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Dasineura sp. near *capsulae* (Diptera: Cecidomyiidae), a candidate for biological control of *Euphorbia esula* complex in North America[1]

P. PECORA, M. CRISTOFARO[2], and M. STAZI

Biological Control of Weeds, Laboratory - Europe, USDA-ARS, Rome, Italy

Abstract:

The gall midge *Dasineura* sp. near *capsulae* Kieffer, whose larvae cause galls on flower buds of *Euphorbia* spp., was selected as a candidate agent for the biological control of leafy spurge, *Euphorbia esula* complex, a plant of Eurasian origin that has become a noxious weed in North America. Studies of a population of this midge associated with *E. esula* at S. Rossore, Pisa, Italy, indicated that this midge is univoltine. The adults appeared from early April until late May, living 2-4 days. Eggs were laid in the inner part of the bracts that cover the cyathium. The neonate larvae migrated into the cyathium, where they caused the formation of galls. The galls, appearing in early May, were produced by the enlargement and distortion of the cyathium and prevented seed production in the infested flowers. In late June and early July, mature larvae left the galls, fell to the ground where they entered the soil, hibernated until the following spring, and pupated a few days before the adults emerged. Host specificity tests of *D.* sp. near *capsulae* were made, using 48 test plants in 17 families. The midge oviposited on 13 test plants (all in the genus *Euphorbia*) and the controls (*E. esula*). Ten of these test plants were in the subgenus *Esula* and one each in the subgenera *Agaloma, Poinsettia,* and *Euphorbium*. The midge completed development only on six of the test plants on which it oviposited, all in the subgenus *Esula*. The restricted host range of this midge suggests it would be safe to use as a biological control agent against leafy spurge in North America.

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[2] Mailing address: Biological Control of Weeds Laboratory-Europe, American Embassy, Agriculture, APO New York 09794.

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Leafy spurge, *Euphorbia esula* L. "complex" (Euphorbiaceae), a plant of Eurasian origin, was accidentally introduced into North America. It has become a serious weed in pasture, range, and non-cropland areas, infesting about one million hectares mostly in Minnesota, North Dakota, Montana, Nebraska, South Dakota, and western Canada (Noble *et al*. 1979). Several insect species have been introduced as biological control agents for leafy spurge in North America (Harris *et al*. 1985). Recently, a European gall midge, *Dasineura* sp. near *capsulae* Kieffer, whose larvae cause capsule-like galls on the flower buds of leafy spurge and interfere with seed production, was selected and studied as a candidate agent for the biological control of this weed.

In the midge complex associated with *Euphorbia* spp. in Europe, four species that produce capsule-like galls – *Dasineura capsulae* Kieffer, *D. loewi* (Mik), *D. cornifex* (Kieffer), *D. euphorbiarum* (Kieffer,) – have been described (Kieffer 1901, 1909; Houard 1908). Recently, Solinas and Pecora (1984) suggested that of this complex, "only two good species (*D. capsulae* and *D. loewi*) may remain." One of these valid species (*D. capsulae*) was recorded from *E. cyparissias* L., *E. esula* L., *E. nicaeensis* Allioni, *E. pithyusa* L. (Kieffer 1901, Houard 1908), *E. falcata* L., and *E. lucida* Waldstein and Kitaibel (Buhr 1964). The other species (*D. loewi*) was found on *E. seguierana* Necker (Buhr 1964). The midges whose biology and host plant range were studied were always collected from capsule-like galls on *E. esula* at S. Rossore (central Italy). According to R. J. Gagné (personal communication), the midges from *E. esula* have a shorter ovipositor than those specimens described by Solinas and Pecora (1984) that emerged from *E. cyparissias* galls collected at S. Rossore. Because the taxonomy of this midge complex associated with *Euphorbia* spp. in Europe is not yet clear, Gagné suggested calling the midge from *E. esula* "*Dasineura* sp. near *capsulae*" until it is properly described and named.

To determine the safety and effectiveness of *Dasineura* sp. near *capsulae* as a biological control agent for leafy spurge, its life history and host range were studied in the field at S. Rossore and in the laboratory at the USDA-ARS facility in Rome, Italy, from 1982 to 1985.

Materials and methods

Life history

To determine the oviposition period of *D.* sp. near *capsulae* and the phenology of the galls produced by this midge, leafy spurge plants growing at S. Rossore were examined at irregular intervals from April to July 1983. Depending on which stage was available, a sample of 100 flower buds or 50 galls of various sizes was collected from 10 randomly chosen plants of *E. esula* at each inspection. These buds and galls were dissected, and the

number of eggs, living and dead larvae, and parasites of the gall midge were recorded. In addition, width and length measurements were taken on 25 mature galls.

Bud galls collected in the second half of June 1982, 1983, and 1984 contained larvae of various stages. Mature larvae emerging from the galls were transferred to 1,600-ml transparent plastic containers with a mixture of moistened peat moss and fine sand in a 2-cm-deep layer on the bottom, which provided a suitable substrate for the diapausing insects. The containers, each with 200 larvae of this midge, were held undisturbed in an outdoor insectary until the following spring when adult emergence started. They were then checked daily, and the emerged adults and associated parasites were collected, counted, and recorded.

To determine adult longevity and egg production per female, 13 pairs of midges were used. Each pair was placed in a transparent plastic, cylindrical cage that had the top covered with nylon screen and the bottoms fitted snugly over cork or balsa wood disks (1.5-2.0 cm thick) that had central holes. *E. esula* flower buds (six to eight per cage) on growing plants were passed through these holes, which were sealed with a cotton plug, allowing the insects to be caged directly on the plant. The cages were inspected daily, and the dead insects were collected and their numbers recorded.

When all the adults had died, the flower buds were dissected, and the number of eggs found on each was counted and recorded. The percentage of egg fertility and the preeclosion period were determined from a sample of 507 newly laid eggs kept in 128-ml acrylic hatching containers.

The development time from neonate to mature larva and the various stages of gall development were determined. Flower buds (n = 15-20) were exposed in a 300-ml cylindrical cage to adults of *D.* sp. near *capsulae* (three females, two males per cage). Eighteen cages were prepared, and exposed flower buds in three of the cages were dissected each week. The number of galls formed was recorded, and the larvae collected were preserved in 70% alcohol. These studies, which started on 20 April and terminated on 25 May 1983, were done in a laboratory room (20-24º C, natural lighting and photoperiod).

Biometric data of all stages of *D.* sp. near *capsulae* were recorded; the width of the preserved larvae was measured at the widest part, and the width of the pupae and adults was measured on the first abdominal segment. The adult length measurements were exclusive of antennae and ovipositor.

Tests of host specificity

To determine the host plant range of this midge, tests were conducted in 1984 and 1985 with 48 plant species or varieties in 17 families. The plants tested included genera and families closely related to *Euphorbia* (order Euphorbiales), plants in other orders of the superorder Rosidae, and plants attacked by other species of *Dasineura*. Heywood (1978) was used as a guide in constructing our list of test plants.

Free-choice host suitability

The object of this experiment was to determine if, in a field situation, feral adults of *D.* sp. near *capsulae* would select any of the exposed test plants as hosts. The experiment was conducted at S. Rossore, where a population of this midge and its host (*E. esula*) oc-

cur naturally. In June 1983, the experimental site (50 by 10 m) was selected in an area with dense stands of *E. esula* plants and was left undisturbed until 16 April 1984, when the experiment started. In the experimental site, three plots (4.00 x 4.00 m each) were established. The experimental design for each plot was a randomized complete block consisting of five treatments (three test plants, and control plants A and B) replicated five times (total, 25 blocks). Leafy spurge biotypes from Nebraska, Montana, Wyoming, and Oregon, *Euphorbia peplus* L., *E. milii* Desmoulins, *E. characias* L., *E. pulcherrima* Willdenow, and *Linum narbonense* L., were included.

The test plants were grown at the Rome Laboratory in plastic pots (22 cm diameter) until the preflower was attained. Then they were taken to the test site, where the pots were buried in the ground with their tops at soil level in the naturally occurring leafy spurge infestation. Control A consisted of *E. esula* plants from S. Rossore transplanted into the same size pots as the test plants; control B was composed of randomly selected *E. esula* plants growing naturally in the test site and left in site. All of the other naturally occurring leafy spurge plants, (except those that were labeled as control B) were removed from the area. The length of time the plants were kept in pots before use in the experiment was not uniform. The leafy spurge plants from Montana, Nebraska, and Wyoming were kept in pots for 6 months before the experiment, the plants from Oregon were in pots for 18 months, and the plants of control A were in pots for 12 months before use. For the other test plants, this period ranged between 4 and 6 months.

To follow the occurrence and development of the galls, weekly observations were made from mid-April to the beginning of June, when the galls had reached maturity. At that time the number of plants present in each block and the number of flowers and galls per plant were recorded.

No-choice oviposition and host suitability

To determine the range of plants that *D.* sp. near *capsulae* would accept for oviposition and plants that would support the development of the midge, 48 test plants in preflower stage were included in the experiment. To provide adults of *D.* sp. near *capsulae* for these laboratory trials, 300 galls (with larvae of various instars) were collected from *E. esula* at S. Rossore in 1983. These galls produced 2,082 mature larvae. In 1984, another 1,275 galls were collected from *E. esula* at S. Rossore and from these, 9,000 mature larvae were collected. The larvae of this midge, placed in 1,600-ml transparent plastic containers, were held in an outdoor insectary, and the newly emerged adults were transferred onto the plants in 300-ml cylindrical cages. Because of shortage of freshly emerged adults, all plants could not be tested simultaneously, so they were divided into nine groups (three to seven test plants plus the plant control per group) and tested using insects that emerged the same day. Eight to 25 flower buds per cage were exposed to five adult midges (three females and two males per cage), and each species of test plant was replicated four times, one cage serving as a replicate. The plants with cages containing the midges were held in a laboratory room at 21-25º C and were left undisturbed until the midges died. Later, to determine if oviposition had occurred, we dissected flower buds until eggs of *D.* sp. near *capsulae* were found in one or two of them. Once we had ascertained that oviposition had occurred, the infested plants in pots were then moved outdoors to a shaded area, and the remaining flower buds were left undisturbed to follow gall development.

To determine the percentage of egg hatch, eggs found during the bud dissections were placed in plastic hatching containers, and as eclosion occurred, the neonate larvae were counted and the number recorded. In April and May 1984 and 1985, the galls produced on the test plants and on controls were counted and dissected, and the number of living and dead midge larvae found in each gall was recorded.

Adults emerging in the spring of 1984 were used to test different North American biotypes of leafy spurge, whereas adults that emerged in the spring of 1985 were used to test the other plants on the list.

Statistical analyses

Data from field and laboratory experiments were subjected to analysis of variance (ANOVA). Means were separated by a Student-Newman-Keuls (a posteriori) test (Sokal and Rohlf 1969).

Voucher specimens

Specimens from this study, labeled BCWLE-87-2, and identified by R.J. Gagné, USDA Systematic Entomology Laboratory, are in the National Museum of Natural History, Washington, D.C., and in the BCWLE collection.

Results

Life history

Adult

The body is reddish yellow with brown sclerotized parts; the female is 2.32 ± 0.09 mm long and 0.41 ± 0.02 mm wide (n = 10), and the male 1.69 ± 0.06 mm long and 0.41± 0.02 mm wide (n = 10).

During 1983-1985, emergence of *D.* sp. near *capsulae* adults started in mid-April, peaked in late April, and continued until mid-May (Fig. 1a). Mating usually occurred on the day of emergence, and females started to lay eggs within 24 hours. Eggs were laid in the inflorescence between the bracts and the cyathium in groups (35.60 ± 7.80 eggs per group; n = 18; range, 15-40). Occasionally eggs were laid inside the cyathium. At S. Rossore in 1983, the oviposition period of *D.* sp. near *capsulae* was from the second half of April until mid-May. There was no significant difference ($P > 0.05$) between the eggs per bud on the 19 April and 2 May samples, whereas significantly ($P < 0.05$) fewer numbers of eggs per bud were found on the samples collected on 11 May (Table 1). Mean fecundity for 13 females was 89.00 ± 35.14 eggs (range, 21-44). Female longevity was 3.07 ± 0.64 days (n = 13), whereas the males lived 2.41 ± 0.69 days (n = 13).

Table 1. Periodic dissections of flower buds and galls of *D.* sp. near *capsulae* collected on *E. esula* at S. Rossore, Italy.

Date	No. flower buds with eggs[a]	No. eggs/bud, $\bar{x} \pm$ SD[b]	Range	Larvae/gall, $\bar{x} \pm$ SD[c]	Range
2 April	0	0		NP	
19 April	12	29.4 ± 10.5a	17-50	NP	
2 May	15	25.9 ± 12.8ab	8-45	13.6 ± 7.2a	4-35
11 May	5	14.4 ± 6.3b	7-21	11.2 ± 5.6ab	3-30
26 May	0	–	–	12.3 ± 6.40a	0-25
15 June	0	–	–	9.3 ± 7.6b	0-24
10 July	NP	0	–	5.00 ± 6.3c	0-25

Means in columns followed by the same letter are not significantly different ($P < 0.05$, Student-Newman-Keuls [a posteriori] test).
[a] Based on sample of 100 flower buds; NP, buds not present.
[b] Calculated on number of flower buds with eggs.
[c] Calculated on a sample of 50 galls; NP, galls not present.

Egg

Freshly laid eggs are white, slightly elongate, with rounded ends, and have a smooth, translucent, soft chorion; they are 0.27 ± 0.02 mm long and 0.07 ± 0.01 mm wide (n = 40). The preeclosion period was 3-5 days; 92.3% of a sample of 507 eggs was fertile.

Larva

In the periodic dissections of field-collected bud and gall samples from *E. esula* in 1983, the first larvae of *D.* sp. near *capsulae* were found in early May either in the inner part of the bracts which cover the cyathium or inside the cyathium, where the process of gall formation was beginning. The mean number of larvae per gall was not significantly ($P > 0.05$) different between the various samples collected in May. Significantly ($P < 0.05$) fewer larvae were found on the samples taken in June and July (Table 1). Four, 12, and 24 empty galls were found in the samples taken on 26 May, 15 June, and 10 July, respectively. The great increase of empty galls recorded in June and July was because at that time several mature larvae had already left the galls and entered the soil to hibernate until next spring.

The larvae of *D.* sp. near *capsulae* completed development in 5 weeks. By the end of the fifth week, the body was yellowish and the sternal spatula was distinct and well formed. The length and width measurements recorded in the periodic observations from neonate to mature larvae are presented in Table 2.

Pupa

Pupae were 2.04 ± 0.18 mm long and 0.59 ± 0.03 mm wide (n = 10), and were light red except for the reddish brown wing and leg appendages. In the laboratory, overwintering larvae moved to the surface of the diapausing substrate in early April and then pupated. The pupal stage lasted 2-4 days.

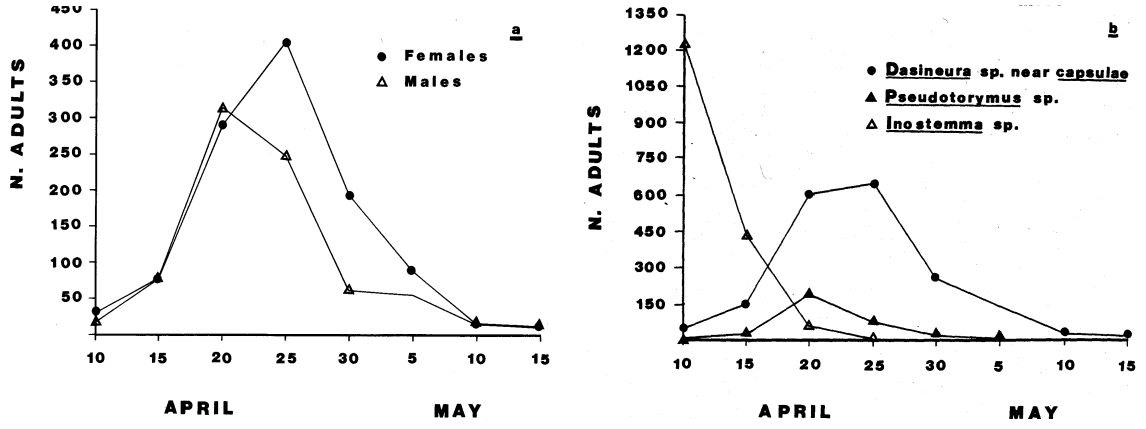

Fig. 1. (a) Emergence of males and females of *D.* sp. near *capsulae* (b) Emergence of *D.* sp. near *capsulae* and its parasites.

Mortality factors

The mean adult emergence was 18.70 ± 3.03% (range, 15.30-21.20%) from 600 mature larvae of *D.* sp. near *capsulae* from galls collected on *E. esula* in 1982 and further collections made in 1983 (2,082 larvae) and 1984 (9,000 larvae). The mean percentage of larvae parasitized by the endoparasite *Inostemma* sp. (Hymenoptera: Platygasteridae) was 30.90 ± 10.90% (range, 19.40-41.30%), whereas the remaining larvae (50.40 ± 8.18%; range: 43.40-59.40%) died as a result of parasitization by the ectoparasite *Pseudotorymus* sp. (Hymenoptera: Torymidae) or by the action of unknown factors (i.e., wrong conditions for hiberation, disease, predation). Because it is not clear how many larvae of the midge are necessary for the complete development of *Pseudotorymus* sp., we could not separate the mortality caused by the ectoparasite from that by unknown factors. In addition, in the dissection of gall samples collected on *E. esula* in 1983, nine larvae of *Pseudotorymus* sp. were found in the June sample and 17 in the July sample. Emergence curves of *D.* sp. near *capsulae* and its parasites for 1985 are presented in Fig. 1b.

Gall formation

Galls produced by *D.* sp. near *capsulae* were formed either by the enlargement and distortion of the cyathium, the deformation of the bracts that cover the cyathium, or a deformation of the leaves of the meristematic tips. At S. Rossore, the majority of the galls (about 80%) produced by this midge were cyathium galls.

In the study following the various stages of gall development, no external modification was observed on the infested flower buds by the end of the first week. In the second week the bracts were still closed, covering the cyathium, but the cyathium showed a thickening of the walls (initiation of gall). By the end of the third week, it was possible to recognize the infested flower buds because the bracts had started to open and the cyathium had become enlarged and reddish (young gall). By the end of the fourth week, the bracts were almost completely opened and well-formed galls were visible (immature

gall), and by the end of the fifth week the bracts had dropped from the inflorescence and some mature larvae of the midge started to leave the galls (mature gall). The mature galls were 8.36 ± 1.51 mm long (range, 5.60-12.40 mm) and 5.06 ± 0.95 mm wide (range, 3.68-6.80 mm; $n = 50$).

Table 2. Measurements of larvae of *D.* sp. near *capsulae* at weekly intervals, April-May 1983.

Intervals	No. specimens examined	Length, mm, $\bar{x}$ ± SD	Width, mm
Week 1	95	0.29 ± 0.03a	0.08 ± 0.0a
Week 2	78	0.33 ± 0.02a	0.08 ± 0.0a
Week 3	63	0.72 ± 0.23b	0.16 ± 0.03b
Week 4	57	1.36 ± 0.31c	0.34 ± 0.09c
Week 5	65	3.10 ± 0.19d	0.79 ± 0.09d

Means in columns followed by the same letter are not significantly different ($P < 0.05$, Student-Newman-Keuls [a posteriori] test).

Host specificity testing

Free-choice host suitability

The data obtained in the multiple choice test conducted at S. Rossore are summarized in Table 3. In Plot 1, galls produced by *D.* sp. near *capsulae* larvae were found only on the controls. The number of galls produced per plant was significantly greater ($P < 0.05$) on control B (*E. esula* growing naturally) than on control A (potted *E. esula* plants).

In Plot 2, the midge caused galls on Oregon and Wyoming biotypes of leafy spurge and on both controls (A and B). The number of galled flowers per plant was significantly greater ($P < 0.05$) on control B than on control A. There was no significant difference ($P > 0.05$) between the number of galls per plant on the potted plants of control A and the potted Wyoming and Oregon biotypes.

In Plot 3, galls were found on the Montana biotype of leafy spurge and on controls A and B, but the number of galls per plant was significantly higher ($P < 0.05$) on control B.

Table 3. Free-choice host suitability test of *D* sp. near *capsulae* conducted at S. Rossore, 1984.

	No. Plants[a]	No. flowers/plant, $\bar{x}$ ± SD	Range	No. flowers galled/plant, $\bar{x}$ ± SD	Range
Plot 1					
Euphorbia esula Italy (Control A)	9	26.2 ± 14.9a	10-60	3.3 ± 2.9a	0-6
E. esula Italy (Control B)	16	61.7 ± 44.1b	16-180	13.1 ± 11.0b	0-40
E. esula Nebraska biotype	6	34.0 ± 18.4ab	8-63	0	–
E. peplus	5	209.0 ± 64.3c	164-320	0	–
E. characias	5	111.0 ± 26.1d	81-150	0	–
Plot 2					
Euphorbia esula Italy (Control A)	11	33.6 ± 19.5a	10-75	5.2± 4.6a	0-17
E. esula Italy (Control B)	16	75.5 ± 43.2b	30-120	15.7 ± 9.1b	0-29
E. esula Oregon biotype	9	30.4 ± 13.7a	20-52	4.4 ± 9.2a	0-28
E. esula Wyoming biotype	9	29.2 ± 12.1a	20-60	2.0± 3.1a	0-7
Linum narbonense	14	17.7 ± 7.0a	7-25	0	–
Plot 3					
Euphorbia esula Italy (Control A)	11	38.5 ± 14.9a	20-73	4.9 ± 4.8a	0-15
E. esula Italy (Control B)	12	63.8 ± 42.7a	25-175	13.7 ± 8.4b	0-26
E. esula Montana biotype	9	37.5 ± 27.3a	18-100	3.2±3.9a	0-11
E. milii	5	77.2 ± 26.5a	35-103	0	–
E. pulcherrima	8	8.9 ± 3.9b	6-12	0	–

Means in columns followed by the same letter are not significantly different ($P < 0.05$, Student-Newman-Keuls [a posteriori] test).
[a] Control A, potted plants of *E. esula;* Control B, plants of *E. esula* growing naturally in the experimental area.

No-choice oviposition and host suitability

Of the 48 plant species or varieties tested, the midge accepted the controls and 13 taxa for oviposition, all in the genus *Euphorbia*. The larvae were able to complete development on the controls and six of the test plants on which oviposition occurred (Table 4).

Table 4. No-choice oviposition and host suitability test of *D.* sp. near *capsulae*[a]; all values are $\bar{x}$ ± SD (*n* = 4).

Test plants[a]	No. exposed flower buds	No. eggs/bud	No. flower buds left for gall development	No. galls	% Infestation[b]
Group I, 1984					
Euphorbia esula Italy (control)	26.2 ± 8.9a	24.7 ±17.6a	21.0 ± 8.4	7.5 ± 6.1a	32.9 ± 31.3a
Leafy spurge Montana biotype	185 ± 6.8a	24.5 ± 9.4a	15.2 ± 5.8	6.7 ± 2.5a	44.9 ± 3.8a
Leafy spurge Wyoming biotype	19.7 ± 4.3a	16.0 ± 7.0a	14.5 ± 5.7	6.0 ± 3.7a	38.4 ± 10.6a
Leafy spurge Nebraska biotype	15.7 ± 3.8a	17.8 ± 13.2a	11.2 ± 2.5	–	–
Group II, 1985					
Euphorbia esula Italy (control)	44.5 ± 6.2a	6.7 ± 6.7a	19.7 ±23.4	6.2 ± 9.4a	35.7 ± 35.1ab
E. amygdaloides	38.2 ± 15.0a	17.7 ± 11.9ab	25.7 ±20.0	–	–
E. marginata	9.0 ± 2.4b	12.0 ± 15.7ab	5.0 ± 3.5	–	–
E. dendroides	15.5 ± 5.9b	31.4 ± 11.6b	13.0 ± 5.7	2.0 ± 2.4a	12.5 ± 14.4a
E. lucida	37.5 ± 6.7a	29.5 ± 16.7b	24.0 ± 17.7	12.2 ± 8.3a	54.5 ± 19.7b
Group III, 1985					
Euphorbia esula Italy (control)	49.7 ± 9.6a	28.2 ± 6.4a	37.7 ± 3.3	6.2 ± 4.3a	15.9 ± 10.8a
E. cyparissias	21.0± 9.5b	9.2 ± 5.7b	12.7 ± 9.6	3.7 ± 4.5a	17.7 ± 20.6a
E. helioscopia	20.0 ± 12.2b	3.7 ± 6.8b	3.2 ± 4.2	–	–
E. peplus	32.5 ± 10.4ab	6.0 ± 7.3b	16.2 ± 19.7	–	–
Group IV, 1985					
Euphorbia esula Italy (control)	31.0 ± 2.6a	12.5 ± 4.1a	25.7 ± 6.1	7.5 ± 5.8a	25.7 ± 19.1a
E. miIii	8.7 ± 4.9b	2.0 ± 4.0b	3.0 ± 6.0	–	–
E. terracina	13.5 ± 11.3b	11.8 ± 17.5ab	6.7 ± 8.9	6.7 ± 8.9a	73.9 ± 8.6b
E. pulcherrima	14.5 ± 3.4b	1.5 ± 3.0b	4.0 ± 8.0	–	–

Means followed by the same letter within a column are not significantly different ($P < 0.05$, Student-Newman-Keuls [a posteriori] test).
[a] Other plants tested are listed in the text.
[b] Percentage of infestation = GP:FBL x 100 (GP, galls present; FBL, flower buds left for gall development).

In Group 1, the mean number of eggs laid on the control and on the North American biotypes of leafy spurge plants was similar ($P > 0.05$). In Group 11, there was no significant difference ($P > 0.05$) between the number of eggs laid on *E. amygdaloides* L., *E. marginata* Pursh, and the control, but a significantly higher ($P < 0.05$) number of eggs were laid on *E. dendroides* L. and *E. lucida* Waldstein and Kitaibel. In Group III, significantly more ($P < 0.05$) eggs were laid on the control than on the test plants (*E. cyparis-*

sias L., *E. helioscopia* L., and *E. peplus* L.). In Group IV, similar ($P > 0.05$) numbers of eggs were laid on the control and *E. terracina* L. but significantly fewer ($P < 0.05$) on *E. milii* and *E. pulcherrima* than on the control plants. The percentage of viable eggs laid on the different test and control plants in any of the groups ranged from 83.3 to 94.1.

There was no significant difference ($P > 0.05$) between the number of galls found on the Italian control plants and the Montana and Wyoming leafy spurge biotypes in Group I, but no galls were found on the Nebraska biotype. The number of galls found on *E. dendroides, E. lucida, E. cyparissias,* and *E. terracina* was not significantly different ($P > 0.05$) from the number produced on control plants in the corresponding groups.

The percentage of infested flower buds on plants was determined using the formula GP/FBL x 100, where GP is the number of galls present and FBL is the number of flower buds left for gall development. Using this calculation, there was no significant difference ($P > 0.05$) between the mean percentages on the test plants and the controls in Groups I, II, and III, but in Group IV the percentage of infested flower buds on *E. terracina* was significantly ($P < 0.05$) greater than on the control plants. Three to seven larvae per gall were found on test and control plants.

The midge did not oviposit on the following test plants: *Euphorbia characias* L., *E. lathyris* L., *E. maculata* L., *E. supina* Rafinesque-Schmaltz, *E. serpyllifolia* Persoon, *Ricinus communis* L., *Mercurialis annua* L. (Euphorbiaceae); *Linum narbonense* L. (Linaceae); Rosa sp., *Crataegus oxycantha* L., *Potentilla fragiformis* Willdenow, *Geum urbanum* L. (Rosaceae); *Alyssum saxatile* L., *Iberis sempervirens* L. (Cruciferae); *Trifolium incarnatum* L., *Cytisus* sp., *Phaseolus vulgaris* L. (Leguminosae); *Clarkia elegans* Douglas (Onagraceae); *Ruta graveolens* L. (Rutaceae); *Pelargonium zonale* Aiton (Geraniaceae); *Anemone* sp. (Ranunculaceae); *Dianthus* sp., *Cerastium tomentosum* L. (Caryophyllceae); *Nerium oleander* L., *Vinca major* L. (Apocynaceae); *Verbena hybrida* Voss (Verbenaceae); *Thymus serpyllum* L. (Labiatae); *Veronica teucrium* L. (Scrophulariaceae); *Tagetes glandulifera* Schrank, *Centaurea cineraria* L., *Carthamus tinctorius* L. (Compositae); *Hordeum vulgaris* L., *Triticum aestivum* L. (Graminae); *Lilium* sp. (Liliaceae).

Discussion

Our studies showed a close synchronization between the period of emergence of *D.* sp. near *capsulae* and the preflower stage of its host plant, *E. esula*. The critical period was from mid-April to mid-May.

We demonstrated under field conditions that this midge would select and could complete development on some North American biotypes of leafy spurge, an important finding supporting the validity of this midge as a biological control agent.

The pot-grown plants of leafy spurge of American origin, as well as control A, had fewer flowers than the plants of *E. esula* (control B) naturally growing in the field. This difference was probably because these plants, having been kept in pots from 6 to 18 months. without fertilization, were not in good vegetative condition. We may assume that the lower infestations recorded on potted plants were because of inferior plant quality.

Observations made in the laboratory indicated that females of this midge (about 95%) selected healthy, vigorous flower buds with the bracts completely covering the cyathium for oviposition. Occasionally, however, we found eggs on weak flower buds or buds on which the bracts had already opened. The larvae of *D.* sp. near *capsulae* rarely completed development on flower buds in poor condition, probably because of the absence of good nutritional tissue.

The lack of oviposition on plants in subgenera other than *Esula* suggests that flower buds of some species may contain deterrent substances or have a floral morphology that limits or negates attraction to the plant for oviposition. Although the number of eggs laid on *E. marginata* was the same as on the control, the midges selected the external part of the bracts for oviposition. This abnormal behavior of *D.* sp. near *capsulae* could indicate that *E. marginata* possesses the proper attractants for oviposition but inappropriate flower buds. The flower buds on the control plant were glabrous, whereas those of *E. marginata* had hairy bracts that may have interfered with ovipositor insertion between bracts. These observations suggest that close synchronization, flower buds in the proper stage, presence of an attractant, and suitable floral morphology are important factors in host selection and oviposition by the midge.

D. sp. near *capsulae* completed its development on only six of the plants on which it oviposited; all were in the subgenus *Esula*. Eggs laid on the other test plants (*E. marginata, E. pulcherrima, E. milii, E. peplus, E. helioscopia*) were fertile, but the larvae did not develop because the flower buds of these plants were so morphologically diverse from those of the normal host plant (control) that normal galls could not form, preventing development of the midge.

No larval development occurred on *E. amygdaloides* or the Nebraska biotype of leafy spurge, even though flower buds of these plants appeared morphologically similar to those of the control. In these cases, larval development of *D.* sp. near *capsulae* may have failed because of the presence of feeding deterrents, lack of essential nutrients, or some other physiological character that make these taxa unsuitable. The restricted host range of *D.* sp. near *capsulae* demonstrated in these tests suggests that it would be safe to release this insect as a biological control agent of leafy spurge in North America.

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