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1 Rue des Grillons,
CH-2800 Delémont, Switzerland

Investigations on potential biological control agents of Scotch broom, *Cytisus scoparius,*

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by

R. Wittenberg & T. Thomann

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Investigations on potential biological control agents of
Scotch broom, *Cytisus scoparius,*
Annual Report, January – December 2000

R. Wittenberg[1] & T. Thomann[2]

[1] CABI Bioscience, c/o CSIRO European Laboratory, 34980 Montferrier-sur-Lez, France
[2] CSIRO European Laboratory, 34980 Montferrier-sur-Lez, France

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Summary

Scotch broom belongs to a group of shrubby legumes, whose members are important weeds in many countries on several continents. Its native distribution covers the western and central part of Europe, but it is widely used as an ornamental plant or planted for soil enhancement outside its native range. In North America, Africa, Asia, and the Australasian Region it has escaped from cultivation and invades mainly deforested areas where it delays or interrupts natural succession by suppressing herbaceous vegetation and the regeneration of pioneer shrubs and trees (Annex, Plate 1). It is also an aggressive invader of agricultural and forestry areas. Natural and human disturbance of forest remnants enhances the establishment of Scotch broom plants in forests and thereby increases their negative impact on biodiversity. A consortium of funding bodies from New Zealand, Australia and U.S.A. supports this programme to study potential biological control agents, which is based at the CSIRO European Laboratory, Montferrier sur Lez, France. The major objectives for the field season of 2000 were the improvement of rearing techniques and preliminary host specificity screening for two gall-forming insects with potential as agents for biological control against the host plant. Another focus of the work was to maintain the broom field plot, for host specificity investigations, established in the laboratory's garden and monitor insect populations.

During March approximately 800 first generation bud galls induced by *Asphondylia sarothamni* (Dipt.: Cecidomyiidae) (Annex plate 2) were collected on field trips to the Cévennes (Annex plate 4) and the foothills of the Pyrenées. Emerging adults were paired and transferred to cages containing potted Scotch broom plants. Unfortunately, due to unknown reasons, not a single gall was produced. Therefore, about 1,100 second generation galls were collected in the Cévennes and adults were released on *C. scoparius* plants in cages as well as in a tent on the field plot. Gall development will be checked in spring 2001 when any development should be evident.

Plants with obvious galls of *Hexomyza sarothamni* (Dipt.: Agromyzidae) (Annex plate 5) were dug out in the field during the winter 1999/2000 and kept in pots in the laboratory's

garden. The plants were placed in a large cage to avoid spread of emerging parasitoids within the laboratory area. When the distinctive windows appeared – a sign that emergence will take place shortly – the plants were transferred into smaller cages inside a CE room. A total of 83 adults (40 males and 43 females) emerged between 11 April and 13 June. Since these galls produced parasitoids too, early attack (in the same year as induction) of the young galls is confirmed. The adults were used for host specificity tests in different designs to identify the best methods. To date several *C. scoparius* plants have developed galls, but no galls have been found on test plant species. The tests will be evaluated in early spring 2001.

During the summer 2000 several additional releases were made on the broom field plot in the laboratory's garden (Annex plate 3). Seven *Gonioctena olivacea* females of the initial introduction in 1999 were recovered and 18 additional adults released on the same plot. All seven females were found on *C. scoparius* and none on French broom (*Genista monspessulana*) or tagasaste (*Chamaecytisus palmensis*), despite beating all plants. On 28 June about 70 *Exapion fuscirostre* adults were released in the plot. Several pairs of *Asphondylia sarothamni* and *Hexomyza sarothamni* were released into a tent placed over the ten central *C. scoparius* plants of the field test. Additionally, some 88 *Agonopterix assimilella* eggs and first instar larvae, reared in a CE room from a collection of pupae, were transferred onto Scotch broom plants in the field plot.

During May an open field multiple-choice oviposition test for *Bruchidius villosus* was set up in a separate garden bed with acid soil. Nine test plant species were arranged randomly in four blocks and 41 adult beetles released. Half of the green pods were collected when their swelling indicated an obvious seed development and number of pods and *Bruchidius* eggs were counted for each plant separately. The second half of the pods was collected after completion of pod development. These pods were dissected and larval development and adult emergence was recorded. It turned out that, due to the open design of the experiment, the plot was infested with three *Bruchidius* species, which developed on five test plant species in the genera *Cytisus* and *Coronilla*.

1 Introduction

This report summarises the investigations on selected potential insect agents for biological control of Scotch broom (*Cytisus scoparius*), a shrubby legume of European origin. It was widely grown as an exotic ornamental and has escaped from cultivation. Today it is a serious pest in New Zealand, Australia, and western parts of the U.S.A., and other countries around the world. Mechanical and chemical control can be applied successfully, but these methods are often too cost intensive, impractical in inaccessible areas, or too unsafe in nature reserves. The broom biological control programme has been running since 1981, initially for New Zealand. In 1991 a project for Australia was started and more recently the U.S.A. became interested and a consortium of the three countries was formed to support the CABI Bioscience Programme based in Montpellier at the CSIRO European Laboratory.

The broom fauna in the south of France has been studied since 1993. Releases of several agents have been made in the participating countries, and the most promising agents still to be studied for biological control of broom are two gall-forming insects, i.e. the agomyzid fly *Hexomyza sarothamni* and the gall midge *Asphondylia sarothamni*, which continued to be the main focus of the programme in 2000 (see Chapters 5 and 6 respectively). These insect species appear to have very specific oviposition requirements and therefore present difficulties for host range testing as well as rearing. Additionally, investigations in the host range of the bruchid beetle, *Bruchidius villosus*, a biological control agent already widely distributed were revitalised due to recent concerns of non-target effects of the beetle.

2 Rearing of *Agonopterix assimilella* (Lep.: Oecophoridae)

In 1999, this moth was released on the broom field plot (see Chapter 7). Searches for the larvae conducted later on in the 1999 season failed to detect any signs of their presence, and so we assume that none survived. Generalist predators such as ants, which frequented the plants because of the presence of aphids, probably killed the very tiny first instar larvae before they were able to establish themselves in shelters. Another possibility is that the larvae are disorientated or otherwise injured due to the handling procedures, and so it was decided to transfer eggs onto the plants in the plot in 2000 to allow a more natural colonisation of the plants by the hatched larvae.

Therefore on 30 May 2000 a field trip was made to a broom infestation at 1250m altitude above L'Esperou (Cévennes) and about 200 mature larvae were collected and brought to the laboratory. Cut branches containing the webs of the mature larvae were stuck into pots of potted *Cytisus scoparius* plants, to allow larvae to enter the fresh plant material. The cage containing the collected material was placed in a CE room (16 hours day with day/night temperatures about 24/18° C). Larvae were allowed to pupate. Despite some unexpected, brief periods of high temperature in the CE room, the rearing of the moth species went well and adult emergence commenced on 20 June. Three males and seven females emerged within one week and were transferred into a cage kept under similar conditions where they were kept during their prolonged aestivation. The cage contained a potted *C. scoparius* plant and a water supply. Food for the adults was offered in form of a bunch of flowers collected in the garden and a supply of a 7% saccharose and vitamin solution. Food and water supplies were replaced at irregular intervals of around 6 days during the aestivation period of the adults.

The first eggs were found on 28 July, and the potted plant in the cage was replaced to provide fresh oviposition substrate. The eggs were allowed to mature before transferring them to the field plot. On 8 August twelve freshly hatched L1 and 59 eggs were released on the nine surviving *C. scoparius* plants in the central block of one field plot (prior to the release one Scotch broom plant had died). The eggs and larvae were transferred onto plants in the plot by attaching a small piece of a branch, harbouring the early

developmental stages of the moth, to the mature plants. The release was made in the evening to avoid the heat of the summer day. The dead plant in the field plot was replaced by a plant taken directly from the *A. assimilella* cage, containing another 17 eggs. Thus, altogether 88 eggs or larvae were released on the ten Scotch broom plants of one field plot. Subsequent surveys revealed several cocoons of *A. assimilella* on these plants. The fate of the larvae and pupae will be monitored in 2001.

3 Host specificity investigations with *Bruchidius villosus* (Col.: Bruchidae)

During May an open-field multiple-choice oviposition and larval development test was set up for this beetle in collaboration with Dr. Andy Sheppard (CSIRO). This bruchid beetle has been released as biological control agent against Scotch broom in New Zealand and Australia in 1987 and 1991 respectively (Syrett *et al*. 1999). The beetles for the release in Australia were obtained from New Zealand, thus belonging to the same tested population. The no-choice and choice oviposition tests prior to the release in New Zealand indicated that *Bruchidius villosus* is highly host specific. However, *B. villosus* is attacking *Chamaecytisus palmensis* (tagasaste) in the field in New Zealand, although the beetles did not oviposit on this plant in choice oviposition tests on cut shoots (Syrett and O'Donnell 1987). These unanticipated field results, throw doubt on other species tested in the same design. Hence this open field plot was set up to compare oviposition data between previous laboratory tests and these experiments under more natural conditions.

B. villosus was established in North-eastern United States of America at the beginning of the 20th century (Bottimer 1968). This population was the source for releases in Oregon in 1998/99. These recent releases justify additional field tests with the beetle, in particular when including North American plant species, in order to reavaluate the risks to non-target plants.

Methods

On 11 May the experiment was set up in the garden of the CSIRO European Laboratory in a separate garden bed filled with acid soil. Four blocks were prepared using 16 plants in each plot. For all test plant species two replicates were used per plot with the exception of the *Coronilla* species. In the latter case one specimen each of *C. emerus* and *C. glauca* were placed into each block. The following potted test plant species were dug into the soil: *Genista monspessulana, Genista tinctoria, Coronilla emerus, Coronilla glauca, Cytisus racemosus, Cytisus praecox, Cytisus striatus, Cytisus scoparius,* and *Spartium junceum*. All plants used were flowering at the beginning of the experiment. Unfortunately, no North American plant could be tested in 2000 as none of the species

available at the laboratory were producing pods at the right time. Only 41 *B. villosus* adults could be collected by three people in two days in the Cevennes by beating *C. scoparius* plants (= 6 person-days). Ten adult beetles were released at the centre of each block on 19 May. However, subsequent results suggest that this number of beetles was swamped by the local resident population of *B. villosus* on *Spartium junceum* at the edge of the garden.

Half of the green pods were collected from the test plants when well-developed seeds were externally visible. The samples were separated by plant species and stored in paper bags in a cold room at about 5° C. The pods were counted for each plant and examined under a stereomicroscope for eggs of *Bruchidius* species. The number of developed seeds and the length of the pod were also recorded. Eggs were classified as fresh (clear), parasitised (opaque), mature (larva visible inside), or hatched. In the case of a hatched egg, the fate of the larva was evaluated by dissecting the pod and seed. Where pod numbers were high, a sub-sample of 50 pods was examined.

The other half of the pods were allowed to fully ripen on the plants, before the brown pods were also collected. This sample was stored for three month to allow complete pod and beetle development. The completion of larval development was investigated by dissection of all pods and the beetles found were mounted and identified to species.

Results and discussion

The presentation of the results for each plant individually has been chosen because of the low number of adults found and the high variability of pod maturation between individual plants (Table 3.1). The numbers of pods in Table 3.1 thus represent half of the total pod number produced, i.e. the pods of the first collection, which were examined for eggs. A total of 229 eggs were counted on six of the nine test plant species. No eggs were found on *Genista monspessulana*, *Cytisus racemosus*, and *Spartium junceum*, although the second species produced the majority of *B. villosus* adults, and the latter plant species is heavily attacked by *B. villosus* in the vicinity of the garden, and in fact, it is thought to be the source of the population found in the broom field plot (cf. Chapter 7). At the time of

examination about half of these eggs (105 of 229) were recognized as parasitised. This would be an underestimate, because very recent parasitised eggs cannot be recognised, and some eggs could have been attacked later on in their development, if the pods had not been harvested.

Table 3.2 summarizes the results by presenting the total numbers of emergence by *Bruchidius* species and test plant species. Forty-two beetles of three *Bruchidius* species emerged out of five of the test plant species. Two genera (*Cytisus* and *Coronilla*) produced adults, whereas no adults emerged from the genera *Genista* and *Spartium*.

Oviposition was expected to have been by *B. villosus* exclusively as it was this species which was released in the open field plot. However, eggs could not be identified to species level, and the emergence of three *Bruchidius* species demonstrated that the plot was invaded by at least two other *Bruchidius* species and makes the value of the release doubtful. The emerging *B. villosus* adults could be the offspring of the released adults or derive from the local population living on *S. junceum*. Moreover the presence of three *Bruchidius* species in the field plot, makes it impossible to know how many eggs of *B. villosus* were laid on the test plant species. Thus, the most important results of the tests are the emergence of adult beetles from the test plants.

Due to the low number of adult *Bruchidius* spp. that emerged from the mature pods collected from the field plot, any conclusions based on these results can only be tentative. However, the results show that *B. seminarius* attacks *Coronilla* spp. and that *Coronilla* spp. are not attacked by the other two *Bruchidius* species. In addition, the observations suggest that *B. villosus* is able to oviposit and successfully develop on species in the genus *Cytisus* and *Chamaecytisus* (see below). Adults of *B. lividimanus* emerged only from *C. scoparius*.

Since some known host species did not produce beetles, the results strongly suggest that some test plant species were not in the right state of pod development during the attack of the beetles. Thus, the local wild-growing population of *S. junceum* was heavily attacked in the vicinity of the garden, but not in the field plot. Similarly, *Genista monspessulana*, a

known host of *B. villosus* may have been flowering too early for a successful attack in the field plot. If one assumes a homogenous population of *B. villosus* with regard to host specificity, then the implications in terms of design and implementation of lab-based choice tests are clear. If potentially suitable host plants are tested at the wrong developmental stage, they are unlikely to be accepted in the trial, whereas if they are presented at the right developmental stage, they will be accepted.

During the course of the open field experiment, pods of *Chamaecytisus palmensis* (tagasaste) and *Genista monspessulana* growing in the adjacent broom field plot and *Spartium junceum* growing naturally in the vicinity of the garden were collected and stored in bags to monitor for emergence of pod-attacking insects. The bags containing tagasaste pods produced 102 *B. villosus* adults, indicating the high suitability of this plant as a host for the broom bruchid. No beetles emerged from the *G. monspessulana* sample, but 29 *B. villosus* were obtained from *S. junceum*. Since *G. monspessulana* is known to be a host of the beetle, these observations seem odd and justify closer observations on the attack rate of *B. villosus* in the broom field plot during the season of 2001.

The specimens of adult *B. villosus* obtained from the garden in 2000, reveal an obvious size difference. The specimens from tagasaste are relatively large probably mirroring the large size of the seeds, whereas the *B. villosus* adult which emerged from *C. scoparius* is very small. This finding indicates that the size of the seed influences the size of the beetle, which develops inside the seed.

Another hypothesis for the unexpected results is that populations of *B. villosus* on different host plants, i.e. *C. scoparius*, *S. junceum*, and *G. monspessulana*, have evolved different host preferences and traits. The results obtained from this experiment do not necessarily show the host specificity of *B. villosus* released as a biological control agent, if the parent population came from *S. junceum*. Thus, additional tests under more controlled conditions using beetle populations collected from different host plants in the field could shed some light on host races and their host specificity. These tests are proposed for 2001.

Table 3.1: Results of the open field plot set up in the laboratory's garden.

Plot 1		First sample					Second sample
	No.	No.	No. eggs				No. and species of
Plant species	rep.	pods	fresh	mature	parasitised	hatched	adult developed
Cytisus scoparius	1	6			1	1	
	2	24	3		15	2	
Coronilla glauca	1	296					4 *B. seminarius*
Coronilla emerus	1	44					14 *B. seminarius*
Cytisus racemosus	1	62					
	2	22					2 *B. villosus*
Cytisus praecox	1	0					
	2	16	4	2	3	1	
Genista monspessulana.	1	84					
	2	10					
Cytisus striatus	1	12					
	2	59	1		1		
Genista tinctoria	1	149	3	4	9	14	
	2	128				1	
Spartium junceum	1	0					
	2	0					

Plot 2		First sample					Second sample
	No.	No.	No. eggs				No. and species of
Plant species	rep.	pods	fresh	mature	parasitised	hatched	adult developed
Coronilla emerus	1	166		1		1	3 *B. seminarius*
Coronilla glauca	1	113	2				
Cytisus praecox	1	4					
	2	1					
Cytisus scoparius	1	3			3	1	1 *B. lividimanus*
	2	9	2		17		
Cytisus striatus	1	50	2		27	5	1 *B. villosus*
	2	55	1	2	2	2	
Cytisus racemosus	1	186					4 *B. villosus*
	2	0					
Genista tinctoria	1	23					
	2	321	2			6	
Spartium junceum	1	0					
	2	0					
Genista monspessulana.	1	0					
	2	0					

Plot 3		First sample					Second sample
	No.	No.	No. eggs				No. and species of
Plant species	rep.	pods	fresh	mature	parasitised	hatched	adult developed
Cytisus striatus	1	72					2 *B. villosus*
	2	27		1		1	
Cytisus scoparius	1	26			4		1 *B. villosus*
	2	6		2		1	
Coronilla glauca	1	57					1 *B. seminarius*
Coronilla emerus	1	41					
Cytisus praecox	1	32	2	8		8	
	2	19	3	2			
Cytisus racemosus	1	52					
	2	64					
Genista monspessulana	1	7					
	2	0					
Genista tinctoria	1	4					
	2	103				7	
Spartium junceum	1	0					
	2	0					

Plot 4		First sample					Second sample
	No.	No.	No. eggs				No. and species of
Plant species	rep.	pods	fresh	mature	parasitised	hatched	adult developed
Cytisus praecox	1	36				4	
	2	1					
Cytisus striatus	1	17				2	
	2	16					
Spartium junceum	1	2					
	2	0					
Cytisus racemosus	1	113					
	2	162					
Coronilla glauca	1	126	3				2 *B. seminarius*
Coronilla emerus	1	672					5 *B. seminarius*
Cytisus scoparius	1	33		3	17		1 *B. lividimanus*
	2	20	2	4	6		1 *B. lividimanus*
Genista monspessulana	1	0					
	2	26					
Genista tinctoria	1	29				5	
	2	182				2	

Table 3.2: Summary of emergence of *Bruchidius* species adults from test plants used in the open field plot.

Test plant species	*B. villosus*	*B. lividimanus*	*B. seminarius*
Cytisus scoparius	1	3	
Cytisus racemosus	6		
Cytisus striatus	3		
Cytisus praecox			
Genista monspessulana			
Genista tinctoria			
Spartium junceum			
Coronilla glauca			7
Coronilla emerus			22

The specimens of adult *B. villosus* obtained from the garden in 2000 reveal an obvious size difference. The specimens from tagasaste are relatively large probably mirroring the large size of the seeds, whereas the *B. villosus* adult which emerged from *C. scoparius* is very small. This finding indicates that the size of the seed influences the size of the beetle, which develops inside the seed.

The eggs of the *Bruchidius* species found in this experiment were always laid directly onto the surface of the pods. The hatching larva bore directly into the pod through the area where the egg touches the pod surface. However, on one occasion an egg on *C. striatus* was found to be attached to the hairs of the pod away from the surface. If the larva needs to penetrate the pod directly from the egg, a dense cover of hair on the pods, as is found in some species, could prevent attack by the beetle. Thus, the eclosion of this egg was observed. The larva hatched and made its way down to the surface of the pod. After some time spent moving around, it did manage to tunnel into the pod successfully. This observation provides evidence that the larva is able to penetrate the pod without the benefit of starting from an egg touching the surface. However, the time spent as a free-roaming larva on the pod will expose the larva to predators and adverse weather, such as heavy rain.

4 *Gonioctena olivacea* (Col.: Chrysomelidae) in the broom field plot

The broom field plot (cf. Chapter 7), in which some 55 adults of *G. olivacea* were released at the end of July 1999, was checked for signs and presence of adults during May 2000. Seven adults, all females, were discovered exclusively on *C. scoparius* plants, whereas no beetles were found on the other test plants. This result is rather surprising, since the beetles could not be found after release in summer 1999. A further 18 adult beetles, which were field-collected on a trip to the Cevennes, were released on the same plot on 15 June 2000.

5 *Asphondylia sarothamni* (Dipt.: Cecidomyiidae)

During March approximately 800 first generation galls of the gall midge were collected on several field trips to the Cévennes and the foothills of the Pyrenées. The galled stems were placed with their stem bases in vials filled with water and held in emergence cages. These cages were stored in a CE room at a temperature regime of night/day (10 hours of light) of 20/25˚C. A total of just 46 adults (20 males and 26 females) emerged over a time span of five weeks (16 March – 25 April) and were transferred into rearing cages placed in a greenhouse. Two or three potted *C. scoparius* plants were exposed to several gall midge pairs per cage. Although eleven plants were exposed to several pairs in different cages, as in 1999, for unknown reasons not a single gall was produced.

Therefore, about 1,100 second generation galls, which are produced in the pods, were collected on field trips to the Cévennes on 30 May and 8 June and kept under various conditions at the laboratory.

Emerging adults were used for single-choice oviposition tests. One potted test plant was placed alongside a Scotch broom plant as control in a cage and exposed to several gall midge pairs. The number of adults used was variable depending upon the adult emergence in the rearing. Due to the extremely short life span of the adults of about one to three days, obtaining pairs was crucial. However, only replicates which received several pairs were included in the test. Four replicates each were carried out using tagasaste and *Lupinus arboreus* and three with *Crotolaria cunninghamii*. After the death of the adults the plants were labelled and stored outside in the laboratory's garden. Unfortunately, checks during the winter for gall development on the plants were not successful and it appears that the gall midges did not induce any galls on the test plants or on the control plants. In conclusion, the requirements for oviposition are still not understood and despite variations of rearing techniques a successful breeding for more than one generation has not yet be achieved.

Two males and seven females were also released on the broom field plot (see also Chapter 7). A sleeve cage was erected enclosing the ten central *C. scoparius* plants of one plot to facilitate oviposition under the most natural conditions possible. The success or

failure of this release will be assessed in spring 2001. If successful, large caged multiple-choice tests should be used for future host specificity tests.

6 *Hexomyza sarothamni* (Dipt. Agromyzidae)

This stem gall inducing agromyzid fly was chosen for further investigations of its potential as a biological control agent against Scotch broom, because it attacks a vacant niche in the exotic range of the weed. Because no other stem-galling agents have been used, this is likely to prevent or reduce competition with other agents, and to have an additive impact on plant health and vigour. Furthermore, many agromyzid flies have the dubious distinction of being "successful" economic pests (i.e. can have significant impact on their host plants) and are in general highly host specific. The feeding of *H. sarothamni* females may also contribute to the impact by opening wounds which allow entry of fungi or other pathogens. The number of such wounds made per female can be surprisingly high. For another agromyzid fly, *Phytomyza vitalbae*, released as biological control agent against old man's beard (*Clematis vitalba*) in New Zealand, the number of feeding punctures made by a female during its life span exceeded 5,000 (Wittenberg & Schroeder 1993).

Rearing

During December 1999 and January 2000 several field trips to the Cevennes were made. The original intention was to cover *Hexomyza sarothamni* stem galls by wrapping them in gauze, in a similar way as the previous winter but earlier, so as to investigate the time of attack by parasitoids. However, on the first trip on 2 December 1999 small plants with galls were discovered, which could easily be dug out and brought to the laboratory, where they were potted and pruned. On four field trips (2 and 9 December 1999 and 11 and 19 January 2000) 128 plants harbouring 293 galls were collected and transferred to the laboratory's garden. The plants were kept in a large cage to prevent emerging parasitoids dispersing and attacking other experimental plants held at the laboratory. The plants were then checked at a weekly interval for the appearance of "windows" in the galls. These "windows" are created by the larvae before entering the pupal stage and provide an exit route for the emerging adults to leave the gall. When windows appeared, the plants were transferred into smaller cages inside a CE room (day/night temperatures of about 24/18° C) for emergence. Two different treatments were used to monitor emergence; half of the potted plants containing galls were placed into one large cage, while the galls of the other

half of the plants were individually enclosed. Small plastic tubes were slipped over the galled branch and the ends were closed using foam stoppers.

Between 11 April and 13 June, 40 males and 43 females emerged from the 293 galls, giving an emergence rate of 28.3 %. Ninety of these galls were kept individually and their emergence could be followed in more detail. Table 6.1 presents the numbers and percentages of the emergence for males, females and parasitoids (as yet unidentified). The emergence period of males and females is illustrated in Figure 6.1.

Table 6.1: Emergence of *Hexomyza sarothamni* adults. Plants with galls were collected during December 1999 and January 2000 in the Cevennes.

	Total number of galls	Emergence of				No Emergence
		Male	Female	Total flies	Parasitoids	
Number	90	21	14	35	25	30
Percentage	100	23.3	15.6	38.9	27.8	33.3

Rather surprisingly, the adults emerged about five weeks earlier than in 1999 as reported in Wittenberg and Thomann (2000).

Emerging adults were either transferred into rearing cages to produce galls for spring 2001 or were used in multiple-choice oviposition tests (see below). In addition, at the beginning of July five males and ten females were released into a tent covering a central Scotch broom plot on the broom field plot (see Chapter 7).

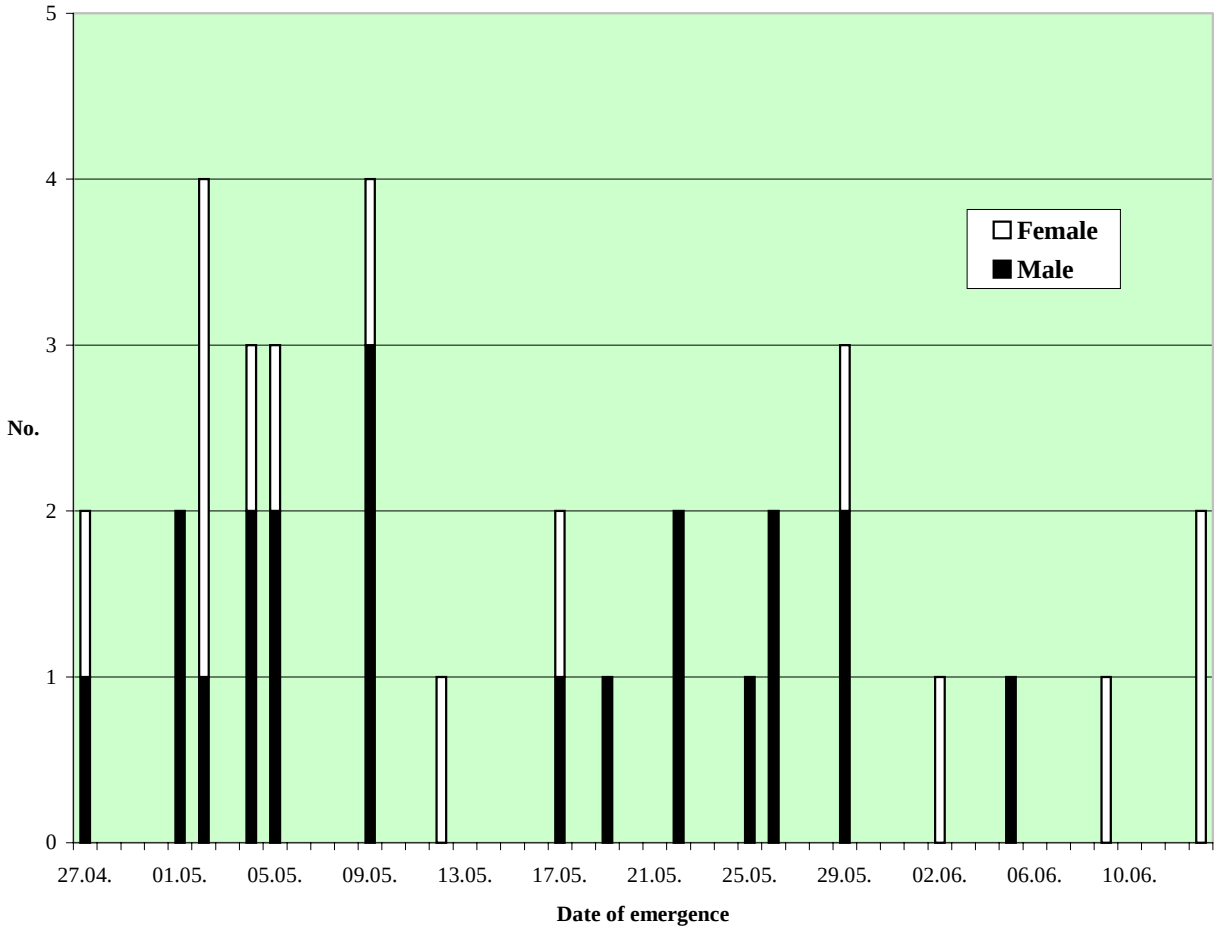

Figure 6.1: Emergence of *Hexomyza sarothamni* adults from galls. Plants with galls were field-collected during December and January in the Cevennes.

Although these galls were collected during the middle of winter, they produced parasitoids as well as flies, thereby confirming that the parasitoids do attack the galls in the year of gall induction (and possibly also the following spring as well).

The plants which were exposed to *H. sarothamni* adults in rearing cages are being kept in the garden of the laboratory. At the latest count on these plants during March 71 galls were evident. The adults are expected to emerge in spring 2001.

Adult beetles emerged about 40 days after the windows had appeared in the gall (male: 39.62±1.85 (n=21) and female 40.60±3.34 (n=15)). This period indicates the length of the pupal stage.

Specificity tests

Specificity trails were set up using potted plants placed in cages in a CE room with a day/night (16 hours of light) temperature regime of approximately 24/18° C. Pairs of the flies were added as emergence made them available, resulting in different numbers of pairs used per trial. While all test plants were used as potted plants, in some cages the control plant *C. scoparius* was not added as a potted plant. A branch of a mature plant was fitted into the cage through a sealed hole in one wall. All plants were labelled and stored outside.

However, gall development has been recorded only in one replicate. This replicate was one where a branch of *C. scoparius* was inserted into the cage, and three galls developed on this branch. The test plants used, three *Lupinus rivularis* and three *L. albifrons*, did not show any sign of attack.

The inconsistent oviposition behaviour of the females presents problems for the specificity testing. Whereas some females readily oviposit on their host plant, many females do not lay eggs. To overcome these irregularities test would have been to set up in many replicates and/or with more flies per replicate. However, the relatively low numbers of adults available so far, mean progress will be slow. Improved culture methods and more extensive field collections, including new field sites, should increase the number of flies available in future seasons.

7 Broom field plot

The broom field plot, as described and drawn as a sketch in Wittenberg and Thomann (2000), was maintained in the laboratory's garden. During the winter the plots were covered with plastic to prevent frost, because tagasaste is not frost-resistant. The plants are developing very well and reached a vigorous state of growth (see Plate 3 of the Annex).

In May every plant in the plots was beaten to check for the presence of *Gonioctena olivacea.* Seven females were found on *C. scoparius,* whereas the other plants showed no sign of the beetle. Moreover, *Aceria genistae* galls (another species released in 1999 - see Wittenberg and Thomann (2000) for details) were found on two *C. scoparius* plants, where they had originally been released, and on one other *C. scoparius* plant in a distant corner of the other plot.

Several additional releases were made during the 2000 season. In addition to the 55 *Gonioctena olivacea* adults released in 1999, another two males and 16 females were added on 15 June (see Chapter 4). On 28 June 70 *Exapion fuscirostre* (Col.: Apionidae) adults were released in block 1. At the end of June two males and seven females of *Asphondylia sarothamni* were released into a tent placed over the ten central *C. scoparius* plants of block 2. Two weeks later five males and ten females of *Hexomyza sarothamni* were released into the same tent. At the beginning of August some 88 *Agonopterix assimilella* eggs and first instar larvae were transferred onto the central Scotch broom plants of block 1 (cf. Chapter 2).

The vigorous growth of the plants makes monitoring of the attack by phytophagous arthropods more and more difficult, but in 2001 monitoring of the establishment of all released organisms will be continued. The plants within the rows touch each other not only intra- but also inter-specifically, allowing less mobile organisms to move around more easily.

8 Work plan for 2001

It seems unlikely that the full four-years work programme presented in 1999 can be followed unless the level of funding improves. The focus in 2001 should therefore be on the prioritised potential agents, on which work is already advanced. Thus, host range testing of *Hexomyza sarothamni* and *Asphondylia sarothamni* will be carried out using as many test plant species as possible. In addition, some specificity tests of *Bruchidius villosus* will be carried out to shed some light on past problems with interpretation of test results.

Biological control agents	2001 field season
Aceria genistae (Eriophyidae)	- Evaluate the spread of the mite population between the plants of the host specificity field plot - Collect material for genetic studies, to help to solve the species complex problem.
Agonopterix assimilella (Oecophoridae)	- Evaluate spread and attack rate on plant specimens used in the field plot
Asphondylia sarothamni (Cecidomyiidae)	- Try to maintain/establish breeding colony at the laboratory - Use both generations for host screening under various conditions
Bruchidius villosus (Chrysomelidae)	- Follow establishment rate, spread, and attack of the beetle on the individual plants used in the field plots - Carry out host specificity tests - Maintain and evaluate an open field plot in the laboratory's garden
Exapion fuscirostre (Apionidae)	- Follow establishment in the field plot
Gonioctena olivacea (Chrysomelidae)	- Follow patterns of spread between plants in the field plot and evaluate host preferences
Hexomyza sarothamni (Agromyzidae)	- Conduct additional cage tests on host specificity and finish testing with plants available - Check attack of plants in field plot

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Annex

Plate 1: Scotch broom (*Cytisus scoparius*) and gorse (*Ulex europaeus*) in a river bed near Darfield, South Island, New Zealand; 17 November 1994, against a backdrop of the majestic Southern Alps.

Plate 2: *Asphondylia sarothamni* female next to galled Scotch broom (*Cytisus scoparius*) pod with emergence hole. Note the stunted growth of the distal end of the pod.

Plate 3: Broom field plots in CSIRO European Laboratory garden at Montferrier sur Lez, France, on 15 September 2000.

Plate 4: The authors examining Scotch broom plants in the Cevennes.

Plate 5: Stem gall of *Hexomyza sarothamni* on *Cytisus scoparius* before forming the "window".

Distribution:

Dr. S. Fowler (5)

Dr. T. Butler (5)

Dr. L.E. Lantz (2)

Dr. M. Pitcairn (3)

Dr. A.W. Sheppard (5)

CABI Bioscience Centres (5)

CSIRO European Laboratory