

**FOREIGN EXPLORATION & HOST
SPECIFICITY TESTING OF
BIOLOGICAL CONTROL AGENTS OF
FRENCH BROOM IN CALIFORNIA**

***INTERNATIONAL BROOM INITIATIVE
PROJECT
2001-2002***

Andy Sheppard
Principal Scientist



Foreign Exploration & Host Specificity Testing of Biological Control Agents of French Broom in California

Final Report

31 January 2002

Andy Sheppard¹ Thierry Thomann² & Sylvie Agret²

¹ CSIRO Entomology, GPO Box, 1700 Canberra ACT 2601

² CSIRO European laboratory, Campus International de Baillarguet, 34980 Montferrier-sur-Lez, France.

Executive summary

This is the final report on work carried out for the biological control of French broom in California by Commonwealth Scientific and Industrial Research Organisation at its European Laboratory in France in collaboration with the USDA European Biological Control Laboratory. Survey trips have been completed to Tunisia, southern Spain and western Portugal. Available resources limited a full suite of planned trips. Morocco and eastern Mediterranean Turkey are the only countries that still need to be surveyed. A recent study of the taxonomy of *Genista* suggests Morocco is the key region for undiscovered potential biocontrol agents. Analysis of the results of previous trips is presented including validation of the sampling regime and the impacts of seed feeding insects on the seed production of French broom and close relatives. Insect biology and culture development studies have been completed for the psyllid *Arytinnis hakani*. This species was found to have 5 nymphal instars and be multi-voltine with a 40-45 day generation time, passing the hot summer months as early instar nymphs. *Lepidapion argentatum* specificity and taxonomic status is unclear from current literature but a PhD project based around this group has recently obtained funding. *Bruchidius villosus* appears to have several host races and a related project is looking at this using molecular techniques.

Completion of host specificity testing of any agent requires an approved test plant list. A revised list is presented to expedite this process. Host specificity testing of *Arytinnis hakani* has been completed against 17 test plants (Objective required 10 test plants), including 7 *Lupinus* species showing this agent to be highly specific. The completion of testing should occur next year, funding permitting. Host specificity testing of *Bruchidius villosus* has been completed against 13 test plants including 7 *Lupinus* sp. Oviposition occurred on *Lupinus elegans* but dissections have shown larval development could not be completed. Several recent scientific developments relevant to this project are outlined and recommendations for further project development are made.

Background

California Department of Transportation, California Department of Forestry and Fire Protection and California Department of Food and Agriculture jointly fund this project. United States Department of Agriculture-Agricultural Research Service disburses funds and contributes to this project through a collaborative agreement with Commonwealth Industrial and Scientific Research Organisation European Laboratory. This project also receives joint funding from the Cooperative Research Centre for Australian Weed Management for aspects of this work relevant to the biological control of French broom in Australia. These latter aspects are outside the scope of this report.

This is the final report in line with agreed reporting requirements associated with this project funded from 1 February 2001 until 30 May 2002. The reporting schedule was for two progress reports on 31 December 2001 and 30 June 2002. The work was completed early and this is the final report. This final report is an expansion of the progress report submitted on 31 August 2001.

This final report, in line with the project proposal, outlines work performed, work status, work progress, difficulties encountered, remedial action, and statement of activity in relation to each agreed objective.

Objective 1: Carryout quantitative field surveys for potential biological control agents for French broom in Portugal, southern Spain, Morocco and Turkey and report on the results in relation to previous surveys. Produce an updated and justified priority list of potential biological control agents for French broom.

Survey trips were successfully carried out to Tunisia in April 2001, Spain and Portugal in June 2001. The additional planned trips could not be made either due to access problems (delays in permits) or due to funding reductions. Further trips are planned to Morocco in spring and summer and a return trip to Spain and Portugal in spring 2002. One trip to the eastern Mediterranean coast of Turkey in spring is also anticipated. This would complete necessary survey trips for *G. monspessulana*.

Survey trip to Tunisia (April/May 2001)

Tunisia was selected as an alternative to Morocco, because *Genista monspessulana* is reported in the mountains of north-west Tunisia (Pottier-Alapetite 1979, Greuter et al. 1989) that are an extension of the Atlas mountains that run across north west Africa including Morocco and because CSIRO has existing connections with the University of Tunis. This facilitated the collection of biological specimens from Tunisia and returning these to France on existing permits. Arranging such permits for Morocco was too long a process to achieve by April 2001. A similar trip to Morocco is now planned from April 2002.

The seven-day trip intensively surveyed all regions of climate relevant to support *G. monspessulana* populations, particularly from Tabarka to La Calle and inland through the Khroumerie mountains around Ain Draham, Beja and Jendouba up to the Algerian border, and around Bulla Regia to as far south as Le Kef. All tarred roads in this region and many dirt roads were surveyed including several hikes on foot into promising areas. Northern Tunisia has intense sheep and goat grazing, restricting non-spiny Genisteae to military bases and fenced properties. The survey located flowering populations of *Cytisus villosus*, *Calicotome villosa*, *Genista ferox*, *Genista microcephala*, *Genista tricuspitate*, from which insects were sampled in the quantitative manner described in (CSIRO 2000) for flowering plants. *Genista monspessulana* and *Spartium junceum*, *Ulex* spp. were not found despite being there in the right season for flowering. While this survey cannot discount the current existence of *G. monspessulana* in Tunisia, its abundance is certainly too low to support a diverse natural enemy community. *Genista ferox* is visually very similar in appearance to *G. monspessulana*, but this and *C. villosus* did not have a diverse insect fauna suggesting we were too far east on the North African coast to be within the evolutionary center of origin for this group. The insect herbivores collected during this trip are in the process of being formally identified. Contacts made at the Universite de Tunis have set the stage for where and when to go to Morocco next year. This trip also highlights and supports literature evidence that further surveys on the eastern North African coast are now unnecessary

Trip to Southern Spain and Portugal (June 2001)

Nine-day trip. In southern Spain the most abundant *G. monspessulana* populations were found in the mountain range between Gibraltar and Ronda in Andalusia. This is the only recorded area of significant abundance in southern Spain. In this region, pods were taken from 10 plants per species at six sites. One site only had *G. monspessulana*, while the other five sites had co-

occurring Genisteae including *Genista linifolia*, *Calicotome villosa*, *Cytisus villosus*, *Cytisus grandiflorus* spp. *Adenocarpus telonensis* and *Ulex* spp. In Portugal, only one plant was found near Sintra (a site for *G. monspessulana* reported in the literature) with most populations found along the axis: Ovido - Coimbra – Porto. Three sites were sampled with *G. monspessulana* alone and three with *G. monspessulana* co-occurring with *Spartium junceum* and *Ulex* spp. Samples taken are currently in storage and will be dissected and analyzed during winter.

Data analysis of previous survey trips

Results are presented of data collected and analyzed on previous survey trips of France northern Spain, Italy and Greece in 2000. The. Survey trips led to quantitative sampling at two sites in NW Spain, three sites in France, ten sites in Corsica, three sites in Sardinia and six sites in Greece. The number of sites roughly corresponds to the abundance of *G. monspessulana* populations found in these geographic units. Species in the Genisteae sampled throughout this survey work are included in Table 1. If species were rarely sampled with other Genisteae (Table 1 column 2) then this was because they were mainly observed growing alone.

Table 1. Species of Genisteae sampled since January 1999 and whether or not arthropods were found. Surveys included took place in Greece, France, Italy, Spain Portugal and Tunisia.

Species	Number of sites sampled alone	Number of sites sampled together with other Genisteae	Total number of sites sampled	Number of sites where arthropods were found on the plant
Natives				
<i>Genista monspessulana</i> *	7	26	33	33
<i>Genista stenopetala</i> °	0	3	3	2
<i>Genista canariensis</i> °	0	1	1	1
<i>Genista corsica</i>	0	2	2	1
<i>Genista ferox</i>	2	0	2	2
<i>Genista microcephala</i>	3	0	3	3
<i>Genista tricuspitate</i>	2	0	2	2
<i>Cytisus villosus</i> ,	5	16	21	21
<i>Cytisus scoparius</i> **°	1	8	9	7
<i>Cytisus arboreus</i>	0	5	5	5
<i>Chamaecytisus proliferus</i>	4	7	11	10
<i>Spartium junceum</i> **°	2	5	7	5
<i>Calicotome spinosa</i> *	0	3	3	3
<i>Calicotome villosa</i>	4	10	14	14
<i>Adenocarpus foliolosus</i> °	0	4	4	2
<i>Adenocarpus telonensis</i>	0	2	2	2
<i>Spartocytisus filipes</i> °	0	1	1	1
<i>Retama raetam</i> **°	1	1	2	0
<i>Ulex europaeus</i> **°	1	4	5	3

* species that are exotics in USA; ° species only (or also) sampled in the Canary Islands (a report for this side trip is in Appendix 1)

The quantitative beating-tray surveys in the northern Mediterranean region have found 82 phytophagous arthropod species on French broom. The rust, *Uromyces genistae* Fuckel., was also observed attacking old leaves in spring/summer (similar sori have been observed by us in California). Of these 82 species, the frequency per sample and the number per sampled plant of

the 50 of the commonest are presented in Table 2. These 50 species were subjected to a focused but not exhaustive literature search. The results of this search suggest that 15 of these species and the pathogen may be restricted in host range to members of the Genisteae (Table 4). Most foliar damage observed was caused by the psyllid *Arytinnis hakani* (Loginova), while the psilid fly, *Chyliza leptogaster* (Panzer), was the only species that was observed killing mature plants, and only at one site in southern France.

Table 2. The abundance and frequency of the 50 most abundant phytophagous arthropod and pathogen species collected during the early beating-tray survey of 18 *G. monspessulana* sites in Spain, France and Italy. Information is also included on a) their likely specificity (from the literature search) b) their phytophagous feeding guild and c) other genera of the Genisteae from which these species were also collected during these surveys.

Species	Specificity ^b	Guild ^c	Insects plant ⁻¹	% frequency ^a	Other Genisteae Genera
Hemiptera					
Mirid sp.*	1	1	0.32	25	<i>Cytisus, Calicotome</i>
<i>Geocoris</i> sp.*	2	1	0.02	3	<i>Calicotome</i>
<i>Gargaria</i> sp. *	1	1	0.08	8	<i>Cytisus, Spartium</i>
<i>Arytinnis hakani</i>	1	1	4.01	61	<i>Cytisus, Calicotome</i>
<i>Cacopsylla ?melanoneura</i> (Foerster)	1	1	0.02	3	<i>Cytisus</i>
<i>Livilla</i> sp, *	1	1	0.01	3	<i>Cytisus</i>
Psyllid sp.*	1	1	0.01	2	
Diptera					
<i>Chyliza leptogaster</i> (Panzer)	1?	7	1.5	16	
Lepidoptera					
<i>Agonopterix nervosa</i> (Haworth)	2	2	0.19	18	<i>Calicotome</i>
<i>Agonopterix scopariella</i> (Heinemann)	1	2	0.09	25	<i>Cytisus</i>
<i>Callophrys rubi</i> (L.)	2	2	0.04	3	
<i>Pseudoterpna pruinata</i> (Hufnagel)	2	2	0.01	2	<i>Cytisus, Calicotome</i>
Pyralid sp. *	2	2	0.02	7	<i>Cytisus</i>
Tortricid sp. *	2	2	0.02	7	<i>Genista, Cytisus, Calicotome</i>
Coleoptera					
Bruchidae					
<i>Bruchidius villosus</i> (F)	2	5	0.26	31	<i>Cytisus, Calicotome, Spartium</i>
<i>Bruchidius lividimanus</i> (Gyll.)	2	5	2.23	48	<i>Genista, Cytisus, Calicotome</i>
Buprestidae					
<i>Anthaxia</i> sp. *	4	7	0.01	2	
Apionidae					
<i>Exapion fuscirostre</i> (F)	1	5	0.01	2	<i>Cytisus, Calicotome</i>
<i>Exapion</i> nr. <i>putoni</i> (Ch. Brisout)	1	5	0.06	5	<i>Genista, Calicotome</i>
<i>Lepiapion argentatum</i> (Gerstäcker)	1	5	0.26	36	
<i>Oryxolaemus ?scabiosus</i> (Weise)	1	3	0.01	2	<i>Cytisus, Calicotome</i>
<i>Pirapion immune</i> Kirby	2	3	0.01	2	<i>Cytisus</i>
<i>Protapion shoehnerri</i> (Boheman)	3	4	0.03	5	<i>Cytisus, Calicotome</i>
<i>Protopirapion attratum</i> (Gemar)	2	4	0.01	2	
<i>Apion</i> sp. a*	1?	5?	0.04	7	

<i>Apion</i> sp. b*	3	5?	0.01	2	
Curculionidae					
<i>Coeliodes erythroleucos</i> (Gmelin)	4	?	0.01	2	
<i>Pachytychius sparsutus</i> (Ol)	2	5	0.15	37	<i>Cytisus, Calicotome</i>
<i>Peritelus senex</i> (Boheman)	2	6	0.14	2	
<i>Pleurodrusus carinula</i> (Olivier)	2	6	0.01	2	<i>Cytisus, Spartium</i>
<i>Polydrusus cervinus</i> (L.)	4	6	0.01	2	
<i>Polydrusus prasinus</i> (Olivier)	4	6	0.01	3	<i>Cytisus, Calicotome</i>
<i>Sitona gressorius</i> (F.)	2	6	0.01	2	
<i>Sitona regensteinensis</i> (Herbst)	1	6	0.14	15	<i>Cytisus, Calicotome, Spartium</i>
<i>Tanymecus</i> sp.	4	?	0.01	2	
Anobid sp.	2	7	0.03	5	<i>Cytisus, Calicotome, Spartium</i>
Cantharid sp. a	4	8	0.02	5	<i>Cytisus</i>
Cantharid sp. b	4	8	0.01	2	
Cantharid sp. c	4	8	0.14	10	<i>Cytisus, Calicotome, Spartium</i>
Cerambycid sp.*	3	7	0.02	5	<i>Cytisus, Calicotome</i>
Clerid sp.	4	8	0.02	5	
Dermestid sp.	4	8	0.01	2	
Latharid sp.	4	8	0.01	2	
Mordellid sp.	4	8	0.04	8	<i>Cytisus, Calicotome, Spartium</i>
Nitidulid sp. a	3	8	0.06	11	<i>Genista, Cytisus, Calicotome</i>
Nitidulid sp. b	3	8	1.01	21	<i>Cytisus, Calicotome, Spartium</i>
Nitidulid sp. c	3	8	0.01	2	
Nitidulid sp. d	3	8	0.01	2	<i>Cytisus</i>
Nitidulid sp. f	3	8	0.75	21	<i>Genista, Cytisus</i>
Ptinid sp.	4	8	0.01	2	
Thysanoptera					
<i>Thrips</i> sp.*	3	1	0.80	28	<i>Genista, Cytisus, Calicotome</i>

* still being identified

^a: % of *G. monspessulana* samples containing this species

^b: specificity; 1 - specific to genus, 2 – specific to tribe, 3 – specific to family, 4 -generalist/tourist

^c: guild; 1- sap sucker, 2 – defoliator, 3 – leaf miner/galler, 4 – flower feeder, 5 – seed feeder, 6 – root feeder, 7 – stem feeder, 8 – flower visitor

The beating tray data were also used to assess the effect of the number of plants sampled per site on the efficiency of catching all the insect herbivores available at that site. A rarefaction curve was constructed from the number of plants sampled and the cumulative number of herbivorous arthropod species detected for that number of samples (Fig.1). The asymptote of the fitted line is an estimate of the total number of herbivorous arthropod species present at that site and the relationship provides an estimate of the sampling efficiency of any given sample size. For example a sample size of 10 plants at the site in Fig. 1 (which had the most diverse range of arthropod species) would be expected to find 93% of the species assuming a total of 30 species at this site. It appears that the sample size chosen was sufficient to collect most species during this survey.

Data from the second visits to each of 14 of the *G. monspessulana* sites was used to look at pre-dispersal seed losses to insects in pods for the different Genisteae species sampled across sites (Table 4) and variation in seed loss within *G. monspessulana* both within and between sites

(Table 5). The overall average seed predation levels in the pods of *G. monspessulana* were 19%. This was higher than for any co-occurring other species in the Genisteae except *C. spinosa* (although data from only one *C. spinosa* population has been analyzed so far). The bruchids were the most damaging species, but the highly specific apionid, *Lepidapion argentatum* (Gerstäcker), also caused significant losses (Table 4). There was large variation in seed losses per *G. monspessulana* plant both with and between sites (Table 5) ranging from 7 to 33% across sites. Further work is needed to sort out the relative importance of the species with larvae that mostly leave the pods prior to collection. The data suggest *Bruchidius villosus* F., a biological control agent already released against Scotch broom is likely to have a significant impact also on French broom.

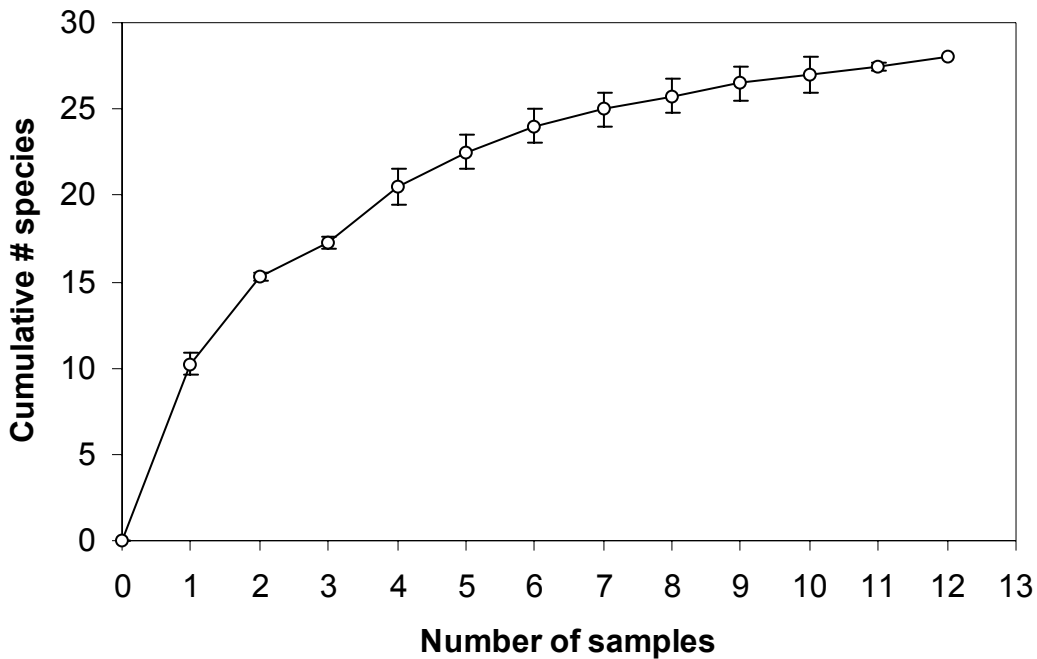


Fig. 1. The rarefaction curve for the number of herbivorous arthropod species detected on *G. monspessulana* against the number of plants sampled using the described beating method at Romanya de la Selva, Sierra de Gavarres, SE Girona SW Spain. This analysis includes all arthropod species collected including specialists and generalists.

Table 3. Phytophagous arthropods collected and identified from French broom during this project for which a literature search indicated that specificity is likely to be restricted to the Genisteae.

Genus	Species	Author	Other Genista sp.	Other Host Genera	References
<u>Hemiptera</u>					
<i>Aytinnis</i>	<i>hakani</i>	(Loginova)		<i>Cytisus</i>	Burckhardt 1989
<i>Aytaina</i>	<i>africana</i>	Heslop-Harrison		<i>Cytisus</i>	
<i>Cacopsylla</i>	<i>?melanoneura</i>	(Foerster)			
<u>Lepidoptera</u>					
<i>Callophrys</i>	<i>rubi</i>	L.	sp.	<i>Cytisus, Ulex, Lotus, Helianthemum, Vaccinium, Rhamnus, Rubus, Cornus etc.</i>	Carter & Hargreaves 1986, Higgins & Riley 1973, Emmet & Heath 1992
<i>Cydia</i>	<i>succedana</i>	Denis & Shiffermuller	<i>anglica, radiata, tinctoria, cinerea,</i>	<i>Cytisus, Ulex, Spartium, Chamaespartium, Lotus, Dorycnium, Ononis,</i>	Emmet 1988, Kloet & Hincks 1972, Suire 1951, 1962
<u>Coleoptera</u>					
<i>Lepidapion</i>	<i>argentatum</i>	Gerstacker	<i>anglica, umbellata</i>	<i>Adenocarpus</i>	Hoffmann 1950, 1958, Alonzo-Zarazaga 1985, Ehret 1990
<i>Bruchidius</i>	<i>lividimanus</i>		sp.	<i>Cytisus, Calicotome etc.</i>	Syrett et al. 1999, Brandl 1981
<i>Bruchidius</i>	<i>villosus</i>	F.	<i>hispanica, tinctoria</i>	<i>Cytisus, Spartium, Laburnum, Petteria, Chamaecytisus</i>	Szentesi Á. and Wink M. (1991), Syrett et al. 1999, Frick 1962
<i>Pachytychius</i>	<i>sparsutus</i>	OI.	<i>cinerea, tinctoria, pilosa, florida</i>	<i>Cytisus, Ulex, Chamaespartium, Echinospartium, Erica,</i>	Caldara 1978, Gurrea Sanz et al. 1988, Hoffmann 1958, Sanz Benito & Gurrea Sanz 1991, Sanz Benito et al. 1989, 1990, Velazquez de Castro et al. 1990, Hoffmann 1958, Syrett & Emberson pers. comm.
<i>Polydrusus</i>	<i>cervinus</i>	(L.)		<i>Quercus, Betula, Dactylis</i>	Hoffmann 1958, Freude et al. 1981
<i>Polydrusus</i>	<i>prasinus</i>	Olivier		<i>Cytisus, Calicotome, Quercus, Alnus, Fagus</i>	Syrett & Emberson pers. comm., Hoffmann 1958
<i>Coeliodes</i>	<i>erythroleucos</i>	(Gmelin)		<i>Lupinus</i>	Hoffmann 1958
<i>Sitona</i>	<i>grossoirius</i>	F	<i>anglica</i>	<i>Ulex, Astragalus,</i>	Hoffmann 1958
<i>Peritelus</i>	<i>senex</i>	Boheman			
<u>Diptera</u>					
<i>Chyliza</i>	<i>leptogaster</i>	(Panzer)			
<u>Uredinales</u>					
<i>Uromyces</i>	<i>genistae</i>	Fuekel	<i>pilosa, sp.</i>	<i>Chamaespartium</i>	Guyot, A.L. and M. Massenet. 1958.

Table 4. Percent seed loss per plant across 5 species of Genisteae at 14 sites containing natural populations of *G. monspessulana* in Spain, France and Italy in the native range.

Genisteae	n	Mean total % seed loss plant ⁻¹	Mean % seed loss plant ⁻¹ to			
			<i>Bruchidius lividimanus</i>	<i>Bruchidius villosus</i>	Apionid ^a spp.	<i>Pachytychius sparsutus^b</i> and/or Lepidoptera
<i>Genista monspessulana</i>	16	17.82 ± 2.60	4.85 ± 1.40	4.04 ± 1.41	2.34 ± 0.73	7.01 ± 1.82
<i>Cytisus villosus</i>	8	7.90 ± 1.66	4.91 ± 1.29	0.06 ± 0.06	3.55 ± 3.35	1.01 ± 1.00
<i>Cytisus scoparius^c</i>	2	1.19 ± 0.26	1.01 ± 0.43	0.00	0.00	0.17 ± 0.17
<i>Calicotome villosa</i>	4	13.36 ± 6.28	3.32 ± 1.30	0.00	9.56 ± 5.53	0.00
<i>Calicotome spinosa</i>	3	12.25 ± 7.30	3.06 ± 0.85	0.00	0.00	9.68 ± 7.90

^a *Lepidapion argentatum* on *G. monspessulana* and *Exapion ?subparallellum* (Dbr.) on *C. villosa* and *Exapion fuscirostre* on *C. villosus*

^b species could not be distinguished as larval damage similar and both have larvae that leave the pods prior to pod maturation.

^c Data only for sites where *C. scoparius* co-occurs with *G. monspessulana* (Corsica), other studies in the native range have estimated seed loss of *C. scoparius* to be 15-23% (Hosking 1995), 0.4-24% (Mazay 1993), 26% (Hinze 1992).

Table 5. Percent seed loss to pre dispersal seed predators of *G. monspessulana* plants at 13 sites spread over two years.

Site	Year	Mean total % seed loss plant ⁻¹	<i>Bruchidius lividimanus</i>	<i>Bruchidius villosus</i>	<i>Lepidapion argentatum</i>	<i>Pachytychius sparsutus</i> and/or Lepidoptera
Spain 1	2000	16.00 ± 4.00	15.00 ± 4.00	0.00	1.00 ± 1.00	0.00
Spain 2	1999	19.31 ± 2.90	15.42 ± 3.15	0.48 ± 0.26	2.48 ± 1.27	0.64 ± 0.43
	2000	25.03 ± 3.72	10.81 ± 2.62	1.12 ± 0.40	3.29 ± 1.05	9.80 ± 1.62
Spain 3	2001	8.31 ± 2.25	0.00	0.00	7.13 ± 2.12	0.00
Spain 4	2001	15.33 ± 5.24	15.33 ± 5.24	0.00	0.00	0.00
France 1	1999	12.26 ± 1.81	0.27 ± 0.20	8.15 ± 1.73	3.14 ± 0.98	0.70 ± 0.38
France 2	1999	3.79 ± 1.29	3.52 ± 1.18	0.10 ± 0.10	0.00	0.17 ± 0.17
Corsica 1	1999	21.00 ± 3.00	1.00 ± 1.00	4.00 ± 2.00	0.00	16.00 ± 4.00
Corsica 2	1999	30.46 ± 3.50	0.61 ± 0.35	5.08 ± 1.09	3.62 ± 1.42	21.16 ± 4.40
Corsica 3	1999	33.00 ± 4.00	7.00 ± 2.00	19.00 ± 4.00	2.00 ± 1.00	5.00 ± 2.00
Corsica 4	1999	27.04 ± 2.90	1.43 ± 0.55	8.24 ± 1.36	10.45 ± 1.81	6.92 ± 2.86
Corsica 5	1999	32.19 ± 6.61	3.27 ± 0.17	9.05 ± 9.05	0.00	19.86 ± 2.62
Corsica 6	1999	11.89 ± 3.34	0.08 ± 0.08	4.43 ± 1.85	2.12 ± 1.39	5.26 ± 1.81
Corsica 8	2000	6.75 ± 1.49	1.64 ± 0.73	1.91 ± 0.53	0.17 ± 0.11	3.03 ± 1.31
Corsica 9	2000	14.30 ± 2.58	1.55 ± 0.53	1.54 ± 0.48	0.62 ± 0.55	10.59 ± 2.40
Sardinia 1	2000	7.59 ± 2.33	1.88 ± 0.79	0.19 ± 0.12	1.28 ± 0.66	4.24 ± 2.04

Prioritizing potential biological control agents for French broom

Survey information has been used to draw up a preliminary list of potential biological control agents (Table 6) and other species that given further work may attain that list. The psyllid, *A. hakani*, and the apionid, *L. argentatum*, are illustrated in CSIRO (2000).

Based on specificity alone it is only natural that a psyllid species should take precedence on a list of potential biological control agents for *G. monspessulana*. Lupines have no recorded psyllid species. Four genera of arytainine psyllids are the only known psyllids to feed on host plants in the Genisteae and they are restricted to this group of hosts (Hodkinson and Hollis 1987). *Arytaina* has hosts in *Cytisus*, *Chamaecytisus*, *Genista*, *Adenocarpus* and *Calicotome*, *Spartocytisus*. *Arytinnis* (Percy, in press) has hosts in the same genera but also in *Retama*. *Livilla* has hosts in *Laburnum*, *Cytisus*, *Chamaecytisus*, *Retama*, *Genista*, *Ulex*, *Adenocarpus* and *Calicotome*, and the psyllid genus *Pseudacanthopsylla* is on *Retama*. In all these genera there is a high degree of specialization. In *Arytinnis* there is also strong parapatry within the Genisteae and within *Genista* in particular. Percy (2001) found that of the 31 *Arytinnis* species studied on 25 host species largely from the Canary Islands (an evolutionary center of diversification for *Genista*), 26 were monospecific, three had two closely related con-generic hosts, one had several con-generic hosts and two host races of one species, *A. modica*, occurred on *G. stenopetala* and *C. proliferus*. Percy carried out an analysis using the molecular phylogeny to date the separation on these two host races of *A. modica* on *G. stenopetala* and *C. proliferus* on the Canary Islands, which suggests divergence occurred between 70,000-121,000 yrs ago (D. Percy pers. comm.).

The appearance of high specificity within the Genistineae is supported by the existence of psyllid-free genera within this subtribe (e.g. *Ulex*), but also needs to be tempered by recognizing high restriction of many of these *Arytinnis* species to particular islands. *Arytinnis hakani* was one of the species studied and has only ever been recorded from *G. monspessulana* and has a relatively wide geographic distribution (north and south coasts of the Western Mediterranean Portugal to Italy and Morocco to Algeria). Molecular and morphological evidence suggests *Arytinnis* probably originates from Macaronesia Percy (2001). That *A. hakani* appears in a genus of probable Macaronesian origin suggests its association with *G. monspessulana* (Continental only) may be relatively recent.

We found *A. hakani* throughout the western Mediterranean distribution of *G. monspessulana*. It is unlikely that other species of psyllid also use *G. monspessulana* as a host. *Arytaina genistae* (Latreille) was found on *C. scoparius* at sites where this co-occurred with *G. monspessulana*, but *A. genistae* was clearly not using *G. monspessulana* (though this species will develop on *Chamaecytisus*; S. Fowler pers. comm.) and has not been recorded from it in the literature. This observation is supported by evidence from California where this psyllid has also been accidentally introduced where attack is observed on *C. scoparius* but not on *G. monspessulana*. Damage by *A. hakani* on *G. monspessulana* in the broom garden at Montpellier has been impressive with heavily attacked plants showing shoot-blackening, reduced growth and flowering.

Table 6. Current short list of potential biological control agents for French broom, based on surveys of half the native distribution.

Current short list of potential biological control agents

- Agent 1. *Arytinnis hakani* (Loginova); – Sap-sucking plant louse
(Only ever recorded from Montpellier broom)
- Agent 2. *Lepidapion argentatum* Gerstaecker; – Small weevil feeding on seeds
(also recorded from *G. anglica*, *G. umbellata*, ?*Adenocarpus* sp.
– taxonomy under revision)
- Agent 3 ?
- Agent 4 ?

Other species which upon further investigation could be added to the short list.

Moths

- Trifurcula serotinella* H.S., – leaf miner
(not seen, specific to tribe - literature search)
- Coleophora trifarella* Zeller; – leaf miner
(not seen, specific to tribe -literature search)

Beetles

- Bruchidius villosus* F. – seed-feeder
(already in the US, also feeds on *Cytisus*, *Spartium*, *Laburnum*,
Chamaecytisus. Further testing against north American lupines
advisable)
- Peritelus senex* Boheman – root feeder
(collected only from target, but host range supposedly includes
Astragalus and *Ulex*)
- Pachytychius sparsutus* (Ol) –seed feeder
(collected from most Gensiteae, but may be the most damaging
seed weevil on *G. monspessulana* and *C. scoparius* (Mazay
1993) – Further investigations necessary

Flies

- Chyliza leptogaster* (Panzer) – stem miner
(seen killing plants, recorded also from *Physocarpus*, *Forsythia*,
Neottia, existence of host races needs investigation)

The seed feeding apionid (*Lepidapion* (*Lepidapion*) *argentatum*) is also likely to be highly specific to *G. monspessulana*. The genus *Lepidapion* has ca. 16 Mediterranean and Canary Island species, and two sub-genera. A major revision is required, and is in part being addressed as a PhD project (Y Jiménez pers. comm.). Hosts in the genus include members of *Genista*, *Teline*, *Ulex*, *Retama*, *Spartocytisus* and *Cytisus* (Alonso-Zarazaga 1985). *Genista umbellata* and *Adenocarpus* sp. have been included in the host range of *L. argentatum*, but this is likely to result from confusion in species definition. Neither of these hosts was found co-occurring with *G. monspessulana* in this study. Five of the six species in the PhD study appear to be monospecific, and confusion reigns between whether *L. argentatum* and *Lepidapion acuminatum* (Schilsky) both attack *G.*

monspessulana, the latter having been recorded from *G. monspessulana* near Cadiz in southern Spain (Alonzo-Zarazaga 1985). Collections need to be made in this area as part of this project and compared with *L. argentatum* specimens from northern Spain and France. This apionid was found to eat up to 10% of seed production at one site in Corsica and has now been introduced into the broom garden in Montpellier.

The other agent with significant potential as a biological control agent for *G. monspessulana* is the stem mining psilid fly (identified as *Chyliza (Chyliza) leptogaster*), largely based on the observation at one site in France of this killing many individuals and appearing to be the main cause of plant population decline at this site. The larvae tunnel under the bark either ring barking whole branches or causing massive necrosis of cambium tissue. Of puparia collected in June 1999, several adults emerged in July, while the vast majority emerged in April 2000. The Psilidae has relatively few genera. *Chyliza* species are considered to be bulb and stem miners and the genus currently have 57 known species (Iwasa 1989), however, very few of these have known host plants. Known host associations include; *Chyliza vittata* Meigen on Orchidaceae (at least 4 genera.), *Chyliza extenuata* Rossi on at least two *Orobanchae* species, and a group of species that have been recorded feeding in wounds of different tree species; *Pinus*, *Ulmus*, *Picea*. *Chyliza leptogaster* has been recorded from nut-like wood galls on *Physocarpus* and *Spiraea* sp. (Rosaceae) in northern Europe, however, Collin (1944), talks about slight morphological differences between his *C. leptogaster* and a “southern form” described by Rondani in Italy in 1876 which the latter called *C. premixta*. Rondani records no host plant for his species. Chandler (1975) comments that this genus had fairly “chaotic taxonomy” when Collin wrote his paper, so it is not clear yet whether the project has picked the host of a southern species. The flies collected, however, did not form anything resembling a “nut-like wood gall”. The *G. monspessulana* host record appears to be the first for the genus within the Fabaceae. Further investigation is needed, with help from alpha and molecular systematists, to clearly determine the potential specificity of this fly, however, a slight concern is the tendency of some species in the genus to appear to only oviposit into existing wounds (e.g. *C. annulipes* on *Pinus*, Lyeborg 1987). A key publication by Dengler (1997) may reveal more when obtained.

It is premature to suggest that the natural enemy species with the greatest potential as biological control agents have already been discovered given that some of the native distribution of *G. monspessulana* still remains to be surveyed.

Objective 2: Obtain clean and viable laboratory cultures of three potential biological control insects for French broom: the plant psyllid, *Arytinnis hakani*, and the seed weevils, *Lepidapion argentatum*, and *Bruchidius villosus*.

Work on these three agents has progressed significantly.

Arytinnis hakani has naturally established in abundance on *Genista monspessulana* in the broom garden and on plants cultivated in pots at the laboratory. A study was undertaken of the life cycle of this psyllid, including generation time from egg to ovipositing adult and the length of the larval instars at constant 20°C/15°C day night temperatures. Male and female psyllid couples in Petri dishes laid eggs on a known date. The 714 eggs were followed through five nymphal instars to adult. Variable survival lead to samples sizes that ranged from 27 to 70 for the different nymphal stages (Fig 2). Generation time was also measured for eggs laid in each month from March to July under laboratory and field conditions at Montpellier using permeable plastic tubes on branches of *G. monspessulana* (Table 7).

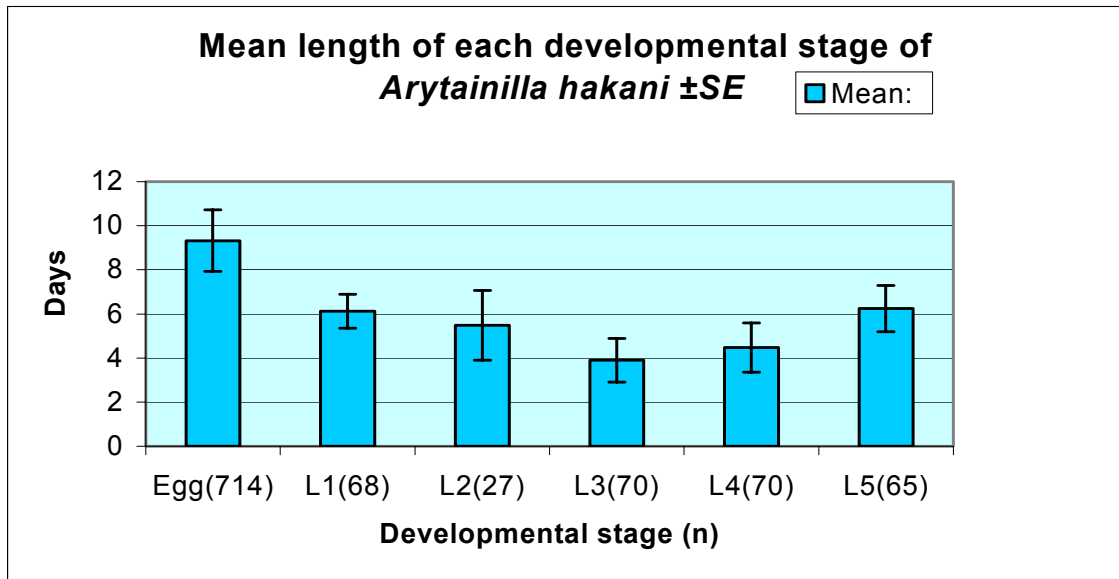


Fig 2. Length of developmental stage of *A. hakani* at 20°C/15°C in March-April

These results show that *A. hakani* is multivoltine with potentially 4 generations in spring and at least two others in autumn (autumn generations still to be confirmed). Observations on *G. monspessulana* plants outside suggest that as external temperature increases through summer there is a decrease in the number of late nymphal instars (L3, L4 & L5) and adults. Adult females also made up only 11% of collections made at the end of July. Survival through the hot dry summer months, therefore, appears to be as early instar nymphs in the youngest leaves.

Table 7. Generation times of *A. hakani* in the lab and field

Date Eggs laid	Génération length (days)	
	Laboratory	Field
14 March	32	
12 March	37	
12 March	40	
13 March	42	
13 March	46	
13 March	47	
25 March	34	
25 March	36	
28 May	No data	59
11 June	42	39
21 June	40	49
02 July	45	42
12 July	41	in progress

Lepidapion argentatum, has been established in the broom garden, but is still only present in small numbers. Lab rearing of this species is not currently possible. As described under Objective 1., further work on this agent would best be preceded by a taxonomic study to better understand the taxonomic status of this species and how it fits within the genus *Lepidapion* attacking Genisteae, particularly *G. monspessulana*. This work has been funded as a PhD project (see Recommendations).

Bruchidius villosus is naturally present on site at Montpellier, feeding within surrounding *Spartium junceum* populations. Starting and maintaining cultures of this species is well understood following the use of this beetle as a biocontrol agent against Scotch broom (*Cytisus scoparius*) in the 1990's. Before we start a new viable culture of this species for the French broom control program, several additional factors require further understanding. There is increasing evidence that there is significant within-species variation with respect to host utilization. Work not directly part of this project has been looking at host race differences using DNA markers and caged choice tests for two populations of *B. villosus*, collected from *C. scoparius* (Germany) and *S. junceum* (Southern France). It was felt that the completion of these studies this year should precede any further culturing activities for this insect. Testing of *B. villosus* against North American test plants has also been carried out (see Objective 3).

Objective 3: Complete laboratory testing of 10 of the 'essential test plants' on the approved plant test list for *A. hakani*, and *B. villosus* that is sufficient to ensure these agents to be ready for importation into a USA quarantine should this project continue.

Progress on development of a draft test plant list for broom biocontrol agents.

Introduction

The system used for selecting test plants for testing host specificity of potential biological control agents for French broom, follows the internationally accepted approach defined by Wapshere (1974). In this phylogenetic approach the test list includes species closely related to the target, species within the same taxonomic tribe, species in the same taxonomic family and then species in other families with chemical, physical similarities to the target. The number of species to be tested in each of these group decreases with the relatedness of the group to the target. The evolutionary arguments for the benefits of this approach for defining host range of potential biological control agents are argued elsewhere (Harris and McEvoy 1995). This approach has recently been refined by Briese and Walker (in press) whereby within the phylogenetic system plant species are selected where possible based on a) similar ecology i.e. life history and b) similar biogeography within the range or potential biogeographic range of the target.

Here it is proposed to restrict testing for the United States to members of the Fabaceae given the size and variety therein, with an emphasis on the most closely related taxa, particularly those containing members of the North American flora not previously exposed.

Taxonomic affinities in the Fabaceae

French broom, *G. monspessulana*, is a member of the *Genista* group of the *Cytisus-Genista* complex within the tribe Genisteae of the Faboideae (= Papilionoideae), one of the subfamilies of the Fabaceae. Apart from the genus *Genista* and *Cytisus* in which there are some ornamental species, only the exotic genera *Lembotropis*, *Petteria* and *Laburnum* are planted as ornamentals in USA. It is also proposed to test common ornamental hybrids of *Genista* (*Cytisus* x *spachianus*, *Cytisus* 'Porlock') as this will give us greater resolution on the specificity of the potential biological control agents as well as assessing likely risks to the Horticultural industry (Aitkinson and Sheppard 2000). Outside the *Genista* group, the *Cytisus-Genista* complex contains the exotic invasive genera *Spartium*, *Calicotome*, *Genista* and *Ulex*. Species of all these genera are weedy in North America.

Within the remainder of the Genisteae, as defined by Polhill (1981), and given the importance of lupines in the native North American flora and as agricultural fodder careful attention has been given to representatives of the subtribe Lupininae. Recent molecular work on the taxonomic affinities within the genus *Lupinus* (Ainouche and Bayer 1999), suggest 5 major clades of relatedness with a biogeographic pattern. Given this it should only be necessary to test representatives of each clade (Briese and Walker in

press). The lupine species included in the list have been chosen on this basis and based on current test plant stocks.

The interpretation of the relationship between the various tribes of Faboideae has varied over time, but some broad groupings are fairly constant. The most recent interpretation is that of Polhill (1981). The Genisteae are seen as one of a basal group of tribes along with the Thermopsidae, Euchresteae, Podalyrieae, Liparieae, Brognartieae, Crotalarieae, Mirbelieae and Bossiaeeae. Polhill (1981) goes on to propose four natural groupings of tribes, the first group being the Sophoreae forming the base or stem of the group, the second group being the Genisteae-Podalyrieae complex and two groups based on the Galegeae and the Tephrosieae.

Of the tribes that could be considered close to the Genisteae, only the Thermopsidae, contains North American species and so *Thermopsis macrophylla* Hook & Arn has been included for testing. Two further native species have also been included as representatives of the tribes Sophoreae (*Sophora secundiflora* (Ortega) DC) and Loteae (*Lotus scoparius* (Torrey & A. Gray) Ottley). Tribes of particular importance to agriculture include the Loteae (*Melilotus*), the Phaseoleae (*Glycine* (soya) and *Phaseolus* (beans)), the Trifolieae (*Medicago* (alfalfa) and *Trifolium* (clovers)), and the Vicieae (*Pisum* (peas), *Vicia* (faba bean), *Lathyrus* (sweet pea)). These too have been included in the test list. Inclusion of sympatric native species from these tribes may also need to be considered e.g. the perennial *Lathyrus jepsonii* E. Greene, the annual *Phaseolus filiformis* Benth., the perennial clover *Trifolium breweri* S. Watson and the native *Vicia americana* Willd. These have not been included in the current test list. Outside this group of tribes, it does not seem necessary to carry out systematic additional testing.

The test plant list (Table 8) has been adapted from a list provided by Dennis Isaacson and published in an ODA report (Isaacson 1998) for the testing of potential biological control agents against Scotch broom (*Cytisus scoparius*). The changes include a) substituting horticultural broom varieties with “*Teline*” parentage for those with *Cytisus* parentage, b) the selection of *Lotus scoparius* as a native representative of that genus sympatric with broom, and considered to be at risk by Californian botanists (Chuck Williams pers. comm.) and c) selecting possible species already available for testing.

Any unexpected long-term agent survival on previously unrecorded host species will lead either to direct rejection of the agent or an extended testing program using species closely related to the ‘new host’ and/or testing conditions more natural to the test organism.

It is important to remember that host specificity testing is aimed at defining potential host range of biological control agents in an academic sense and should not be used to screen all related rare and endangered plant species. Where possible the species were selected to come from an area sympatric to the distribution of the target.

Table 8. List of plants proposed for testing of potential biological control agents for French broom *Genista monspessulana* for the Western USA.

SPECIES	Life History ^a	Sympatry with target ^b
Tribe Genisteae		
Subtribe Genistinea		
<i>Cytisus scoparius</i> (L.) Link	P	S
<i>Cytisus striatus</i> (Hill) Rothm.	P	S
<i>Cytisus</i> 'Porlock' ⊗	P	S
<i>Cytisus</i> x <i>spachianus</i> ⊗	P	S
<i>Genista monspessulana</i> (L.) L. Johnson	P	S
<i>Ulex europeus</i> L.	P	S
<i>Spartium junceum</i> L.	P	S
Subtribe Lupininae		
Clade A (Eastern New World Lupines)		
<i>Lupinus texensis</i> Hook*!	A	A
Clade B (Smooth-seeded Old World Lupines)		
<i>Lupinus angustifolius</i> L.!	A	S
Clade C (Rough seeded Old World Lupines)		
<i>Lupinus pilosus</i> Murr.!	A	S
Clade D (Other Old World Lupines)		
<i>Lupinus micranthus</i> Guss!	A	A
Clade E (Western New World Lupines) ¹		
<i>Lupinus arboreus</i> Sims*!	P	S
<i>Lupinus albifrons</i> Benth*!	P	S
<i>Lupinus concinnus</i> J. Agardh*!	A	S
<i>Lupinus elegans</i> H.B.K.*!	P	A
<i>Lupinus luteolus</i> Kellogg*!	A	S
<i>Lupinus microcarpus</i> Sims		
var. <i>densiflorus</i> (Benth.) Jepson*!	A	S
<i>Lupinus polyphyllus</i> Lindley*!	P	S
<i>Lupinus pusillus</i> Pursh*!	A	A?
<i>Lupinus rivularis</i> Lindley*!	P	S
<i>Lupinus succulentus</i> Koch*!	A	S
Tribe Sophoreae		
<i>Sophora secundiflora</i> (Ortega) DC*	P	A
Tribe Phaseoleae		
<i>Glycine max</i> (L.) Merr.!	A	A
<i>Phaseolus vulgaris</i> L.!	A	A
Tribe Loteae		
<i>Lotus scoparius</i> (Torrey & A. Gray) Ottley*	P	S
<i>Melilotus alba</i> Medikus !	A/B	A

¹ These 5 species come from different groupings in the Ainouche & Bayer (1999) analysis

Tribe Viciaeae		
<i>Pisum sativum</i> L.!	A	S
<i>Vicia faba</i> L.!	A	S
<i>Lathyrus odoratus</i> L.!	A	S
Tribe Trifolieae		
<i>Medicago sativa</i> L.!	P	S
<i>Trifolium repens</i> L.!	P	S
Tribe Thermopsidaeae		
<i>Thermopsis macrophylla</i> Hook & Arn.*	P	S

*native species

! species available for testing

^a A = Annual, B = biennial, P = perennial

^b S = sympatric with target in either native or exotic range, A = allopatric with target

⊗ Note on horticultural varieties: The horticultural varieties presented are considered to have the following parentage (Aitkinson and Sheppard 2000, Cooke 1997). *Cytisus* x *spachianus* has a range of varietal names (*Cytisus racemosus* Hort, *Cytisus* ‘*Racemosus Nana*’, *Cytisus praecox* ‘*Nana*’, *Cytisus* ‘*Racemosus Scoparius Nanus*’, *Genista racemosa* Hort, *Genista hispanica* sensu Macoboy, *Genista* x *spachiana* and is considered to be a hybrid between *Genista canariensis* and *Genista stenopetala*). *Cytisus* ‘*Porlock*’ is considered to be a hybrid between *Genista monspessulana* and *Cytisus* x *spachianus*.

Host Range testing of *Arytinnis hakani*

Progress against this objective is nearing completion. Testing of *A. hakani* has been completed using no-choice tests. Seventeen of the plant species on the test plant list in Table 8 have been tested. Five replicate cages were set up with one plant of each of 5 or 6 test plant species. Two parallel cages were set up with one *G. monspessulana* control plant within. Five adult *A. hakani* couples (5 male and 5 female) per test plant were introduced into each cage with test plants and into the control cages. After 5 days the adults were removed. After 5 days exposure to *A. hakani*, all plants were observed for eggs, nymphs and adults once a week for 8 weeks. Three series of tests were performed. The results are given in Table 9. Eggs were observed on *G. monspessulana*, *Cytisus* “*Porlock*” (a horticultural cultivar of *G. monspessulana*, *Genista canariensis* and *Genista stenopetala* hybrid origin), *Spartium junceum* and *Lupinus arboreus*. Survival beyond early instar nymphs and greater than one week only occurred on *G. monspessulana* and *Cytisus* “*Porlock*”. We were able to complete more testing than defined in the objective because of parallel funding from the Cooperative Research Centre for Australian Weed Management as several species occur on Australian and American test plant lists. It should be possible to complete testing of *A. hakani* in 2001 if a final test plant list can be approved.

Table 9. Results of host specificity testing completed for *A. hakani* to date

Test Plant	Reps	Reps that received eggs	#Eggs per attacked plant	#Early instar Nymphs per attacked plant	#Late instar Nymphs per attacked plant	#Adults developed per attacked plant	Survival weeks
<i>Cytisus scoparius</i>	5	0	0.0	0.0	0	0	0
<i>Cytisus striatus</i>	5	0	0.0	0.0	0	0	0
<i>Cytisus porlock</i>	5	5	95.0	100.0	100	50	8
<i>Genista monspessullana</i>	5	5	162.5	76.7	43.3	33.3	8
<i>Ulex europeus</i>	5	0	0.0	0.0	0	0	0
<i>Spartium junceum</i>	5	3	20.0	20.3	13.3	1.6	6
<i>Lupinus arboreus</i>	5	4	3.8	1.0	0	0	1
<i>Lupinus polyphyllus</i>	5	0	0.0	0.0	0	0	0
<i>Lupinus rivularis</i>	5	0	0.0	0.0	0	0	0
<i>Lupinus concinnus</i>	4	0	0.0	0.0	0	0	0
<i>Lupinus densiflorus</i>	5	0	0.0	0.0	0	0	0
<i>Lupinus luteolus</i>	5	0	0.0	0.0	0	0	0
<i>Lupinus pusillus</i>	5	0	0.0	0.0	0	0	0
<i>Pisum sativum</i>	5	0	0.0	0.0	0	0	0
<i>Vicia faba</i>	5	0	0.0	0.0	0	0	0
<i>Medicago sativa</i>	5	0	0.0	0.0	0	0	0
<i>Trifolium repens</i>	5	0	0.0	0.0	0	0	0

Host range testing of *Bruchidius villosus*

A similar testing protocol to that used for *A. hakani* was used for the testing of *B. villosus* against American test plants. Five replicate cages were set up each containing one plant of *Lupinus arboreus*, *Lupinus elegans*, *Lupinus pilosus*, *Lupinus pusillus*, *Lupinus luteolus*, *Lupinus succulentus*, *Lupinus concinnus*, *Pisum sativum*, *Vicia faba*, *Cytisus Porlock*, *Viminaria juncea*, *Cytisus striatus*, and *Medicago sativa* with flowers and immature pods. On 1 June 2001, *Bruchidius villosus* adults (73 males and 73 females) collected from *C. scoparius* only stands at Lüneburger Heide / North Germany were added to each cage. *B. villosus* from *C. scoparius* was used because a) the *B. villosus* already in North America primarily uses *C. scoparius* as its host and b) *B. villosus* from *G. monspessulana* are active much earlier in the season when many of the test plants would not be available with flowers and pods. After twelve days the pods were checked for eggs, and the number noted. The plants were then left in the tents, and *C. scoparius*, *S. junceum*, and *G. monspessulana* were added as control plants. Six days later all pods were checked for eggs to verify that the beetles were still ovipositing and to identify any differences in egg numbers on test plants without and with control hosts.

Table 10. Oviposition by *B. villosus* in the no-choice/choice cage test

Plant species	Mean pods per plant	#Eggs Day 12	# Eggs Day 18	Pods with eggs Day 12	Pods with eggs Day 18	Survival to adult
<i>Cytisus scoparius</i>	7.6		1.2		1.2	+
<i>Genista monspessulana</i>	34.8		1.6		1.8	+
<i>Spartium junceum</i>	3.8		4.6		2	+
<i>Lupinus arboreus</i>	2.2	0	0	0	0	-
<i>Lupinus elegans</i>	23.4	6.2	3.2	2.2	3.2	-
<i>Lupinus pilosus</i>	8.4	0	0	0	0	-
<i>Lupinus pusillus</i>	16	0	0	0	0	-
<i>Lupinus luteolus</i>	39.2	0	0	0	0	-
<i>Lupinus succulentus</i>	10.2	0	0	0	0	-
<i>Lupinus concinnus</i>	14.6	0	0	0	0	-
<i>Pisum sativum</i>	13.8	0	0	0	0	-
<i>Vicia faba</i>	6.8	0	0	0	0	-
<i>Cytisus</i> "Porlock"	132.8	5.8	7.6	4.6	7.6	+
<i>Viminaria juncea</i>	38.4	0	0	0	0	-
<i>Cytisus striatus</i>	87	6.2	10.4	5.2	8.4	+
<i>Medicago sativa</i>	56	0	0	0	0	-

The results of this experiment are presented in Table 10. Oviposition occurred on the three known hosts and *Lupinus elegans*, *Cytisus* "Porlock" and *Cytisus striatus*. When the three control plant species were included within the cage, there was no apparent increase in the host species range over which eggs were laid. Eggs were laid on *L. elegans* for example, whether or not the control plants were present. Pod dissections subsequently showed that larval development to adult was only successfully completed on test plants in the subtribe Genistinea. These results are significant in that this is the first host range test of *B. villosus* in which eggs have been observed on any *Lupinus* species (Syrett and O'Donnell 1987, Isaacson 1998). These tests provide conventional host specificity testing evidence, for the first time, supporting the conclusion that risk to North American lupines from *B. villosus* is likely to be negligible.

Two further host choice experiments were carried out using *B. villosus* at CSIRO's European laboratory at the same time as this experiment on separate funding. They did not specifically address the objectives of this project. These experiments looked at a) host range of the local *Spartium junceum* origin *B. villosus* population against a wide range of potential European host plants, repeating the experiment carried out last year and reported in CSIRO (2000) and b) the host preference of two separate *B. villosus* populations, one from *Spartium junceum* and one from *C. scoparius*. These experiments are not reported here because they did not include American test plants. They are reported in CABI Bioscience Switzerland Center, June 2001 Quarterly Report (CABI 2001). A footnote from this work, however, was that *B. villosus* eggs on *Coronilla emerus* reported in CSIRO (2000) were subsequently found not to belong to *B. villosus* (CABI 2001).

Difficulties encountered and Remedial action

The main problem this year has been an invasion of the broom garden by the pyralid *Uresiphita polygonalis* Hb. It started to defoliate *G. monspessulana* stands in preference to adjacent stands of *C. scoparius* and *Chamaecytisus proliferus*. We took the opportunity to check its apparent broad host range in the literature and it showed the capacity to complete development on *Lupinus arboreus*. The difficulty was that it threatened the persistence of our stocks and garden stands of *G. monspessulana*. We were subsequently forced to apply a BT based Lepidoptera-specific insecticide to our field culture plots. The plants now appear to be recovering.

Recent developments

A recent study on the molecular phylogeny of legume-feeding psyllids and their Genisteae host plants within the Western Mediterranean and Macaronesian regions has contributed significantly to this project (Percy 2001). This study shows that the group of plant species that have previously been included under the genus *Teline* are themselves divided into two clear lineages that have separate origins within the large genus *Genista*. The lineages have been termed the *G. linifolia* group and the *G. monspessulana* group. This clearly supports the redundancy of *Teline* as a distinct genus, but also more clearly defines those species of *Genista* closely related to *G. monspessulana*. The *G. monspessulana* group includes 7 species (with several subspecies) on the Canary Islands and Madeira, where *G. monspessulana* does not occur and two species from the Mediterranean region, namely *G. monspessulana* and *Genista osmariensis* Cosson (the latter occurring only in Morocco). This group is also more closely related to other species of *Genista* in Spain and North Africa than to the *G. linifolia* group. These include *G. cinerea*, *G. ramosissima*, *G. clavata* and *G. umbellata*, although Percy sampled relatively few species of the genus *Genista* (9 species, excluding the '*Teline*' members, of > 70 spp.).

The study also showed that there could be a high level of genetic variation within one species. *Genista stenopetala* (which has 5 subspecies in the Canaries and also grown for fodder) is not a clearly defined species; different subspecies group geographically, being more closely related to other taxa from the same island. Where one subspecies of *G. stenopetala* has been moved between islands in the Canaries it has not picked up the psyllid species attacking another sub-species of *G. stenopetala* endemic to that island, but adults of a psyllid typically feeding on *G. microphylla* have been found on cultivated and naturalized *G. stenopetala* on Gran Canaria. Reports of within island hybridization may act to confound taxonomic distinction. This begs the question of whether similar variation exists for *G. monspessulana*. Great morphological variation has been described (Maire 1987) and most parts of the distribution of *G. monspessulana* are reproductively isolated from each other. What exactly is the French broom found in California? *Genista monspessulana* has not been subjected to a molecular analysis of within-species variation, and the full geographic range of this species around the Mediterranean has not been sampled for the presence of psyllids.

Percy's study also explores the massive diversification of psyllids on their Genisteae hosts within Macaronesia. A visit made to the Canary Islands last year, as part of this project, suggests that this is perhaps the only plant herbivore group that has undergone such diversification on these island groups. Other insect groups typically found on Genisteae in Europe e.g. the apionids and *Sitona* weevils were poorly represented there. Given that *G. monspessulana* does not occur on these islands, all this suggests that the remaining evolutionary center of origin for the *G. monspessulana* group and related species relevant to locating potential biological control agents for French Broom is both the northern and southern coasts of the Western Mediterranean.

Recommendations

Work this year has highlighted a number of recommendations

- 1) A separate taxonomic study of the Apionidae on the Genisteae. The time is now ripe for this given recent advances in molecular taxonomy and studies already completed that have characterized relatedness within and across key genera in the Genisteae. A PhD project as part of this project based at CSIRO's European Laboratory and its recently completed molecular lab is the most economic approach to completing this task. Funds have since been secured from the Cooperative Research Centre for Australian Weed Management to fund this project based in France.
- 2) Approval of a test plant list to complete the testing in 2002. Table 8 provides a recommended final test plant list based on the list in last years report. This list should be sent out for consideration and approval within the United States.
- 3) Rapid completion of agent selection to allow fast progress towards the testing and importation of potential agents.
- 4) Recent studies have highlighted the relevance of molecular studies of intra-specific variation in French broom across both its native and introduced range.
- 5) Expansion of the project to include other weeds in the Genisteae relevant to USA and Australia from Federal funding sources.

References

- Ainouche A.-K. and Bayer R.J. (1999) Phylogenetic relationships in *Lupinus* (Fabaceae: Papilionoideae) based on internal transcribed spacer sequences (ITS) of nuclear ribosomal DNA. *American Journal of Botany* 86: 590-607
- Aitkinson I. and Sheppard A.W. (2000) Broom biocontrol: conflicts of interest with the Australian Nursery Industry. *Plant Protection Quarterly* 15: 176-178
- Alonso Zarazaga M.A. (1985) Revisión de los taxones supraespecificos de la familia Apionidae en la region Paleártica (Coleoptera Curculionoidea). Doctoral thesis, University de Málaga 881 pp. (unpublished)
- Brandl, P. (1981) Bruchidae. In: Freude, H. (ed.) Die Kafer Mitteleuropas 10: 7-21.

- Briese D.T. and A Walker (in press) A New Perspective on the Selection of Test Plants for Evaluating the Host-Specificity of Weed Biological Control Agents: The Case of *Deuterocampta quadrijuga*, a Potential Insect Control Agent of *Heliotropium amplexicaule*. *Biological Control*
- Burckhardt D. (1989) Les psylles (Insecta, Homoptera, Psylloidea) de l'Algerie. *Archives des Sciences* 42: 367-424
- CABI Bioscience Switzerland Centre, June (2001) Quarterly Report.
- Caldara R. (1978) Revisione der *Pachytychius* Palearctici (Coleoptera: Curculionidae) *Estratto dalle Memorie della Società Entomologica Italiana* 56: 216 pp.
- Carter D.J. and Hargreaves B. (1986) A field guide to the caterpillars of butterflies and moths in Britain and Europe. Collins, London. 296 pp.
- Chandler P.J. (1975) Observations on plant associations of the Psilidae (Diptera). *Entomological Record and Journal of Variation* 87:13-17.
- Collin J.E. (1944) The British species of Psilidae. *Entomological Monthly Magazine* 80: 214-224.
- Cooke D. (1997) Draft list of horticultural brooms in Australia. Unpublished report, Animal and Plant Control Commission, Adelaide, South Australia.
- CSIRO Entomology (2000) Selection and testing of Biological Control Agents for Control of French Broom *Genista monspessulana* (L.)L. Johnson. Contracted Research Report Number 58
- Dengler K. (1997) On the life cycle of *Chyliza leptogaster* Panzer and *Chyliza nova* Collin (Psilidae, Diptera) and their importance as producer of phloem-necroses on deciduous trees. *Studia Dipterologica* 4: 143-164
- Ehret J.-M. (1990) Les Apions de France: clés d'identification commentées (Coleoptera Curculionidae Apioninae) *Bulletin mensuel de la Société Linneenne de Lyon* 59: 209-292
- Emmet A.M. (1988) A field guide to the smaller British Lepidoptera. 2nd edit. The British Entomological and Natural History Society, London UK 288 pp.
- Emmet A.M. and Heath J. eds. (1992) The moths and butterflies of Great Britain and Ireland Vol. 7 (2): Lasiocampidae – Thyatiridae with life history chart of the British Lepidoptera. Harley Books, UK, 400pp.
- Freude H., Harde K.W. and Lohse G.A. (eds) (1981) Die Kafer Mitteleuropas. Band 10. Bruchidae, Anthribidae, Scolytidae, Platypodidae, Curculionidae. Goecke & Evers, Krefeld, Germany. 310 pp.
- Frick K.E. (1962) A three-week study of *Bruchidius villosus*, the seed beetle that attacks the seeds of *Cytisus scoparius* in the eastern US. Unpublished file report, USDA, Agricultural Research Service, 10 pp.
- Greuter, W. et al. (Eds.) (1989) Med-Checklist Vol. 4 (published)
- Gurrea Sanz, P., de los Mozos Pascual M., Sanz Benito M.J. and Lucianez Sanchez M.J. (1988) Curculionidos (Coleoptera : Curculionoidea) asociados a las semillas de las Genisteas en la Sierra de Gredos. *Actas de Gredos Boletín Universitario* 7: 65-72
- Guynot A.L. and Massenot, M. (1958) Les Rouilles des Gênets et des Cytises [*Uromyces genistae-tinctoriae* (Pers.) Wint. Sensu lato]. *Uridineana* 5: 507-523
- Harris P. and McEvoy P. 1995. The predicability of insect host plant utilisation from feeding tests and suggested improvements for screening weed biological control agents. In . Proceedings of the VIIIth International Symposium on Biological

- Control of Weeds (eds. E.S. Delfosse and R.R Scott). Canterbury New Zealand, DSIR/CSIRO. Melbourne. pp. 125-131
- Higgins L.G. and Riley N.D. (1973) A field guide to the butterflies of Britain and Europe. Collins, London. 381 pp.
- Hinz H.L. (1992) Studies on the two seed feeding beetles *Apion fuscirostre* F. (Coleoptera: Apionidae) and *Bruchidius villosus* F. (Coleoptera: Bruchidae) : potential biological control agents for broom (*Cytisus scoparius*). M.Sc. thesis, Applied Entomology, Imperial College, London. 72 pp.
- Hodkinson I.D. and Hollis D. (1987) The legume feeding psyllids (Homoptera) of the weste Palaearctic region. Bulletin of the British Museum of Natural History (Entomology) 56: 1-86.
- Hoffmann A. (1950) Coléoptères Curculionides (1). *Faune de France* 52: 1-486
- Hoffmann A. (1958) Coléoptères Curculionides (3). *Faune de France* 62:1209-1841
- Hosking J.R. (1995) The impact of seed- and pod-feeding insects on *Cytisus scoparius*. In . Proceedings of the VIIIth International Symposium on Biological Control of Weeds (eds. E.S. Delfosse and R.R Scott). Canterbury New Zealand, DSIR/CSIRO. Melbourne. pp.45-51
- Isaacson D. (1998) A petition to import *Bruchidius villosus* from North Carolina to the western U.S. for biological control of Scotch broom (*Cytisus scoparius*). Unpublished report, Oregon Dept of Agriculture.
- Iwasa M. (1989) The Japanese species of the genus *Chyliza* (Diptera, Psilidae). Japanese Journal of Entomology 57: 148-162.
- Kloet G.S. and Hincks, W.D. (1972) A checklist of British insects, 2nd edit. Lepidoptera. Handbooks for the identification of British insects. Volume 11, part 2. Royal Entomological Society of London, 153 pp.
- Lyneborg L. (1987) On the life history of *Chyliza annulipes* Macquart, 1835 (Diptera: Psilidae). Entomologiske Meddelelser 55:27-29.
- Maire, R. (Quezel, P., Ed.) (1987) Flore de l'Afrique du Nord, Vol. 16. Dicots. Leguminosae
- Mazay S. (1993) Etude de la faune entomologique s'attaquant à la reproduction du cytise à balais, *Cytisus scoparius* (L.) Link (Fabaceae) en Cévennes méridionales. Ingenieur Agronome thesis Universite Libre de Bruxelles, Bruxelles. 67 pp.
- Percy, D.M. (2001) Diversification of legume-feeding psyllids (Hemiptera, Psylloidea) and their host plants (Leguminosae, Genisteae). PhD Thesis University of Glasgow pp. 317..
- Percy, D.M. (in press) Legume-feeding psyllids (Hemiptera, Psylloidea) of the Canary Islands and Madeira. *Journal of Natural History*.
- Polhill, R.M. (1981). Papilionoideae. In: Advances in Legume Systematics Part 1, eds. R.M. Polhill and P.H. Raven, Royal Botanic Gardens, Kew. pp. 191-208
- Pottier-Alapetite G. (1979) Flore de la Tunisie 1:310
- Sanz Benito M.J. and Gurrea Sanz P. (1991) Inventario y analysis biogeografico preliminar de las especies de Curculionoidea (Coleoptera) de Genisteae en Las Sierras del Sistema Central (Peninsula Iberica). *Graellsia* 47: 117-127
- Sanz Benito M. J., Gurrea Sanz P. and García Oejo Izquierdo A. (1989) Aspectos sobre la biologia y fenologia de *Pachytychius sparsutus* Ol. 1807 (Coleoptera: Curculionoidea) en el Macizo Central de la Sierra de Gredos (Sistema Central).

Actas de Gredos Boletín Universitario 9: 1-8

- Sanz Benito M.J., Gurrea Sanz, P., de los Mosos Pascual, M., Martín Cano, J. and Munguira M. L. (1990) Distribución del nicho ecológico de los Curculionidae (Coleoptera) en los *Cytisus* en la Sierra de Alcaraz. *Jornados sobre el medio natural Albacense, Albacete*. pp. 203-5.
- Suire J. (1951). Microlepidopteres des plantes caracterisant les zones naturelles de la costiere. Memoires de la Societe d'Etudes des Sciences Naturelles de Nimes N°8, Contribution à la mise en valeur de la Costiere du Gard (Etude du Milieu)
- Suire J. (1965) Microlepidopteres de la Costiere du Gard. Doctoral thesis, Ecole de L' Agriculture Montpellier, France.
- Syrett P. and O'Donnell D.J. (1987) A seed-feeding beetle for biological control of broom. A.J. Popay, (ed.). Proceedings of the fortieth New Zealand Weed and Pest Control Conference, 11-13 August 1987. Nelson, New Zealand. pp. 19-22.
- Syrett P., Fowler S.V., Coombs E.M., Hosking J.R., Markin G.P., Paynter Q.E. and Sheppard A.W. (1999) The potential for biological control of Scotch broom (*Cytisus scoparius*(L.) Link) and related weedy species. *Biocontrol News and Information* 20:17-34
- Szentesi Á. and Wink M. (1991) Fate of quinolizidine alkaloids through three trophic levels: *Laburnum anagyroides* (Leguminosae) and associated organisms. *Journal of Chemical Ecology* 17: 1557-1573
- Velázquez de Castro A.J., Alonzo Zarazaga M.A., and Outerelo R.(1990) Curculionidae (Coleoptera) de Navacerrada, Sierra de Guadarrama (España). *Boletín de la Real Sociedad Española de Historia Natural (Sección Biológica)* 85: 17-37
- Wapshere A.J. (1974) A strategy for evaluating the safety of organisms for biological weed control. *Annals of Applied Biology* 77:201-211