. There is a need for such studies as part of future classical weed biocontrol programs using insect agents (Wan and Harris, 1997).

Native North American plant species outside the genus *Cynoglossum* that were included in the tests were not accepted by *L. quadriguttatus* and thus are not at risk of being attacked after release of the flea beetle. The inclusion of Boraginaceae species native to the area of release largely improved the predictability of the experimental host range of *L. quadriguttatus.* In cases in which test-plant species of genera different from the one to which the target weed belongs are susceptible to attack by a potential biocontrol agent, it is advisable to include plant species from such genera native to the area of release in host-range investigations. For this reason *L. ruderale* was included in host-specificity tests with *L. quadriguttatus.* Also for the same reason, the two *Amsinckia* species were included in investigations: They represent an abundant and species-rich genus, which is closely related to *Cynoglossum* but is not present in Europe. In light of the recently demonstrated nontarget effects of weed biocontrol agents and the debate following the safety of classical weed biocontrol in general (e.g., Simberloff and Stiling, 1996; Louda *et al.*, 1997; Strong, 1997; Thomas and Willis, 1998), the subsequent change of the original test-plant list based on earlier host-specificity results was inevitable.

Selection Criteria for Test-Plant Species and the Centrifugal–Phylogenetic Approach

In addition to the inclusion of test-plant species from the release area, the adaptation of the centrifugal–phylogenetic approach (Harris and Zwölfer, 1968; Whapshere, 1974) for meeting the changed regulatory and public requirements on host specificity of insect agents was emphasized in this study. In a comparable program aimed at the biological control of Paterson's curse (*Echium plantagineum* L.) in Australia (Vayssieres and Wapshere, 1983), a total of 69 plant species was tested against two weevil species but only 10 of these test-plant species belonged to the family Boraginaceae. In contrast, 36 of the 57 plant species used for host-range investigations with *L. quadriguttatus* were Boraginaceae species (Jordan, 1997 and data presented in this study). Moreover, 13 of the 36 Boraginaceae species belong to the genus *Cynoglossum*. The use of more test-plant species that are closely related to the target weed and fewer species that belong to different plant subfamilies or families allows for a more precise prediction of the physiological and ecological host range of *L. quadriguttatus.*

The Potential of L. quadriguttatus for the Biological Control of Houndstongue

In summary, it can be stated that *L. quadriguttatus* is host specific to species within the genus *Cynoglossum*, with a strong preference for its field host, *C. officinale.* Native North American *Cynoglossum* species were accepted only to a small degree. A few plant species from other genera, i.e., *E. vulgare, S. grandiflorum,* and *L. officinale,* might support development of the flea beetle to a limited degree, but related native North American species within the respective genera do not exist or were not accepted as host plants. In addition, none of the three susceptible species is reported as field host of *L. quadriguttatus* in Europe (Mohr, 1966; Koch, 1992). In a field population in which *E. vulgare* and houndstongue were growing sympatrically, no *E. vulgare* plant showed signs of *L. quadriguttatus* herbivory (Jordan, 1997).

Currently, there are no satisfactory means of effectively controlling *C. officinale* in North America. Measures applied are expensive and/or not persistent. Biological control of houndstongue might offer the possibility of a permanent control of this weed in forests and rangelands. *C. officinale* has a tap root that facilitates water uptake in dry habitats and serves as a storage organ for metabolic products during winter. Thus, priority was given to below-ground herbivores for controlling houndstongue. The weevil species *M. cruciger* preferentially attacks bolting rosettes after hibernation (Schwarzlaender, 1997). However, it is known for houndstongue that the young rosette is a much more sensitive life stage (de Jong and Klinkhamer, 1988; de Jong *et al.*, 1990) and insect herbivores attacking the most sensitive plant phenostages are expected to impair the plant population dynamics (Crawley, 1989). *L. quadriguttatus* attacks young rosettes during summer and fall before hibernation. Adult flea beetles feed on leaves, negatively affecting photosynthesis of plants, and larvae mine in the cortex of rootlets, thereby hampering the water uptake of plants and facilitating secondary infections by pathogens and bacteria (Jordan, 1997). It is assumed that herbivory by *L. quadriguttatus* prior to *M. cruciger* attack will further weaken the competitive ability of houndstongue and might prevent the further spread of the weed in North America. The introduction of *L. quadriguttatus* into Canada was approved by the Biological Control Review Committee in 1998 and the agent was first released the same year.

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