CABI Bioscience

A Division of CAB International

Annual Report

Investigations on potential biological control agents of Scotch broom, *Cytisus scoparius,* January – December 1999

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Sponsored by: Landcare Research, New Zealand, CSIRO and NSW Agriculture, Australia, and the Oregon Department of Agriculture and the Washington State Noxious Weed Control Board, USA

Weed Biological Control Programme

Biological Pest Management

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February 2000

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Summary

The main objectives of the work programme in 1999 were
to initiate laboratory breeding colonies of one or both of the two gall forming insects

1 Introduction

This report summarises the investigations on selected potential arthropod agents for biological control of Scotch broom (*Cytisus scoparius*), a shrubby legume of European origin. It was widely grown as an ornamental and has escaped from cultivation. Today it is a serious pest in New Zealand, Australia, parts of the U.S.A., and other countries around the globe. Mechanical and chemical control can be applied successfully, but these methods are often too cost intensive and unpractical 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.

At the end of June a report was prepared covering specific work required by the Washington State Noxious Weed Control Board. At the same time a progress report was written reporting on the work programme and the progress until the International Weed Conference in Bozeman, Montana. It would be desirable to prepare only one technical report a year presenting all work done in one year.

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 yet to be studied for biological control of broom are two gall-forming insects, which were the main focus of the programme in 1999 (chapters 4 and 5). These insects proved to be difficult to rear in previous years, thus the main focus was to establish breeding colonies of these two insect species at the laboratory. These breeding stocks will be used for host range testing in order to validate host records of the literature.

2 *Aceria genistae* (Acarina: Eriophyidae)

Previous host screening with this gall forming mite has produced extremely promising results (Paynter & Jourdan 1998). However, during these tests some survival occurred on tagasaste plants, which is a plant of growing economic importance in Australia and New Zealand. Hence additional field tests are crucial. It should be noted that there are some indications that we are dealing with a complex of highly specific sibling species, rather than a single species. This taxonomic problem needs to be addressed.

Galls were field-collected near Mandagout from *C. scoparius* plants on August 2 and left to dry for five hours in the laboratory before they were tied onto the Scotch broom plants of field plot 2 (cf. chapter 8). The plants were still in pots, thus, the entire plants were brought inside a CE-room for four days to avoid direct sunlight. The galls were cut from the branch and with the aid of a stereo microscope checked for *A. genistae* and predatory mites. The latter were removed with a fine paintbrush, as thoroughly as possible, but they could not be removed when they were actually inside the galls. Five galls containing *A. genistae* were tied to each plant. The entire branch with the attached gall was held in a plastic bag, the inner surface of which was misted with a water sprayer, and sealed. The plants were kept for four days under these humid conditions to allow the mites to wander onto the new host plant. Thereafter the plastic cover and the original gall were removed and the plant was replaced in the same spot in the field plot. Three days later tagasaste plants from the same plot but a different row were infested with the mite using the same method. The success rate of the transfer can only be monitored in spring 2000, since the galls will not be obvious before that time.

3 *Gonioctena olivacea* (Col.: Chrysomelidae)

At the end of July, 55 adults of this chrysomelid beetle were collected in the field near Mandagout (for details of site cf. page 8) by beating *C. scoparius* bushes. After return to the laboratory five or six adult beetles were immediately released on each Scotch broom plant of the central row in plot 1 of the field test. The beetles were not found again on the plants, when the plot was searched for their whereabouts. It is possible that the beetles left the plants after their release, because the *C. scoparius* plants were still rather small.

4 Rearing of *Asphondylia sarothamni* (Dipt.: Cecidomyiidae)

4.1 Field collections and rearing

Material and methods

Starting in February and continuing through April several field trips to the Cevennes north of Montpellier were made to collect *Asphondylia sarothamni*. Attack by this species is reasonably easy to recognise as the galled flower buds are deformed and swollen. Branches on which galls were found were cut in the field and transferred to the laboratory. The stems were inserted into vials filled with water, and the branches kept in cages and checked for emergence of adult gall midges several times a day. The freshly emerged adults were transferred immediately onto potted broom plants in cages, preferably several pairs per cage. In addition, in order to provide varying conditions for the adults two mature broom plants planted in the garden were each covered with gauze cloth and several gall midge pairs released. Honey bees and bumble bees obtained from the garden were added to ensure pollination of the flowers, since the second generation galls of *A. sarothamni* are formed in the pods, thus, a normal development of

the flowers is required. Unfortunately, for unknown reasons no galls were found on any of the exposed plants.

Second generation pod galls were collected in the Cevennes at the beginning of June and brought to Montpellier for emergence of second generation adults. The emerged adults were transferred onto potted plants in CE rooms with a day/night temperature regime of about 26/21º C, since it was suspected that the fluctuating temperature with low and high peaks might have played a crucial role in inhibiting first generation oviposition.

Results

A total of 29 males and 67 females emerged out of the bud galls between May 3 and 22. This number was sufficiently high to put several pairs together on plants in four cages and the two tents erected in the garden, although the adult life span was extremely short at just one or two days. At that time there was a shortage of plants in the suitable stage, i.e. flowering plants, but some flowers were exposed to the gall midges in all cages. However, for unknown reasons no galls were produced on the plants.

Second generation adults, obtained by collections of pod galls, were kept on plants of different age and size to vary the conditions for the gall midges. Unfortunately, many plants died rapidly in the CE room after exposure to the gall midges. The reason for the mortality remains obscure. The surviving plants are being kept at the institute and will be checked for bud galls early 2000.

Discussion

There are several possible reasons why the establishment of a breeding colony of *A. sarothamni* in the first generation failed. There was a shortage of suitable plants at the institute with a timely flower development. A wide variety of plant ages and stages of potted broom plants were chosen and exposed to adults obtained from field-collected bud galls to provide adults with several different potential oviposition sites, since it is not known in detail which stage of the reproductive organs females prefer for oviposition. The plant health and chemical composition using potted plants is also a concern. However, the reason why not a single gall was produced despite the effort in exposing various plants to several pairs of the gall midge remains obscure. It is not yet known whether the females of the second generation produced eggs, because the bud galls will not develop before early spring 2000.

4.2 Behavioural studies

Periods of continuous observation of *A. sarothamni* adults were made as well as casual observations between other work activities. These observations were to improve understanding of the gall midges' basic needs so as to improve the rearing methods. Behavioural studies conducted on May 4 for a period of 24 hours revealed a marked increase in

adult activity during the evening and also the morning. Three males and one female were released into a cage containing a small Scotch broom plant and observed under natural light conditions in a temperature-controlled room for 24 hours. The flies' behaviour was constantly monitored during this time, except for the darkness when no observation was possible. There was a considerable increase in activity from 20:00 onwards until the light ceased. At 5:30 of the following morning the light was bright enough to allow observations. The gall midges were highly active again (or still). Activity decreased at around 10:00 a.m., but the difference in activity was not as clear-cut and obvious as in the previous evening. The question as to whether the adults are active all night remains uncertain. During the observation described here it was decided against the use of light during the night so as not to disturb the natural behaviour. An attempt to use a red light source to observe nocturnal activities will be made during summer 2000.

One copulation was observed at 07:08 in the morning on the roof of the cage. It lasted for 15 minutes and 2 seconds. It was a back-to-back position with the bodies in an angle of 50º to each other. On May 7 oviposition or oviposition attempts were observed. The female walked up and down stems and probed into small buds. She used her antennae straight down on the surface to locate the right spot for insertion of the ovipositor. Females always probed with their head facing downwards. Probing took about 10 seconds. These are only preliminary studies; more observations are needed to quantify the results presented here.

5 Breeding of *Hexomyza sarothamni* (Dipt.: Agromyzidae)

The two gall producing insects were identified as a major focus of the current and future broom work, since this niche is not yet occupied in the areas of introduction. Agromyzid flies in general are highly host specific and many economic pest species cause considerable damage to their respective host plants. Additionally, many agromyzid species are controlled very effectively by natural enemies in their natural distribution, and released from these parasitoids they have the potential to build up high populations in a comparatively short period. The chosen species for biological control of broom, *H. sarothamni*, induces stem galls on the fresh growth of *C. scoparius*. In addition, females damage the plant by their feeding and it is anticipated that the small feeding holes made enhance penetration by pathogenic fungi.

Field collections

In order to initiate a breeding colony of *Hexomyza sarothamni* the first objective was to collect this insect species in the field. Galls were thought to be the most appropriate stage and were collected at several field sites in the Cevennes about 2 hours drive north of Montpellier. Most of the sites were alongside the road D 329 gradually climbing the mountains from Pont d'Herault (Alt. 190m) via Mandagout (44º 01'N, 3º 38'E) to L'Esperou (Alt. 1230m). In addition, on one occasion galls were collected in large stands of Scotch broom adjacent to the D 113 (43º 96'N, 3º 36'E) at an altitude of 530m just east of Montdardier. Since there is a continuum of host plants along the roads, the field sites are not regarded as separate populations of the insects but rather as one population with exchange between the sites. Although *H. sarothamni* is rather rare, collections were quite successful with four to seven galls collected per person-hour, since the mature galls are easily recognisable.

No distribution pattern such as preferences for specific oviposition sites could be assessed due to the comparatively small number of galls, which could be found. However, often two or more galls per attacked plant were found when adjacent plants did not show any signs of attack, indicating a clumped distribution of galls. Once one gall was spotted, up to six galls could be found in its close vicinity. It is speculated that the females stay for a certain time in a small area within a bush and oviposit therein before moving to another place. This behaviour was observed in another agromyzid species on *Clematis vitalba* (Wittenberg & Schroeder 1993). Since moving between plants comprise a higher risk, the strategy to lay eggs in a clumped pattern is likely to reduce this risk of detection by predators and thus increases the number of offspring. On the other hand the increasing likelihood for aggregated galls to be found by a parasitoid will select for spreading the eggs more equally. Detection of galls (for humans) becomes more difficult as the season proceeds because the growth of new plant material conceals the previous year's stems. On the other hand young galls are less conspicuous and subsequently harder to find.

During the month of June some 200 stem galls from different altitudes were collected in the field and placed either in a small cage or in individual boxes in water-filled vials to allow adult emergence. The crucial point for collection is to cut the stems with galls at the right moment. It was supposed that flies in galls exhibiting a small, round area of thin tissue resembling a window (apparently just the layer of epidermis cells) are in the pupation stage and would not suffer from stem cutting. The larvae seem to prepare this area for subsequent emergence before they pupate. If the material were collected too early the cut stems would not support the development of fly larvae in the galls. On the other hand a late collection would result in many empty galls from which adult flies had already emerged. The occurrence of the agromyzid in the mountains is an advantage because the developmental stages vary corresponding to the altitude, i.e. a collection of galls from a higher altitude will yield earlier stages of the fly, and vice versa.

Experiment to reduce parasitism

On several field trips to different sites near Montpellier between the end of February and the beginning of March, 106 young *H. sarothamni* stem galls were detected and sleeved by wrapping them in gauze to try to prevent or reduce parasitism. The protection by the gauze wrapping should prevent access to the galls or inhibit attack by parasitoids during spring and early summer until pupation of the larvae takes place. The details of the sites with sleeved galls were as follows:

⇒ Les Aires near Lamalou (43º 35'N, 3º 06'E; Alt. 180m): 47 galls on 15 plants.
⇒ South of Mandagout (cf. page 8): 9 galls on 5 plants.
⇒ Mandagout (as above): 22 galls on 12 plants.
⇒ North of Mandagout (as above): 28 galls on 14 plants.

During field trips in June the sleeved galls were collected and brought to the laboratory. Most of the galls were held in emergence boxes to obtain adult flies, but some were dissected to assess the situation inside the galls. From 16 galls dissected only one exhibited the normal emergence signs, i.e. the specific opening of the puparium by an *H. sarothamni* adult, whereas four died in an early stage, chalcidoid parasitoids emerged out of another four, three were inhabited by a weevil (*Pirapion immune*?), an unknown parasitoid emerged and left the destroyed puparium behind in one case, and three were either not galled at all or died very early. From these dissections it is apparent that wrapping the galls did not prevent parasitism. Either attack by parasitoids occurred at a very early stage of gall formation in the previous year (1998) or parasitoid behaviour and performance was not significantly altered by the wrapping.

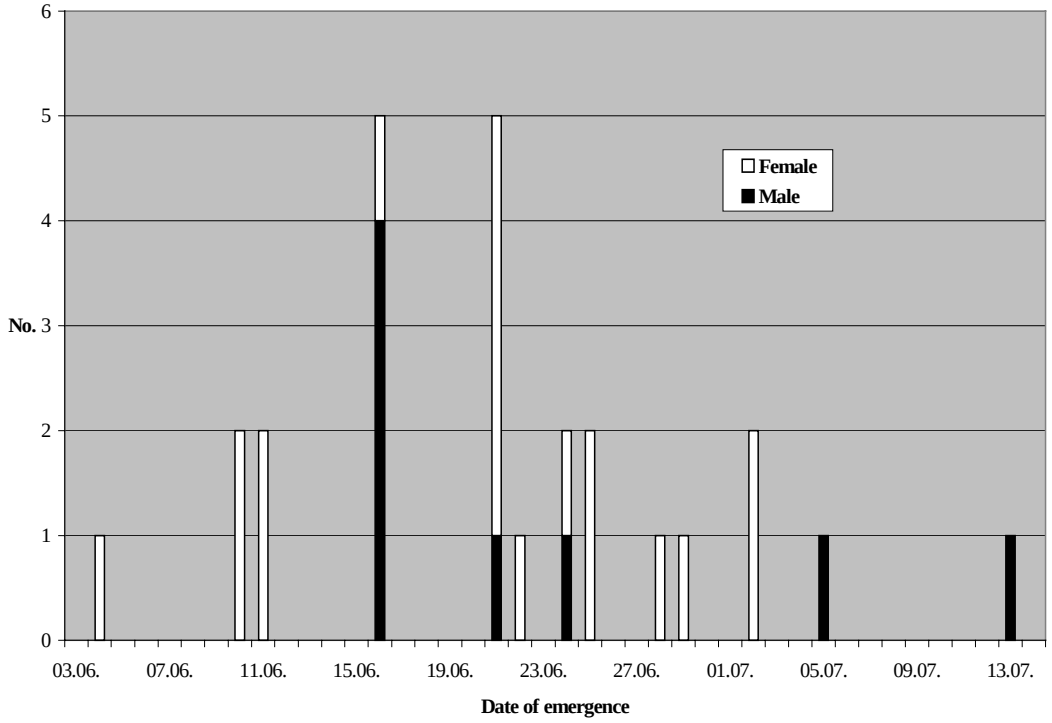
During November, galls were again protected in the field, this time using two different methods, and plants with galls were transferred into the laboratory, to investigate the failure for protection in spring. Besides the gauze wrapping described above, galls were protected by a plastic tube closed at both ends with a foam stopper, which allows the normal growth of the stem and prevents any access to the galls by parasitoids. Seventeen of these wrapped galls, collected at Les Aires on the 22nd of June, were kept individually in small containers (cf. next paragraph). Only one female emerged seven days later.

Emergence of adults from field collected material

The cut stems with galls were put into small vials filled with water and were kept in plastic containers (33x42x42cm) covered with gauze. These cages were placed in a CE room with a day/night temperature regime of about 24/18º C. In order to obtain more information about the fate of the individual galls a second design was also used for emergence of adults. Individual cut stems were placed into a vial with water and kept in small plastic

cylinders (13 cm in height and 10 cm diameter). Emergence of adults was checked several times a day. A total of 27 adults (8 males and 19 females) emerged out of both container types between June 4 and July 13 (Figure 5.1).

An evaluation of the emergence rate was possible for the individually stored galls, whereas no detailed results were obtained from the larger containers. One hundred and sixty-four galls, collected at several sites described earlier, were kept individually. Unfortunately only nine adults emerged (about 0.5%) – seven females during June and two males in July. After emergence had ceased and no further emergence was expected, all individually kept galls were dissected. The results of the dissections are summarised in Table 5.1. In the majority of cases it was possible to determine the fate of the gall, however in some cases due to poor condition of the plant material it was impossible to trace what had happened. In addition to the nine adults emerged during the rearing, 13 flies had emerged prior to collection in the field, and another eight flies died before or during emergence. Since the adult flies do not always create an obvious hole in the gall during emergence, galls showing a slit in the window of the gall were collected rather than discarded, this led to the collection of empty galls. The occurrence of dead flies inside and outside the puparium suggests a natural mortality during emergence increased by artificial conditions in the rearing facility, such as humidity. At the end of the emergence period the humidity was probably too high, judging by the mould on the plant material. Therefore the rearing boxes will be changed slightly for rearing the agromyzid fly in the 2000 season, thereby providing a drier environment.



Empty puparia found in the galls could be used to determine what emerged, because agromyzid flies and parasitoids have a different way of opening the puparium. The flies, belonging to the suborder Cyclorrhapha, develop a specific structure, an inflatable membranous sac called ptilinum, with which they break open the puparium at a specific

circular slit, allowing a cap to lift from the head end, whereas the parasitoids don't have this structure and bite their way out of the puparium. Two different parasitoids emerged out of the *H. sarothamni* galls, i.e. unidentifed braconid and chalcid species. In 32 cases the fate could not be determined for various reasons. The early deaths of three *H. sarothamni* larvae in their young galls suggest that a defence mechanism of the plant may have caused some mortality.

Table 5.1: Results from dissection of individually kept *Hexomyza sarothamni* galls, collected in the field.

Male emerged	2
Female emerged	7
Fly emerged prior to collection	13
Fly dead in puparium	6
Fly died during emergence	4
Puparium dead	17
Parasitoid emerged prior to collection	16
Braconid emerged	26
Chalcid emerged	14
Fly or parasitoid emerged	4
Parasitoid dead in puparium	2
Parasitoid larva in puparium	14
Gall empty	4
Larvae dead in early stage	3
No gall	6
Undeterminable fate	32

To summarize:
Total of galls 164
From the 104 whose fate could be determined 72 (69%) were parasitised and 32 (31%) agromyzid flies emerged
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Rearing of *H. sarothamni*

Emerged adults were paired and put either into plastic boxes (33x42x42cm) covered with a gauze top and containing between two and six *potted C. scoparius* plants, or into similar but larger cages (33x42x52cm) using a single older host plant. In the latter design only the plant was covered by the cage whereas the pot was placed into a hole in the base of the cage. The soil surface around the plant was covered with a thin layer of plaster of Paris to prevent ants and other predatory arthropods in the soil ascending onto the plant. Rearing was carried out in CE rooms with a day/night temperature regime of approximately 24/18º C. The plants used in these trials were exchanged at irregular intervals to offer fresh oviposition sites to the females.

Pollen, paper strips dipped into honey-water solutions, and water supplies were added to the cages to allow feeding and drinking by the adult flies. After two days, several dead flies were found. Several hours of observation suggested that the flies were not finding the food and water supplied. Therefore the flies were taken out of the cages and transferred into Petri dishes for a daily feeding period of one to two hours. When the flies were placed in the

Petri dishes for the first time, they immediately commenced feeding on the honey-water strips. This procedure was repeated daily and the survival of the flies improved. The longevity of the adults however could not be evaluated due to the difficulties in finding the tiny flies in the cage.

Some preliminary observation studies were made of the adult behaviour to assess their needs and preferences for food, shelter and oviposition sites. These observations will help to optimise the rearing. In one cage copulation was observed. Feeding of the females on the broom plants was also observed. The female bores a hole into the fresh tip of a broom plant with her ovipositor, then moves backwards and imbibes the sap exuding from the plant surface. This feeding may be important for the egg development (oogenesis) and subsequent host choice for oviposition, as shown for an agromyzid fly species in Schwarzländer *et al.* (1996). Long-term observation of adult behaviour is required to locate the preferred oviposition site.

The plant material that was exposed to the flies is being kept under near natural conditions at the laboratory. Until December only seven well-developed galls could be detected. It is hoped that some more galls will start developing in early 2000.

Host specificity investigations

No oviposition choice tests have been carried out, because too few adult pairs were available. It was decided to concentrate on starting a breeding colony rather than disturb the females at this critical step.

Discussion and conclusions

Gall formers in general, including members of the agromyzid family, are normally narrowly host specific. Literature records for this particular species seem to justify optimism for a suitably restricted host range. An agent feeding on shoots is probably the best addition to the agents already introduced which attack pods and foliage. Gall formation is considered to be damaging to the plant and can kill stems (Syrett *et al.* 1999). If released from its high parasitism rate, the agromyzid fly may well be more numerous as an introduced species than it is in its native range – although adaptation by indigenous parasitoids cannot be ruled out.

For the first time galls were collected in sufficient numbers that several fly pairs could be obtained at the same time. Behaviour such as feeding on the host plant by females was apparently not affected by the cages used, but more importantly, copulation was observed under cage conditions. At this stage the question of to what extent the females accepted the plants for oviposition remains unsolved. However, it is hoped that a breeding colony has been successfully started and will be maintained for emergence from parasitoid-free galls in 2000.

6 Host specificity tests with *Agonopterix assimilella* (Lep.: Oecophoridae)

Material and Methods

At the end of May some 200 *Agonopterix assimilella* larvae were collected at L'Esperou in the Cevennes (for details of site cf. page 8) and brought to the laboratory. Branches with cocoons of the mature larvae were stuck into pots containing *C. scoparius* plants to allow larvae to wander onto the fresh plant material or pupate in the soil of the pots. The pots were kept in rearing cages in a CE room. Emerged adults were transferred into a cage containing potted broom plants, where they were kept until they commenced oviposition after a prolonged aestivation time. The cages were supplied with cut flowers, paper strip dipped in honey solution and water at three-days intervals. The first eggs were found at the beginning of August. Some of the eggs were left in the cage, whereas others were transferred into Petri dishes and kept in different places to spread the risk of fatal incidences. Freshly hatched larvae were used to perform L1 starvation tests with the only test plant outstanding for this agent, i.e. *Clianthus puniceus*. The tests were set up in similar way as last year (Paynter *et al*. 1998) to keep the results comparable. Three newly hatched L1 were transferred, using a fine paint brush, onto a cut sprig of either *C. puniceus or C. scoparius*, which was placed in a Petri dish on top of a layer of damp filter paper. A separate Petri dish and sprig was used for each replicate. Five replicates each were carried out. It was planned to carry out the tests until the larvae would have completed their development. However, three days after the tests were started, on 29 August, when the larvae were checked for the first time, the results were so convincing that the tests were terminated.

Results

Three days after test begin, the fate of the larvae, as well as damage to the plant and frass production was recorded. The results (Table 6.1) were clear-cut. All larvae on the control plants were alive, feeding under a web and producing frass. All larvae transferred to a *C. puniceus* sprig were dead (one was not found) and with the exception of two larvae there was no sign of frass or feeding damage. Two larvae seemed to have eaten to avoid starvation but were not able to use the plant as valuable food and died subsequently. Since all larvae on the test species were dead, the specificity tests were terminated.

Table 6.1: Results of L1 no-choice tests with *Agonopterix assimilella* (after three days).

Test plant	Replicate	Frass	Damage	No. larvae	
				dead	alive
Cytisus scoparius	1	+	+	-	3
Cytisus scoparius	2	+	+	-	3
Cytisus scoparius	3	+	+	-	3
Cytisus scoparius	4	+	+	-	3
Cytisus scoparius	5	+	+	-	3
Clianthus puniceus	1	-	-	3	-
Clianthus puniceus	2	+	+ (2 larvae)	3	-
Clianthus puniceus	3	-	-	3	-
Clianthus puniceus	4	-	-	3	-
Clianthus puniceus	5	-	-	2	-

+ = yes; - = no

Discussion

First instar larvae of *A. assimilella* were not able to survive on *Clianthus puniceus* and died quickly. These results together with previous starvation tests (Paynter *et al*. 1998) confirmed that the experimental host range of this moth species is restricted to the tribe Genistae.

Host specificity investigation under open field conditions

The moth species *A. assimilella* was included in the field trial (cf. chapter 8) in the garden of the laboratory. Young larvae not used for the laboratory specificity tests were collected and transferred onto the Scotch broom plants in the field test. L1 or L2 were dissected out of ties found in the rearing. Five larvae were transferred with a fine brush onto each of the *C. scoparius* plants in the central row of one plot. A total of 45 larvae was released on August 31, since one plant was found dead. To avoid predation by ants and drying up in full sunshine the larvae were released in the evening. However, the larvae could not be found on a subsequent search, thus, it is assumed that they did not survive. If the insects are not established it is planned to release mature larvae on the plot during summer 2000.

7 Other phytophagous species on Scotch broom

Several other species were encountered on field trips, two of higher importance in regard to future biocontrol. Two *Phyllonorycter scopariella* adults (Lep.: Gracillariidae) were found at two sites in the Cevennes. This stem-boring moth species is recorded only from *C. scoparius* and is expected to be highly specific as typical for this genus (Emmet 1988).

Several sawfly larvae were collected in the field, transferred to the laboratory, and fed on Scotch broom until they

entered the soil provided in a plastic box. These boxes are kept for hibernation and emergence of adults will hopefully reveal the identification of the species. Two *Rhogogaster* spp., regarded as highly host specific, are known to occur on Scotch broom (Syrett *et al.* 1999).

8 Field trial in CSIRO garden at Montferrier

A field trial for investigation of the host range of several agents and potential agents for the biocontrol programme against *Cytisus scoparius* was designed and prepared in the garden at the CSIRO laboratory at Montferrier. This experiment was set up in close co-operation with Dr. Andy Sheppard (CSIRO), who also rendered it possible with additional funding. The soil in the garden was prepared (an acidic garden soil was mixed with sand in a proportion of 2:1 and filled in the rows for about 60cm depth) as two plots consisting of three rows of flowerbeds each – the rows are approximately 11m long. The soil at Montferrier is rather calcareous and therefore not suitable for most broom species. Three test plant species, i.e. *Chamaecytisus palmensis* (tagasaste), *Genista monspessulana* (French Broom), and *Cytisus scoparius* (Scotch broom) were chosen. The design includes 60 potted individuals of each plant species (30 per plot). The potted plants were put into the soil in a block design of three blocks - one block of each species with ten individual plants - per flowerbed row (Figure 8.1). The space between the rows is 1.5m. The single plants are spaced 70cm apart in one row. There is a gap of 1.1m between the blocks of the different plant species. The two plots are identical in design and separated by only 3.5m. It is planned to release insects always on the central block of *C. scoparius* and follow the spread of the insects into the other blocks. During winter the field test is covered with two horticultural tunnels to allow tagasaste to survive during the cold time of the year. At the beginning of the tests the plants were put into the soil in pots, but before the tunnel were erected the plants were planted directly into the soil at the same spot they were placed before.

Forty-five L1 and L2 of *Agonopterix assimilella* were released in plot one on the 31st of August. Unfortunately the moth larvae were not found again on a subsequent search several days later. There is a high likelihood that ants, which are common on plants, consumed the very tiny larvae. The larvae were released in the evening to reduce the threat from ants and dry sunny conditions. If the insects are not established it is planned to release mature larvae on the plot during summer 2000.

Fifty-five adults of the beetle *Gonioctena olivacea* were released on the same plot at the end of July.

The mite *Aceria genistae* was released on the central *C. scoparius* block on plot 2 on August 10. Due to the small size of the mite, rather than transfer individuals, five entire galls were tied on each of the ten plants (for details see chapter 2). Because the mites are comparatively slow to spread, it was decided to release the mite on tagasaste as well. Using the same method as before they were released on the same plot but in a different row on *C. palmensis* (on August 13). Induced galls on the plants will not be obvious before next spring. Hence an evaluation of the release has to be postponed until then.

Cytisus scoparius	*Genista monspessulana*	*Chamaecytisus palmensis*
Chamaecytisus palmensis	*Cytisus scoparius*	*Genista monspessulana*
Genista monspessulana	*Chamaecytisus palmensis*	*Cytisus scoparius*

9 Priorities for future biological control research for *Cytisus scoparius*

Annex 1 summarises the proposed work programme for the next three seasons for the biological control project of *C. scoparius*. As discussed and endorsed at Bozeman in July 1999, a full-time entomologist with technical support for a period of at least three more years is planned, in order to make significant progress and reach conclusions about the suitability of new agents. On this basis we propose this work programme which stretches over the next three seasons, i.e. until the end of December 2002. In the following part, the work programme is described in a descending priority order from essential work to opportunistic, as time and material allows.

Top priority of future research will be the two gall-forming species *Hexomyza sarothamni* and *Asphondylia sarothamni*. Both species need to be cultured as a first step. Thereafter oviposition choice tests will be carried out, since the behaviour of females rather than larvae is of critical importance for host choice by internal feeders. In 2001 oviposition tests will be continued under different conditions. Additional tests will be carried out during 2002 as necessary and it is hoped that the experimental host range of these two top priority agents will be demonstrated by the end of the three years proposal. In addition to the host specificity screening, behavioural studies and other experiments about the biology of the agents will be carried out as basic knowledge for rearing and prediction of behaviour and performance after release. If the two Diptera species are considered to be suitably host specific for release, a release petition would be prepared with national collaborators.

The field trial in the garden of the institute will provide information on host range under near-natural conditions for several potential biological control agents. The mite *Aceria genistae* is released on one plot and the preference will be monitored. These open-field results are considered to be crucial as an addition to the laboratory testing carried out at the CSIRO in Australia. The two gall-forming Diptera will be tested on the field plots as well. *Bruchidius villosus* will be included in the tests, because beetles were found on tagasaste in New Zealand, despite test results which suggested a high host specificity. Since tagasaste does not occur on mainland Europe, this will be the first field test with tagasaste and Scotch broom growing in close proximity and therefore of potential relevance for other biocontrol agents. *Gonioctena olivacea* and *Agonopterix assimilella* are already released in the field plot. The resultant information on whether they will attack tagasaste under these field conditions will be a valuable addition to laboratory testing.

Another item of high priority for the broom biocontrol programme in general is the study of the *Aceria genistae*

species complex. If these studies demonstrate the presence of several highly host specific species, and the species adapted to Scotch broom is not yet present in the U.S.A., this mite should be the next agent considered for release in the USA. Material from different hosts in the study area will be collected to support the necessary taxonomic studies. These are intended to be carried out by a CRC student in Australia (CABI Bioscience has capability in this area but would need additional funding to do this work).

Some additional host specificity tests with the beetle *Exapion fuscirostre* will be conducted for Australia, as needed.

Species, which will be investigated on an opportunistic approach, will include sawfly species and the moth *Phyllonorycter scopariella*. Literature records indicate a high specificity of these species, but, since their impact is probably of less importance, biology and host range of these species will only be investigated if there is enough time and material available.

The root feeding curculionid *Polydrusus confluens* has been identified for further study in spite of concerns about its host specificity. This will only be pursued if material becomes unexpectedly available at the project field sites.

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11 Acknowledgements

The authors are grateful to Manaaki Whenua - Landcare Research, New Zealand, CSIRO and NSW Agriculture, Australia, and the Oregon Department of Agriculture and the Washington State Noxious Weed Control Board, USA for financial support. Dr. Andy Sheppard's (CSIRO) collaboration on practical work as well as fruitful discussions made an important contribution to the project. Discussions about broom work with Dr. Matthew Cock (CABI Bioscience) were very stimulating and gave the work the right direction. We wish to thank all colleagues at the CSIRO European Laboratory for their support of the work, in particular the Director, Dr. John Scott, for his interest

in the project and hosting our programme. Many people involved in the biological control of broom programme gave helpful suggestions and support throughout the season.

Biological control agents 1999 field season

(done or underway)	2000 field season
Aceria genistae (Eriophyidae)	
Release of mites in the host specificity field test plot, Make collections from several host plants and sites for additional genetic studies?	Monitor establishment of colony in the host specificity field plot at Montpellier
Agonopterix assimilella (Oecophoridae)	Collect mature larvae at the end of May
Produce first instar larvae in the laboratory	
Complete L1 tests for NZ	
Release L1 in the host specificity field plot	
Repeat release in autumn with older larvae	Check establishment of species in the field plot
In case of failed establishment attach eggs to plants in field plot after rearing adults from field-collected larvae	
Asphondylia sarothamni (Cecidomyiidae)	Collect first generation galls in the field
Attempt at rearing second generation adults failed	Collect first generation galls in the field
Initiate and maintain a breeding stock at the laboratory	
Conduct specificity tests with first generation adults and flowering test plant species under multiple-choice conditions	Release mated females in host specificity field trial at Montpellier
Bruchidius villosus (Chrysomelidae)	Collect adults in the field and release on host specificity field plot at Montpellier
Monitor establishment *Exapion fuscirostre* (Apionidae)	Some additional host specificity tests
Gonioctena olivacea (Chrysomelidae)	Release adult beetles on the host specificity field plot at Montpellier, Check establishment, If failed to establish, collect adults, rear on host plant and release young larvae
Hexomyza sarothamni (Agromyzidae)	Collect galls and commence a breeding stock at the institute
Protect galls found during field trips in November with sleeve cages against parasitoid attack	Carry out combined oviposition and larval development tests under multiple- and no-choice conditions in cages on potted plants

Check sleeve cages in the field and evaluate impact on attack by parasitoids	Release adults on the host specificity field plot
Carry out behavioural studies in order to understand preference and choice of the adult flies and to optimise conditions for rearing	Maintain breeding colony at the institute
Phyllonorycter scopariella (Gracillariidae)	Few individuals were collected during a field trip into the Cevennes, Collect adults on field trips and allow them to oviposit in cages at the institute
Polydrusus confluens (Curculionidae)	Attempt to find a field colony
Sawfly species	Collect larvae and allow them to hibernate, Check emergence of adult sawflies from the rearing and identify the species, If species identification indicates that the species is rather oligophagous to monophagous, initiate a breeding programme
Other items	Design and set up the host specificity field plots including *Cytisus scoparius*, *Genista monspessulana*, and *Chamaecytisus palmensis* for investigation of the specificity of several biocontrol agents
Finalise test plant lists for consortium countries	
Check availability of plants needed and receive material from release countries	
Seed and grow all test plant species available	Continue field experiment on host specificity of several biocontrol agents
Culture all available test plants in garden and green house space at the laboratory	Re-seed annual plants and maintain perennials at the station

Biological control agents

2001 field season	2002 field season
Aceria genistae (Eriophyidae)	Evaluate the spread of the mite population between the plants of the host specificity field plot
If problems about species complex of this mite are clarified by genetic studies, draft a petition for release of the species specific to *C. scoparius* in the U.S.A.	
Conduct additional specificity screening required for the U.S. environment	Monitor the population levels of the mite in the field plots, Send shipments to the U.S.A.
Agonopterix assimilella (Oecophoridae)	Evaluate spread and attack rate on plant specimens used in the field plot, Monitor infestation rates of individual plants in the field plot
Asphondylia sarothamni (Cecidomyiidae)	Maintain breeding colony at the laboratory
Use both generations for host screening under various conditions	If results of tests from 2001 are promising, carry out final testing with the gall midge and prepare a release petition
Bruchidius villosus (Chrysomelidae)	Follow establishment rate, spread, and attack of the beetle on the individual plants used in the field plots, Evaluate attack and preferences in the field plots
Exapion fuscirostre (Apionidae)	
Gonioctena olivacea (Chrysomelidae)	Follow patterns of spread between plants in the field plot and evaluate host preferences, Evaluate preferences of adult feeding and oviposition sites on the field plot
Hexomyza sarothamni (Agromyzidae)	Conduct additional cage tests on host specificity and finish testing with plants available
Check attack of plants in field plot	Take a decision on basis of the host specificity tests to conduct some more specific tests with crucial plant species under various conditions, If tests show a narrow host range and suggest a high degree of security that American native plants and Tagasaste will not likely be attacked after release, contribute to petitions for release in New Zealand, Australia, and the U.S.
Phyllonorycter scopariella (Gracillariidae)	Commence and maintain a breeding stock
Carry out multiple-choice oviposition tests	Maintain a breeding colony, Conduct host specificity tests
Polydrusus confluens (Curculionidae)	Rear field-collected beetles, if beetles could be detected in sufficient numbers in the field
Conduct oviposition tests, if beetles available	Host screening of the beetle, Decide whether this beetle species is a promising species in light of specificity
Sawfly species	Carry out larval development tests under no-choice conditions on all plants available, Conduct oviposition tests if required by results of previous tests
Other items	Decisions to be taken depending on progress