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Investigations on the *Pegomya argyrocephala* Complex of Species (Diptera: Anthomyiidae) to Select Candidate Biological Control Agents for Leafy and Cypress Spurge in North America

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Abstract

Leafy spurge (*Euphorbia* x *pseudovirgata*) and cypress spurge (*E. cyparissias*) are perennial herbs of European origin which have become serious weeds of grassland in Canada and the United States. Two of five *Pegomya* species (Diptera: Anthomyiidae) which attack spurges in Europe, appear to be promising control agents of leafy and cypress spurge. *P. euphorbiae* prefers *E. cyparissias* but can also attack *E. virgata* and Canadian leafy spurge. *P. curticornis* is restricted to *E. virgata* in the field, but in laboratory tests Canadian leafy spurge was found equally acceptable. The host ranges of these two *Pegomya* spp. are restricted to a few plants within the subsection *Esulae* in the genus *Euphorbia*. The larvae mine down through the stems and pupate in the stem base where a gall is formed. The larvae of *P. euphorbiae* and *P. curticornis* cause significant damage to their host plants. Attacked shoots mostly dry up prematurely and those surviving attack remain small and generally do not flower.

Introduction

Leafy spurge, *Euphorbia* x *pseudovirgata* (Schur) Soo, and cypress spurge, *E. cyparissias* L. (Euphorbiaceae), are herbaceous perennials of European origin that dominate and exclude other herbaceous plants in North American grasslands. They contain a toxic latex that makes them unpalatable to most grazing animals. Neither spurge is abundant enough to be a noxious weed in Europe.

The taxonomy of leafy spurge is still under investigation, and the exact European origin is not known. The name *Euphorbia* x *pseudovirgata* is used to distinguish the North American leafy spurge from the closely-related Eastern European species *E. virgata* Waldst. & Kit. There are presently about 400,000 ha infested by the weed in the USA and over 60,000 ha in the Canadian prairies.

Cypress spurge is most prevalent on limestone soil in eastern Canada. Some 14,000 ha are infested and 300,000 ha are threatened by the weed (Harris 1983).

Hennig (1973) treats the *Pegomya* species (Diptera: Anthomyiidae) attacking *Euphorbia* spp. under the name *P. argyrocephala* and places it in the *hyoscyami*-group of species (*Pegomya sensu stricto*) which are all leaf-miners. However this taxonomic position is questionable (Griffith 1982). From a taxonomic point of view the *argyrocephala* group has a unique position and is not closely related to any other species in the genus *Pegomya*.

During 1983-6 flies were reared at the C.I.B.C. European Station, from the known host plants *E. cyparissias* and *E. amygdaloides* L. and from three new host plant species, *E. virgata*, *E. lucida* Waldst. & Kit and *E. hyberna* L. According to Michelsen (1988) who has closely examined our material, five species of *Pegomya* have been recognized. *P. euphorbiae* (Kieffer) is predominantly associated with *E. cyparissias* and is apparently the most common and widespread species in Europe. This species has been reared also from *E. virgata* and *E.*

lucida in Hungary. Tosevsky (pers. comm., 1987) reports the species from *E. seguieriana* Necker in Yugoslavia. *P. lucidae* sp.n., a large and distinct species associated with *E. lucida*, has so far only been collected in Hungary. *P. curticornis* (Stein), apparently closely-related to *E. virgata* was found in central and eastern Hungary. *P. hybernae* sp.n. (reared from *E. hyberna* and collected in central France) and *P. amygdaloides* (Meigen) (reared from *E. amygdaloides*) are probably western European species.

Because of ecoclimatic mismatching, *P. amygdaloides* and *P. hybernae* sp.n. (infesting wood spurges collected in central France) were excluded as candidate agents for the biological control of leafy and cypress spurge in North America.

To estimate the suitability of *P. euphorbiae*, *P. curticornis* and *P. lucidae* sp.n. as candidate biological control agents of leafy and cypress spurge in North America, the host preference, host-specificity and effect of the flies on infested shoots of *Euphorbia* spp. were investigated.

Materials and Methods

Host Preference

Oviposition preference of *P. euphorbiae*, *P. curticornis* and *P. lucidae* sp.n. collected in Hungary was studied in simultaneous choice tests with potted plants in cages (40 x 40 x 70 cm) in a greenhouse. *E. cyparissias*, *E. virgata*, *E. lucida* and Canadian leafy spurge were offered in each cage to two females. Eight replicates were made with *P. euphorbiae*, four with *P. curticornis* and nine with *P. lucidae* sp.n. The plants were checked for oviposition daily or every second day and replaced by fresh plants when oviposition occurred.

The host plant suitability for larval development was determined by percent larval survival. During dissections, only full-grown living third instar larvae, older than 55 d, and puparia were counted. Percent larval survival is based on the number of shoots infested by newly-hatched larvae. Larval mortality due to intra-specific competition is not considered.

Host-specificity

The host-specificity of *Pegomya* was mainly based on oviposition tests since the larvae complete their development inside the shoot on which they hatch. Since it was expected that little oviposition could occur on any test plant in simultaneous oviposition tests, single-choice oviposition tests were made in small containers to enhance the significance of the tests. Two females were used for each test which were in close contact with the test plant. The tests were run in the laboratory. Alternative exposure to the test and control plants in a 3- to 4-d cycle was used to ascertain female fecundity. Two series of tests were run to avoid the deleterious effects of possible adult conditioning on first oviposition and a possible oviposition cycle. In the first series, the control was offered first to the flies, and to the test plant in the second series. Test plants on which occasional oviposition occurred were used for simultaneous-choice tests in the presence of the field host plant. The majority of oviposition tests were made with *P. euphorbiae* from which the greatest number of pupae could be collected from *E. cyparissias*, since all the flies reared from various spurges were believed to be the same species until the end of 1986. However, flies reared from different host plants were kept separate and tested separately with the most critical test plant species because it was suspected that host races of *P. "argyrocephala"* may occur. The test plant list was proposed by Harris (1983).

Larval development and survival was checked by dissection of shoots 3 to 8 wks after oviposition. The plants were kept in the Institute's garden.

Life History and Effect on the Host Plant

The life history of *Pegomya* was investigated in the laboratory. The effect of *Pegomya* on the attacked shoots was studied predominantly on *E. cyparissias* and on Canadian leafy spurge, with plants infested during the course of the various oviposition experiments. Data on survival, growth and flowering of attacked and healthy control shoots were collected. The angle of the shoots was measured after oviposition (in April) and again at pupation time (June).

Results and Discussion

The life histories of *P. curticornis*, *P. lucidae* sp.n., *P. argyrocephala* and *P. euphorbiae* are very similar. Adults emerge at the end of March to early April. Eggs are laid between developing leaves or in the closed floral bracts on the shoot tip. Potential fecundity is about 80 eggs. There are three larval instars. Immediately after hatching the larvae mine into the pith and downwards to the shoot base, which is reached within 25 to 30 d. Chewing of the vascular bundles by the third instar larvae at the shoot base induces gall formation. Only a single larva develops/shoot, except on *E. lucida*. Pupation occurs in June. There is one generation/yr. The life history of *P. hybernae* sp.n. is different: the few larvae collected in early September were just pupating. A detailed description of the life history of the *Pegomya* species is given by Gassmann (1987).

Effect on the Host Plant

The mining of *P. curticornis* and *P. euphorbiae* has a significant effect on the survival, growth and flowering of *E. x pseudovirgata* and *E. cyparissias*, respectively (Table 1). Shoot growth is stopped almost immediately after hatching, by feeding of the newly-hatched larvae on the meristematic tissues of the shoot apex. Death of the shoot occurs when the third instar larvae chew the vascular bundles at the shoot base. Observations at collection sites indicate that 85% of the attacked shoots of *E. cyparissias* dried up before the end of May. About 50% of the shoots of *E. virgata* are being killed. Remaining shoots are much shorter than healthy shoots, distorted and usually without inflorescence. Most probably dry habitats enhance the effect of *Pegomya* on *Euphorbia* and also prevent regrowth of shoots. The robust shoots of *E. lucida* are also affected by the larvae of *P. lucidae* sp.n. together with other specialized insects (Gassmann 1987).

Host Preference

P. euphorbiae and *P. lucidae* sp.n. significantly preferred to oviposit on their field host plants - *E. cyparissias* and *E. lucida*, respectively - than on the three other spurge species, and particularly *E. x pseudovirgata* (Table 2). In contrast, *P. curticornis* did not show any significant preference between its field host plant (*E. virgata*) and Canadian leafy spurge.

Larval survival of *P. euphorbiae* and *P. lucidae* sp.n. were significantly higher on their field host plants, compared with Canadian leafy spurge, but equal survival rates were observed for *P. curticornis* on *P. virgata* and *E. x pseudovirgata* (Table 3). Ecological adaptation of the adults (oviposition preference) is correlated with the physiological adaptation of the larvae (larval survival).

P. curticornis is the most suitable species for biological control of North American leafy spurge. *P. euphorbiae* could possibly be adapted to *E. x pseudovirgata* in pure stands. *P. lucidae* sp.n. is not a suitable agent for biological control of leafy or cypress spurge.

Table 1. Effect of *Pegomya curticornis* (Stein) and *P. euphorbiae* (Kieffer) on survival, growth and flowering of shoots of potted *Euphorbia x pseudovirgata* (Schur) Soo and *E. cyparissias* L. Numbers in brackets are the total number of shoots. Percent of dead shoots, shoots with growth and shoots flowering differed significantly between shoots infested by the two *Pegomya* species compared with control shoots (Chi square test, $P < 0.001$).

Treatment	Percent shoots dead	Percent shoots alive with growth	Percent shoots alive flowering
Leafy spurge shoots mined by *Pegomya curticornis*	37.5 (88)	12.7 (55)	1.8 (55)
Control shoots	7.7 (182)	91.1 (168)	25.6 (168)
Cypress spurge shoots mined by *P. euphorbiae*	37.8 (180)	24.1 (112)	31.2 (112)
Control shoots	15.3 (98)	81.9 (83)	71.1 (83)

Table 2. Oviposition preference in simultaneous-choice tests by *Pegomya euphorbiae* (Kieffer), *P. curticornis* (Stein) and *P. lucidae* sp.n. (n = number of replicates). Underlined values are mean numbers of eggs laid on the natural host plant for each fly species. Oviposition on the natural host plant by each fly species is compared to the oviposition on the three other spurge species using the Mann-Witney U-test, corrected for the number of tests. Values in rows followed by the same letter are not significantly different ($P > 0.05$).

Pegomya species	Mean number of eggs/replicate laid on *Euphorbia* species			
	cyparissias L.	*virgata* Waldst. & Kit	*lucida* Wadlst.	Canadian leafy spurge
P. euphorbiae ($n = 8$)	28.9	4.0	1.9	8.8
P. curticornis ($n = 4$)	7.3	19.8 *a*	5.8	15.5 *a*
P. lucidae sp.n. ($n = 9$)	0.0	4.7	35.4	2.3

Host-specificity

The results of sequential choice tests are summarised in Table 4. Oviposition, partly by unmated females, occurred on 10 *Euphorbia* spp., six of which belong to the subsection *Esulae*.

Outside the genus *Euphorbia* another eight species in the family Euphorbiaceae were tested. Oviposition occurred only once on *Ricinus communis* L. (Euphorbiaceae), but none of the four larvae which hatched started feeding. Outside the family Euphorbiaceae, regular oviposition occurred on *Linum usitatissimum* L. (Linaceae), a plant morphologically similar to spurge. Occasional oviposition of a few eggs occurred on another five test plant species when these plants were first offered to flies which were apparently under oviposition stress.

The results of simultaneous oviposition tests are summarized in Table 5. Occasional oviposition occurs on several *Euphorbia* spp. and on *L. usitatissimum* but, with the exception of *E. peplus* L., was much less frequent than in sequential choice tests. Establishment of a few larvae were observed in shoots of seven *Euphorbia* species and *L. usitatissimum*, but all larvae died except on *E. segetalis* L. and *E. myrsinites* L., where a single living larva each was found 3 wks after oviposition.

Table 3. Percent larval survival of *Pegomya euphorbiae* (Kieffer), *P. curticornis* (Stein) and *P. lucidae* sp.n. Numbers in brackets are the total number of shoots infested. The number of larvae surviving on the field host plant of each of the three *Pegomya* species is compared with the number of larvae surviving on Canadian leafy spurge, using the Chi-square test.

Pegomya species	% larval survival on *Euphorbia* species			
	cyparissias L.	*virgata* Waldst. & Kit	*lucida* Wadlst.	Canadian leafy spurge
P. euphorbiae	77.3 (119)	-	-	35.7 (42)***
P. curticornis	-	58.3 (12)	-	55.6 (54) n.s.
P. lucidae sp.n.	-	-	70.0 (10)	7.3 (41)***

*** = significant at 0.1% level; n.s. = not significant.

Sequential and simultaneous oviposition tests with *P. curticornis* and *P. lucidae* sp.n. on critical plants used for *P. euphorbiae* gave similar results. The experimental host range of *P. curticornis* seems to be even slightly narrower than that of *P. euphorbiae*. *E. lathyris* L. was well accepted in the oviposition test of *P. lucidae* sp.n. and is probably suitable for larval development. The record of Baudys (1954) of a *Pegomya* species attacking *E. lathyris* may refer to *P. lucidae* sp.n. or to another undescribed species.

Conclusions

Work on the *P. argyrocephala* group of species for biological control of leafy and cypress spurge has largely contributed to solving the taxonomy of the group and to the description of the life history of the species. The *Pegomya* species, in particular *P. euphorbiae* and *P. curticornis*, cause significant damage to their host plants, especially in dry habitats. Attacked shoots mostly dry up prematurely and those surviving attack remain small and generally do not produce flowers.

The laboratory investigations of the potential host range of *P. euphorbiae* and *P. curticornis* demonstrate that the two species are restricted to a few closely-related species of *Euphorbia* in the subsection *Esulae*. Under field conditions, *P. euphorbiae* predominantly attacks *E. cyparissias*, but occasionally develops on *E. virgata*, *E. lucida* and *E. seguieriana*. *P. curticornis* seems to be restricted to *E. virgata*. *P. lucidae* sp.n. has been found exclusively on *E. lucida* but possibly attacks *E. lathyris*.

Host preference studies indicate that both *P. euphorbiae* and *P. curticornis* accept Canadian leafy spurge. *P. curticornis* does not discriminate between its original European host plant, *E. virgata*, and Canadian leafy spurge. *P. lucidae* sp.n. hardly accepts leafy spurge. *P. curticornis* is the most suitable species for biological control of North American leafy spurge.

P. euphorbiae and *P. curticornis*, collected in central Hungary, should be climatically preadapted to most of the infestation areas of leafy spurge in North America. Their effect on the target weed should be enhanced in dry habitats.

Table 4. Results of sequential choice oviposition tests with *Pegomya euphorbiae* (Kieffer).

Test plant	*E. cyparissias*		Test plant				
	No. females days	No. eggs	No. females days	No. eggs	Total No. of shoots with eggs	No. of shoots with fertile eggs	Larval development
Euphorbiaceae							
Euphorbia							
Sect. Aniophyllum							
Subsect. Chamaesyceae							
E. maculata L.	10	35	9	0			
E. serpyllifolia Pers.	34	76	23	0			
Sect. Adenopetalum							
Subsect. Petaloma							
E. marginata Pursh.	27	45	21	0			
Subsect. Tithymalopsis							
E. corollata L.	7	19	7	0			
Subsect. Tricherostigma							
E. antisyphilitica Zucc.	25	36	14	0			
Sect. Poinsettia							
E. pulcherrima Willd.	30	92	37	0			
E. heterophylla L.	23	114	24	0			
Sect. Euphorbium							
Subsect. Tirucalli							
E. tirucalli L.	19	70	12	0			
Subsect. Dicanthium							
E. milii Ch. des Moulins	53	138	38	24	8 ("flowers")		
E. trigona Haw.	16	95	12	0			
E. grandialata Dyer	15	47	13	0			
Subsect. Meleuphorbia							
E. meloformis Ait.	22	60	18	0			

Table 4. Continued.

Test plant	*E. cyparissias*		Test plant				
	No. females days	No. eggs	No. females days	No. eggs	Total No. of shoots with eggs	No. of shoots with fertile eggs	Larval development
Sect. Tithymalus							
Subsect. Decussatae							
E. lathyris L.	31	103	26	0			
Subsect. Galarrhaei							
E. hyberna L.	46	153	29	5	3	2	2 shoots mined (4/9 cm), 1 L3 dead
E. polychroma L.	18	94	18	6	5	-	
Subsect. Esulae							
E. amygdaloides L.	50	261	50	50	15	7	No shoot mined
E. characias L.	17	100	24	0			
E. wulfenii Hoope	11	44	10	0			
E. purpurea (Raf.) Fern	43	101	26	1	1	1	1 shoot mined (10 cm)
E. seguieriana Neck	40	103	26	35	6	5	2 shoots mined (8/6 cm), 2 L3 dead
E. oblongata Griseb.	57	194	36	25	11	8	4 shoots mined ($x = 6$ cm), 3 L3 dead
E. peplus L.	40	143	41	46	11	6	6 shoots mined ($x = 13$ cm), 4 L3 dead
E. segetalis L.	42	65	22	46	6	2	2 shoots mined (15/5 cm), 1 L3 alive*
Subsect. Myrsinitae							
E. myrsinites L.	88	231	62	31	9	6	2 shoots mined (9/5 cm), 1 L3 alive* 1 short mine (2 cm), 1 L2 dead
Pedilanthus macrocarpus Benth.	27	140	20	0			
P. tithymaloides (L.) Poit.	12	24	9	0			
Manihot esculenta Crantz.	16	48	14	0			
Acalypha hispida Burm. f.	18	47	30	0			
Aleurites fordii Hemsl.	47	204	46	0			
Croton variegatum Blume	12	55	14	0			
Mercurialis perennis L.	17	32	21	0			
Ricinus communis L.	25	53	20	4	1	4	4 L1 hatched and died, no mine
Chenopodiaceae							
Beta vulgaris L.	29	69	27	0			

Table 4. Continued.

Test plant	*E. cyparissias*		Test plant				
	No. females days	No. eggs	No. females days	No. eggs	Total No. of shoots with eggs	No. of shoots with fertile eggs	Larval development
Umbelliferae							
Daucus carota L.	14	61	18	0			
Solanaceae							
Lycopersicon esculentum Mill.	31	102	25	0			
Cruciferae							
Raphanus sativus L.	21	56	18	0			
Polygonaceae							
Rheum rhaponticum L.	23	49	26	0			
Gramineae							
Zea mays L.	19	48	23	0			
Cucurbitaceae							
Cucumis sativa L.	25	108	29	0			
Lauraceae							
Persea americana Mill.	37	107	30	3	1	1	1 L1 hatched and died, no mines
Leguminosae							
Pisum sativum L.	29	135	33	0			
Geraniaceae							
Pelargonium sp.	-	-	-	-			
Linaceae							
Linum usitatissimum L.	72	207	57	47	10	7	6 shoots mined (x = 8 cm), 4 L3 dead
L. flavum L.	11	54	4	1	1	-	

Table 4. Continued.

Test plant	*E. cyparissias*		Test plant				
	No. females days	No. eggs	No. females days	No. eggs	Total No. of shoots with eggs	No. of shoots with fertile eggs	Larval development
Lythraceae							
Lythrum salicaria L.	21	29	24	0			
Cistaceae							
Helianthemum nummularium Mill.	18	24	21	1	1	-	
Rosaceae							
Rosa sp.	32	67	35	2	2	-	
Rubus sp.	27	133	31	0			
Crassulaceae							
Sempervivum tectorum L.	11	34	12	0			
Liliaceae							
Hemerocallis sp.	20	45	26	0			
Iridaceae							
Iris sibirica L.	26	53	25	0			
Scrophulariaceae							
Linaria vulgaris L.	36	111	20	4	3	1	1 L1 hatched and died, no mine
Apocynaceae							
Vinca minor L.	25	82	25	0			
Asclepiadaceae							
Asclepias syriaca L.	33	58	21	0			
Compositae							
Cichorium intybus L.	21	57	16	0			
Lactuca sativa L.	13	45	18	0			
Sonchus arvensis L.	13	41	9	0			
Parthenium argentatum Gray	16	110	19	0			

Table 4. Continued.

Test plant	*E. cyparissias*		Test plant				
	No. females days	No. eggs	No. females days	No. eggs	Total No. of shoots with eggs	No. of shoots with fertile eggs	Larval development
Papaveraceae							
Papaver nudicale L.	37	69	22	0			
Convolulaceae							
Calonyction aculeatum House	10	47	12	0			

* Plants dissected 3 wks after oviposition.

Table 5. Results of simultaneous-choice oviposition tests with ***Pegomya euphorbiae*** (Kieffer).

Euphorbia species	No. females days	No. eggs	Total no. of shoots attacked	No. shoots with fertile eggs	Larval development
E. myrsinites L.	179	10	4	3	1 shoot mined (15 cm), no larva 2 short mines (< 1, 5 cm), 1 L2 dead
E. amygdaloides L.	44	0	-	-	
E. milii Ch. des Moulins	137	0	-	-	
E. oblongata Griseb.	86	2	1	1	
E. polychroma L.	93	5	3	1	
E. peplus L.	74	17	12	10	6 shoots mined ($x = 8$ cm), 2 L3 dead
Ricinus communis L.	86	0	-	-	
Rosa sp.	44	0	-	-	
Linum usitatissimum L.	102	5	3	2	1 shoot mined (14 cm), L3 dead
Canadian leafy spurge	53	15	3	3	1 puparium
E. cyparissias	219	261	67	56	3 puparia, only 12 shoots dissected

P. euphorbiae and *P. curticornis* have a narrow host range and should be regarded as potential biological control agents of cypress, and leafy spurge, respectively. Their release in North America is recommended.

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