FINAL REPORT

DEVELOPMENT OF BIOLOGICAL CONTROL

FOR

GARLIC MUSTARD (ALLIARIA PETIOLATA)

bу

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INTRODUCTION

Garlic mustard (*Alliaria petiolata*) is one of the most serious invasive species in the Northeast, Southeast and Midwest replacing native spring wildflowers in forest communities. Physical, mechanical, and chemical means have failed to provide long-term control, and the development of biological control appears the only viable option for ecologically sound management of garlic mustard. During the Natural Areas Association meeting in Chicago from 10/24-10/26/96 an informal group of participants from 7 states discussed possibilities for developing a biological control program for garlic mustard. Fundraising efforts continued through 1997 and by spring 1998 a contract was signed with CABI Bioscience Center, Switzerland (formerly known as the International Institute of Biological Control) to begin explorations for potential biological control agents in the native range of garlic mustard in Europe. The work in Europe is jointly sponsored by Departments of Natural Resources in Minnesota, Illinois, Indiana, Kentucky, the Hoosier National Forest, Native Plant Societies of Illinois and Indiana, and Hennepin Parks in Minneapolis and the overall program coordinated by Cornell University. Only the joint funding has made it possible to achieve results described in the following.

OBJECTIVES

The objectives for the work in Europe during the first 2 years were to:

1. Identify field sites for investigations of potential biological control agents in Europe

- 2. Identify potential biological control agents through literature searches and field surveys
- 3. Begin preliminary investigations of promising potential biological control agents

WORK PROGRAM

The following information is a summary of progress reports and of the 1998 Annual Report received from CABI Bioscience, Switzerland. A formal Final Report from CABI summarizing the 1999 field season is due at the end of 1999 year. Because of the timing (the field work in Europe continues) only limited new information can be provided at this point.

In April and May 1998 field surveys in southern Germany and Switzerland were conducted and several promising populations of garlic mustard were identified within a range of 100km around Delémont. Lack of funding in the first year prevented more extensive surveys particularly to Central and Eastern Europe, however, these surveys were conducted in early 1999. At each site abiotic (shade, moisture) and biotic (number of garlic mustard plants, cover, number of rosettes, shoots per plant etc.) factors were recorded. This will prove helpful in comparing European sites with sites in North America and in determining whether any of the potential biological control agents shows microhabitat preferences.

Garlic mustard is essentially herbivore free in North America whereas 69 insect herbivores and 7 fungi are reported to attack the plant in Europe. Of these, nearly 50% were coleopterans (beetles), mainly chrysomelids (leaf beetles) and curculionids (weevils). A detailed list was provided by CABI in their 1998 Annual Report. Most of the European species are not hostspecific enough to consider their introduction to North America, however, several species were found to have potential as biological control agents. Among these are four weevil species, a flea beetle, and a leaf miner which have formed an exclusive relationship with garlic mustard as their food plant (Table 1). Field surveys showed that attack by these insects reduced seed output and may lead to premature plant death.

Table 1: Potential biocontrol agents associated with Alliaria petiolata in Europe

Species	Plant structure attacked
Coleoptera, Curculionidae Ceutorhynchus constrictus Ceutorhynchus roberti Ceutorhynchus alliariae Ceutorhynchus scrobicollis Coleoptera, Chrysomelidae	seeds stems, petioles stems, petioles roots
Phyllotreta ochripes Diptera, Agromyzidae Ophiomyia alliariae	roots leaves

The larvae of *C. roberti* and *C. alliariae* mine the shoots of garlic mustard, larvae of *C. constrictus* attack the seeds, and as suspected in the 1998 annual report it has now been confirmed that *C. scrobicollis* develops in the rosettes during the fall, winter and spring. At present it is unclear how many generations this species produces annually but colonies of *C. scrobicollis* are now established at the facility in Delémont, Switzerland and life-history investigations are continuing. The species appears to have a more eastern distribution and was located during extensive field surveys in spring 1999.

At the selected field sites close to Delémont, regular (usually every 2 weeks) samples were taken for qualitative and quantitative assessments of the insect and fungal community associated with garlic mustard in Europe. At each site, plants were visually inspected for feeding attack, samples were dissected and rosettes excavated and dissected. Attacked plant parts or immature stages were returned to the laboratory and reared to the adult stage. Life history observations were made and adults sent to specialists to verify species identifications. A summary of these observations was provided with the 1998 Annual Report from CABI and will not be repeated here (a copy of the annual report is provided as an attachment).

The work program at CABI for 1999 includes extensive field surveys in eastern and central Europe (which were completed in May and resulted in the location of *C. scrobicollis*) as well as a continuation of field surveys and regular dissections of plants at the permanent sites closest to Delémont (see CABI report). Additional attempts are being made to establish colonies for rearing of all promising potential biocontrol agents (see Table 1). This would allow easier manipulations for future host specificity testing. Particular emphasis is being placed on the *Ceutorhynchus* species and on *Phyllotreta ochripes*.

In North America a preliminary test plant list was distributed to scientists and natural areas managers in winter 1999. Comments received will be incorporated and a final test plant list

will be submitted to the Technical Advisory Group for the introduction of biological control agents (TAG, within USDA, APHIS) for their approval. In addition, field collections of seeds, bulbs, corms etc. of spring ephemerals have been planned to provide material for the European researchers for host specificity tests that are planned to start in 2000 (if sufficient funds for the work can be obtained).

In summary, the development of a biological control program targeting garlic mustard is making significant progress. Promising biological control agents have been located and their impact on plant performance appears substantial. Researchers at CABI are prepared to continue this work and all indications suggest that we will have a viable program that can move the garlic mustard biocontrol research into the next phase: detailed life history investigations and host specificity tests for a select group of promising control agents (Table 1).

CABI Bioscience

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Investigations on potential biological control agents of garlic mustard, Alliaria petiolata (Bieb.) Cavara & Grande

ANNUAL REPORT 1998

H.L. Hinz & E. Gerber

CABI Bioscience integrating:

International Institute of Biological Control **IIBC**

IIE International Institute of Entomology IIP International Institute

International IMI Mycological Institute Investigations on potential biological control agents of garlic mustard, *Alliaria petiolata* (Bieb.) Cavara & Grande

H.L. Hinz & E. Gerber

Period under report: April - November 1998

Sponsored by:

Illinois State Nature Preserves Commission Indiana Division of Nature Preserves Minnesota Scientific and Natural Areas Kentucky State Nature Preserves Commission

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Abstract

Garlic mustard is a biennial forest understory herb, which was introduced from Europe and is presently recorded in 28 states in the USA and three provinces in Canada. It invades natural forest communities and is regarded as a serious problem because it displaces the indigenous undergrowth flora. Conventional control methods, such as fire, cutting, uprooting and herbicide applications have been successfully used to reduce the density of garlic mustard in natural areas. However, these methods are labour intensive, expensive and require follow-up treatments in order to eliminate plant recruitment from the seed bank. In April 1998, a project aiming at the biological control of garlic mustard was initiated.

The project started with a literature review that revealed 69 phytophagous insect species and 7 fungi to be associated with garlic mustard in Europe. During field surveys carried out in Switzerland and Southern Germany, 26 species were collected from garlic mustard and 17 of those species were successfully reared.

Six species were selected as potential biological control agents. With the exception of *Phyllotreta* ochripes, the species selected are recorded as monophagous on garlic mustard. The two shootmining weevils, *Ceutorhynchus alliariae* and *C. roberti*, are sibling species which were found to attack rosettes and bolting plants of garlic mustard. Heavily attacked plants were observed to die back without producing seeds. The larvae of *Ceutorhynchus constrictus* develop in the seeds of garlic mustard, each destroying one to two seeds during their development. The flea beetle *Phyllotreta ochripes* is recorded only from *Roripa amphibia* in addition to garlic mustard. The larvae were found at all field sites surveyed. The shoot-mining agromyzid *Ophiomyia alliariae* and the weevil *Ceutorhynchus scrobicollis* are two additional potential agents which were not found during this first field season.

Further surveys and more detailed investigations on the biology and phenology of the selected biological control agents are planed for 1999.

1 Introduction

Garlic mustard is a biennial plant of European origin, which was presumably introduced into North America for medicinal use and as a green vegetable (CAVERS et al. 1979). It was first recorded in 1868, on Long Island NY (NUZZO 1993). Since then, the plant has spread throughout northeastern and midwestern North America and is now recorded in 28 states in the USA and three provinces in Canada (NUZZO 1993). Garlic mustard is disturbance adapted and both naturally and anthropogenically disturbed habitats are the primary dispersal corridors for this invasive exotic (NUZZO 1993). Garlic mustard invades natural forest communities and is regarded as a serious problem because it displaces the indigenous undergrowth flora. Toothwort (*Dentaria lacinata*), for instance, has declined significantly in cover after only four years cooccurrence with garlic mustard (NUZZO 1992). いたのためになるのであるとないためのである

Garlic mustard overwinters as a basal rosette and is therefore well established when spring ephemerals germinate. It is assumed to compete largely by occupying physical space (McCARTHY 1997). In addition, competition might occur due to its ability to interfere with the production of mycorrhizae (ROBERTS 1997) or through allelopathic effects (McCARTHY 1997).

Several methods have been used to control the proliferation of garlic mustard in natural areas. For small infested areas, hand removal may be an effective method to limit its invasion into previously unoccupied habitats (McCARTHY 1997). In large infested areas, fire, cutting and herbicide applications have been successful in reducing the density of garlic mustard. However, all methods require follow-up treatments in order to eliminate plant recruitment from the seed bank (NUZZO 1991, NUZZO 1996a, NUZZO 1996b). The lack of specialized natural enemies of garlic mustard in North America is assumed to be one of the reasons for its success in invading its new habitats. First investigations into the prospects of classical biological control started in April 1998 on the initiative of Bernd Blossey (Cornell University, Ithaca NY). The project is currently being sponsored by the states of Illinois, Indiana, Kentucky and Minnesota.

2 Garlic mustard

2.1 Taxonomy and morphology

Family:	Cruciferae (Brassicaceae)
Tribe:	Arabideae
Subtribe:	Arabidinae
Genus:	Alliaria Scop.
Species:	petiolata (Bieb.) Cavara & Grande
Synonyms:	Arabis petiolata M.B., Erysimum Alliaria L., Sisymbrium Alliaria Scop., Hesperis Alliaria Lam., Alliaria alliaria Huth, Crucifera Alliaria E.H.L. Krause, Erysimum alliaceum Salisb., Alliaria alliacea Britt. et Rendle, Alliaria officinalis Andrz. (according to HEGI 1986)

According to the classification in HEGI (1986), the subtribe Arabidinae consists of 3 genera in Central Europe, i.e. Arabis, Aubretia and Alliaria. The genus Alliaria consists of only two species, Alliaria petiolata and A. brachycarpa M.B., the latter is recorded from the Caucasus (HEGI 1986).

The stems of garlic mustard are erect, simple or little branched, glabrous or with few simple hairs and grows up to 1.5 m high. Lower leaves are reniform, the others deltoid. Both types are coarsely toothed and glabrous. Numerous small flowers form racemes at the apex of the stems. Seeds are black, nearly cylindrical and situated in a single row in pods. The following chromosome numbers are recorded: 2n = 36; 42; 43 (GOHIN & KAUL 1976)

2.2 Biology and ecology

The name garlic mustard refers to the strong odor of garlic released when the plant is crushed. The plant grows preferentially in humid, shady habitats, but can also be found in dry locations and in full sun (HEGI 1986). It is a common plant in the undergrowth and at the edge of riverside forests. Garlic mustard is well adapted to disturbance and is a common component of ruderal communities and disturbed forests.

The flowers of garlic mustard are usually cross-pollinated by flies and short-tongued bees but self-pollinate in the absence of pollinators (CRUDEN et al. 1996). Garlic mustard reproduces exclusively by seeds; the major means of dispersal is probably by epizoochory (CAVERS et al. 1979). Germination occurs only after 60 -120 days of cold stratification (pers. comm. Blossey). Seeds can stay viable in the soil for up to 5 years but most germinate within the first two years (ROBERTS & BODDRELL 1983).

Garlic mustard is a biennial or perennial plant, perennating through the formation of adventitious buds. The latter has only been recorded in Europe (CAVERS et al. 1979). The seeds germinate in early spring and form rosettes which overwinter. In the spring of the second year of growth all plants bolt and flower between April and June. From May onwards, pods are formed and by mid-June, pods are ripe and release their seeds throughout summer and early fall (CAVERS et al. 1979).

3

Plant species in the family Cruciferae are rich in glucosinates, a sulfur-containing group of molecules (ETTLINGER et al. 1968). Sinigrin is the most abundant glucosinate found in garlic mustard. It acts as a feeding or oviposition stimulant for various insect species (NIELSEN et al. 1989, RENWICK 1989).

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2.3 Geographic distribution

The natural distribution area of garlic mustard includes the whole of Europe south of the 68th parallel but the species is less common in the extreme south (TUTIN et al. 1964). It also occurs in North Africa, India and Sri Lanka (CAVERS et al. 1979). Garlic mustard has been recorded at altitudes of up to 1800 m in the southern Alps, while in the northern valleys, it is only found up to 800 m (HEGI 1986).

3 Literature survey

A survey was carried out using CAB-Abstracts and CAB-Pest on CDROM, as well as evaluating host plant records in taxonomic literature on herbivorous insects. A total of 69 species in 20 different families as well as seven fungus species were found to be associated with garlic mustard (Appendix 1). Some 50% of the insect species recorded belong to the order Coleoptera, mainly to the families Curculionidae and Chrysomelidae. Four species in the genus *Ceutorhynchus* (Coleoptera; Curculionidae) and *Ophiomyia alliariae* (Diptera; Agromyzidae) are cited as monophagous on garlic mustard.

Some species names on the list have been updated according to information we received from taxonomists (pers. comm. Behne, Sauter). The species *Phytomyza atricornis* is now divided into two species, *Chromaomyia syngenesiae* and *Chromaomyia horticula* (pers. comm. von Tschirnhaus). The latter is known to feed on garlic mustard.

4 Field survey

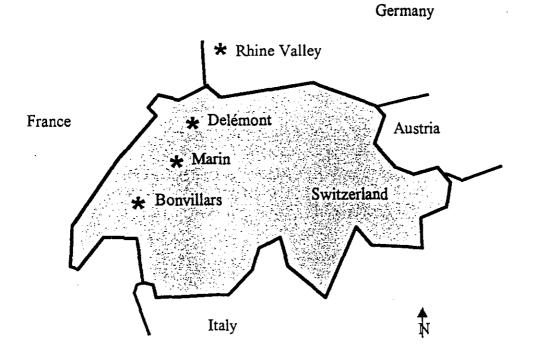
4.1 Field sites

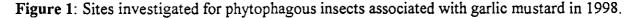
Four sites were chosen to investigate the phytophagous insect community on garlic mustard (Figure 1). According to small-scale habitat differences, two of the sites were further divided into substations. Between 29 April and 12 May, ten plots (50×50 cm) were laid out at random along a transect in each substation. The number of plants and the number of shoots per plant were recorded in each plot. Based on shoot density, the substations were divided into density classes. In addition, the light conditions were recorded for each substation. At most sites garlic mustard was associated with *Galium aparine* and *Urtica dioica*.

Switzerland

The site at Bonvillars (B), on the southern slopes of the Jura Mountains, is situated in a relatively dry oak forest. Garlic mustard is found scattered in the underwood. A particularly dense stand (17 $\times 2$ m) was chosen along a roadside for sampling.

4





The site at Marin (M) is in the immediate vicinity of Lake Neuchâtel in a former riverside forest. Garlic mustard is a common plant in the undergrowth, but is particularly dense in recently disturbed areas. A relatively uniform patch $(25 \times 15 \text{ m})$ was chosen for sampling.

The site at Delémont (D) is close to a river. It has been divided into three substations. D1 (15×2 m) is situated along a roadside shaded by trees where garlic mustard grows as a monospecific stand. In D2 (10×5 m), garlic mustard grows in a meadow on a steep slope in full sun. In the immediate surroundings of these two substations, further small patches of garlic mustard, denoted as D3, were included for some of the sampling. No data on density of garlic mustard were collected from these patches.

Germany

The site in the Rhine Valley (R) is in a former wetland area close to the Rhine. The site was divided into 4 substations. R1 ($12 \times 5 \text{ m}$) and R2 ($12 \times 7 \text{ m}$) are situated in a maple tree plantation. R3 ($40 \times 5 \text{ m}$) is located by a roadside on a slope, partially shaded by trees (*Robinia pseudoacacia*). In R4 ($15 \times 9 \text{ m}$), garlic mustard grows together with sparse vegetation on a gravel slope in full sun.

In addition to these four sites, garlic mustard plants were collected in Eastern Austria during a field trip for insects associated with scentless chamomile.

The substations differed considerably in density of garlic mustard plants (Table 1). The number of shoots per plot, visually perceived as the density of a garlic mustard stand, is influenced by the

number of plants as well as by the number of shoots per plant. The highest densities were measured in shady garlic mustard stands.

				Mean number ± SE						
Site	Light level	Density	Rosettes	Bolting plants	Shoots	Shoots/plant				
В	half-shade	sparse - medium	1.0 ± 0.4	9.4 ± 1.3	12.2 ± 1.5	1.3 ± 0.1				
M	half-shade	sparse	60.2 ± 3.1	3.3 ± 0.7	5.3 ± 1.4	1.6 ± 0.2				
Dl	shady	medium	0	7.7 ± 1.3	24.9 ± 3.7	3.2 ± 0.3				
D2	sunny	sparse	0	7.7 ± 1.5	9.6 ± 2.5	1.3 ± 0.1				
R 1	shady	dense	1.1 ± 0.5	36.9 ± 5.4	77.9 ± 7.0	2.1 ± 0.1				
R2	shady	medium -dense	3.0 ± 0.8	13.1 ± 1.5	37.5 ± 6.3	2.9 ± 0.2				
R3	half-shade	medium	-	5.4 ± 1.8	23.9 ± 9.6	4.4 ± 0.5				
R 4	sunny	sparse	0	2.1 ± 0.5	9.1 ± 2.6	4.3 ± 0.7				

Table 1: The number of plants and shoots and the number of shoots per plant for all field sites and substations investigated. Data are means of 10 plots (0.25m²).

-, not recorded

4.2 Sampling

Between 29 April and 12 May, garlic mustard plants were sampled for adult insects. Forty sweeps were carried out per substation with a sweep net. During the same period, 10 plants were randomly collected along a transect for later dissection. From D2, 20 plants were collected and dissected. Prior to dissection, the number of shoots per plant, shoot height and shoot-base diameter were recorded. In addition, the dry weight of above- and below-ground plant parts was recorded. All plant material was dried at 70°C for 24 hours.

Between 23 June and 1 July, a random sample of 10 shoots with ripening seeds was collected from each substation. Prior to dissection, the number of pods per shoot and the number of seeds per pod were recorded. The pods in R2, R3 and R4 were already dry and releasing seeds so data from these substations have been omitted. Regular sampling of rosettes for dissection started at the end of August. Random samples of rosettes were carried out at 3-4 week intervals throughout fall to find further potential agents. At some of the sites investigated, rosette density was very low. Therefore, additional sites in the area of Delémont and in the Rhine Valley were included in this investigation. On several occasions throughout the growing season, plants, pods and rosettes with obvious signs of insect damage were collected and dissected. All larvae found upon dissection were either transferred into 70% alcohol for morphological measurements and as reference material or reared on plant material. Mature larvae were offered soil for pupation. The rearings were either kept in an underground insectary or at fluctuating temperatures in a wooden shed.

4.3 Insects recorded

Of the 39 species collected or reared from garlic mustard, 26 have been identified to species level (Appendix 2). Most of these are not specific enough to be considered as potential biological control agents. Some species, which were found developing on garlic mustard, are known pests of cultivated crucifers (FINCH & THOMSON 1992; DIECKMANN 1972; HOFFMANN 1954). The following pest species are cited for Europe and do not occur in North America: *Phyllotreta atra* (F.), *P. undulata* Kutsch. and *P. nigripes* (F.) (Coleoptera; Chrysomelidae), *Baris cuprirostris* (F.) and *B. coerulescens* (Scop.) (Coleoptera; Curculionidae), *Ceutorhynchus contractus* (Marsham) and *C. pallidactylus* Mrs. (Coleoptera; Curculionidae), *Pieris brassicae* (L.) (Lepidoptera; Pieridae). The pest species also recorded for North America are: *Ceutorhynchus pictitarsis* Gyllenhal and *C. obstrictus* Mrs. (Coleoptera; Curculionidae), *Delia radicum* (L.) (Diptera; Anthomyiidae), *Pieris rapae* (L.) (Lepidoptera; Pieridae). *Plutella xylostella* (L.) (Lepidoptera; Plutellidae) and *Evergestis forficalis* (L.) (Lepidoptera; Pyraustidae).

Ceutorhynchus floralis (Paykull) (Coleoptera; Curculionidae) is recorded to develop in the seeds of wild crucifers. DIECKMANN (1972) suggested that the two North American species, *Neosirocalus septentrionalis* (Gyllenhall) and *Neosirocalus puberulus* (Leconte), should be synonymous with *C. floralis*.

A subspecies of *Pieris napi*, *P. napi oleracea* (Lepidoptera; Pieridae) is found in North America. Garlic mustard is accepted for oviposition by this species but the larvae subsequently die (HUANG et al. 1995). In contrast, the European subspecies can develop on garlic mustard (LEES & ARCHER 1974) and rearing of several larvae at the institute was successful.

One species in family Pieridae, Anthocharis cardamines (L.), might be of potential interest as a biological control agent. The larvae feed on a range of wild crucifers but Cardamine pratensis and Alliaria petiolata are considered their main host plants (COURTNEY & DUGGAN 1983). The larvae were observed to feed mostly on ripening seeds.

5 Potential biological control agents

According to the information on host-specificity available in the literature, six species were selected as potential biological control agents. Of these, four species belong to the genus *Ceutorhynchus* in the subfamily Ceutorhynchinae (Coleoptera; Curculionidae). Within this subfamily, the degree of host-specificity is generally high and several monophagous species are noted (DIECKMANN 1972). L. Behne (Entomological Institute, Eberswalde) confirmed the identification of all *Ceutorhynchus* species.

5.1 Ceutorhynchus alliariae Brisout and Ceutorhynchus roberti Gyllenhal

The two sibling species C. alliariae and C. roberti are both considered to be monophagous on garlic mustard. In the past, C. alliariae was regarded as a subspecies of C. roberti (HOFFMANN, 1954). Today, their species status is generally accepted, supported by different penis shapes as well as sympatric distribution and different habitat preferences (DIECKMANN 1972). Adult beetles can be distinguished by the color of their tarsi; while all tarsal segments of C. alliariae are red, the ones of C. roberti are black (DIECKMANN 1972, pers. comm. Behne). Since the

identification of the material sampled could only be confirmed by a taxonomist in August most of the following information comprises data for both species.

Both species are frequently found in Central Europe. Several authors assume that the two species may differ in habitat preferences (STREJCEK 1969, PENECKE 1928). They state that *C. alliariae* is more frequent in shady places (e.g. woods), whereas *C. roberti* occurs on garlic mustard growing in open habitats. The distribution of the two species found during our investigation does not support this assumption (Table 2).

At most sites investigated, both species were found. However, one species clearly dominates at each site (Table 2). Further investigations are needed to better understand the sympatric occurrence of the two species and their interaction.

	C. al.	liariae	C. roberti		
Site	Reared	Sampled	Reared	Sampled	
В	0	2	0	0	
D1	0	0	7	0	
D2	0	0	0	2	
D3	1	1.	16	3	
Μ	19	15	1	2	
R1	0	1	32	3	
R2	1	0	5	0	
R3	0	1	12	1	
R4	0	2	4	4	
Kaumbach (Austria)	7	0	0	0	
Traisch (Austria)	1	Q	0	0	

 Table 2: Number of sampled and reared C. alliariae and C. roberti at the sites and substations investigated.

Both species are described as univoltine shoot-miners (DIECKMANN 1972). Adult beetles appear in mid April on garlic mustard and oviposition starts by the end of April. We found eggs in the stems of bolting plants as well as in stalks of large rosettes. The larvae were found mining the whole length of the shoot and sometimes the mines extended down to the root crown. The headcapsule diameters of the larvae indicate three larval instars. For more precise data, the larvae of the two species must be separated. To date, no morphological differences between the larvae of these two species have been found.

Since the larvae of the two species could not be distinguished, the data in Table 3 covers both species. The percentage of shoots attacked was high at all sites investigated, exceeding 90% at two sites (Table 3). The mean number of larvae per attacked shoot also reached high levels. The highest number was observed in the Rhine Valley in substation R2, where up to 30 larvae mined

in one shoot. Plants with such high attack levels showed clear signs of damage. The attacked shoots dried up and did not produce any seeds. In some cases, the whole plant died.

Site	n	Shoots attacked (%)	Mean no. larvae/attacked shoot
В	10	92	2.5 ± 0.2
М	10	68	2.0 ± 0.3
D1	10	70	6.0 ± 1.1
D2	20	48	2.4 ± 0.7
R 1	10	92	8.4 ± 1.5
R2	10	73	11.2 ± 1.7
R3	10	90	2.6 ± 0.5
R4	10	75	5.1 ± 0.8

 Table 3: Percentage of shoots attacked by C. alliariae and/or C. roberti and mean number of larvae per attacked shoot.

n, number of plants dissected

The correlation between above-ground biomass and number of larvae of C. alliariae and/or C. roberti per plant was significant for all sites and substations investigated (Fig. 2). However, the regression coefficients (b) differed significantly between sites ($F_{7,74} = 14.75$, P < 0.001). Slopes were shallower for Bonvillars, Marin and D2, where a plant with a biomass of 5 g was only attacked by one larva, while at R1 (the dense site in the Rhine Valley) a plant with the same biomass was attacked by 64 larvae. The shoot base diameter of single shoots was also positively correlated with degree of larval attack, and the slopes again differed between sites ($F_{7,245} = 4.78$, P < 0.001).

The larvae leave the plant and pupate in the soil (DIECKMANN 1972). At the institute, emergence occurred at the end of July/beginning of August. Rearing of these two species was successful with more than 50% of the sampled larvae developing to adults. Even larvae found in plant material stored for 5 weeks at a temperature of 4 °C developed normally into adults.

Two species of parasitoids belonging to the families Pteromalidae (Hymenoptera) and Braconidae (Hymenoptera) were reared from larvae collected in the Rhine Valley substations. Both parasitoids will be sent to taxonomists for identification.

After emergence, the weevils fed on leaves of garlic mustard. The amount of garlic mustard eaten by the beetles declined steadily in the weeks following emergence and feeding stopped completely at the end of August. Further, no adults were observed at the field sites during summer. This indicates that the weevils aestivate. On 3 November, a very mild day, a single C. roberti was observed on a rosette.

The beetles overwinter in leaf litter or in the soil (DIECKMANN 1972). At present, 58 C. roberti and 23 C. alliariae are being kept on rosettes covered with gauze bags in the institute's garden for overwintering.

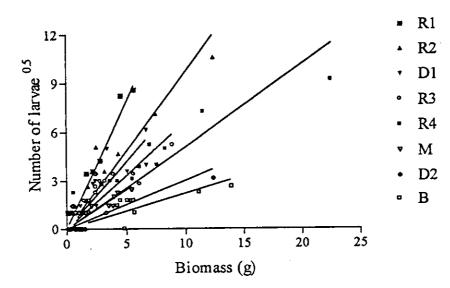


Figure 2: Relationship between above-ground biomass and the number of *C. alliariae* and/or *C. roberti* per plant for all sites and substations investigated in 1998. The number of larvae was square-root transformed. Regression lines were forced through the origin. R1: b = 1.595, r = 0.9858; R2: b = 0.975, r = 0.9670; D1: b = 0.843, r = 0.9498; R3: b = 0.670, r = 0.9594; R4: b = 0.512, r = 0.9655; M: b = 0.494, r = 0.9658; D2: b = 0.304, r = 0.7518; B: b = 0.222, r = 0.9350 (n as in Table 3).

5.2 Ceutorhynchus constrictus (Marsham)

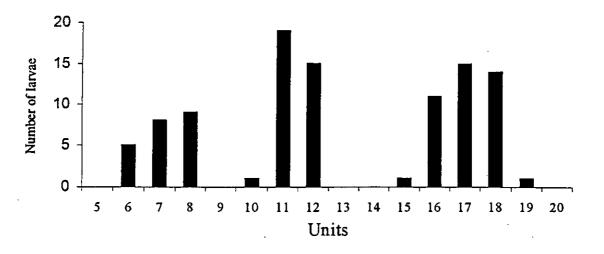
C. constrictus is considered to be monophagous on garlic mustard (DIECKMANN 1972). It is a univoltine species, commonly found all over western and Central Europe. Adults appear in April and were observed to feed on leaves of garlic mustard. Eggs are laid into the developing seeds during May and June (DIECKMANN 1972). We found that a single egg is laid into each seed, but several seeds can be attacked within the same pod (Table 4). The larvae feed on the ripening seeds. One larva consumes one to two seeds before leaving the pod to pupate in the soil. Three larval instars can be distinguished by their head-capsule diameters (Figure 3).

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In contrast to information given by DIECKMANN (1972), C. constrictus did not emerge during summer in our rearing. We found fully developed living adults in earthen cocoons in October. Thus, we assume that emergence will occur only after overwintering.

Host plant recognition and specificity in feeding responses of adult C. constrictus were tested by NIELSEN et al. (1989) who showed that as yet unidentified water soluble compounds are the most important feeding stimulants. Sinigrin, the most abundant glucosinate in garlic mustard, was also acting as a feeding stimulant. Of the 41 plant species tested (Fam. Cruciferae, Resedaceae and Tropaeolaceae), only four species, Brassica nigra, Sinapis alba, Thlaspi arvensis and Descurainia sophia, were accepted to the same extent as garlic mustard for adult feeding.

C. constrictus was found at all sites investigated (Table 4). However, the number of seeds attacked was not particularly high at any site.



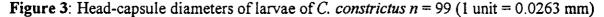


Table 4: The number of pods per shoot and seeds per pod, as well as the total number of seeds investigated and the percentage of seeds attacked by larvae of *C. constrictus* for different sites and substations. Data are from 10 randomly sampled shoots per site or substation.

	Mean n	$b. \pm SE$	Total	
Site	Pods/shoot	Seeds/pod	no. seeds	Attacked seeds (%)
B	44.1 ± 7.6	14.0 ± 0.2	6168	1,13
М	23.7 ± 4.7	10.3 ± 0.3	2492	3.41
D1	20 ± 2.3	18.0 ± 0.3	2775	0.29
D2	28.8 ± 3.3	13.4 ± 0.2	2912	1.00
R1	11.3 ± 0.9	6.5 ± 0.4	706	6.37

Larvae collected in the Rhine Valley were attacked by ectoparasitoids (Hymenoptera; Pteromalidae). Parasitoid adults were reared and will be sent to a taxonomist for identification.

5.3 *Phyllotreta ochripes* (Curtis)

The identification of *P. ochripes* (Coleoptera; Chrysomelidae) was confirmed by M. Döberl. Adults of *P. ochripes* are recorded to feed on a limited range of wild crucifers. Larval development is only known to occur on two species: *Roripa amphibia* (DOGUET 1994) and *Alliaria petiolata* (our investigation). The distribution range of this species covers most of Europe and parts of northwestern Asia (GRUEV & DOEBERL 1997). It is predominantly recorded in humid habitats (DOGUET 1994).

Little specific data on the biology of this species is available in the literature. Adult beetles appear in April (DOGUET 1994). It is known from other species of the same genus, that the eggs are laid into the soil close to the host plants and that the newly hatched larvae make their way to the roots (BALACHOWSKY 1963). We found larvae of *P. ochripes* mining underneath the

epidermis of the root and root-crown of both bolting plants and rosettes of garlic mustard. In rosettes, larvae were also observed to feed within the stem-base. Though adults were sampled from all sites, larvae in bolting plants sampled at the end of April/beginning of May were only found in the Rhine Valley. At the other sites, larvae of *P. ochripes* were found in rosettes sampled at the end of May and in June.

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At the institute, larvae pupated in the soil and the new generation appeared in July. The freshly emerged beetles fed on leaves of garlic mustard. The amount of leaf material eaten declined rapidly and feeding stopped completely 3 - 4 weeks after emergence. No adults were observed at the field sites during summer. This indicates that *P. ochripes*, like *Ceutorhynchus alliariae* and *C. roberti*, also aestivates. Single beetles were found on rosettes in fall. *P. ochripes* is reported to overwinter in leaf litter or in the soil (DOGUET 1994). At present, 27 individuals are being kept on garlic mustard covered with gauze bags in the institute's garden for overwintering.

Interestingly enough, living larvae were found in rosettes collected on 31 August, and on 10 November two *P. ochripes* emerged. This late development could indicate that a partial second generation occurs as has been recorded for *Phyllotreta undulata*, *P. aerea* and *P. consobrina* (BALACHOWSKY 1963).

5.4 Ceutorhynchus scrobicollis Nerensheimer und Wagner and Ophiomyia alliariae Hering

C. scrobicollis (Coleoptera; Curculionidae) and O. alliariae (Diptera; Agromyzidae) are considered to be monophagous on garlic mustard (DIECKMANN 1972, SPENCER 1990). However, neither of the two species were found during our field surveys. In fact, C. scrobicollis has not been recorded to occur in Switzerland or Southern Germany (pers. comm. Scherler, Behne). According to the information available, its area of distribution is in Eastern Europe (pers. comm. Behne, DIECKMANN 1972). A field trip is planned 1999 in order to find this species. Little is known about the biology of C. scrobicollis and no information is available about its feeding niche on garlic mustard. According to DIECKMANN (1972), this species may develop in garlic mustard during fall and winter. Ophiomyia alliariae mines and pupates in the shoots of garlic mustard. The species is known to be bivoltine and is recorded from Central Europe and Great Britain (HERING 1957).

6 Work programme 1999

Based on the work carried out in 1998, the following work programme is proposed for 1999:

- Carry out field surveys:
 - Continue sampling phytophagous insects at the sites investigated in 1998;
 - Extend field surveys to northeastern Europe to find additional potential biological control agents, especially *Ceutorhynchus scrobicollis*;
- Continue life-history studies of Ceutorhynchus alliariae, C. constrictus, C. roberti and Phyllotreta ochripes:
 - Carry out regular dissections of plants to study their phenology;
 - Investigate oviposition behaviour, fecundity and larval development in the laboratory;
 - Carry out studies on the biology and niche separation of the two sibling species *Ceutorhynchus alliariae* and *C. roberti* using artificially infested plants;
- Develop suitable techniques for host-specificity tests;
- Develop a test-plant list in cooperation with B. Blossey and relevant organizations in the USA.

Acknowledgements

We would like to thank the following taxonomists for identifications of the sampled insects: L. Behne (Entomological Institute, Eberswalde: Curculionidae), M. Döberl (Chrysomelidae), W. Marggi (Carabidae), V. Michelsen (University of Copenhagen: Anthomyiidae), W. Sauter (Lepidoptera) and M. von Tschirnhaus (University Bielefeld, Germany: Agromyzidae). We also wish to thank P. Scherler for information about *Ceutorhynchus scrobicollis* in Switzerland and A. Gander for indicating garlic mustard field sites at Marin and Bonvillars.

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Distribution List

B. Blossey (20)

L. Behne

G. Markin

P. Mason

H. Müller-Schärer

CABI Bioscience Centres (5)

CABI Bioscience (5)

Phytophagous species	Source	Specificity	Plant part	Guild	Distribution
Coleoptera	_				
Nitidulidae					
Melingethes aeneus (F.)	LIPA et al. (1977)	o	flower	ecto	CE
Melingethes coracinus (Sturm)	KOCH (1989)	Р	flower	ecto	CE
Melingethes viridescens (F.)	LIPA et al. (1977)	р	-	ecto	CE
Chrysomelidae					
Phaedon cochleariae (F.)	LIPA et al. (1977)	0	leaf, shoot	ecto	E
Phyllotreta astrachanica Lop.	KOCH (1992)	0	leaf, shoot, root	ecto, endo	
Phyllotreta atra (F.)	LIPA et al. (1977)	0	leaf, shoot, root	ecto, endo	CE
Phyllotreta cruciferae (Gz.)	LIPA et al. (1977)	o	leaf, shoot, root	ecto, endo	CE
Phyllotreta nemorum (L.)	LIPA et al. (1977),	o	leaf, shoot, root	ecto, endo	CE
	HERING (1957)				
Phyllotreta ochripes Curt	LIPA et al. (1977)	0	leaf, shoot, root	ecto, endo	CE
Phyllotreta undulata Kutsch.	LIPA et al. (1977)	o	leaf, shoot, root	ecto, endo	CE
Phyllotreta vittata (F.)	LIPA et al. (1977),	0	-	ecto?	CE
	KOCH (1992)				
Psylliodes cuprea Koch	LIPA et al. (1977)	0	leaf, shoot	ecto	CE, SE
Psylliodes cupreata (Dufl.)	LIPA et al. (1977)	o	leaf, shoot	ecto	CE
Psylliodes napi (F.)	LIPA et al. (1977)	o	leaf, shoot	ecto	CE
Byturidae					
Byturus aestivus (L.)	KOCH (1989)	p	flower	ecto	CE
Curculionidae					
Ceutorhynchus alliariae Brisout	DIECKMANN (1972),	m	shoot	endo?	CE, WE
	HOFFMANN (1954)				
Ceutorhynchus atomus Boheman	HOFFMANN (1954)	0	leaf, shoot	endo?	CE, SE, NAf
Ceutorhynchus chalybaeus Germar	HOFFMANN (1954),	0	shoot	endo	Р
	DIECKMANN (1972),	-			
	BUHR (1964)				
Ceutorhynchus constrictus (Marsham)	DIECKMANN (1972),	m	pođ	ecto, endo	CE
······	HOFFMANN (1954)		-		
Ceutorhynchus contractus (Marsham)	HOFFMANN (1954)	Р	leaf, shoot	endo	Е
Ceutorhynchus erysimi (F.)	HOFFMANN (1954)	0	shoot, root	endo	Е
Ceutorhynchus floralis	LIPA et al. (1977),	o	leaf, shoot	ecto, endo	E, ME,
(Syn: Neosirocalus floralis Paykull)		-	- •		NAf, NA
Ceutorhynchus napi Gyllenhal	HOFFMANN (1954)	0	leaf, shoot	endo, ecto	CE, SE, NAf
Ceutorhynchus obstrictus	LIPA et al. (1977),	0	leaf, shoot, pod	ecto, endo	Е
(Syn: Ceutorhynchus assimilis (Paykull))		-		,	
Ceutorhynchus pleurostigma (Marsham)	HOFFMANN (1954)	0	leaf, root	endo, ecto	Е
Cealority news prositioning in a final containing	BUHR (1964)	•	••••••		
Ceutorhynchus pallidactylus	LIPA et al. (1977),	р	leaf, shoot	endo, ecto	E, ME, NAf
(Syn.: Ceutorhynchus quadridens (Panzer))		F			
Ceutorhynchus rhenanus (Schultze)	HOFFMANN (1954)	o	-		CE, NE, EE
(Syn.: Neosircalus rhenanus)	. ,				
Ceutorhynchus roberti Gyllenhal	DIECKMANN (1972),	m	shoot	endo	E, UdSSR
	HOFFMANN (1954)				
Ceutorhynchus scrobicollis	TEMPERE et al. (1989),	m	•	-	WE, CE, EE
Neresheimer & Wagner	DIECKMANN (1972)				

Appendix 1: Phytophagous species associated with Alliaria petiolata: literature survey

Phytophagous species	Source	Specificity	Plant part	Guild	Distributio
Ceutorhynchus thomsoni Kolbe	TEMPERE et al. (1989), DIECKMANN (1972)	0	•	-	CE, NE
Lixus ochraceus Boheman	TEMPERE et al. (1989),	0	root?	endo?	E, ME, NAf
	DIECKMANN (1983)				
Otiorhynchus veterator Uyttenboogaart	DIECKMANN (1980),	р	leaf, bud, seed	ecto	se, we
· · · ·	HOFFMANN (1958)	•			
Peritelus sphaeroides German	DIECKMANN (1980),	р	leaf, root	ecto	CE, WE
	HOFFMANN (1958)	-			
Hymenoptera					
Tentredinidae					
Athalia liberta (Klug)	LORENZ & KRAUS (1957)	o	leaf	ecto	EA
Athalia glabricollis Thomson	LORENZ & KRAUS (1957)	0	leaf	ecto	EA
Diptera					
Agromyzidae					_
Liriomyza strigata (Meigen)	HERING (1957)	Р	leaf	endo	E
Liriomyza crucifericola Hering	HERING (1957)	р	leaf	endo	CE
Ophiomyia alliariae Hering	HERING (1957)	m	shoot	endo	CE, GB
Phytomyza rufipes Meigen	LIPA et al. (1977),	o	leaf	en do	E
	HERING (1957)				_
Phytomyza atricornis Meigen	HERING (1957),	Р	leaf	endo	E
	SEGUY (1934)				
Drosophilidae					
Scaptomyza flava Meigen	LIPA et al. (1977)	P	leaf	endo	-
Scaptomyza flaveola Meigen	HERING (1957)	-	leaf	endo	-
Thysanoptera					
Thripidae					
Thrips angusticeps Uz.	LIPA et al. (1977)	р	flower, leaf, shoot	ecto	E
Lepidoptera					
Incurvariidae					
Nematopogon panzerella (F.)	HEATH et al. (1976)	0	•	•	E
Adela rufimitrella (Scopoli)	HEATH et al. (1976)	0	leaf	ecto	E, Udssr
Plutellidae					
Plutella xylostella L.	LIPA et al., 1977,	0	leaf, shoot	ecto	cos.
(Syn.: Plutella maculipennis Curtis)	HERING, 1957			_	<u></u>
Rhigognostis incarnatella (Streudel)	HEATH et al. (1996)	٥	•	ecto?	CE, EE, W
Tortricidae					
Argyroploce variegana Hbn.	LIPA et al. (1977)	p	leaf, shoot	ecto	-
Cacoecia reticulana Hbn.	LIPA et al. (1977)	р	leaf, shoot	ecto	-
Cnephasia asseclana Denis & Schiffermüller	LIPA et al. (1977)	P	leaf, shoot	ecto	-
(Syn.: Cnephasia virgaureana (Treit.))					
Pyraustidae					AF -F
Evergestis limbata L.	HANNEMANN (1964)	0	leaf	ecto	CE, SE
Evergestis fortficalis L.	HANNEMANN (1964)	0	leaf	ecto	E
Geometridae					_
Larentia fluctuata	BLASCHKE (1914)	-	•	edo	E
Xanthorhoe fluctata (L.)	HEATH et al. (1991)				-
Pionea forficalis L.	LIPA et al. (1977)	0	leaf, shoot	ecto	-

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Phytophagous species	Source	Specificity	Plant part	Guild	Distribution
Pieridae					-
Pieris brassicae (L.)	FERNANDO, 1971	0	leaf	ecto	E
	(in CAVERS et al 1979)				
Pieris rapae L.	HEATH et al. (1989)	0	leaf, shoot	ecto	H.
Pieris napi L.	LEES (1974)	0	leaf	. .	H
Anthocharis cardamines L.	HEATH et al. (1989)	0	flower, seed, leaf	ecto	E
Noctuidae					
Mamestra brassicae (L.)	SZENTESI & JERMY (1991)	P	leaf	ecto	•
Heteroptera					
Pentatomidae					CD
Eurydema oleracea L.	LIPA et al. (1977),	0	leaf, shoot, seed	ecto	GB
	SOUTHWOOD				
	& LESTON (1959)				
Miridae			leaf, shoot	ant 0	_
Lygus rugulipennis Popp.	LIPA et al. (1977)	Р	leal, shoot	ecto	-
Homoptera					
Psyllidae		·			
Bactericera nigricornis Forst.	LIPA et al. (1977)	р	leaf, shoot	ecto	-
Aphididae					
Brevicoryne brassicae (L.)	LIPA et al. (1977)	0	leaf, shoot	ecto	COS.
Lipaphis erysimi (Kaltenbach)	LIPA et al. (1977)	0	leaf, shoot	ecto	CE, WE
	BUHR (1964)				CE, WE
Macrosiphum kaltenbachii Schouteden	THEOBALD (1926)	0	leaf	ecto	
Myzuss persicae (Sulzer)	LIPA et al. (1977)	0	leaf, shoot	ecto	cos.
	BUHR (1964)				
Cercopidae					5 314
Philaenus spumarius L	BUHR (1964)	р	shoot	endo, ecto	E, NA
Fungi					
Albugo candidae (Pers.) Ktze.	BUHR (1964)	-	-	-	-
Alternaria alliariae officinalis	SAVULESCU &	•	-	-	-
	SANDU-VILLE (1933)				
	(in CAVERS et al. 1972)				
Erysiphe communis (Wallr.)	KHAN et al. (1975)	· •	-	-	-
Peronospora parasitica	NICOLAS et al. (1949),	-	-	-	-
	(in CAVERS et al. 1979)				
Peronospora niessleana Berlese	BUHR (1964)	•	-	-	-
Plasmodiophora brassicae Woron.	BUHR (1964)	-	-	-	-
Ramularia armoraciae	RAI et al. (1972)	-	-	-	•

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m, monophagous (only garlic mustard recorded as host plant); o, oligophagous (host plants within the family Cruciferae); p, polyphagous (host plants in several families)

CE, Central Europe; cos, cosmopolitan; E, Europe; EA, Eurasia; EE, Eastern Europe; GB, Great Britain; H, Holoarctic; ME, Middle East; NA, North America; NAf, Northern Africa; NE, Northern Europe; P, Palearctic; SE, Southern Europe; UdSSR, former Soviet Union; WE, Western Europe.

Phytophagous species	Source	Specificity	Plant part	Guild	Distribution
Coleoptera					
Carabidae					
Amara similata (Gyll.)	s	p	pod	ecto	CE
Amara ovata (F.)	s	p	•	-	CE
Elateridae	U	F			
1 species	S	-	-	-	-
Nitidulidae	U				
	S	_	-	-	-
1 species	3	-	_		
Mordellidae	-		_	_	_
Anaspis sp.	S	-	-	-	. –
Chrysomelidae	_				
3 species	S	-	-	-	CE
Phyllotreta astrachanica Lop.	S	0	-	-	CE
Phyllotreta atra (F.)	S	0	-	-	CE
Phyllotreta nigripes (F.)	S	0	- shoot, root	endo	CE
Phyllotreta ochripes Curt.	r, s	0	511001, 1001		CE
Phyllotreta undulata Kutsch.	S	0	-	-	CL
Bruchidae					
1 species	S	-	-	-	-
Curculionidae			10		NE CENAS
Baris coerulescens (Scop.)	r (?), s	0	root?	endo ?	ME, SE, NAf
Baris cuprirostris (F.)	S	0	-	-	ME, NAf
Ceutorhynchus alliariae Bris.	I, S	m	shoot, root	endo	CE, WE
Ceutorhynchus contractus (Marsh.)	r, s	0	leaf	endo	E CE
Ceutorhynchus constrictus (Marsh)	r , s	m	pod	endo	E, ME, NAf,
Ceutorhynchus floralis (Payk.)	S	o	•	•	NA
Contractor observations Mar	¢	0	_	-	E
Ceutorhynchus obstrictus Mrs.	S S	~ 0	_	-	E, ME, NAf
Ceutorhynchus pallidactylus Mrs.	s	0	-	-	E,
Ceutorhynchus pictitarsis Gyll. Ceutorhynchus roberti Gyll.	r, s	m	shoot, root	endo	E, UdSSR
	1, 5				_,
Hemiptera					
5 species	S	-	-	-	-
Hymenoptera					
Tentredinidae					
Athalia glabricollis Thomson	r	0	leaf	ecto	EA
Diptera					
Agromyzidae					
Chromatomyia horticola (Gourreau)	r	р	leaf	endo	E
Anthomyiidae					
Delia radicum (L.)	r	0	root	endo	E, NA

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Appendix 2: Phytophagous species associated with *Alliaria petiolata*: field survey (Information on "plant-part" and "guild" according to our investigation, for larval development only)

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Phytophagous species	Source	Specificity	Plant part	Guild	Distribution
Lepidoptera		· · · · · · · · · · · · · · · · · · ·		•	
Plutellidae					
Plutella xylostella L.	r	ο	leaf	ecto	cos.
Tortricidae					
Cnephasia asseclana Denis & Schiffermüller	г	р	leaf	ecto	-
Pyraustidae					
Evergestis fortificalis L.	г	0	leaf	ecto	E
Pieridae					
Anthocharis cardamines (L.)	r	· o	leaf, pod	ecto	Е
Pieris brassicae (L.)	r	0	leaf	ecto	E
Pieris napi (L.)	r	0	leaf	ecto	Н
Pieris rapae (L.)	r	0	leaf	ecto	Н
Geometridae					
Xanthorhoe fluctuata L.	r	0	leaf	ecto	-
Fungi					
mildew	S	-	-	-	-

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r, reared; s, sampled

m, monophagous (only garlic mustard recorded as host plant); o, oligophagous (host plants within the family Cruciferae); p, polyphagous (host plants in several families); -, no information

CE, Central Europe; E, Europe, H, Holoarctic, cos, cosmopolitan, ME, Middle East; NA, North America; NAf, North Africa; SE, South Europe; UdSSR, former Soviet Union.

Certificate of Expenditures

According to the terms of your grant with the Illinois Conservation Foundation, your entity agreed to provide a minimum of 50% in matching funds that were to be expended on additional fees and/ or expenditures.

Please refer to the attached report that reflects ACTUAL and IN-KIND expenditures. If your project shows an UNDERMATCH, you must spend the balance shown.

This report should now cover the ACTUAL costs incurred for the reporting period.

Direct Costs for the time period of: May 1, 1998 - December 30, 1999

-----Current Period-----

Cumulative	Τo	Date
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Line Item	Total Costs	Federai Share	Match	Total Costs	Federal Share	Match
Person Services						
Fringe Benetits						
Travel						
Equipment						
Supplies						· · ·
Contractual	\$30,000	\$5,000	\$25,000	\$30,000	\$5,000	\$25,000
Other						

(Must Define)

Costs on this document must meet the test of necessity, reasonableness, and allowability in accordance with applicable cost principles applicable to this award. All costs charged are subject to audit. Recipients are responsible to insure proper management and financial accountability of federal funds.

Backup for these expenses are to include the attached time sheet, and copies of invoices and/or cancelled checks for other expenses. All financial reports of costs are to be maintained by your entity for possible audit by the Illinois Conservation and the Auditor General of the State of Illinois and the U.S. Department of Forest Service and any other audits conducted by your entity including the Single Audit.

I certify that to the best of my knowledge and belief the expenditures above are correct and that all outlays were made in accordance with the grant conditions or other agreements.

Authorized Official Signature

12-20-

Date

