Application to release the gorse pod moth, *Cydia succedana* (Lepidoptera: Tortricidae) for the biological control of gorse, *Ulex europaeus* L. (Fabaceae)

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Application submitted by the
Department of Primary Industries Victoria
P.O. Box 48, Frankston Victoria, 3199

Technical contact
John Ireson
Tasmanian Institute of Agriculture
University of Tasmania
John.Ireson@utas.edu.au

Administrative contact:
Jean-Louis Sagliocco
Invertebrate Sciences, Biosciences Research
Department of Primary Industries, Victoria
Jeanlouis.Sagliocco@dpi.vic.gov.au

State Government Victoria Department of Primary Industries

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Appendix 2 - Hill, R.L. and Gourlay, A.H. (2002). 'Host-range testing, introduction and establishment of *Cydia succedana* (Lepidoptera: Tortricidae) for biological control of gorse, *Ulex europaeus* L., in New Zealand,' *Biological Control* 25, 173-86.

Appendix 3 - Application for approval of the release of gorse pod moth, *Cydia succedana* (Denis and Schiffermüller), a potential biological control agent for gorse, *Ulex europaeus* L. (submitted 2001).

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Appendix 5 – Withers, T.M, Watson, M. and Gresham, B. (2012). Assessing gorse pod moth (*Cydia succedana*) infestation levels in *Lupinus* spp. at Tikitere Forest, New Zealand. Unpublished report, Scion, Rotorua, New Zealand.

Acknowledgements
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1. Executive Summary

Background

The gorse pod moth, *Cydia succedana* (Lepidoptera: Tortricidae), was approved for release in Australia in 2001 following host specificity studies on 79 species or cultivars of plants. However, the moth's release in Australia was postponed when field surveys in New Zealand revealed that it could exploit the weedy perennial *Lupinus arboreus* and some *Lotus* species. Subsequent New Zealand studies from 2003 to 2006 found that the release of untested moths from Portugal, coupled with asynchrony between the flight period of gorse pod moth and gorse flowering, explained the unanticipated non-target attack in New Zealand. Furthermore, the results of repeated host testing on *Lotus* and other species, using moths from England, concurred with the original tests and suggested that the English populations would be unlikely to exploit non-target species. To confirm that gorse pod moth from England would not be a major risk to commercial lupin species or cultivars grown in Australia, a host specificity study was conducted on selected cultivars in quarantine at Frankston, Victoria, over a three-year period from 2009-2011. A comparison of the phenology of gorse pod moth, gorse and the lupins grown commercially in Australia and their susceptibility to attack under field conditions in New Zealand was also undertaken in 2011/12.

Additional host testing on lupins and phenological differences to gorse

Commercial cultivars of lupins chosen for the quarantine host specificity testing of gorse pod moth in Australia were *Lupinus luteus* L. cv. 'Pootalong,' *Lupinus albus* L. cv. 'Kiev' and *Lupinus angustifolius* L. cv. 'Wonga'. Standard no-choice larval starvation tests provided additional confirmation that English populations of gorse pod moth display a preference for gorse, *Ulex europaeus*, over the test plant species. Tests conducted during 2009/10 showed that English populations of gorse pod moth would be unlikely to survive on cultivars of *L. angustifolius* and therefore support the earlier tests on this species. However, a higher level of development on *L. albus* and *L. luteus* of 20% and 14% respectively in the no-choice starvation tests, although significantly lower than the 44% that survived on gorse, suggested that some low level impact on cultivars of these species could occur. Although there was no significant difference between numbers of eggs laid on gorse, *L. albus* and *L. luteus* in tests conducted in 2011, none of the larvae hatching from these eggs survived to the pupal stage on lupins. However, 24% of the larvae that hatched from the eggs laid on gorse developed to the pupal stage and emerged as adults. Paynter *et al.* (2008) showed that virtually all non-target attack in New Zealand by the Portuguese population of gorse pod moth was recorded when gorse was not in flower during summer. The lupin species on which gorse pod moth was recorded in New Zealand was the perennial weedy species *Lupinus arboreus* that flowers mostly in summer, after the peak flowering period of gorse. Commercial cultivars of lupins grown in Australia are annuals. These are usually planted from mid-April until early June. Flowering and immature pod and seed development in these lupins occurs in late winter and spring and corresponds with flowering and immature pod and seed development in gorse which occurs over a longer period. Cultivars of the commercial lupin species are harvested for their seed in summer, however, mature pods and seeds are not attacked by larvae of gorse pod moth. The phenology of commercial lupin species therefore negates the risk of any non-target attack by gorse pod moth.

Recommendation for release

A recent study by Withers *et al.* (2012) in New Zealand using commercial cultivars of *L. angustifolius*, *L. albus* and *L. luteus* imported from Australia confirmed the unlikelihood that these cultivars would be attacked during their growing season in Australia. Withers *et al.* (2012) found that no lupin pods of commercial Australian cultivars directly exposed to gorse pod moth under field conditions were attacked during spring when gorse was flowering. As expected, any non-target pod moth infestations were recorded when gorse was not flowering. Therefore, in Australia, it is unlikely that commercial cultivars of lupins will be attacked and any risk that larvae could survive on commercial species/cultivars of lupins in numbers large enough to inflict significant damage is very low. The release of gorse pod moth for the biological control of gorse in Australia is therefore recommended.

2. Information on the Target, *Ulex europaeus* L.

2.1 Taxonomy

Order: Fabales
Family: Fabaceae (= Leguminosae)
Sub-family: Papilionoideae
Tribe: Genisteae
Genus/Species/Author: *Ulex europaeus* Linnaeus, 1753
Common name: Gorse, Furze

2.2 Description

Gorse is a prickly, perennial evergreen shrub which, if left undisturbed, can grow to a height of about 4 m but is usually less than 2.5 m high and up to 3 m in diameter (Richardson and Hill 1998). Leaves are 1-3 cm long and spine-like changing from grey green when young to dark green as they mature. Flowers are pea-like and bright yellow, 1.5-2 cm long and borne mostly in leaf axils and terminal clusters. Seed is about 3 mm long and changes in colour from green to brown to black depending on maturity (Parsons and Cuthbertson 2001). The seed is produced in hairy, ovoid pods 1-2 cm long. Seed dispersal is primarily by pod dehiscence. The seed can be ejected up to 5 m (Moss 1959) although Hill *et al.* (1996) found that most seed fell within 2.5 m, usually in or near the canopy of mature bushes. Seed densities have been measured in a number of studies both in and on the soil ranging from 2,660 to 10,000/m^2 (Richardson and Hill 1998). Seeds can remain viable in the soil for at least 25 years (Moss 1959).

2.3 Native range and centre of origin

Gorse is a native of central and Western Europe and the British Isles (Parsons and Cuthbertson 2001) where it occurs in native heathland (Tubbs 1974) and on disturbed or neglected farmland and forests (Zwölfer 1962). The centre of origin of the genus *Ulex* is the West Iberian Peninsula which includes western Spain and Portugal from where 15 species and six sub-species are now recognised (Cubas 1999).

2.4 Australian and overseas distribution

Gorse is found across temperate Australia (Fig. 1) and infests up to 1 million hectares (Anon 2009). Potential distribution based on climate is 87 million hectares which includes most agricultural land in Victoria, Tasmania, coastal South Australia and much of south west Western Australia. The main problem regions are principally in Victoria and Tasmania. In Tasmania it grows from sea level to 800 m in altitude within an annual rainfall area of 500-1500 mm. The heaviest infestations covering *ca.* 30,000 ha occur in midland areas on pastures grazed mainly by sheep (Ireson *et al*. 1999). Isolated heavy infestations also occur on the West Coast near Zeehan and along the East Coast. It is also present on King Island.

In Victoria, gorse is distributed throughout the state except for the Mallee and parts of Gippsland (Anon 2009). Lane *et al.* (1980) listed gorse as Victoria's sixteenth most widespread weed. Their surveys showed that gorse occupied an estimated total area of 948,000 ha with scattered infestations found on 805,000 ha and medium to dense infestations on 143,000 ha. Some of the heaviest infestations have been recorded in the Central Highlands around Ballarat where an estimated 8,000 ha of public and private land were reported to be infested in 1999 (Miller *et al.* 1999).

In South Australia it has a scattered distribution over several thousand hectares in the higher rainfall areas of the state, particularly in the Mt. Lofty ranges, Barossa and Clare Valleys, the Eyre, Fleurieu and Yorke Peninsulas, Burra, Jamestone and Wakefield. It also occurs on

Kangaroo Island. In New South Wales its distribution is limited to about 2,000 ha mainly in the south east and southern Tablelands, Blue Mountains and around Lithgow (Anon 2009).
Gorse is uncommon in Western Australia, Queensland and the ACT. In Western Australia gorse has become the focus of an intensive control programme as the total area infested is less than 100 ha spread over 360 locations mostly within 50 km of Albany (Moore and Williams 2008).
Gorse occurs in most temperate areas of the world and is now considered a weed in more than 30 countries. Apart from many European countries it is also found in Argentina, Brazil, India, Iran, New Guinea, South Africa and Trinidad (Holm *et al.* 1997). It is regarded as a serious weed in New Zealand, Hawaii, Chile and North America in the Pacific Coast States of Washington, Oregon, and California (Hill *et al.* 2008).

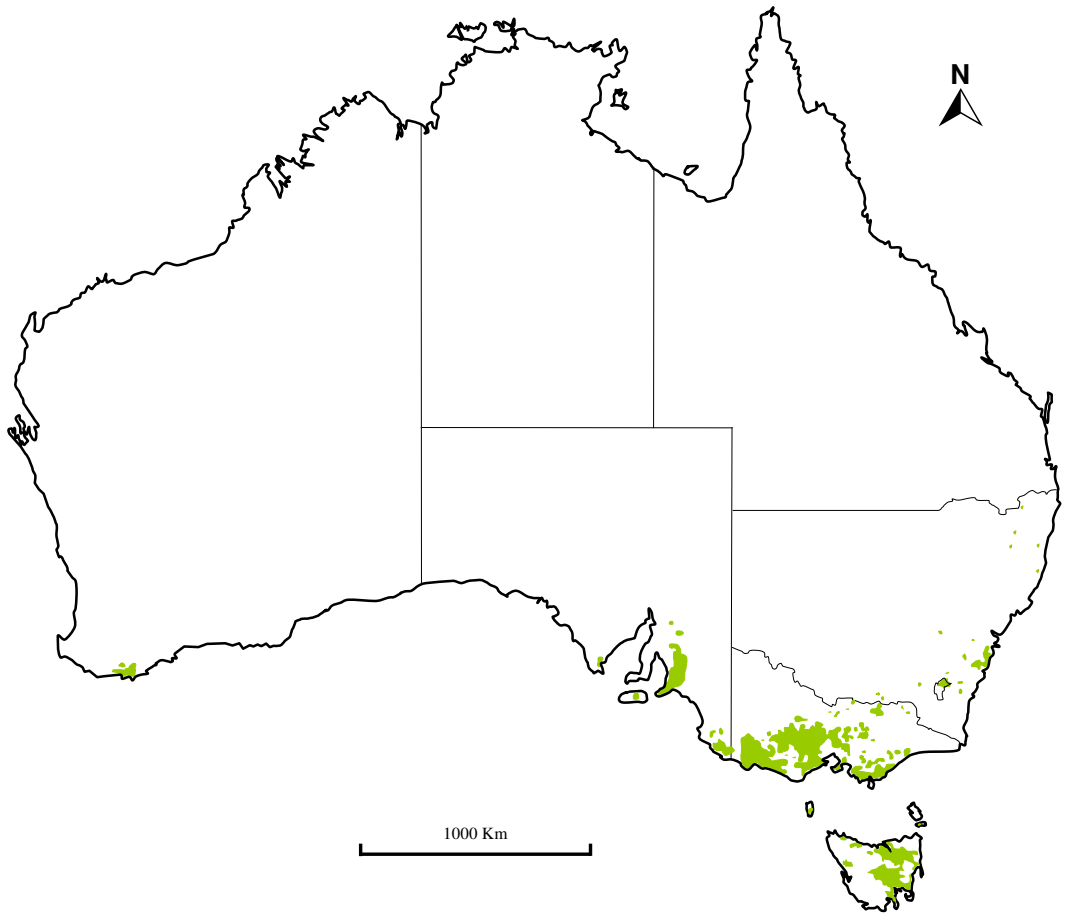

Figure 1. Current distribution of gorse in Australia (Tasmanian Institute of Agriculture).

2.5 Native and introduced related species

Ulex europaeus belongs to the order Fabales, family Leguminosae, subfamily Papilionoideae, tribe Genisteae, sub-tribe Genistinae. The relationships of the different tribes and the position and origin of their genera was redefined by Lewis *et al.* (2005) (Figures 2-3, Table 1).

Table 1. List of exotic genera in the tribe Genisteae naturalised in Australia and their regions of origin (Lewis *et al.* 2005)

Subtribes	Genera	Region of origin
Lupinae	*Lupinus*	Europe, Africa and South America
Cytisinae	*Calicotome*	Southern Europe, North Africa
Cytisinae	*Cytisus*	North Africa, Europe
Genistinae	*Genista*	North Africa, Europe
Genistinae	*Retama*	Southern Europe, North Africa
Genistinae	*Spartium*	Southern Europe
Genistinae	*Ulex*	Europe

In Australia, there are several exotic genera within the tribe Genisteae that contain species that are naturalised and invasive (Table 1) but there are no native Australian species within this tribe (Hosking *et al.* 1998).

The genera that contain species naturalised in Australia are discussed as follows:

Lupinus

Lupins are the largest of the legume crops grown in Australia and are used by pastoralists as a seed and fodder crop. In 2010, the total area sown to lupins in Australia was 692,000 ha which produced 823,000 tonnes of grain. About 77% of this production was in the south west of Western Australia, 10% in New South Wales, 9% in South Australia and 4% in Victoria (Australian Bureau of Statistics 2011). There are currently no significant areas used for growing lupins in Tasmania or Queensland. Although widely cultivated in Australia, some lupin species also have significant potential as weeds of pastures, crops, roadsides and other disturbed sites (Groves *et al.* 2005; Richardson *et al.* 2006) (Table 2). *Lupinus angustifolius* L. (narrow leafed lupin), *L. albus* L. (white or albus lupin) and *L. luteus* L. (yellow lupin), are the primary commercial species grown in Australia. Production is dominated by *L. angustifolius* which constitutes over 95% of all tonnage, with *L. albus* and *L. luteus* making up most of the remainder (Glencross 2007).

Table 2. Distribution and status of most common lupin species in Australia

Species	NSW	QLD	SA	TAS	VIC	WA	Status
Lupinus albus L. (white lupin)	-	+			+	+	Used for cropping
L. angustifolius L. (narrow-leaf lupin)	+	+	-	+	+	+	Most widely used cropping species but also listed as an environmental weed in WA (Groves *et al.* 2005)
L. arboreus Sims (tree lupin)				+	+		Listed as a sleeper weed in Tasmania and Victoria (Groves *et al*. 2005)
L. cosentinii Guss. (sand plain lupin, blue lupin)	+	+					Used for cropping but also listed as a significant environmental weed in WA (Groves *et al.* 2005)
L. luteus L. (yellow lupin)	+		+			+	Used for cropping but also listed as an environmental weed in WA (Groves *et al.* 2005)
L. pilosus L. (blue lupin)	+					+	Used for cropping
L. polyphyllus Lindley (Russell lupin)	+		+		+		Grown as an ornamental, but also listed as a sleeper weed in Victoria (Groves *et al.* 2005)

Calicotome

The only species recorded in this genus in Australia is *Calicotome spinosa* Link (spiny broom) which is native to the Mediterranean region. It was originally used as a garden or

hedge plant in Victoria where it has become a weed in higher rainfall areas (Richardson *et al.* 2006). It has not been recorded as a weed in other states (Parsons and Cuthbertson 2001).

Cytisus

Cytisus scoparius (L.) Link subsp. *scoparius* (English broom, Scotch broom) is native to Europe and has become a serious weed in parts of New South Wales, Victoria, South Australia and Tasmania where it is distributed over at least 200,000 ha (Hosking *et al.* 1998). Two other species within the *Cytisus* group are also naturalised in Australia. *Cytisus palmensis* (Christ) Hutch. (=*Chamaecytisus palmensis* (Christ) Bisby and Nicols =*Chamaecytisus prolifer* (L. f.) Link) (tagasaste, tree lucerne) is native to the Canary Islands. Although promoted as a fodder plant mainly in Western Australia it is also an environmental weed (Hosking *et al.* 1998) and is found in all states (Richardson *et al.* 2006). *Cytisus multiflorus* (white Spanish broom), a native of western Europe, is a popular garden plant that has now become a potentially serious agricultural and environmental weed in South Australia and Victoria (Richardson *et al*. 2006). It is on the Federal Government's alert list of 28 environmental weeds.

Genista

Genista monspessulana (L.) L.A.S. Johnson (Cape broom, Montpellier broom) is native to Europe and the Mediterranean and a serious environmental weed in Australia, occurring in all states. *Genista linifolia* L. (=*Teline linifolia* (L.) Webb & Berth) (flax-leaf broom) is native to the western Mediterranean and is a garden plant that has become weedy in parts of Victoria, South Australia, New South Wales and Western Australia but is not naturalised in other states (Parsons and Cuthbertson 2001). Other Genista species considered to be naturalised in Australia and native to either Europe and the Mediterranean or western Asia are: *G. horrida* (Vahl) DC., *G. monosperma* (L.) Lam., *G. stenopetala* Webb & Berth. (=*G. maderensis* (Webb & Birth.) and *G. tinctoria* L. (Hosking *et al.* 1998; Richardson *et al.* 2006). There are also several species hybrids of Genista developed by the nursery trade that have the potential to become weeds.

Retama

Retama raetam (Forssk.) Webb & Berthel. (white weeping broom) is native to the Mediterranean region, the Middle East and Northern Africa. It was brought to Australia as an ornamental shrub and is now an invasive threat in the drier regions of South Australia and Western Australia (Richardson *et al.* 2006). It is on the Federal Government's alert list of 28 environmental weeds. A closely related species, *Retama monosperma* (L.) Boiss. (bridal broom), although once a popular ornamental in Australian gardens, is no longer considered suitable because it poses a similar environmental threat (CRC for Weed Management 2003).

Spartium

Spartium junceum (L.) (Spanish broom) is native to the Mediterranean region and is a weed of roadsides and bushland in New South Wales, Victoria, South Australia and Tasmania (Richardson *et al.* 2006).

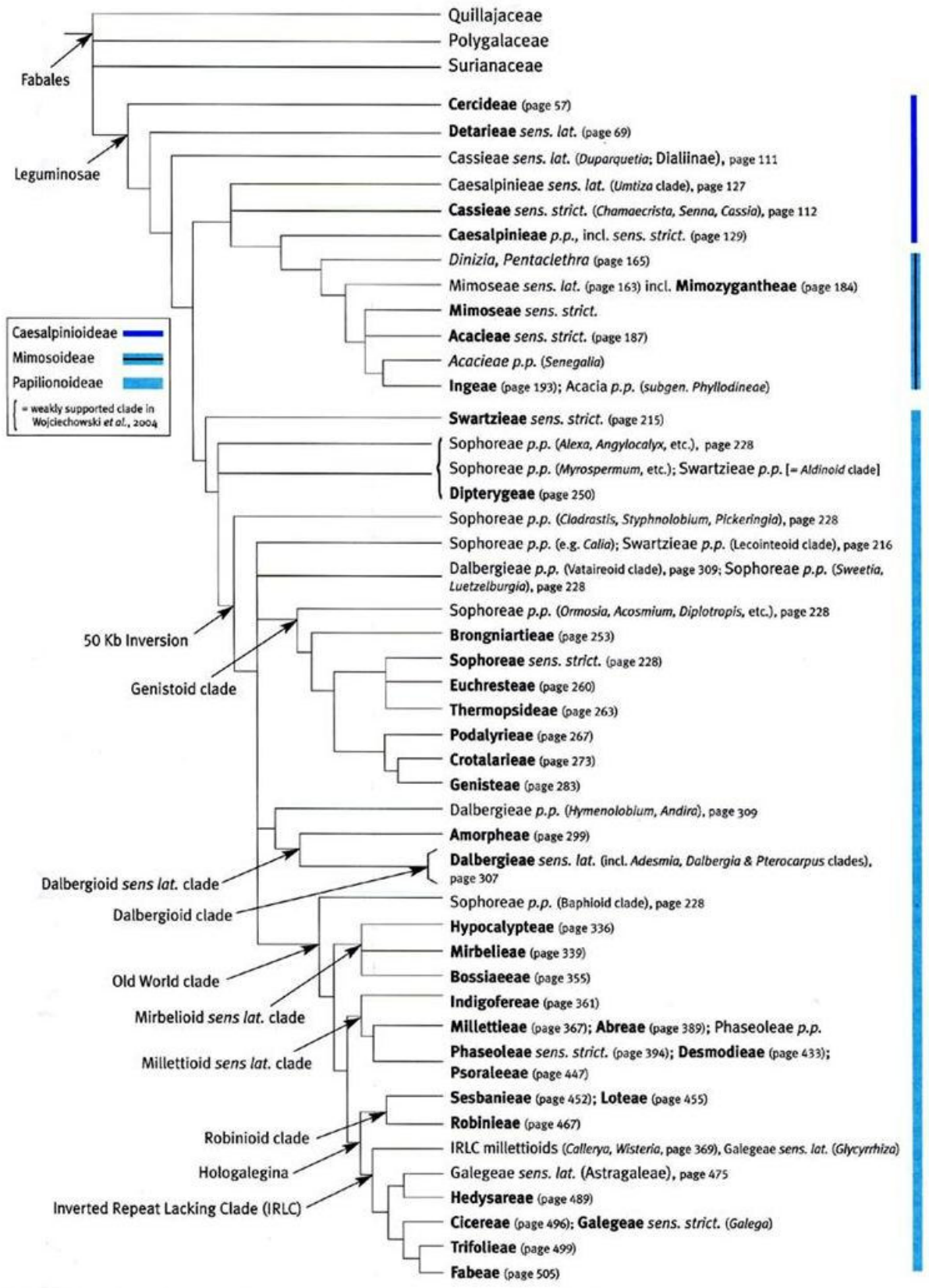

FIG. 1 **Phylogeny of Leguminosae compiled as a supertree, based on analyses by Doyle *et al.* (2000); Crisp *et al.* (2000); Wojciechowski *et al.* (2000; 2004); Pennington *et al.* (2001); Kajita *et al.* (2001); Herendeen *et al.* (2003a); Luckow *et al.* (2003); Wojciechowski (2003). The 36 tribes dealt with in this volume are in bold type. Page references are either to the beginning of the tribe or to the diagram of relationships following the introduction to that tribe**

Figure 2. Phylogeny of the Leguminosae family (reproduced from Lewis *et al.* 2005)

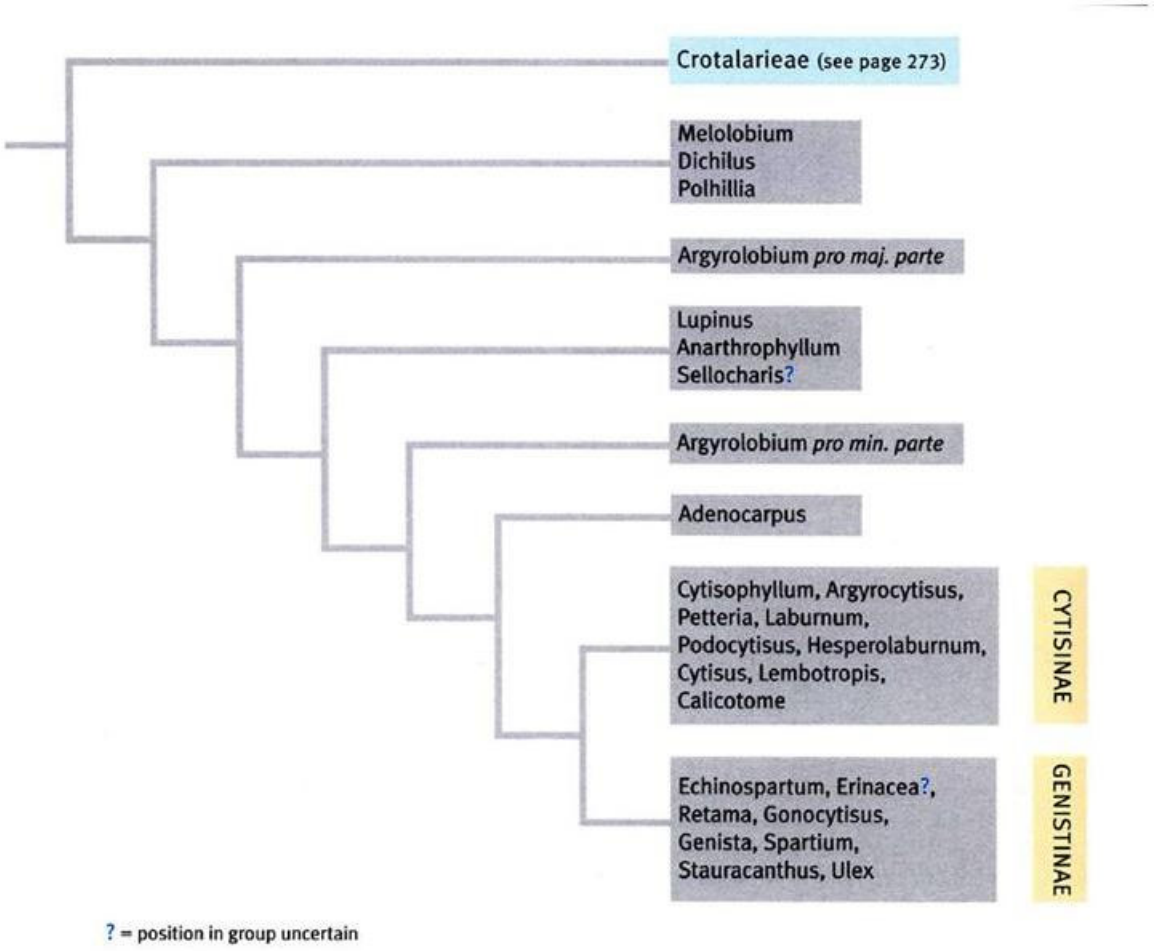

FIG. 38 **Diagram of relationships in tribe Genisteae after Käss & Wink (1997); Pyne (1999); Crisp *et al.* (2000); Cubas *et al.* (2002); Wink & Mohamed (2003); Pardo *et al.* (2004)**

Figure 3. Diagram of relationships in the tribe Genisteae showing position of genera in sub-tribes (reproduced from Lewis *et al.* 2005).

2.6 Approval as target for biological control

Gorse was approved as a target for biological control in July, 1995, following nomination by the Department of Primary Industries and Fisheries Tasmania (Ireson *et al.* 1999).

2.7 Pest status

Gorse is a Weed of National Significance (Thorp and Lynch 2000). In the main problem areas of Tasmania and Victoria, gorse is considered a serious weed because it invades pastoral land and significantly reduces pasture and animal productivity, and provides habitats and shelter for vertebrate pests. In forestry plantations it reduces tree growth and survival and is a significant fire hazard. It invades bushland reducing access and conservation values, increasing fire hazards and threatening the survival of rare and endangered plants and plant communities. It is also a fire hazard in urban areas. Gorse is difficult and expensive to control with currently available methods and necessary control by public authorities along roadsides and railways lines involves high financial inputs.

The annual costs of gorse management to agricultural and forest industries across Australia have been estimated at $7 million (Thorp and Lynch 2000). In Tasmania, the annual loss of

productivity of animal industries due to the presence of gorse has been estimated at $1 million per year in the central and northern midland areas alone (Ireson *et al.* 1999). This figure would be much higher if other areas of Tasmania were included as infestations occur on rural land in all parts of the State. In Victoria, an economic analysis on the costs of gorse to the community in the central highlands region (Miller *et al.* 1999) found that an ongoing 'do nothing' strategy would result in $7 million in tangible and intangible costs to the community over five years. The analysis also showed that the implementation of a control strategy in the region over a five year period would provide a total economic benefit of approximately $2.1 million. No figures are available on losses attributable to gorse from other States.

2.8 Other methods of control available

Traditional control methods most commonly used are:

Herbicides

A range of herbicides are registered for gorse control with costs ranging from $300/ha up to $1,660/ha depending on the height and density of the infestation (Anon. 2009). Follow up spraying may be necessary after 12 months.

Mechanical clearing

Mechanical clearing is the best method for controlling large infestations on land that is suitable for sowing down to pasture. Costs can range from $200/ha up to $2,900/ha. (Anon. 2009). The aim is to reduce the above-ground mass of gorse before follow-up methods are applied which can include spraying, restoring pasture, grazing or cultivation. Bulldozers with rippers, or medium or heavy tractors with dozer blades and rippers attached are used. Since the object of mechanical grubbing is to rip out as much of the root system as possible, this work is usually done when the ground is soft. Gorse mulching, using a heavy duty rotary hoe pulverises the gorse and incorporates the plant material into a form of mulch that provides suppression of seedlings. Crushing with a tractor-mounted "Meri Crusher" breaks bushes into small pieces and incorporates broken material in the top 10 cm of soil, usually resulting in less regrowth than other mechanical methods (Anon. 2009).

Fire

Burning alone will not adequately control gorse and it must be used in combination with other control options. By itself, burning is only a stopgap measure as regrowth of established bushes and seedling establishment is generally rapid after burning. Burning reduces the amount of foliage drastically and produces green shoots, which are far more attractive to goat or sheep browsing than mature shoots. Burning can be a useful way to remove dead gorse at least 12 months after spraying. Because burning live gorse destroys competitive cover and stimulates regenerative growth and seedling germination, it must be followed-up with spraying, establishment/maintenance of pasture and grazing. Burning living infestations will also germinate seed. The resulting seedlings can then be controlled by herbicides or heavy grazing (Anon 2009).

Grazing

Grazing by sheep is the best method for controlling gorse seedlings. After a dense gorse infestation has been removed and the area sown to pasture it can be grazed heavily by sheep during the spring and summer to prevent the establishment of gorse seedlings. Sheep will browse established gorse bushes during spring or when alternative feed is in short supply. However, they prefer to eat pasture species so that significant control cannot be achieved by sheep grazing unless large numbers are confined to gorse patches for most of the year.

Harradine and Jones (1985) showed that Angora goats are ideal for gorse control. Goats prefer to browse young gorse shoots rather than graze actively growing pasture. They remove flowers and defoliate bushes, browsing them back to stumps when the stocking rate is high enough. However, well-established gorse bushes are not readily killed by browsing and are capable of recovery after several years of browsing if the goats are removed from the area.

Management
Irrespective of the control methods employed, the prevention of reinfestation by gorse or of infestation by other weeds as a result of the removal of gorse cover is a matter of great importance. Before control or eradication is attempted there should be a clear idea of how the land is to be used and treated afterwards. For instance, the establishment of a vigorous, correctly fertilised permanent grass and clover sward will do much to suppress seedlings and will also allow heavier stocking rates. Grazing is an important factor in preventing recolonisation in cleared areas. Regrowth and any surviving young plants can be spot sprayed.

2.9 Effectiveness of current control methods

The difficulties and cost of controlling gorse by traditional methods has resulted in the investigation of classical biological control as an additional option that could be used in conjunction with current methods as part of a long term integrated control strategy.
A combination of traditional methods i.e. the use of herbicides, burning, cultivation and grazing can contain the problem on agricultural land and other mainly accessible areas. However, gorse is also a serious environmental weed in disturbed areas of a variety of vegetation types (Wells 1991; Anon. 1997). The use of traditional control methods to contain its spread into areas of native vegetation is more difficult because of the risk of damage to surrounding desirable species and limited accessibility.
Biological control offers an alternative solution to the problem if the introduction of a guild of agents can reduce gorse vigour to a stage where it can be controlled more easily by traditional methods at a much lower cost, its spread is restricted due to reduced seed output, and/or native vegetation is able to compete with it more readily.

3. Information on potential agent, *Cydia succedana* Denis & Schiffermüller

3.1 Taxonomy

Class: Insecta
Order: Coleoptera
Family: Tortricidae
Sub-family: Olethreutinae
Tribe: Grapholitinae
Genus/Species/Author: *Cydia succedana* (Denis & Schiffermüller, 1775)
Common name: Gorse pod moth

3.2 Biology

The moth ranges from 5-8 mm in length and has a wing span of 12-16 mm. The ground colour of the forewing is white or greyish white with grey or brownish grey markings. Eggs are white, flat and *ca*. 1 mm in diameter. Neonate larvae are white with black heads. Mature larvae are pale yellow with yellowish brown head capsules. Gorse pod moth is considered a bivoltine species, usually completing two generations each year in Europe and New Zealand (Emmet 1988; Suckling *et al*. 1999; Sixtus 2004; Hill *et al.* 2008; Paynter *et al*. 2008), although it can be univoltine in cooler localities such as Scotland (Emmet 1988; Razowski

2003). In New Zealand, gorse pod moth adults emerge in spring and oviposit on spring-flowering gorse. Larvae feed inside seed pods and emerge to pupate outside the pod in mid-late summer Some of these pupae overwinter and emerge the following spring but a significant percentage of new adults emerge in late summer and oviposit on autumn-flowering gorse (Withers *et al*. 2008). Second generation larvae overwinter in a cocoon and pupate in spring (Razowski 2003). New Zealand studies (Sixtus 2004) have shown that adult pod moth numbers are either low or zero in mid-winter (June-August). First generation adults emerge in spring from mid-September increasing to a maximum between November and January. There is variation between sites, depending on weather conditions (Sixtus 2004) and considerable generation overlap. Second generation adults probably emerge as early as late December at some sites, with peak emergence occurring somewhere between February and May (Sixtus 2004). Larvae of first generation gorse pod moth would be feeding on gorse seeds mainly from October with second generation larvae possibly starting to feed in January.

3.3 Native range

According to Razowski (2003) gorse pod moth is known from western Europe to Transcaucasia, Asia Minor, Iran, Afghanistan, Kazakhstan and Mongolia. The probable centre of origin of the species is Western Europe.

3.4 Related species and a summary of their host range

Cydia is a large genus with a worldwide distribution. Emmet (1988) lists 33 British species of *Cydia*, most of which attack buds, flowers or fruit of their host plants. Some generalist species have become pests in many parts of the world. In Australia, these include the codling moth, *Cydia pomonella* (L). and the closely related oriental fruit moth, *Grapholita molesta* (Busck). However, most species have been recorded from only a few closely related hosts. There has been some disagreement between authorities on the distinction between gorse pod moth (*C. succedana*) and *Cydia ulicetana* (Haworth). Danilevsky and Kuznetzov (1968) recognised them as separate species, but Bradley *et al.* 1979 and Emmet 1988 considered *C. ulicetana* to be an inferior synonym of gorse pod moth. Although Razowski (2003) reinstated the separation between the two species, Paynter *et al.* (2008) pointed out genitalia differences that had been used to separate gorse pod moth and *C. ulicetana* were not a reliable identification feature. More significant was their molecular analysis on *Cydia* specimens collected from New Zealand, England and Portugal which showed they were identical and concordant with any natural variation within a single species. Paynter *et al.* (2008) concluded that the species involved in the New Zealand biological control program was gorse pod moth as well as providing evidence that intra-specific host races of gorse pod moth may exist which could differ in their host specificity. Apart from *Ulex* spp. there are literature records of gorse pod moth feeding on several other members of the Genisteae and on Lotus (Loteae) in the moths native range (Hill and Gourlay 2002). However, host range tests on gorse pod moths sourced from England indicated that the English populations were highly host specific (Hill and Gourlay 2002) suggesting that records of gorse pod moths from other hosts could be erroneous (Paynter *et al.* 2008).

3.5 Proposed source of the agent

It is proposed that the collection and consignment of moths will be from England and conducted by the Commonwealth Agricultural Bureau International.

3.6 Mode of action

Larvae bore into pods and consume the developing seeds. Once all seeds are consumed, larvae can emerge through holes chewed in the pod and seek another pod. Individual larvae have been found to destroy two to three pods in the course of their development (Hill and Gourlay 2002).

3.7 Potential for control

This agent has high potential as a biological control agent in Australia. In its native range, gorse pod moth can have two generations per year, with adults of the first generation flying in spring and a second generation flying in late summer/autumn. In New Zealand, where gorse pod moth is now widely established, the majority of the damage is done to seeds produced from the spring/early summer flowering period and not from the second flowering period in late summer/autumn (Hill *et al* 2008). Gourlay *et al.* (2004) showed that gorse pod moth, in combination with the already established gorse seed weevil, *Exapion ulicis*, recorded an overall 81% loss in spring seed production at one site. A modelling study by Rees and Hill (2001) showed that the annual gorse seed crop needed to be reduced by around 75-85% in order to reduce recruitment of gorse below replacement levels. Therefore, if gorse pod moth is established in Australia, it is expected to be a significant control agent in cooler locations where there is only one major flowering period during spring/early summer.

3.8 Non-target organisms at risk

See section 3.10 and appendices 1-4.

3.9 Possible interactions with existing biological control agents

The release of gorse pod moth will complement the other four biological control agents now established on gorse in Australia. The first of these agents to be released was the gorse seed weevil, *Exapion ulicis* (Forster), which was first introduced to Tasmania from New Zealand (via England), in 1939. A second biological control programme involving host testing and importing of European agents via New Zealand has been underway in Australia since 1995 (Ireson *et al.* 1999). This programme has since resulted in the establishment of three foliage feeders, the gorse spider mite, *Tetranychus lintearius* Dufour, released in 1998, the gorse thrips, *Sericothrips staphylinus* (Haliday), released in 2001 and the gorse soft shoot moth, *Agonopterix umbellana* (Fabricius) released in 2007 (Ireson and Davies 2012). Efficacy studies have shown that this combination of three folivores and one seed feeder will contribute to the biological control of gorse in Australia (Davies *et al.* 2005; Davies *et al.* 2007; Davies *et al.* 2008), but these agents are constrained by predation and the effects of the phenology and seasonality of gorse (Ireson *et al.* 2003; Ireson *et al.* 2008a; Ireson *et al.* 2008b; Hill *et al.* 2008). An additional agent or agents is still required. Release of gorse pod moth will complement the seed feeding activities of *E. ulicis*. Hill (1982) predicted that the combined seed predation by the gorse seed weevil and the gorse pod moth would be complementary rather than strongly competitive, if the moth was introduced to New Zealand. Following the moths release in New Zealand in 1992 (Hill and Gourlay 2002), a subsequent study (Gourlay *et al.* 2004) confirmed this prediction by showing that the combined effects of the two agents on seed production was greater than either alone. If a combination of these two seed feeding agents can reduce annual seed production above 75% as it has at some sites in New Zealand (Hill and Gourlay 2002; Gourlay *et al.* 2004), this should be enough to reduce seed banks below critical replacement levels at some sites in Australia.

3.10. Host specificity studies

Gorse pod moth was released as a biological control agent for gorse in New Zealand in 1992 after detailed tests on 44 species of plants by Hill and Gourlay 2002 (see Appendix 2) who concluded that the agent posed no significant threat to plants of economic or environmental value. Apart from *Ulex* spp., European host records note gorse pod moth feeding on several other members of the Genisteae including weedy species of *Genista* sp., *Sarothamnus* (=*Cytisus*) sp., *Spartium* sp. as well as *Lotus* spp. (Loteae) (Bradley 1979; Emmet 1988). However, larvae of gorse pod moth did not survive to pupation on any of these other genera during host testing by Hill and Gourlay (2002) who concluded that literature records from these hosts were unreliable. Between March 2000 and May 2001, additional tests were carried out with gorse pod moth on an approved list of 35 species or cultivars of Australian plants (see Appendix 3). These tests, which were carried out in New Zealand by Landcare Research New Zealand Ltd., confirmed the earlier tests by Hill and Gourlay (2002) that gorse pod moth was host specific to *Ulex* spp. and that the risk that other species would be attacked was low. Based on the outcome of these tests, gorse pod moth was approved for release in Australia in 2002. Although it is currently on the 'List of Specimens taken to be Suitable for Live Import' under the Environment Protection and Biodiversity Conservation Act 1999, its release has been postponed since field surveys in New Zealand revealed that the host-range of gorse pod moth actually mirrored host-range records from the native range and could possibly exploit some exotic species in the genera *Lupinus* and *Lotus* (Withers *et al.* 2008).
The apparent failure to predict the field host range of gorse pod moth was investigated by Paynter *et al*. (2008)(see Appendix 4) who noted that all the original host specificity studies were conducted on moths sourced from England but the populations of gorse pod moth released in New Zealand were sourced both from England and Portugal. Paynter *et al.* (2008) conducted additional host specificity studies on *Lotus* and concluded that populations of gorse pod moth sourced from Portugal have a different host range than those sourced from England. Their tests on moths sourced from England concurred with the original New Zealand tests by Hill and Gourlay (2002) and indicated that gorse pod moth would be unlikely to exploit the non-target species. Therefore, gorse pod moth sourced from England should be safe to release in Australia. However, although gorse pod moth is still approved for release in Australia, additional host specificity studies on lupin species were conducted in quarantine in Australia during 2010 and 2011 using moths sourced from England. The results of these tests are presented in the following report (Appendix 1) in order to enable a new risk assessment of the release of gorse pod moth in Australia.

4. Proposed release procedure

4.1 Release from quarantine

Imported populations will be bred through at least one generation by DPI Victoria in a quarantine culture which will be tested to ensure the culture is free of hyperparasites and disease. If quarantine authorities give approval for release, the insects will be removed to glasshouses outside quarantine for mass rearing by DPI Victoria and the Tasmanian Institute of Agriculture.

4.2 Field release

Mass rearing cultures will be used to make releases in Tasmania, Victoria and eventually South Australia. All release sites will be recorded with GPS co-ordinates and released populations will be monitored for field establishment.

4.3 Field establishment and evaluation

Confirmation of field establishment may take several years. If the agent does establish successfully, monitoring will continue to measure dispersal and assess the impact of the population on gorse. Surveys will also be conducted to monitor any unexpected non-target effects. If populations increase to sufficiently high densities at any site, it will be used to collect and transfer the moths to new sites in order to accelerate dispersal.

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APPENDIX 1

Host testing of the gorse pod moth, *Cydia succedana* (Lepidoptera:Tortricidae), for the biological control of gorse in Australia: Further tests on lupins

Ireson, J., Relf, M., Sagliocco, J-L., Kwong, R., Holloway, R., Bruzzese, A. and Chatterton, W. (2011)

CONTENTS

1. Executive Summary

Background

The gorse pod moth, *Cydia succedana* (Lepidoptera: Tortricidae), was approved for release in Australia in 2001 following host specificity studies on 79 species or cultivars of plants. However, the moth's release in Australia was postponed when field surveys in New Zealand revealed that it could exploit the weedy perennial *Lupinus arboreus* and some *Lotus* species. Subsequent New Zealand studies from 2003 to 2006 found that the release of untested moths from Portugal, coupled with asynchrony between the flight period of gorse pod moth and gorse flowering, explained the unanticipated non-target attack in New Zealand. Furthermore, the results of repeated host testing of *Lotus* and other species, using moths from England, concurred with the original tests and suggested that the English populations would be unlikely to exploit non-target species. To confirm that gorse pod moth from England would not be a major risk to commercial lupin species or cultivars grown in Australia, a host specificity study was conducted on selected cultivars in quarantine at Frankston, Victoria, over a three-year period from 2009-2011. A comparison of the phenology of gorse pod moth, gorse and the lupins grown commercially in Australia and their susceptibility to attack under field conditions in New Zealand was also undertaken in 2011/12.

Additional host testing on lupins and phenological differences to gorse

Commercial cultivars of lupins chosen for the quarantine host specificity testing of gorse pod moth in Australia were *Lupinus luteus* L. cv. 'Pootalong,' *Lupinus albus* L. cv. 'Kiev' and *Lupinus angustifolius* L. cv. 'Wonga'. Standard no-choice larval starvation tests provided additional confirmation that English populations of gorse pod moth display a preference for gorse, *Ulex europaeus*, over the test plant species. Tests conducted during 2009/10 showed that English populations of gorse pod moth would be unlikely to survive on cultivars of *L. angustifolius* and therefore support the earlier test results on this species. However, a higher level of development on *L. albus* and *L. luteus* of 20% and 14% respectively in the no-choice starvation tests, although significantly lower than the 44% that survived on gorse, suggested that some low level impact on cultivars of these species could occur. Although there was no significant difference between numbers of eggs laid on gorse, *L. albus* and *L. luteus* in tests conducted in 2011, none of the larvae hatching from these eggs survived to the pupal stage on lupins. However, 24% of the larvae that hatched from the eggs laid on gorse developed to the pupal stage and emerged as adults. Paynter *et al.* (2008) showed that virtually all non-target attack in New Zealand by the Portuguese population of gorse pod moth was recorded when gorse was not in flower during summer. The lupin species on which gorse pod moth was recorded in New Zealand was the perennial weedy species *Lupinus arboreus* that flowers mostly in summer, after the peak flowering period of gorse. Commercial cultivars of lupins grown in Australia are annuals. These are usually planted from mid-April until early June. Flowering and immature pod and seed development in these lupins occurs in late winter and spring and corresponds with flowering and immature pod and seed development in gorse which occurs over a longer period. Cultivars of the commercial lupin species are harvested for their seed in summer, however, mature pods and seed are not attacked by larvae of gorse pod moth. The phenology of commercial lupin species therefore negates the risk of any non-target attack by gorse pod moth.

Recommendation for release

A recent study by Withers *et al.* (2012) in New Zealand using commercial cultivars of *L. angustifolius*, *L. albus* and *L. luteus* imported from Australia confirmed the unlikelihood that these cultivars would be attacked during their growing season in Australia. Withers *et al.* (2012) found that no lupin pods of commercial Australian cultivars directly exposed to gorse pod moth under field conditions were attacked during spring when gorse was flowering. As expected, pod moth feeding on the non-target plants was only recorded when gorse was not flowering. Therefore, in Australia, it is unlikely that commercial cultivars of lupins will be attacked and any risk that larvae could survive on commercial species/cultivars of lupins in numbers large enough to inflict significant damage is very low. The release of gorse pod moth for the biological control of gorse in Australia is therefore recommended.

Introduction

Three folivores and one seed feeder have already been released for the biological control of gorse, *Ulex europaeus* L., in Australia. These are the gorse seed weevil, *Exapion ulicis* (Coleoptera: Brentidae), the gorse spider mite *Tetranychus lintearius* Dufour (Acari: Tetranychidae), the gorse thrips, *Sericothrips staphylinus* Haliday (Thysanoptera: Thripidae) and the gorse soft shoot moth, *Agonopterix umbellana* (Fabricius) (Lepidoptera: Oecophoridae). Studies have shown that, although these four agents have established and will contribute to gorse control, an additional agent or agents will still be required to significantly reduce gorse vigour (Ireson *et al.* 2006). The seed-feeding gorse pod moth, *Cydia succedana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), is widely established in New Zealand and, in combination with the gorse seed weevil, has resulted in seed destruction at levels ranging from 75-85% at some sites. Modelling studies (Rees and Hill 2001) have indicated this is the level of seed destruction necessary to cause a decline in gorse densities. Gorse pod moth therefore has the potential to play a significant role in the biological control of gorse in Australia.

Gorse pod moth was approved for release in Australia in 2001 following host specificity studies conducted on 44 New Zealand plant species (Hill and Gourlay 2002) and additional tests on 35 species or cultivars of Australian plants (see Appendix 3). However, its release in Australia was postponed when field surveys after the release of the moth in New Zealand (Withers *et al*. 2008) revealed that the moth could exploit other species of exotic Genisteae (C*ytisus scoparius* L. (Link), *Genista monspessulana* (L.) L.A.S. Johnson and *Lupinus arboreus* Sims) as well as *Lotus pedunculatus* Cav. (Loteae). Populations of gorse pod moth released in New Zealand were collected from both Portugal and England and have now interbred. Paynter *et al.* (2008) found that the release of untested moths from Portugal, coupled with asynchrony between the moths flight period and gorse flowering explained the unanticipated non-target attack in New Zealand. Host specificity tests by Paynter *et al.* (2008) on *Lotus corniculatus*, *Genista monspessulana* and *Cytisus scoparius*, using moths collected from Yately Common in southern England, supported the original New Zealand tests and indicated that the English population would be unlikely to exploit the non-target species. Therefore, gorse pod moth collected from England should be safe to release in Australia.

Although earlier host testing enabled approval for the release of gorse pod moth in Australia in 2001, this report presents the results of additional host specificity studies on commercially valuable lupin species or cultivars grown in Australia to investigate whether the agent is still considered safe to release. *Lupinus angustifolius* (narrow leafed lupin), *L. albus* (white or albus lupin) and *L. luteus* (yellow lupin), are the primary commercial species grown in Australia (Glencross 2007). Host specificity studies on cultivars of these species were conducted by the Department of Primary Industries Victoria (DPI Victoria) in the quarantine facility at Frankston during 2009, 2010 and 2011 using larvae produced from annual consignments of adults collected in England.

Methodology

Selection of lupins for host testing

Many of the commercial varieties of lupins in Australia are spring flowering annuals that produce pods in summer. Flowering in some cultivars of the narrow leafed lupin, *Lupinus angustifolius,* is known to be accelerated by an increase in the photoperiod (Rahman & Gladstones 1974). Species and cultivars of lupins chosen for the quarantine host specificity testing of gorse pod moth in Australia were based on studies conducted by DPI Victoria at Frankston and by the Tasmanian Institute of Agriculture (TIA) at New Town Laboratories near Hobart (Ireson *et al.* 2011). These studies showed that *L. luteus* cv. 'Pootalong,' *L. albus* cv. 'Kiev' and *L. angustifolius* cv. 'Wonga' grown from seed could be induced to produce

flowers and immature pods in about eight weeks under glasshouse conditions of 20-24°C and a minimum photoperiod of 16L:8D. Flowering and pod production in these cultivars was therefore easier to synchronise with the importation of moths during the European spring; for other species and cultivars, flowering and pod production took longer than 12 weeks.
Seed plantings for the host specificity studies from 2009-2011 study commenced each February and were continued every two weeks. Most of the plantings were conducted at Frankston, however, plantings of *L. angustifolius* cv. 'Wonga' were also conducted at New Town Laboratories. General propagation methods used at the two centres were previously described by Ireson *et al.* (2011).

Importation of gorse pod moth
From 2009-2011, seven consignments imported under Australian Quarantine and Inspection Service permits (Table 1) were received from Dr Richard Shaw, Principal Investigator at the Commonwealth Agricultural Bureau International (CABI), Bakeham Lane, Egham, Surrey.
Each consignment was sent in ventilated, crush-resistant plastic tubes with a sprig of gorse and a cotton wool ball soaked in honey. All adults from the 2009 and 2010 consignments were immediately collected from the tubes then sexed (Table 1) and placed in plastic storage containers to enable mating and oviposition. Newly hatched larvae were used for no-choice starvation tests. Moths received in 2011 (Table 1) were immediately collected from the consignment tubes, sexed and placed in large perspex cages for choice oviposition tests followed by no-choice starvation tests on hatched larvae.

Table 1. Gorse pod moth consignments imported from England during 2009, 2010 and 2011

Consignment no.	Quarantine entry no.	Date received	Moths received in consignments*				
			Total moths	No. dead	No. live	No. ♂	No. ♀
1	AAN9WFRJ6	21/05/2009	55	2	53	21	32
2	AAPFAMFPY	11/06/2009	73	6	67	40	27
3	AAT9KT9X7	21/05/2010	186	65	121	102	19
4	AAWE73F7L	10/06/2010	156	28	128	68	60
5	AAY7XPC9P	2/05/2011	123	6	117	84	33
6	AA3CHRPFW	13/05/2011	257	35	222	139	83
7	AA3GN3LTK	30/05/2011	89	27	62	34	28

* Consignment 1: Adults separated into 10 (5 Litre) oviposition containers; containers 1-8 with 2 ♂: 3 ♀, container 9 with 2 ♂: 4 ♀, container 10 with 3 ♂: 4 ♀.
Consignment 2: Adults separated into 10 (5 Litre) oviposition containers; containers 1-7 with 4 ♂: 3 ♀, containers 8-10 with 4 ♂: 2 ♀.
Consignment 3: Adults separated into 10 (5 Litre) oviposition containers; containers 1-9 with 4 ♂: 2 ♀, container 10 with 2 ♂: 1 ♀.
Consignment 4: Adults separated into 15 (5 Litre) oviposition containers; containers 1-8 with 5 ♂: 4 ♀, containers 9-15 with 4 ♂: 4 ♀.

Egg production and larva collection for no-choice host specificity testing (2009-2010)
Oviposition containers (5 Litre) were provided with a cut branch of gorse approximately 10-15 cm in length (with at least four flowers and/or pods) and a container with tissue soaked in a 5% honey/water solution for the moths. These were placed in a controlled environment room at 18-20^{o}C and a photoperiod of 16L:8D. The gorse was removed from each container every four or five days and examined for eggs. Eggs found on flowers, pods and gorse stems were collected and put into sterile Petri dishes lined with moist filter paper. At least 1600 eggs were obtained from the four consignments. The eggs were kept at 18-20^{o}C and a

photoperiod of 16L:8D until hatching. Only larvae that were collected within two hours of hatching were used for host testing.

No choice starvation tests 2009-2010

No-choice starvation tests were used to assess the ability of gorse pod moth larvae to feed and develop on flowers and pods of test plants using the standard Petri dish method of previous tests (Ireson and Gourlay 2001; Hill and Gourlay 2002; Paynter *et al.* 2008).

Replicates were set up in sterile Petri dishes with moist filter paper. Five flowers or green pods of each test species and a gorse control were placed in separate Petri dishes and five neonate larvae placed in each dish. This procedure was replicated a minimum of seven times for each test species as well as for gorse controls (Table 3). Neonate larvae were transferred to flowers or pods using a fine-point paint brush (one larva per flower or pod). The Petri dish replicates were checked every four or five days and feeding damage together with numbers of live and dead larvae was recorded. Flowers and/or pods were added to the replicates when necessary. The number and weight of all pupae collected were recorded

Pupae from the 2009 consignments were sterilised in 0.1% sodium hypochlorite solution for 30 seconds, then rinsed in sterilised water for 30 seconds. A sample of the rinsing water from each shipment was sent to DPI Victoria's Knoxfield centre for fungal and bacterial pathogen testing together with first generation adults.

Choice oviposition tests and no-choice starvation tests 2011

Moths were placed in perspex test cages (45 cm x45 cm x 75 cm) (length x width x height) with a 5% honey and water solution. Each cage contained two cut non-flowering branches (each 25 cm in length) of gorse, *Lupinus albus* cv. 'Kiev', *Lupinus luteus* cv. 'Pootalong' and *Lupinus angustifolius* cv. 'Wonga' in separate vials of water covered with Parafilm® with a small hole for the stem. Flowering plants could not be used in these tests. Unseasonally warm weather conditions in southern England caused the emergence of adult gorse pod moth much earlier than expected and the glasshouse test plants were still about a fortnight away from flowering at the time the first consignment was received. The vials containing the non-flowering specimens of gorse and the lupin test species were placed randomly in each cage. Tests were terminated after 72 hours and all cut branches were examined for the presence of eggs. It was intended to replicate each test five times for each of the three scheduled consignments of moths. Larvae that hatched on eggs laid on each test species and gorse were then used for a second round of starvation tests on the same plant species using the standard Petri dish method.

Analysis

For the four consignments received in 2009/10, there were not enough larvae available to set up a complete set of replicates for each test species on one date. For the analysis, the data were combined as if all the tests had been done together. Because the sodium hypochlorite treatment may have affected adult emergence, only the results for numbers of larvae surviving to the pupal stage were analysed. To examine whether any differences in the number of pupae developing on the test species compared to gorse were significant or if there was a difference in pupal weights, the data were logarithmically transformed and an analysis of variance performed using Genstat (2009). Means were compared using the Tukey test. A similar analysis was performed on tests conducted in 2011. This was to determine differences in the number of eggs laid on different test plants compared to gorse and differences in the development of hatched larvae to the pupal stage in a repeat of the no-choice larval starvation tests that followed.

Examination of phenological synchronicity between gorse and commercial lupin species and susceptibility to damage by gorse pod moth

The gorse pod moth has two generations each year. Paynter *et al.* (2008) showed that all the non-target attack recorded in New Zealand occurred during summer (December to February) when gorse had no pods or flowers present and moths were still flying and ovipositing. The phenology of gorse and the commercial lupin species grown in Australia was examined and this relationship compared to the life cycle of gorse pod moth known from studies conducted in New Zealand (Suckling *et al.* 1999, Paynter *et al.* 2008, Sixtus 2004). Both gorse pod moth and the gorse seed weevil invade young green pods to feed only on immature, green seeds. Studies conducted on the phenology of the gorse seed weevil in Tasmania (Davies *et al.* 2008) were used to indicate the period when immature gorse seeds would be most vulnerable to attack by the gorse pod moth in Australia. A field study to test damage susceptibility of commercial lupin cultivars grown in Australia to gorse pod moth populations during and outside their growing season was conducted in New Zealand during 2011/12 by Withers *et al.* (2012) (see Appendix 5).

Results

Importation of gorse pod moth 2009/10

Field collection of moths in England in late May and early June 2009 was difficult; poor seasonal conditions resulted in only 120 live moths being received in two consignments. A third consignment, scheduled for early June 2009, was cancelled because of low moth numbers. Further attempts to collect moths for a third consignment in September, during the second flight period of the moth in the European autumn, were also unsuccessful. It was then decided to postpone the third consignment until May 2010. Although consignment 3 arrived as scheduled on 21 May 2010, sexing of the 121 live moths showed the sex ratio to be 102 males to only 19 females. A fourth consignment was therefore forwarded in June 2010 to ensure the provision of enough larvae for the completion of the tests. The mean egg fertility for the female moths in the four consignments was 50.8 (SE ± 2.1) (Table 2).

Table 2. Fertility of gorse pod moth females received from England, 2009/2010 and larval numbers used for tests

Consignment no.	No. eggs produced	No. larvae produced	No. hatching (%)
1	296	149	50.3
2	453	204	45.0
3	316	174	54.0
4	577	310	53.7

No-choice starvation tests 2009/10

In the Petri dish tests 10 of the 50 larvae (20%) placed on *L. albus* cv. 'Kiev', seven of the 50 (14%) placed on *L. luteus* cv. 'Pootalong' and only one of the 35 (2.9%) placed on *L. angustifolius* cv. 'Wonga' survived on pods to pupal stage (Table 3). Sixty of the 135 larvae (44.4%) placed on the gorse pods in the controls survived to the pupal stage. The treatment effect on the number of larvae surviving to the pupal stage on the different host plants was significant ($F\ 3,\ 50 = 12.1,\ P<0.001$) (Fig. 1). The mean number of larvae surviving to the pupal stage on the gorse controls was significantly higher than on *L. albus* ($P< 0.05$), *L. luteus* ($P<0.01$), or *L. angustifolius* ($P<0.001$). The difference in larval survival between the three lupin cultivars was not significant. There was also no significant difference in the mean weight of pupae collected from gorse or any of the lupin species.

Pathogen tests conducted on pupae and adults did not detect any pathogens or internal parasites.

Table 3 Survival of first-instar larvae to pupae in no-choice tests

Species tested	No. of replicates	No. of larvae	No. of pupae	Development of larva to pupa (%)
Ulex europaeus (control)	27	135	60	44.4 (0.007)
Lupinus albus cv.'Kiev'	10	50	10	20 (0.007)
Lupinus luteus cv. 'Pootalong'	10	50	7	14 (0.006)
Lupinus angustifolius cv. 'Wonga'	7	35	1	2.9 (0.008)

Note: Figures in parentheses are the mean pupal weights (grams)

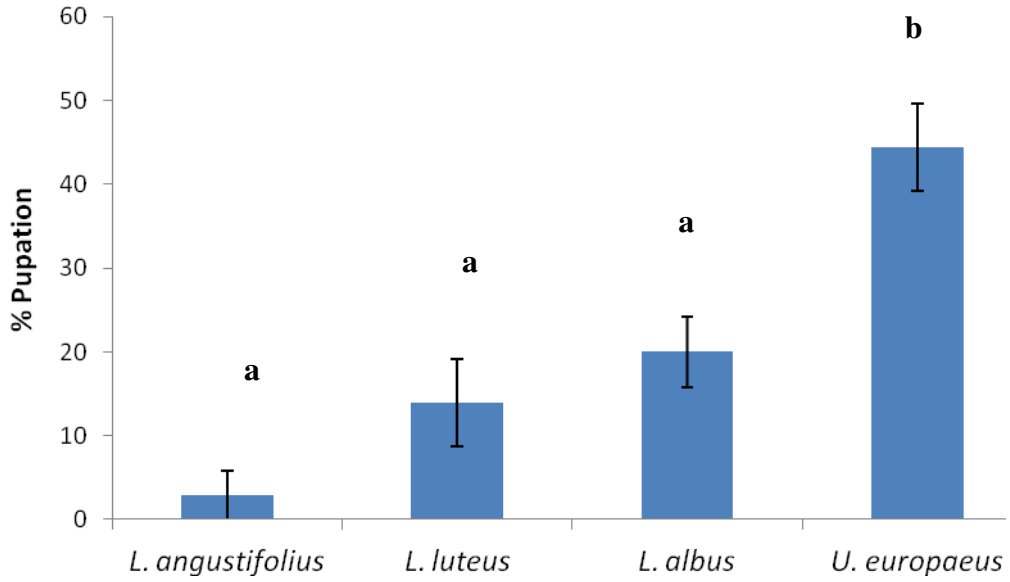

Figure 1. Mean (± SE) number of larvae surviving to the pupal stage (expressed as a percentage) on gorse compared to the number surviving on *L. angustifolius*, *L. luteus* and *L. albus* in no-choice larval starvation tests (means with the same letter are not significantly different).

Importation of gorse pod moth 2011

Unseasonally warm spring weather in England in 2011 caused moths to emerge about one month earlier than expected and caused problems for the scheduled tests. The collections were made a fortnight apart as in the previous collection years, however, moths in the second and third consignments laid a total of only 30 eggs (Table 3) so the results could not be

analysed. Furthermore, the comparatively high moth mortality in each of these two consignments compared to the first (Table 1) suggested that the moths had laid most of their eggs by the time they were collected and were approaching the end of their life cycle. Again, this was probably hastened by the unseasonably warm conditions. The first consignment had a skewed sex ratio with twice as many males as females (Table 1) and there were only enough females for three replicates instead of the scheduled five. Even so, sufficient eggs were laid during this series of tests to enable analysis of the results (Table 4).

Choice oviposition tests and no-choice starvation tests 2011

The treatment effect for the level of oviposition on the different test and gorse by moths from consignment 1 was significant (*F 3, 8 = 12.4, P<0.002*). There was no significant difference in the mean number of eggs deposited on either gorse, *L. albus* cv. 'Kiev' and *L. luteus* cv. 'Pootalong' but only one egg was laid on *L. angustifolius* cv. 'Wonga' which was significantly lower than on either gorse or the other two lupin cultivars.

Of the eggs oviposited on gorse, 41% (21) hatched compared to the 59% (20) and 68% (26) that hatched on *L. albus* cv. 'Kiev' and *L. luteus* cv. 'Pootalong' respectively. However, in these tests, 21% (5) of the larvae that fed on gorse survived to the pupal stage with each pupa successfully producing an adult. None of the larvae that fed on the test plants survived to the pupal stage. The treatment effect for larval survival was significant (*F 2, 64, 6.9, 0.01<P>0.001*), there being a significant difference in the mean number of larvae surviving to the adult stage on gorse compared to the test plants (Table 4, Figure 2).

Table 3. Comparative oviposition levels by gorse pod moth received from England in 2011 on each test plant species compared to gorse

Consignment no.	Replicate no.	Test date	No. ♂:♀/ replicate	No. eggs per test plant			
				Gorse	*L. albus* cv. 'Kiev'	*L. luteus* cv. 'Pootalong'	*L. angustifolius* cv. 'Wonga'
1	1	02/05/11	10:10	6	9	8	0
	2	02/05/11	10:10	25	21	17	1
	3	02/05/11	10:10	20	4	13	0
2	1	13/05/11	10:10	0	0	0	0
	2	13/05/11	10:10	0	0	3	1
	3	13/05/11	10:10	1	6	3	0
	4	13/05/11	10:10	5	1	3	4
3	1	30/05/11	12:10	0	0	0	0
	2	30/05/11	11:9	0	0	0	0
	3	30/05/11	11:9	3	0	0	0

Table 4. Comparative survival to the adult stage of larvae emerging from eggs deposited on gorse and lupin test species in consignment 1

Plant species	No. eggs laid	No. eggs hatched (%)	No. larvae developing to pupa (%)	No. larvae developing to adults (%)
Gorse (control)	51	21 (41%)	5 (24%)	5 (24%)
L. albus cv.'Kiev'	34	20 (59%)	0	0
L. luteus cv.'Pootalong'	38	26 (68%)	0	0
L. angustifolius cv. 'Wonga'	1	0	0	0

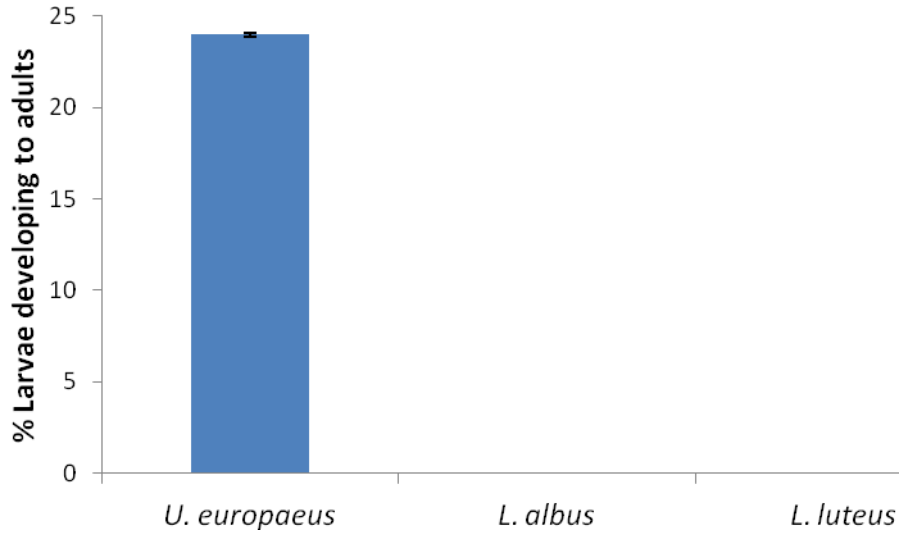

Figure 2. Mean (± SE) number of larvae surviving to the adult stage (expressed as a percentage) on gorse compared to the number surviving on *L. luteus* and *L. albus* in no-choice larval starvation tests following egg hatch on each plant species.

Examination of phenological synchronicity between gorse and commercial lupin species and susceptibility to damage by gorse pod moth

A comparison of the phenology of gorse and the commercial lupin species in relation to adult flight periods and larval damage by the gorse pod moth shows that off-target infestations of the gorse pod moth on commercial lupin species would be unlikely. Paynter *et al.* (2008) noted that virtually all non-target attack in New Zealand was recorded when gorse was not in bloom. The phenology of gorse in Australia follows similar patterns to that recorded in New Zealand as no flowers are present in the summer months when gorse pod moth adults would still be active. In New Zealand, the other Fabaceae that were infested, albeit at much lower levels than that recorded for gorse (Paynter *et al.* 2008), progressively come into bloom after gorse has finished flowering. Studies in New Zealand have shown that pod moth numbers are low or zero in mid-winter (June-August). First generation moths emerge in spring from mid-September, increasing to a maximum between November and January, with most first generation moths having emerged by early January. Generation overlap occurs, with second generation adults probably emerging as early as late December at some sites, with peak emergence occurring somewhere between February and May (Sixtus 2004; Paynter *et al.* 2008). Larvae of first generation gorse pod moth would be feeding on gorse seeds mainly from October.

The first generation of gorse pod moth is therefore well synchronised with spring flowering gorse. The problem of non-target attack occurs with the second generation of moths, because adults emerge and females oviposit before gorse starts to flower again in early autumn. This is when all the non-target attack on some closely related Fabaceae has been recorded (Paynter *et al.* 2008). However, no non-target attack has been recorded in spring when all the commercial cultivars of lupins flower and produce immature pods at the same time as gorse. It is the flowering and immature pod stage of the lupins which would be vulnerable to attack. The study by Withers *et al.* (2012) (Appendix 5) in New Zealand using commercial cultivars of *L. angustifolius*, *L. albus* and *L. luteus* imported from Australia has now provided further confirmation that these cultivars will not be attacked during their growing season in Australia. Withers *et al.* (2012) (Appendix 5) found that no lupin pods of commercial Australian cultivars were attacked during spring/early summer when gorse was flowering. As expected, any pod moth infestations were recorded when gorse was not flowering, particularly during February and March. Therefore, in Australia, commercial cultivars of lupins will not be susceptible to attack.

In Australia, the phenology of commercial cultivars of *L. angustifolius*, *L. albus* and *L. luteus* varies depending on where they are being grown. However, because they are usually planted over a period extending from mid-April until early June (Walker *et al.* 2011), periods of flowering and immature pod and seed development in lupins are overlapped by flowering and immature pod and seed development in gorse which occurs over longer periods (Davies *et al.* 2008). For instance the earliest flowering of commercial lupin cultivars commences from the end of July and can continue until mid-October and the green pods and young seeds would be present from September but mainly during October and November although immature seed from late plantings may be present into early December at the latest (Walker *et al.* 2011). This is inclusive of the period when immature seeds of gorse are attacked by the gorse seed weevil (*E. ulicis*) and would also be the period when larvae of first generation gorse pod moth would be feeding on gorse seeds. Cultivars of the commercial lupin species are harvested for their mature seed from early December and this can extend into January and early February, however, mature pods and seed of any plant are not attacked by larvae of gorse pod moth.

Discussion

Hill and Gourlay (2002) and Paynter *et al.* (2008) concluded that gorse pod moth populations sourced from Portugal appeared capable of exploiting a broader range of plants than populations sourced from England which would be unlikely to exploit non-target species of Genisteae and Loteae. The additional no-choice starvation tests conducted during 2009/10 that are presented in this submission show that English populations of gorse pod moth would be unlikely to survive on cultivars of *L. angustifolius* and therefore support the earlier tests on cultivars of this species (see Appendix 3). However, the higher level of development on *L. albus* and *L. luteus* in the 2009/10 no-choice starvation tests, although significantly lower than on gorse, suggested that some low level impact on cultivars of these species could occur. There was no significant difference between numbers of eggs laid on gorse, *L. albus* and *L. luteus* in tests conducted in 2011, but this result needs to be interpreted with caution. Indiscriminate oviposition behaviour in the host specificity testing of phytophagous insects under caged conditions is well known and was reviewed by Withers and Barton Browne (1998). They discussed the possibility of oviposition being more indiscriminate by females which have experienced the target weed prior to entering the test arena (which these females had) than that shown by newly emerged females. Perhaps the oviposition tests may have been more conclusive if the lupins and gorse had been in flower when the tests were performed, as originally intended. Even so, the subsequent starvation tests, in which none of the larvae fed

on lupins survived to the adult stage, again provided evidence that the lupin species/cultivars tested would not be favoured hosts for and English population of gorse pod moth. However, perhaps of most significance are the field results of Withers *et al.* (2012). These confirm the earlier observations by Paynter *et al.* (2008) by demonstrating that no non-target attack on commercial cultivars of lupins grown in Australia is expected because gorse is in flower at the same time as the commercial lupin cultivars are producing flowers and immature pods and seeds. The risk that larvae could survive on commercial species/cultivars of lupins in numbers large enough to inflict significant damage is therefore very low. The release of gorse pod moth for the biological control of gorse in Australia is therefore recommended.

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APPENDIX 2

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Host-range testing, introduction, and establishment of *Cydia succedana* (Lepidoptera: Tortricidae) for biological control of gorse, *Ulex europaeus* L., in New Zealand

R.L. Hill[a,*] and A.H. Gourlay[b]

[a] *Richard Hill and Associates, Private Bag 4704, Christchurch, New Zealand*
[b] *Landcare Research, P.O. Box 69, Lincoln 8152, New Zealand*

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Abstract

Cydia succedana Denis and Schiffermüller (Lepidoptera: Tortricidae) has been introduced to New Zealand as a biological control agent to attack the seeds of gorse (*Ulex europaeus*; Fabaceae). Gorse is a major weed in New Zealand and in other temperate parts of the world including Oregon and California (USA), at high elevations in Hawaii (USA), Chile, and Australia. This paper describes the host-range tests conducted to assess the risk that *C. succedana* posed to nontarget plants, and to gain approval for the introduction of this moth into New Zealand. The release and establishment of *C. succedana* are recorded. First-instar larvae transferred onto excised pods of 39 leguminous test plants completed development on gorse controls (40.0%), *Pisum sativum* (7.2 and 8.0%), and the rare native species *Clianthus puniceus* (10.0%). Larvae also fed on pods *of Lens culinaris* and *Sophora* spp. but none completed development. Excised shoots bearing flowers and pods of 33 leguminous plants were exposed to female moths in small cages. No eggs were laid on 17 species. Oviposition on the other 16 plants never exceeded 10% of that on controls. Eggs were laid on *C. puniceus* and *Sophora microphylla*, but not on *P. sativum* or *L. culinaris*. Tests were conducted in larger cages outdoors using whole plants of 17 leguminous species. Moths were more selective in this arena. No eggs were laid on *C. puniceus*, but occasional eggs were laid on *S. microphylla* in both "choice" and "no-choice" tests. Behavioral observations suggested that larvae tend to actively seek out gorse pods in preference to pods of *S. microphylla*. It was concluded that *C. succedana* posed no significant threat to *Sophora* spp., or to any other plants with economic or environmental value in New Zealand. *C. succedana* was released in 1992, and since then has been distributed at 134 sites in New Zealand. It has established at 78% of the sites that have been adequately assessed. There appears to be no geographic establishment pattern, and this species may establish wherever gorse occurs. The potential effect of *C. succedana* on the population dynamics of gorse in New Zealand is discussed. Introduction of this species to Hawaii and Australia is being considered.

Keywords: Biological control; Gorse; Gorse pod moth; New Zealand; Host-range testing; *Cydia succedana*; *Ulex europaeus*; Weeds

1. Introduction

Cydia succedana Denis and Schiffermüller (Lepidoptera: Tortricidae) is commonly called the gorse pod moth. It was introduced to New Zealand from Europe in 1992 as a biological control agent for gorse, *Ulex europaeus* L. (Fabaceae), and has established there (Harman et al., 1996). This paper describes the research that was conducted before this species was released in New Zealand, and assesses how the insect has performed since its introduction.

Gorse is a native of western and central Europe and the British Isles and now occurs in most temperate areas of the world. It is a serious weed in Chile, Australia, USA (Oregon, northern California, Washington state), and New Zealand (Richardson and Hill, 1999). Markin and Yoshioka (1996) state that gorse is common on 14,000 ha of pastureland and open forest on the islands of Hawaii and Maui. Gorse remains one of New Zealand's most serious weeds (Hill and Sandrey, 1986). It is a woody perennial legume shrub that can grow to 4 m tall. It can be found on approximately 5% of the land

* Corresponding author. Fax: +64-3-325-2074.
E-mail address: hillr@crop.cri.nz (R.L. Hill).

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not occupied by indigenous or alpine vegetation, and commonly forms impenetrable, spiny thickets. It is an intractable problem that causes significant production losses in agriculture and forestry, and poses a serious fire risk to forests and peri-urban areas (Richardson and Hill, 1999). The outstanding ability of gorse to reinvade sites from which it has been cleared is directly related to its large and persistent seed bank. Its ability to colonize new sites is related to the amount of seed produced and dispersed.

Biological control of gorse was first attempted in 1931, when *Exapion ulicis* (Forst.) (Coleoptera: Apionidae) (Alonso Zarazaga, 1990) was introduced to attack gorse seed in pods. Julien and Griffith (1998) record this species as *Apion ulicis* (Forst.). At that time some farmers valued gorse as a living fence to contain grazing stock, and the introduction of further biological control agents that damaged plants was not considered (Miller, 1970). In 1988 it was finally determined that the costs of gorse outweighed its benefits (Hill, 1988), and six further control agents have been introduced since then (Hill et al., 2000). *C. succedana* was the fifth of these (Harman et al., 1996).

Exapion ulicis is univoltine in spring. Gorse produces seed pods in both spring and autumn, and the proportion of annual seed production that occurs in each season varies with elevation and latitude (Hill et al., 1991). Where most gorse seed is set in autumn, annual seed production is not greatly affected by the weevil, even though infestation levels in spring pods can exceed 90% (Miller, 1970). Conversely, where most seed is produced in spring, the pod infestation levels tend to be lower, and any seed produced in autumn escapes attack. There is also variation in the impact of the weevil between years (Hill et al., 1991). Cowley (1983) estimated that the weevil destroyed approximately 30% of annual seed production at one site near Auckland. *Exapion ulicis* is rare or uncommon in parts of the country such as the west coast of the South Island, and the proportion of seeds destroyed there is small (R.L. Hill and A.H. Gourlay, unpublished data). This also limits the success of *E. ulicis* as a biological control agent for gorse in New Zealand.

Zwölfer (1962) lists the insect species known to attack gorse in Europe. *C. succedana* was selected from these candidates to decrease the annual seed production of gorse in New Zealand, and to reduce the geographic, seasonal, and temporal variation in seed predation. *Cydia* is a large genus with a worldwide distribution. Some *Cydia* species attack the shoots of the host plant, but most attack the reproductive structures, particularly fruits and pods. For example, Emmet (1988) lists 35 British *Cydia* species, 26 of which attack buds, flowers, or fruits. Emmet's list also suggests that these species have narrow host-ranges. Nine of the 12 species recorded from species of the Fabaceae have been reported from only one or two closely related hosts. Several species in this genus are cosmopolitan pests, including *Cydia pomonella* (L.) (codling moth).

Zwölfer (1962) recorded that *C. succedana* was bivoltine in Europe. It lays eggs on *U. europaeus* pods in spring, and adults emerge to lay eggs on the pods of *U. minor* Roth and *U. gallii* Planch. in late summer and autumn. As *U. europaeus* sets seeds in both spring and autumn in New Zealand (Hill et al., 1991), it was assumed that both generations of *C. succedana* would attack *U. europaeus* seeds in New Zealand. *C. succedana* larvae consume *E. ulicis* larvae encountered in pods. However, a study of gorse seed predation in England showed that fewer than 20% of *U. europaeus* pods occupied contained both species together (Hill, 1982). This suggested that the effects of these two agents in spring may be complementary rather than strongly competitive. Hence, *C. succedana* was selected for introduction to New Zealand as part of a balanced suite of biological control agents for this weed (Hill et al., 2000).

Apart from gorse, the literature records *C. succedana* from *Sarothamnus* (= *Cytisus*) sp., *Genista* sp., *Spartium* sp. (all species belonging to the tribe Genisteae), and *Lotus* sp. (Bradley et al., 1979; Emmet, 1988; Zwölfer, 1962). The validity of these records is difficult to assess, but it has become clear that populations currently identified as belonging to *C. succedana* are present in parts of Europe where gorse is uncommon (Dr. Peter Witzgall, Swedish University of Agricultural Sciences, personal communication), and certain populations may utilize plants other than *Ulex* spp.

The primary aim of this paper is to present research into the host range of *C. succedana* that was conducted to support the successful application for permission to release this species into New Zealand. However, it also describes the introduction of *C. succedana* to New Zealand, and its current distribution there. The potential importance of *C. succedana* as a biological control agent for gorse in New Zealand is discussed.

2. Materials and methods

2.1. Origin of populations

Cydia succedana was first introduced to New Zealand in 1988, when 198 moths were imported into secure containment for evaluation and rearing in quarantine. The moths were collected from Yateley Common, Hampshire, England, by stalking and capturing individual moths that rested on the spines of gorse or on neighboring heathland vegetation. All host-range tests described in this paper were conducted on moths from the population collected at Yateley Common, or nearby Chobham Common. The population that was released into New Zealand also contained moths reared from a

population that was collected from Viana do Castello, Portugal in 1992.

2.2. Rearing

Preliminary experiments were conducted to develop this rearing regime, but the results are not presented in detail. All stages of the moth were reared continuously at 16 °C and 16L:8D photoperiod. The optimum rearing method developed was as follows. Three *C. succedana* moths (two females and one male) were placed in a closed, clear plastic cylinder (30 × 30 cm) with a dental roll soaked in a dilute solution of honey and a few grains of pollen in water. Fresh 15-cm-long gorse stems bearing young pods and/or mature flowers were cut, and arranged in the cylinder in a glass vial (10 × 2.5 cm) of water sealed with Parafilm. Shoots were replaced after 3–5 days, and the container was placed at 13 °C for 3 days before returning it to 16 °C. This maximized both adult lifespan and lifetime oviposition. Flowers and pods bearing eggs were removed from the stems, along with any eggs on spines, and pods were separated from the calyces. Calyces and spines were placed in a dry, closed, clear plastic box (20 × 12.5 × 10 cm), and larvae hatched. One hundred to 150 small, immature pods were collected from the field and placed on filter paper in a plastic box (20 × 12.5 × 10 cm). Thirty to 50 larvae were transferred onto these pods within 24 h of hatching, using a camel-hair brush. Alternatively, bare pods with eggs were added to the boxes containing immature pods. Each box was closed with fine gauze. To maintain the quality of the delicate pods and first-instar larvae, a square of damp filter paper was laid across the top, and the box was placed in an open plastic bag. After 5–7 days, the filter paper cover was removed. After 2–3 weeks, boxes were checked every 2 days for second- and third-instar larvae emerging to seek a new pod in which to complete development. Fifty such larvae were transferred using a camel-hair brush to another box containing 100–200 mature green pods containing well-developed seeds. These boxes were allowed to dry out slowly. Larvae completed development and moths emerged within the boxes. Alternatively, late-stage larvae were transferred to plugs of a "general purpose diet" and Brinton's diet in polycarbonate tubes (7.5 × 1 cm) (Ashby et al., 1985). Larvae pupated and adults emerged successfully within stoppered tubes.

2.3. Host-range tests

The potential host-range of *C. succedana* was estimated experimentally by measuring the ability of moths to lay eggs on various plants in the presence and absence of gorse, and by measuring the mortality and development rate of larvae when fed on plants other than gorse. Replication varied within and between experiments, but is recorded in Tables 1–3. Data are presented as means and standard errors, and the analysis presented is qualitative rather than statistical.

Selection of test plants. The principles summarized by Wapshere (1974) were used to select the 44 plants tested. Particular emphasis was given to plants closely related to gorse within the tribe Genisteae, plants that had previously been recorded as hosts (*Sarothamnus* (= *Cytisus*) spp., *Genista* spp., *Spartium* sp., and *Lotus* spp.), related plants not previously exposed to the agents (in particular the New Zealand native species such as *Carmichaelia* spp.), legumes of economic importance in New Zealand, and a small selection of more distantly related plants. Since *C. succedana* larvae develop to maturity within the pods of their host plant, the plants tested were largely limited to those capable of forming such pods. The exceptions to this were *Malus* sp. and *Pinus* sp., both of which are the hosts of related *Cydia* spp. and are economically important in New Zealand. Not all test plant species were used in all test designs.

First-instar starvation tests. The ability of larvae to feed and develop on pods of a range of plants was assessed using first-instar starvation tests. Two to five young pods were picked from test plants and placed in petri dishes on damp filter paper, one species per dish. Young gorse pods were set up in the same way to serve as controls. Female *C. succedana* moths prefer to lay eggs on or near fertilized gorse flowers. Hatching larvae move to developing pods and bore into them. To mimic this behavior, five newly hatched, unfed larvae were placed on the pods in each petri dish. Dishes were stacked in plastic bags to minimize desiccation and stored at 18 °C and 16L:8D photoperiod. Pods were checked at 5-day intervals, and the number of larvae surviving was noted. Percentages of survival to the end of the second observation period and to pupation were calculated.

Oviposition tests. The ability of adult female *C. succedana* to lay eggs on various plants was measured in small-cage and field-cage tests. Small-cage experiments were conducted in a secure insect containment facility at Lincoln, New Zealand from September 1988 to March 1989. Fresh stems of approximately 10 cm in length bearing young pods and/or mature flowers were cut from test plants. Each stem was arranged in a glass vial of water (10 × 2.5 cm) sealed with Parafilm. Choice tests (i.e., gorse present) and no-choice (i.e., gorse absent) tests were carried out (this terminology equates to the "choice plus target" and "choice minus target" of Heard (2000)). One shoot each of two to four plants randomly selected from the list of test plants was randomly placed in a closed, cylindrical, clear plastic arena (30 × 30 cm), with a dental roll soaked in a dilute solution of honey in water. An additional gorse shoot was added in choice tests. Two male and three female moths were added to each test arena. Tests were randomly placed on a bench

Table 1
Survival of first-instar *Cydia succedana* larvae when placed on pods of a range of test-plant species in petri dishes[a]

FAMILY SUBFAMILY Tribe *Species*	No. of tests	Mean LD50 (days)	Age of last larva at death (days)	Mean % survival to 10 days	Mean % survival to pupation	Mean % survival of control larvae at end of test	Experiment site	Notes
FABACEAE								
FABOIDEAE								
Genisteae								
Ulex europaeus L.	41	–	–	56.1	40.0	–	NZ	Across all completed controls
	10	–	–	86.0	40.0	–	UK	Across all completed controls
Chamaecytisus palmensis (H. Christ) F.A. Bisby and K.W. Nicholls	2	7.5	23	30.0	0	30.0	NZ	Significant attack on pod exterior
Cytisus scoparius L. (Link)	5	6.5	8	0	0	76.0	NZ	
	3	2.5	3	0	0	92.0	UK	
Genista hispanica L.	3	2.5	3	0	0	92.0	UK	
Laburnum anagyroides Medik.	3	2.5	8	0	0	90.7	UK	
Lupinus arboreus Sims	4	5.0	8	0	0	60.0	NZ	
	3	2.5	18	20.0	0	88.0	UK	
L. polyphyllus Lindl.	3	4.2	8	0	0	60.0	NZ	
Spartium junceum L.	5	7.5	8	0	0	76.0	NZ	
Teline monspessulana (L.) K. Koch	2	7.5	23	30	0	35.0	NZ	
	3	2.5	18	33.3	0	72.0	UK	
Carmichaeliae								
C. arborea (G. Forst.) Druce	3	5.8	13	20	0	80.0	NZ	
C. arenaria G. Simpson	2	2.5	8	0	0	80.0	NZ	Significant attack on pod exterior
C. astonii G. Simpson	2	2.5	3	0	0	80.0	NZ	
C. crassicaule Hook. F.	4	3.8	8	0	0	86.5	NZ	Significant attack on pod exterior
C. enysii Kirk	2	2.5	3	0	0	80.0	NZ	
C. fieldii Cockayne	4	3.8	8	0	0	70.0	NZ	Significant attack on pod exterior
C. kirkii Hook.	2	2.5	3	0	0	80.0	NZ	
C. muiratai (A.W. Purdie) Heenan	2	5	8	0	0	80.0	NZ	Significant attack on pod exterior
C. torulosa (Kirk) Heenan	2	2.5	3	0	0	80.0	NZ	
C. virgata Kirk	2	2.5	3	0	0	80.0	NZ	
Galegeae								
Clianthus puniceus (G. Don) Sol. ex Lindl.	2	7.5	–	20.0	10.0	30.0	NZ	Significant attack on pod exterior
	5	2.5	–	20.0	4.0	69.2	UK	Significant attack on pod exterior

Colutea arborescens L.	3	2.5	3	0	0	96.0	UK	
Glycininae								
Glycine max (L.) Merr.	3	2.5	3	0	0	92.0	UK	
Loteae								
Lotus peduncultatus Cav.	5	6.5	8	0	0	53.3	NZ	
Phaseolineae								
Phaseolus vulgaris L.	2	2.5	8	0	0	90.0	NZ	
Sophoreae								
Sophora microphylla Aiton	3	2.5	23	13.3	0	77.3	UK	Significant attack on pod exterior
Sophora sp. Hybrid	4	3.8	18	25.0	0	50.0	NZ	Significant attack on pod exterior
Trifolieae								
Medicago arborea L.	2	7.5	8	0	0	50.0	NZ	
M. sativa L.	5	5.5	8	0	0	56.0	NZ	
Trifolium pratense L.	4	6.3	8	0	0	60.0	NZ	
T. repens L.	5	3.8	8	0	0	No controls	NZ	
Trifolium sp. 'Alexandrina'	3	2.5	8	0	0	60.0	NZ	
Trifolium sp. cv. 'Zigzag'	3	2.5	8	0	0	No controls	NZ	
Vicieae								
Lathyrus odoratus L.	5	6.5	8	0	0	76.0	NZ	
Lens culinaris Medik.	3	8.3	18	26.7	0	60.0	NZ	Pods penetrated, seeds eaten
	3	2.5	3	0	0	96.0	UK	
Pisum sativum L.	11	8.4	–	27.3	7.2	59.4	NZ	Pods penetrated, seeds eaten
	5	2.5	–	16.0	8.0	68.0	UK	Pods penetrated, seeds eaten
Vicia faba L.	3	2.5	3	0	0	92.0	UK	
MIMOSOIDEAE								
Mimoseae								
Acacia dealbata Link	3	2.5	3	0	0	93.3	NZ	
PINACEAE								
Pinus sp.	3	2.5	3	0	0	96.0	UK	
ROSACEAE								
Malus sp.	3	2.5	3	0	0	96.0	UK	

[a] Experiments were conducted in New Zealand (NZ) and in the United Kingdom (UK). Five first instar larvae were used in each test. LD50 describes the time taken for half of the larvae in each test to die. Unless noted, larval feeding was restricted to occasional nibbling of pod valves.

Table 2
Oviposition by *Cydia succedana* moths on shoots of a range of test plants in the presence and the absence of gorse in small-cage tests in New Zealand

FAMILY SUBFAMILY		Choice tests (gorse present)			No-choice tests (gorse absent)		
Tribe *Species*	No. of shoots presented	Test plant Eggs/shoot ± SE	Gorse control Eggs/shoot ± SE	No. of tests	Test plant Eggs/shoot ± SE	No. of tests
FABACEAE						
FABOIDEAE						
Genisteae						
Ulex europaeus L.	–	–	–	–	40.4 ± 5.1	10
Chamaecytisus palmensis (H. Christ) F.A. Bisby and K.W. Nicholls	2	0	15.3 ± 1.0	6	0	7
	4	0	16	1	0	1
	5	1.7 ± 0.3	35.0 ± 1.4	3	4.5	2
Cytisus scoparius L. (Link)	2	2.0 ± 0.2	10.0 ± 1.9	4	2.8 ± 1.6	12
	3	0	20.0 ± 5.6	3	–	–
Lupinus arboreus Sims	2	1.0 ± 1.0	16.1 ± 2.8	7	7.8 ± 4.7	10
	3	1.0 ± 0.8	20.0 ± 5.6	5	–	–
L. polyphyllus Lindl.	2	4.2 ± 3.6	28.7 ± 6.8	6	2.8 ± 1.0	11
	3	0	14.6 ± 3.8	5	–	–
Spartium junceum L.	3	1.0 ± 0.7	25.5 ± 4.9	10	4.6 ± 3.8	10
Carmichaelieae						
Carmichaelia arborea (G. Forst.) Druce	2	0	21.0 ± 4.7	3	0	1
	3	0	12.3 ± 2.7	3	0	3
C. arenaria G. Simpson	2	0	24.8	2	0	2
	3	0	21.5 ± 5.2	4	0	1
C. astonii G. Simpson	2	–	–	0	0	2
	3	0	15.1 ± 3.6	7	0	1
C. corrugata Col.	3	0	18.8 ± 4.2	4	0	3
C. crassicaule Hook. f.	2	1	63.5	2	–	–
	3	0.5 ± 0.4	24.2 ± 5.2	11	0	3
C. enysii Kirk	3	0	16.7 ± 6.7	3	0	3
C. fleldii Cockayne	3	0.8 ± 0.4	21.8 ± 4.5	10	0.7 ± 0.3	3
C. kirkii Hook. f.	2	0	13.0	2	0	3
	3	0	17.3 ± 9.8	3	0	1
C. muratai (A.W. Purdie) Heenan	3	0	14.5 ± 2.7	6	0.7 ± 0.7	3
C. torulosa (Kirk) Heenan	3	0.7 ± 0.4	29.0 ± 7.6	6	0	3
C. virgata Kirk	3	0	21.2 ± 6.6	6	0	3
Galegeae						
Chamthus puniceus (G. Don) Sol. ex Lindl.	2	1.1 ± 0.6	25.7 ± 3.6	12	3.0 ± 1.9	7

Loteae							
Lotus pedunculatus Cav.	3	0.2 ± 0.2	18.1 ± 4.0	14	0.9 ± 0.6	8	
Phaseolineae							
Phaseolus coccineus L.	3	0	19.8 ± 3.2	10	0	5	
P. vulgaris L.	2	0	9	1	0	1	
	3	0	14.7 ± 1.9	3	0	1	
	5	0	23.5 ± 7.9	3	–	–	
Sophoreae							
Sophora microphylla Aiton	2	3.3 ± 2.6	17.1 ± 4.7	11	2.8 ± 1.4	10	
	4	0	16	2	–	–	
	5	3	21.5	2	1.3 ± 1.3	3	
Trifolieae							
Medicago arborea L.	2	0	0	4	0	3	
	3	0	14	2	–	–	
	5	0	35.0 ± 13.5	3	0	2	
M. sativa L.	2	0	15.8 ± 2.2	5	0.4 ± 0.4	8	
	3	0.2 ± 0.2	18.7 ± 3.7	6	–	–	
	4	–	–	0	2	1	
	5	0	41.5	2	0	1	
Ononis campestris Koch	2	0	28.0 ± 4.6	5	0	3	
Trifolium pratense L.	3	1.0 ± 0.4	17.3 ± 4.4	8	0	5	
T. repens L.	3	0.4 ± 0.4	22.9 ± 3.7	9	1.3 ± 0.5	4	
Trifolium sp. cv. 'Alexandrina'	3	0.8 ± 0.8	30.8 ± 7.7	6	0	5	
Trifolium sp. cv. 'Zigzag'	3	0	25.2 ± 3.9	5	0	4	
Vicieae							
Lathyrus odoratus L.	3	0	14.7 ± 2.9	10	0	4	
Lens culinaris Medik.	2	0	19.3 ± 1.3	3	0	4	
Pisum sativum L.	2	0	18.7 ± 3.4	3	0	7	
	3	0	31.3 ± 5.8	11	–	–	
Vicia faba L.	2	0	19	1	0	3	
	4	0	45	1	–	–	
	5	0	13	1	–	–	
MIMOSOIDEAE							
Mimoseae							
Acacia dealbata Link	2	0	23.7 ± 4.8	3	0	3	

Table 3
Oviposition by *Cydia succedana* moths on a range of potted test-plant species in the presence and the absence of gorse in outdoor cage tests in the UK

FAMILY SUBFAMILY	Choice tests (gorse present)			No-choice Tests (gorse absent)	
Tribe *Species*	Test plant Eggs/shoot ± SE	Gorse control Eggs/shoot ± SE	No. of tests	Test plant Eggs/shoot ± SE	No. of tests
FABACEAE					
FABOIDEAE					
Genisteae					
Ulex europaeus L.					
Chamaecytisus palmensis (H. Christ) F.A. Bisby and K.W. Nicholls					
Cytisus scoparius L. (Link)	0.8 ± 0.8	20.0 ± 3.8	5	0	5
Genista hispanica L.	0.8 ± 0.8	29.2 ± 5.7	5	2.0 ± 2.0	5
G. 'Lydia'	0	29.2 ± 5.7	5	0	5
Laburnum anagyroides Medik.	0	29.2 ± 5.7	5	0	5
Lupinus arboreus Sims	0	20.8 ± 4.1	5	0	5
Teline monspessulana (L.) K. Koch	0	20.8 ± 4.1	5	0	5
Carmichaelieae					
C. compacta Petrie	0	20.0 ± 3.8	5	–	–
Galegeae					
Clianthus puniceus (G. Don) Sol. ex Lindl.	0	20.4 ± 2.6	10	0	5
Colutea arborescens L.	0	16.0 ± 3.2	5	0	5
Glycininae					
Glycine max (L.) Merr.	0	13.0 ± 1.1	5	0	5
Phaseolineae					
Phaseolus vulgaris L.	0	13.0 ± 1.1	5	0	5
Sophoreae					
Sophora microphylla Aiton	0.2 ± 0.2	24.3 ± 3.4	10	0.6 ± 0.6	5
Viciae					
Lathyrus odoratus L.	0	29.2 ± 5.7	5	0	5
Lens culinaris Medikus	0	13.0 ± 1.1	5	0	5
Pisum sativum L.	0	29.2 ± 5.7	5	0	5
Vicia faba L.	0	29.2 ± 5.7	5	0	5
ROSACEAE					
Malus sp.	0	24.3 ± 3.4	5	0	5

at 18 °C and 16L:8D photoperiod for 3 days, when the number of eggs laid on each shoot was counted. For each set of moths, choice tests were alternated with no-choice tests. The presence of eggs on gorse shoots in each choice test was used as a control for the quality of the same moths in the previous no-choice test. Arenas containing gorse alone were set up as controls for no-choice tests, and data from these controls were pooled. Consistent oviposition in these controls indicated that ambient conditions were suitable during the experimental period. The number of eggs laid allowed comparison of the relative acceptability of plants for oviposition.

To clarify results obtained in small-cage tests, the susceptibility of 17 plant species to *C. succedana* oviposition was examined again in near-natural conditions. Six plastic mesh cages (1 × 1 × 1 m) were erected outdoors at CABI Bioscience, Silwood Park, Ascot, UK in April 1990. Five potted plants bearing flowers and pods were randomly arranged in each cage. The choice of which of the 17 species to include in each test was random, within the constraint that each species was presented five times [10 times for *Sophora microphylla* Aiton and *Clianthus puniceus* (G. Don) Sol. ex Lindl.]. Choice and no-choice tests were carried out in each experimental period. Gorse was one of the five plants presented in choice tests. Gorse plants in the choice experiments provided a control for concurrent no-choice experiments. A dental roll soaked in a dilute solution of honey in water was lodged in each plant. Six male and nine female *C. succedana* moths were released into each cage. After 3 days the number of eggs laid on each plant was counted and recorded.

The response of larvae hatching on test plants was monitored. Two shoots bearing pods of *Sophora* sp., two of *C. puniceus*, and two of gorse were exposed to high densities of moths in small containers until eggs were laid on them. Pods were checked 7 days after hatching and the performance of larvae was recorded.

Late-instar migration tests. Second- or third-instar larvae consume the contents of one pod, and then emerge to seek another in which to continue development. The later instar larvae of oligophagous insects are generally considered to be less host-specific than first-instar larvae (Cullen, 1990). The risk that itinerant larvae might pose to *S. microphylla* growing near gorse was assessed experimentally in the laboratory. Sixty-centimeter-tall gorse plants without pods were placed on the floor of an evenly lit room at a constant temperature of 15 °C and long photoperiod (16L:8D). Thirty-centimeter-long shoots of gorse and *S. microphylla* bearing pods were collected from the field and mounted in water in 250-ml flasks. As far as possible, the volume of vegetation and pods on each shoot was equivalent. Two *S. microphylla* and two gorse shoots were randomly arranged around, and touching, each gorse plant. Twenty large (mostly third-instar), mobile *C. succedana* larvae were placed on the central potted gorse bush. After 3 days, pods were removed from all stems and dissected, and the position of all larvae was noted. Preliminary experiments showed that 80–90% of larvae released on the central plant could find and inhabit new gorse pods on cut shoots in this time. In two tests, only immature *S. microphylla* pods were presented, and in another two, pods containing well-formed seeds were presented. In one further test, one shoot of each plant species was presented. Tests were conducted simultaneously, and data from like shoots were pooled.

2.4. Release and monitoring

Fifty moths emerging from culture were collected into a 30-cm Mylar tube that was sealed with plastic at one end, and cloth at the other. Moths died quickly when dehydrated. A sprig of gorse was lodged in the tube, along with a dental roll soaked in a dilute solution of honey and water. This provided a humidity gradient along the tube. Two tubes were packed in a polystyrene box with a padded freezer pad, and shipped to the release point. Moths were released by shaking moths out the tubes in at least 1 ha of flowering gorse. Once *C. succedana* established, moths were collected for re-distribution by stalking, or by disturbing bushes and netting moths that took flight. Releases were later increased in size from 100 to 500 as moths became more freely available. At 6–12 month intervals, release sites were visited by local staff of client organizations to check for agent establishment. The staff was trained to distinguish *C. succedana* from other day-flying moths. An effective synthetic pheromone lure for male *C. succedana* was developed by 1996 (Suckling et al., 1999), and sex-attractant-based traps were deployed at many release sites in 1999/2000 to determine if populations persisted.

3. Results

3.1. Rearing

A combination of two female moths plus one male moth produced the best yield of eggs per female within the oviposition boxes used. Adults survived 3–10 days at 20 °C and 5–15 days at 13 °C. Lifetime fecundity under these conditions was approximately 30 eggs/female. Most eggs were laid on the inside surface of the browning petals or sepals surrounding the developing pod. Some were laid on pods, and occasionally on spines or stems near flowers. Eggs hatched approximately 12 days after deposition. First-instar larvae were susceptible to desiccation, but also to condensation within hatch boxes. Otherwise, a high proportion of eggs hatched successfully under a variety of regimes. It was noted that

larvae could complete the first instar without penetrating a pod by feeding on detritus within the degenerating flower. Once all seeds were consumed, larvae emerged through round holes chewed in the side of the pod and sought another pod. Each larva destroyed two or three pods in the course of its development. The larval period was 6–7 weeks, and the pupal period was 3–4 weeks. Approximately 80% of first-instar larvae transferred to pods survived to seek a second pod, 70% of those larvae pupated successfully, and 75% of those pupae produced moths. The yield of this intensive rearing technique was therefore approximately 30 moths per 100 eggs, or 10 moths per parent female.

Larvae that completed early instars feeding on gorse pods and then transferred to artificial diets, both routinely used to rear codling moth, *C. pomonella* (Ashby et al., 1985), developed successfully to become fertile adults. All insects used for host-range tests were fed on gorse pods. Moths reared on both diet and pods were released widely in the first year of the release program.

3.2. Host-range tests

First-instar starvation tests. Thirty-nine plant species were tested. Most of the first-instar larvae placed on young gorse pods in both New Zealand and UK experiments survived beyond 10 days (56.1% and 86%). Forty percent of all larvae completed development and successfully produced moths (Table 1). By comparison, performance of larvae on other plants was considerably depressed. Eight of the plants tested were closely related to gorse within the tribe Genisteae. When fed young pods of these plants, several first-instar larvae survived to 10 days. However, feeding was limited, and no larvae completed development. Nine endemic New Zealand *Carmichaelia* spp. were tested, but when fed on these pods, no larvae survived to 10 days. Feeding was restricted to the valves of the pod, and no larvae entered the lumen or attacked seeds. However, larvae survived for at least 10 days on pods of several other species within the pea family (Table 1).

Two of the 35 *C. succedana* larvae placed on pods of the rare New Zealand native legume shrub *C. puniceus* survived to produce moths in tests conducted in both New Zealand and the UK (10% and 4% of larvae tested). When mated, these moths were fertile. Feeding was restricted to the pod valves. *Clianthus* pods on which larvae had fed were maintained for several weeks, and seeds appeared to ripen normally.

Some feeding was observed on pods of *S. microphylla*, but no larvae completed development. Feeding was restricted to the pod valves, and no larvae were found attacking seeds. Larvae fed successfully on the pods of several domesticated legumes within the tribe Vicieae, and a small proportion of moths completed development on pods of *Pisum sativum* in New Zealand (7.2%) and UK (8.0%) experiments. In this case, larvae penetrated the pod lumen and consumed seeds. Larvae also successfully penetrated lentil pods, *Lens culinaris*, and fed on seeds before dying. Peas and lentils were the only plants other than gorse where feeding on seeds was observed. First-instar larvae survived poorly on *Cytisus scoparius*, *Genista hispanica*, *Spartium junceum*, and *Lotus pedunculatus*, species or genera recorded as hosts in Europe.

Oviposition tests. Thirty-three plants were tested in choice and no-choice oviposition tests in small cages. The design of experiments was not entirely consistent as a varying number of cut shoots were presented to moths in random combinations of species (Table 2). However, there appeared to be no discernible difference in the host-range as revealed by the different tests. In most no-choice tests, two or three (but occasionally five) cut shoots were presented in random combinations of species. At the same time, single gorse shoots were presented to the same number of moths in separate boxes. Eggs were laid on control shoots in every experimental period at an average of 40 eggs per shoot (Table 2), indicating that ambient conditions were suitable for oviposition throughout this experiment. The number of eggs laid in these arenas allowed comparison of the relative acceptability of test plants. No eggs were recorded on 17 of the 33 plants tested. Few eggs were laid on the pods of the test plants presented, either in the presence or the absence of gorse. The most acceptable plants for oviposition were those most closely related to gorse. In the absence of gorse, there was significant oviposition on *Lupinus* spp., *S. junceum*, and *C. scoparius*. A small number of eggs were laid on pods of *C. puniceus* and *S. microphylla*. Moths laid most eggs on the five species that were most closely related to gorse within the tribe Genisteae (Table 2). Oviposition on the other plants tested was spasmodic, and never exceeded 10% of the oviposition recorded on controls. Eggs were laid on *C. puniceus* and an ornamental hybrid *S. microphylla*, but no eggs were laid on *L. culinaris* or *P. sativum*.

A similar pattern was observed in choice tests. Eggs were laid on species within the tribe Genisteae, but rarely on endemic *Carmichaelia* spp. Occasional eggs were laid on crop legumes tested (Table 2). Once again, eggs were laid on *C. puniceus* and *S. microphylla* in the presence of gorse. In 12 paired tests, oviposition on *C. puniceus* averaged 4% of that recorded on control shoots presented in the same box. Oviposition on *S. microphylla* was almost 20% of that on the control shoots, but of the 36 eggs laid in 11 tests, 29 were laid in one test. No eggs were laid on *L. culinaris* or *P. sativum*.

Seventeen plants were tested in outdoor field cages in the UK, including most plants on which eggs were laid in small-cage tests conducted indoors. Eggs were laid on only three of these species. *Cytisus scoparius* attracted

eggs in the presence of gorse, but there was no oviposition when gorse was absent (Table 3). Another closely related species, *G. hispanica*, also attracted eggs, but there were fewer eggs on this host than on the gorse control (Table 3). Occasional eggs were laid on *S. microphylla*, but in choice tests, only two eggs were laid on this species compared to 243 eggs laid on control plants within the same cage. In the absence of gorse, three eggs were laid on the five *Sophora* plants.

Although marginally attractive in small-cage tests, no eggs were laid on *C. puniceus* in the 10 choice tests and 5 no-choice tests carried out in large cages. *C. succedana* did not lay eggs on peas or on lentils in large-cage tests (5 choice and 5 no-choice tests).

Larvae that hatched on the pods behaved differently from those transferred onto pods in starvation tests. Seven days after hatching, most larvae on gorse pods had successfully established inside the pods (10 of 10, 5 of 7). On *S. microphylla*, all larvae moved off the pods and died on the surrounding filter paper (10 of 10, 6 of 6). On *C. puniceus*, one larva nibbled the pod and died, and the remainder migrated off the pod and died (7 of 8, 3 of 3).

Late-instar migration tests. When 40 late-instar *C. succedana* larvae were given a choice of migrating to shoots bearing immature *S. microphylla* pods or to shoots with gorse pods, 33 of the 40 larvae released were recovered from within gorse pods, and one was found in the sepals of a *S. microphylla* pod (Table 4). When the choice was between shoots bearing gorse pods or *S. microphylla* pods that contained developing seeds, 6 of the 32 larvae recovered were on *S. microphylla* pods and 26 were in gorse pods. The proportion of mature pods presented that sustained damaged was similar. However, while all larvae recovered from gorse shoots had occupied pods, only one had penetrated a *S. microphylla* pod. The other larvae recovered from the *S. microphylla* shoots were feeding externally on pod valves.

3.3. Release and monitoring

Since 1992, moths have been released at 134 sites throughout New Zealand (Fig. 1) as part of an ongoing technology transfer program (Hayes, 2000). The use of sex-attractant-based traps enabled establishment of *C. succedana* to be confirmed at sites where moth numbers were too low to be observed, or where conditions were not conducive to moth flight at the time sites were visited. Moths have been released only recently at 41 of the 134 sites. These were not checked, as it was too early to judge establishment success. Of the 93 older sites, only 64 have been adequately assessed by diurnal observation or pheromone-trapping. *C. succedana* has established at 50 (78%) of these. The number of moths released at these sites varied from 100 to 500, and there is no evidence that release size has influenced establishment success. There appears to be no geographical pattern to success or failure of establishment.

4. Discussion

Colonization of a new host plant requires a specialist phytophage to locate the plant, lay eggs on it, and for the developing larvae to produce fertile adults. Safety-tests seek to predict which plants would allow an insect species to fulfil these requirements, but which test methods do this best is debated (Sheppard, 1999). In this study, the physiological capability of *C. succedana* to utilize plant species of conservation or significant economic value was estimated by testing representative species of all legume genera in New Zealand, in both the presence and the absence of gorse. Behavioral observations and experiments were then used to infer the likely field host-range from within the physiological host-range (Van Klinken, 2000).

Cydia succedana larvae hatched from eggs laid on or adjacent to gorse pods, selected pods, and burrowed into the lumen. As this is a critical behavior to ensure developmental success, first-instar larval starvation tests were considered appropriate (Heard, 2000). Tests were conducted within secure insect containment, and there was no alternative to using pods that had been removed from plants for these tests. Starvation tests indicated that first-instar larvae of *C. succedana* could damage the pods of *L. culinaris*, *S. microphylla*, *C. puniceus*, and *P. sativum*, but completed development only on pods of *C. puniceus* and *P. sativum*.

Table 4
Distribution of 40 late-instar *Cydia succedana* larvae 3 days after release into arrays of pod-bearing shoots of *S. microphylla* Aiton and *U. europaeus* L.

	Shoots presented	*Sophora microphylla* Aiton			*Ulex europaeus* L.		
		Pods presented per shoot	Pods damaged per shoot	Larvae recovered per shoot	Pods presented per shoot	Pods damaged per shoot	Larvae recovered per shoot
Immature pods	4	27 ± 4	0	0.25 ± 0.25	143 ± 3	11 ± 3	8 ± 3
Mature pods	4	32 ± 3	2 ± 0.8	1.5 ± 0.6	120 ± 18	9.5 ± 2	6.6 ± 1
Gorse only	2	–	–	–	195.5	9.5	8.5

Mean number ± SE.

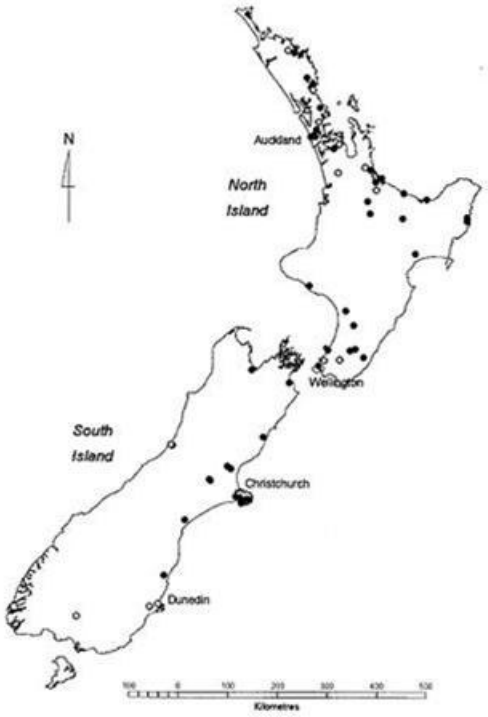

Fig. 1. Sites in New Zealand where *Cydia succedana* has been released (o) and where establishment has been confirmed (•).

While *P. sativum* proved an adequate host for first-instar larvae to complete development, *C. succedana* did not lay eggs on this species in either small-cage tests or field cages. For this reason it is unlikely that first instar larvae would ever encounter pea pods in New Zealand, and unlikely that a permanent population of *C. succedana* could develop on peas. However, *C. succedana* larvae move between pods in the course of their development. There is a possibility that migrating larvae might find and damage pea pods in crops that grow adjacent to gorse, near a gorse hedge for example. There is little evidence for this. *C. succedana* has not been reared from pea pods in England (Dr. C. Wall, Bunting Biological Control, Colchester, UK, personal communication), despite long-term studies on the ecology of the closely related *Cydia nigricans* (F.), (e.g., Graham, 1988), a common pest of pea pods. A similar argument can be made regarding the risk of damage to *L. culinaris* by *C. succedana.*

Cydia succedana moths were more selective in choosing oviposition sites when faced with whole plants in large cages outdoors than in small-cage tests conducted indoors, confirming the view that tests conducted in small arenas tend to overestimate potential host-range (Cullen, 1990). A small number of eggs were laid on pods of *C. puniceus* in small-cage tests, but none were laid on pods on whole plants under more natural conditions in field-cage tests. As with peas, these experiments indicated that it is unlikely that eggs will be laid on *C. puniceus* in New Zealand, and therefore unlikely that seeds will be destroyed. This conclusion is reinforced by the observation that whereas larvae hatching on excised gorse pods immediately entered the pod, larvae hatching on *C. puniceus* pods tended to actively reject them. This suggests that the pods were relatively unattractive to the first-instar larvae and that even if eggs were laid on pods, this would not necessarily result in pod infestation.

There was no evidence that first-instar larvae of *C. succedana* could complete development on pods of *S. microphylla*, although significant numbers of larvae survived beyond 10 days, and attacked the pod valves. Larger larvae could bore into pods and damage seeds, but none completed development on *Sophora* spp. Both first- and later-instar larvae showed a lower preference for *S. microphylla* pods, even when these were placed in close proximity to gorse. Only two larvae initiated feeding on *S. microphylla* pods in the presence of gorse. In small cages, this species attracted a small number of eggs. In larger cages, in more natural conditions, moths showed much stronger oviposition preferences, and were less inclined to oviposition *S. microphylla*, either in the presence or absence of gorse. It is likely that *C. succedana* would show even better discrimination in the field (Cullen, 1990). From these results we predict that small larvae may be found on *Sophora* species in New Zealand from time to time, but there is no evidence that hatching larvae will complete development on these species or establish viable populations. Although the species tested was nominally *S. microphylla*, Allan (1961) records that *S. microphylla* commonly hybridizes with *Sophora tetraptera* J.S. Mill, and with *Sophora prostrata* Buchanan. These latter species were not tested separately.

The data suggest that there is a low probability that *C. succedana* will colonize or damage any of the plants tested, or any other legumes present in New Zealand. Based on these results, Hill (1990) concluded that the introduction of *C. succedana* to New Zealand posed no significant threat to valued plant species, and following application, permission to release *C. succedana* in New Zealand was granted in 1991. This species is now firmly established in New Zealand. There appears to be no discernible geographic pattern in the success or failure of *C. succedana* to establish at individual sites (Fig. 1), and it seems likely that this species will establish at sites throughout New Zealand.

Cydia succedana moths are diurnal. The primary method for assessing establishment was to search for flying moths from late morning. A sex attractant for male moths was isolated and developed for monitoring the establishment and flight phenology of *C. succedana* (Suckling et al., 1999). This is one of only three examples

where lepidopteran pheromones have been used for monitoring agent establishment (Stanley et al., 2000).

The value of seed-feeding insects as biological control agents for weeds has been debated for many years. How will *C. succedana* contribute to the control of gorse in New Zealand? Paynter et al. (1996) suggested that severe reduction of seed production by biological control agents would likely reduce the ability of *C. scoparius* (Scotch broom) to invade new areas of its exotic range. This is likely to be true in New Zealand, where Scotch broom has yet to occupy its full range. The same argument could be made for gorse in the relatively few regions of New Zealand that have not been exposed to gorse infestation in the past. It would also be true where land management has excluded gorse for long periods, and where seed banks are largely exhausted. While the role of seed-feeders in limiting invasion is acknowledged, the ability of seed-feeding insects alone to reduce the populations of weeds is more controversial. Crawley (1990) and Myers and Risley (2000) suggest that density-dependent compensation by weed populations makes seed-feeding biological control agents ineffective, except at unrealistically high levels of seed predation. Myers and Risley (2000) considered the mortality of seedlings and small plants to be of greater importance in reducing populations. T. Partridge et al. (Landcare Research, Lincoln, New Zealand, personal communication) recently showed that inter-specific competition, especially by grasses, can reduce gorse seedling survival to low levels. They concluded that where seedling survival was low (2 seedlings per m^2), reduction in the annual seed crop using seed-feeding species could further reduce the recruitment of gorse below replacement levels, leading to population decline. Recent population dynamics models for broom (Rees and Paynter, 1997) and gorse (Rees and Hill, 2001) predicted that high levels of disturbance, low seedling survival, and low fecundity could lead to population decline in these weeds. When the survival of gorse seedlings beyond the first year was set at 1%, the model suggested that reduction in annual seed crop by 90% to 2500 seeds per m^2 resulted in gorse population decline, even in the absence of major disturbance (Rees and Hill, 2001). T. Partridge et al. (personal communication) measured the impact of *C. succedana* and *E. ulicis* on seed production at one site. Flowering phenology varied with altitude at the site. At the high level, plants produced seed only in fall, and in the absence of attack by the univoltine, spring-feeding *E. ulicis*, seed production was reduced by only 8%. At the lower end of the site, where plants seeded largely in spring, the combined effects of the two agents reduced annual seed production by 92% to 14 seeds per m^2. This is below the level that the model predicted would be necessary for population maintenance.

Recent studies in New Zealand have shown that there is considerable spatial variation in seed production (T. Partridge et al., personal communication), longevity of seeds in the seed bank (Hill et al., 2001), levels of mortality from plant competition (Partridge et al., personal communication), and levels of seed predation by *C. succedana* and *E. ulicis* (Partridge et al., personal communication). Gorse populations may be under pressure in some places. It remains to be seen whether seed predation by *E. ulicis* and *C. succedana* leads to a decline in gorse populations in such areas, but Rees and Hill (2001) have shown that, in combination with complementary management practices, this is possible.

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APPENDIX 3

Application for approval of the release of gorse pod moth, *Cydia succedana* (Denis and Schiffermüller), a potential biological control agent for gorse, *Ulex europaeus* L.

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Tasmanian Institute of Agricultural Research
13 St. John's Avenue, New Town, Tas. 7008
Ph.: (03) 6233 6821; Fax: (03) 6278 2716
email: john.ireson@utas.edu.au

CONTENTS

Summary
Gorse, *Ulex europaeus*, was declared a target for biological control in Australia in 1995 and declared a weed of national significance in 1999. This application presents the results of tests to determine the suitability of the gorse pod moth, *Cydia succedana*, as a potential biological control agent for gorse in Australia. *C. succedana* was released as a biological control agent for gorse in New Zealand in 1992 after detailed tests on 44 species of plants. Studies of European host records had previously indicated *C. succedana* had a narrow host range and was host specific to *Ulex* species. Between March 2000 and May 2001, additional tests were carried out with *C. succedana* on an approved list of 35 species or cultivars of Australian plants. Field surveys were also conducted in New Zealand during this period to confirm the predicted host range of *C. succedana* at New Zealand release sites. The tests and surveys confirmed that *C. succedana* is host specific to *Ulex* spp., and safe to release in Australia. The aim of this application is to obtain approval for the importation of *C. succedana* to Australia for field release.

Part 1 Information on the Target Species, *Ulex europaeus* L.

1.1 Taxonomy
Order: Fabales
Family: Fabaceae
Tribe: Genisteae
Genus/Species/Author: *Ulex europaeus* Linnaeus, 1753
Common name: Gorse, Furze

1.2 Native range
Gorse is a native of central and Western Europe and the British Isles (Parsons and Cuthbertson 2001) where it occurs in native heathland (Tubbs 1974) and on disturbed or neglected farmland and forests (Zwölfer 1962).

1.3 Australian and overseas distribution
In Australia, gorse occurs in all States except the Northern Territory (Parsons and Cuthbertson 2001). Its weed status appears to be related to latitude as the main problem regions are principally in Victoria and Tasmania. In Tasmania it grows from sea level to 800 m in altitude within an annual rainfall area of 500-1500 mm. The heaviest infestations covering *ca.* 30,000 ha occur in the central and northern midlands on pastures grazed mainly by sheep (Ireson *et al*. 1999). Isolated heavy infestations occur on the West Coast near Zeehan, in the far north west in the Circular Head district, on the East Coast, the far north east around Gladstone and in the George Town area. It is also present on King Island.

In Victoria, Lane *et al.* (1980) listed gorse as the sixteenth most widespread weed. Their surveys showed that it occupied an estimated total area of 948,000 ha. over which scattered infestations were found on 805,000 ha. and medium to dense infestations on 143,000 ha. It is common along roadsides and on disturbed land in the central highlands region, south west Victoria and parts of Gippsland. It also extends into the eastern, south eastern and south western fringes of the grain belt. In South Australia it has a scattered distribution in the higher rainfall areas of the state, particularly in the Mt. Lofty ranges, and has also been recorded on Kangaroo Island.

Gorse is uncommon in Western Australia, Queensland and the ACT. In Western Australia it is reported from a total of 175 locations covering an estimated area of 185 ha. The main areas affected are around Albany. The only known infestation of gorse in Queensland occurs

over a small area near Toowoomba. In NSW it has a very limited distribution but is locally common on the north and central coasts, central tablelands and central and south west slopes (Parsons and Cuthbertson 2001).

Gorse now occurs in most temperate areas of the world. Holm *et al.* (1979) categorise it as a serious weed in New Zealand and Hawaii, a principal weed in Australia and Chile and a common weed in Iran, Italy and Poland. Apart from many European countries it is also found in Brazil, India, New Guinea, South Africa, Trinidad and North America where it is a serious weed in the Pacific Coast States of Washington, Oregon, and California (Parsons and Cuthbertson 2001).

1.4 Native and introduced related species

There are no native *Ulex* spp. and no native species in the tribe Genisteae in Australia.

1.5 When approved as a target species, and proposing organisation

Gorse was approved as a target for biological control in July 1995 (Ireson *et al*. 1999), following nomination by the Department of Primary Industry and Fisheries, Tasmania.

1.6 Details of pest status

1.6.1 Nature of damage caused

In the main problem areas of Tasmania and Victoria, gorse is considered a serious weed because it invades pastoral land and significantly reduces pasture and animal productivity, and provides habitats and shelter for vertebrate pests. In forestry plantations it reduces tree growth and survival and is a significant fire hazard. It invades bushland reducing access and conservation values, increasing fire hazards and threatening the survival of rare and endangered plants and plant communities. It is also a fire hazard in urban areas. Gorse is difficult and expensive to control with currently available methods and necessary control by public authorities on roads and railways lines involves high financial inputs

1.6.2 Extent and value of losses

Gorse was declared a Weed of National Significance in 1999. In Tasmania, the annual loss of productivity of animal industries due to the presence of gorse has been estimated at $1 million per year in the central and northern midland areas alone (Dept of Primary Industry and Fisheries unpubl. data). This figure would be much higher if other areas of Tasmania were included as infestations occur on rural land in all parts of the State.

No costs are available for the other types of losses due to gorse as listed above. However, the damage caused to property in several serious urban and rural fires (eg. Zeehan, Knocklofty Reserve) has been greatly increased by gorse infestations. Furthermore, gorse was the weed most often considered of significance in bushland and riparian environments in all regions of Tasmania by participants at a series of environmental weeds workshops held in August 1992 (Young 1992).

In Victoria, an economic analysis on the costs of gorse to the community in the central highlands region (Anon. 1999) found that an ongoing ‘do nothing’ strategy would result in $7 million in tangible and intangible costs to the community over 5 years. The analysis also showed that the implementation of a control strategy in the region over a 5 year period would provide a total economic benefit of approximately $2.1 million. No figures are available on losses attributable to gorse from other States. However, there is much concern regarding its capacity to spread and biological control is seen as a method that would be useful in restricting the weed.

1.6.3 Current control methods available

Chemical

Extensive trial work in Tasmania has shown that the most effective herbicide for gorse control is a mixture of triclopyr and picloram (Grazon DS Herbicide®). Where thorough coverage of the bush can be achieved, one application will give complete control with no regrowth. However, it is recommended that treated bushes be checked 12 months after application and the re-growth treated. Because of the sensitivity of clover, and horticultural crops and trees to the picloran component of Grazon, the chemical is not recommended for use in orcharding, or horticultural cropping areas or where desirable tree species are present. Triclopyr alone or alternate herbicides such as metsulfuron-methyl, amitrole or glyphosate, although less effective than Grazon, are recommended when the use of Grazon is inappropriate. Grazon can be applied throughout the year.

Burning

Burning alone will not adequately control gorse bushes. By itself, burning is only a stopgap measure as regrowth of established bushes and seedling establishment is generally rapid after burning. Burning reduces the amount of foliage drastically and produces green shoots, which are far more attractive to goat or sheep browsing than mature shoots. Burning is also useful if done several months after spraying when, under the best conditions, it reduces even the heaviest of woody stems to ashes.

Cultivation

Mechanical clearing is the best method for controlling large infestations on land that is suitable for sowing down to pasture. Bulldozers with rippers, or medium or heavy tractors with dozer blades and rippers attached can be used. Since the object of mechanical grubbing is to rip out as much of the root system as possible, this work should be done when the ground is soft. Gorse mulching, using a heavy duty rotary hoe that pulverises the gorse and incorporates the plant material into a form of mulch, has found to be an effective form of control but is restricted to stone-free ground, requires a follow-up spray and pasture cover needs to be rapidly established.

Grazing

Grazing by sheep is the best method for controlling gorse seedlings. After a dense gorse infestation has been removed and the area sown to pasture it can be grazed heavily by sheep during the spring and summer to prevent the establishment of gorse seedlings. Sheep will browse established gorse bushes during spring or when alternative feed is in short supply. However, they prefer to eat pasture species so that significant control cannot be achieved by sheep grazing unless large numbers are confined to gorse patches for most of the year.

Harradine and Jones (1985) have shown that Angora goats are ideal for gorse control. Goats prefer to browse young gorse shoots rather than graze actively growing pasture. They remove flowers and defoliate bushes, browsing them back to stumps when the stocking rate is high enough. However, well-established gorse bushes are not readily killed by browsing and are capable of recovery after several years of browsing if the goats are removed from the area.

Subsequent Management

Irrespective of the control methods employed, the prevention of reinfestation by gorse or of infestation by other weeds as a result of the removal of gorse cover is a matter of great importance. Before control or eradication is attempted there should be a clear idea of how the land is to be used and treated afterwards. For instance, the establishment of a vigorous, correctly fertilised permanent grass and clover sward will do much to suppress seedlings and

will also allow heavier stocking rates. Grazing is an important factor in preventing recolonisation in cleared areas. Regrowth and any surviving young plants can be spot sprayed.

1.6.4 Effectiveness of current control methods

A combination of currently used methods i.e. the use of chemicals, burning, cultivation and grazing can contain the problem on agricultural land and other mainly accessible areas. However, gorse is also a serious environmental weed in disturbed areas of a variety of vegetation types (Wells 1991; Anon. 1997). The use of traditional control methods to contain its spread into areas of native vegetation is more difficult because of the risk of damage to surrounding desirable species and limited accessibility.

Biological control offers an alternative solution to the problem if the introduction of a guild of agents can reduce gorse vigour to a stage where it can be controlled more easily by traditional methods at a much lower cost, its spread is restricted due to reduced seed output, and/or native vegetation is able to compete with it more readily.

1.6.5 Costs of current control methods

While gorse can generally be effectively controlled on arable and grazing land, the high cost of control precludes much activity. For example, in Tasmania, a chemical control program for a dense infestation followed by pasture establishment may range from $700 up to $1,500 per hectare which exceeds current land values in some areas. Unless the pasture establishment is successful and the subsequent grazing management is correct, gorse may re-establish in the area within a few years.

In 1996, the annual cost of reclaiming land currently infested with gorse in central and northern midland rural areas of Tasmania alone was estimated at around $45 million (Dept of Primary Industry & Fisheries unpubl. data). This figure would be much higher if the costs to reclaim land in other rural areas were included together with the cost to public authorities for control and reclamation of land along roadsides, railway lines, recreation areas and disturbed areas of natural vegetation. The additional costs for control in these areas would also be expected to total several million dollars.

In Victoria, a total of $37,500 was made available for the control of gorse on public land for the 1993/94 financial year in the regions of Ballarat, Portland, Alexandra, North East, Bendigo and Geelong. An unknown additional cost towards the control of gorse in Victoria is incurred annually by farmers (and others) who wish to control the weed on their own properties. Anon. (1999) state that the cost of gorse control in Victoria can range from $175 per hectare to $445 per hectare. In NSW, councils alone spend around $10,000 each year on gorse control.

1.6.6 Undesirable side effects of current control methods

All herbicides used for control of gorse are severely damaging to pasture legumes and desirable trees and shrubs. Damage to eucalypts, wattles and other non-target species is common where gorse is controlled by foliar application of herbicides in bushland. Picloram, one of the component herbicides of the most commonly used product (Grazon DS), can persist in the ground for up to two years and prevent re-establishment of pasture legumes in treated areas.

The major level of soil disturbances associated with mechanical removal of gorse leaves treated areas susceptible to soil erosion and reinvasion by gorse or other weeds.

1.6.7 Beneficial aspects

Gorse was once used extensively as a hedge plant (Richardson and Hill 1998) and can provide shelter and nesting sites for native animals where no native understorey remains. It can also provide shelter and fodder for livestock (Harradine and Jones 1985) and is regarded as a useful pollen source by bee-keepers (Parsons and Cuthbertson 2001). In some instances, particularly along creek lines, gorse has been useful in controlling erosion (Anon. 1999).

Part 2 Information on the Potential Biological Control Agent - Gorse Pod Moth, *Cydia succedana* (Denis and Schiffermüller)

2.1 Taxonomy

Order: Lepidoptera
Family: Tortricidae
Tribe: Olethreutinae
Genus/Species/Author: *Cydia succedana* (Denis and Schiffermüller) 1836
Common name: Gorse pod moth

2.2 Summary of agent biology and ecology

2.2.1 *Description and life cycle*

The moth ranges from 5-8 mm in length and is pale brown in colour. Eggs are white, flat and *ca*. 1 mm in diameter. Neonate larvae are white with black heads. Mature larvae are pale yellow with light brown head capsules. *C. succedana* is a bivoltine species, completing two generations each year in Europe and New Zealand (Hill 1990; Suckling *et al*. 1999). In New Zealand, *Cydia succedana* adults emerge in spring and oviposit on spring-flowering gorse. Larvae feed inside seed pods and emerge to pupate outside the pod in late summer. Some of these pupae overwinter and emerge the following spring but a significant percentage of new adults emerge in late summer and oviposit on autumn-flowering gorse (Anon. 1998).

2.2.2 *Feeding damage and estimate of efficacy*

Larvae enter the pods and feed on the seed in spring and autumn. The seed damage is expected to complement that caused by the larvae of the already established and widespread gorse seed weevil, *Exapion ulicis* (Forster), which only attack the spring seed crop. Preliminary studies at a site in Canterbury, New Zealand have shown that the two species were jointly destroying *ca*. 60% of the annual seed crop with *C. succedana* taking *ca*. 15% of the autumn/winter seed crop when *E. ulicis* larvae were not active (Partridge *et. al*. submitted).

2.2.3 *Native range, related species and summary of their host range*

The native range of *C. succedana* is Europe. *Cydia* is a large genus with a worldwide distribution. Emmet (1988) lists 33 British species of *Cydia*, 26 of which attack buds, flowers or fruit of their host plants. Some generalist species have become pests in many parts of the world. In Australia, these include the codling moth, *Cydia pomonella* (L). and the oriental fruit moth, *Cydia molesta* (Busck). However, most species, including *C. succedana*, have a very narrow host range, being reported from only one or two closely related hosts.

Zwölfer (1963) discussed three *Cydia* species recorded from gorse in Europe. Of these *C. succedana* was recorded as bivoltine in Europe feeding on *Ulex europaeus* in spring and *U. minor* and *U. gallii* in late summer and autumn. *Cydia internana* Haworth appears entirely restricted to *U. europaeus* in Europe, has one generation a year—in late spring, and overwinters as a pupa. The third, *Cydia latyrana* (Hübn.), was reared from gorse shoots but was never reared to adult.

2.2.4 *Proposed source of agent*

C. succedana was released in New Zealand in 1992 with stock collected in Cornwall UK and Viana do Castello, Portugal, the populations being mixed prior to release (Suckling *et al.* 1999). It is now becoming widely established in New Zealand and material is readily

available for importation into Australia. It is proposed that material be supplied by Landcare Research New Zealand Ltd. based at Lincoln. This will be air freighted to Victoria and be opened in the quarantine facility at Keith Turnbull Research Institute at Frankston. All packaging and extraneous material will be autoclaved. The insect will then be bred through one complete generation and the progeny tested for freedom from hyperparasites and disease prior to field release, as per quarantine regulations.

2.3 Non-target organisms at risk

C. succedana is host specific to *Ulex* spp. (gorse) so non-target organisms will not be at risk from this species (see section 3 on host specificity).

2.4 Interaction with existing control program

It is expected that *C. succedana* will be one of several agents required to collectively reduce the vigour and reproductive capability of gorse to a stage where it can be more easily controlled in combination with traditional methods as part of an integrated management program. *Cydia succedana* will complement the action of the widely established gorse seed weevil, *Exapion ulicis*, (Forster) that was released in Australia in 1939 and is now widely established. Flowering and pod production of gorse varies considerably not only between sites but on individual bushes within sites. At some sites, particularly those in cool, high altitude localities most gorse bushes flower in late winter/spring. At other sites, such as those in warmer, coastal localities, flowering occurs in autumn and winter as well as in spring. The larvae of the weevil only feed on a proportion of seed produced in spring and summer and, as they are not present during the autumn/winter period, a significant proportion of the annual seed crop escapes attack (Cowley 1983; Hill *et. al*. 1991). As *C. succedana* is active in autumn and spring, this species is expected to play a significant role in reducing the annual seed crop. These seed feeding agents will both complement the action of two foliage feeding agents, the gorse spider mite, *Tetranychus lintearius* Dufour, which feeds on mature foliage, and the gorse thrips, *Sericothrips staphylinus* Haliday, which feeds on young growth and seedlings. These latter two agents were first released in Australia in December 1998 and January 2001, respectively.

2.5 Collaborators and nature of collaboration

The Tasmanian Institute of Agricultural Research (TIAR), Keith Turnbull Research Institute (KTRI) in Victoria and Landcare Research New Zealand Ltd. (LRNZ) based at Lincoln have already successfully collaborated in the host testing, introduction, mass rearing, release and monitoring of the gorse spider mite, *Tetranychus lintearius*, and the gorse thrips, *Sericothrips staphylinus*, in Australia. Staff at LRNZ carried out the host specificity tests that enabled the introduction of *T. lintearius* and *S. staphylinus* into Australia and CSIRO Division of Entomology, Canberra, have assisted in the supply of some of the plant material used in the host specificity tests. This collaborative work is continuing in the work program for *C. succedana*. LRNZ was contracted by TIAR to carry out the host testing of *C. succedana* on an approved list of Australian plants. If *C. succedana* is approved for release, TIAR, KTRI, CSIRO and LRNZ will continue their collaboration on the biological control of gorse.

Part 3 Host Specificity

3.1. Plant test list and previous tests

The approved host specificity test list (Table 1), contains 35 species or cultivars (including gorse). The list was approved on the basis of tests already carried out on 44 species or cultivars of plants that enabled *C. succedana* to be released in New Zealand (Table 2 and Hill 1990, see appendix). Landcare Research New Zealand Ltd. at Lincoln carried out host testing of the additional Australian plant species.

The choice of Australian species was based on the strategy detailed by Wapshere (1974) and by using a more recent interpretation of the relationship between the various tribes of the Fabaceae (Sub-family Papilionoidae (Faboideae)) by Polhill (1981). The selection of species (other than gorse) in the sub-tribe Genistinae included in the Australian test list (Table 1) was based on their use as ornamentals and, in the case of *C. palmensis* (tagasaste), its use as a fodder shrub. Although *C. palmensis* was previously tested by Hill (1990), additional tests were carried out to confirm that the plant would not be an alternative host for *C. succedana*. Three commercial cultivars of *Lupinus augustifolius* (Lupininae) were also included in the list due to the importance of this species as a fodder crop.

As defined by Polhill (1981) the Genisteae are seen as one of a basal group of tribes along with the Thermopsideae, Euchresteae, Podalyrieae, Liparieae, Brognartieae, Crotalarieae, Mirbelieae and Bossiaeeae. Polhill (1981) goes on to propose four natural groupings of tribes, the first the Sophoreae forming the base or stem of the group, the second 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, the Thermopsideae, Euchresteae, Podalyrieae, Liparieae, and Brognartieae contain no Australian species or economically important species. However, the Crotalarieae contain two Australian genera and the Mirbeliae and Bossiaeeae contain many Australian genera. Species representing genera from these tribes were tested on the basis of their affinity with the Genisteae. Outside these tribes one Australian species from the tribe Indigofereae was included on the Australian list (Table 1) as it occurs within the Australian distribution of *Ulex europaeus*. The tests carried out by Hill (1990) make up a representative selection across the other groups.

The NZ test species (Table 2) also include a number of leguminous plants of economic and environmental importance to Australia. However, because of the importance of plants in the Phaseoleae and Trifolieae to Australian agriculture, four species representing four genera of tropical legumes and four cultivars of the temperate legume *Phaseolus vulgaris*, as well as the native ornamental species *Hardenbergia violacea*, were included in the Australian test list together with *T. subterraneum*.

Outside the Faboideae the genus *Acacia* in the sub-family Mimosoideae is important to Australia and the common Australian species and *A. dealbata* and *A. mearnsii* were included in this test list.

Table 1 – Approved Australian host specificity test list for gorse pod moth, *Cydia succedana* (Denis and Schiffermüller), a potential biological control agent for gorse, *Ulex europaeus* L.

Plant Classification	Scientific Name	Common Name	Origin
1. Related plants (same Family)			
Family Fabaceae			
Sub-family Faboideae			
Tribe Bossiaeeae	*Bossiaea riparia* A. Cunn ex Benth.	River Leafless Bossiaea	Native
	Goodia lotifolia Salisb.	Golden-tip	Native
	Hovea corrickiae J. H. Ross	Corrick's Hovea	Native
	Platylobium formosum Sm.	Handsome Flat Pea	Native
Tribe Crotalarieae	*Crotalaria cunninghamii* R. Br.	Green Bird Flower	Native
Tribe Genisteae			
Sub-tribe Genistinae	*Ulex europaeus* L.	Gorse, Furze	Temperate legume
	Chamaecytisus palmensis (Christ.) Bisby & Nichols	Tagasaste, Tree Lucerne	Temperate legume
	Genista monspessulana (L.) L.A.S. Johnson	Montpellier Broom	Garden ornamental
Sub-tribe Lupininae	*Lupinus angustifolius* L. cv. 'Gungurru'	Lupin, New Zealand Blue Lupin	Temperate legume
	Lupinus angustifolius L. cv. 'Merrit'	" "	"
	Lupinus angustifolius L. cv. 'Yandee'	" "	"
Tribe Galegeae	*Swainsona laxa* R. Br.		
Tribe Indigofereae	*Indigofera australis* Willd.	Austral Indigo	Native
Tribe Loteae	*Lotus australis* Andrews	Australian Trefoil	Temperate Legume
Tribe Mirbelieae	*Aotus ericoides* (Vent.) G. Don	Common Aotus	Native
	Daviesia latifolia R. Br.	Hop Bitter Pea	Native
	Dillwynia glaberrima Sm.	Smooth Parrot-pea	Native
	Eutaxia microphylla (R.Br.) J. Black	Eutaxia	Native
	Gompholobium huegelii Benth.	Common Wedge Pea	Native
	Kennedia prostrata R.Br.	Running Postman	Native
	Oxylobium ellipticum R. Br.	Golden Rosemary	Native
	Pultenaea juniperina Labill.	Prickly Beauty	Native
Tribe Phaseoleae	*Centrosema pubescens* Benth.	Centro	Tropical legume
	Hardenbergia violacea (Scheev.) Stearn.	False Sarsaparilla	Native
	Lablab purpureus (L.) Sweet	Lablab bean	Tropical legume
	Macroptilium atropurpureum (DC.) Urban	Purple bean	Tropical legume

Table 1 (continued) – Approved Australian host specificity test list for gorse pod moth, *Cydia succedana* (Denis and Schiffermüller), a potential biological control agent for gorse, *Ulex europaeus* L.

Plant Classification	Scientific Name	Common Name	Origin
Tribe Phaseoleae (continued)	*Phaseolus vulgaris* L. cv. Broker	Common Bean	Temperate legume
	Phaseolus vulgaris L. cv. Flo	" "	"
	Phaseolus vulgaris L. cv. Labrador	" "	"
	Phaseolus vugaris L. cv. Rapier	" "	"
	Vigna radiata (L.) Wilczek	Mung Bean	Tropical legume
Tribe Psoraleeae	*Psoralea pinnata* L.	African Scurfpea	Temperate legume
Tribe Trifoliae	*Trifolium subterraneum* L. var. 'Denmark'	Subterranean Clover	Temperate legume
Sub-family Mimosoideae			
Tribe Acacieae	*Acacia dealbata* Link.	Silver Wattle	Native
	Acacia mearnsii De Wild.	Black Wattle	Native

Table 2 - List of plant species previously tested against gorse pod moth, *Cydia succedana* (Denis and Schiffermüller), to enable the agents introduction into New Zealand.

Plant Classification	Scientific Name	Common Name
1. Related Plants (same family)		
Family Fabaceae		
Sub-family Faboideae		
Tribe Genisteae		
Sub-tribe Genistinae	*Ulex europaeus* L.	Gorse, Furze
	Cytisus scoparius (L.) Link	Scotch Broom
	Chamaecytisus palmensis (Christ.) Bisby & Nichols	Tagasaste, Tree Lucerne
	Genista hispanica L.	Spanish Broom
	Genista lydia Boiss.	Lydia Broom
	Genista (Teline) monspessulana (L.) L.A.S. Johnson	Montpellier Broom
	Spartium junceum L.	Spanish Broom
	Laburnum anagyroides Medicus	Golden-chain
Sub-tribe Lupininae	*Lupinus arboreus* Sims	Tree Lupin
	Lupinus polyphyllus Lindle**y**	
Tribe Carmichaelieae	*Carmichaelia arborea* (Forst. f.) Druce	Tree Broom
	Carmichaelia arenaria Simpson	Native Broom
	Carmichaelia astonii Simpson	" "
	Carmichaelia. compacta Petrie	" "
	Carmichaelia corrugata Col.	" "
	Carmichaelia enysii Kirk	Prostrate Dwarf Broom
	Carmichaelia fieldii Ckn.	Native Broom
	Carmichaelia kirkii Hook. f.	Climbing Broom
	Carmichaelia virgata Kirk	Native Broom
	Chordospartium muratai	Weeping Broom
	Corallospartium crassicaule (Hook. f.) J.B. Armst.	
	Notospartium torulosum Kirk	
Tribe Galegeae	*Colutea arborescens* L.	Bladder Senna
	Clianthus puniceus (G. Don.) Sol. ex Lindi.	Kaka Bill
Tribe Loteae	*Lotus pedunculatus* auct. non cav.	Birdsfoot Trefoil
Tribe Phaeseoleae	*Phaseolus coccineus* L.	Scarlet Runner Bean
	Phaseolus vulgaris L.	Common Bean
	Glycine max (L.) Merr.	Soybean
Tribe Sophorae	*Sophora microphylla* Ait.	
	Sophora sp. (hybrid)	

Table 2 (continued) - List of plant species previously tested against gorse pod moth, *Cydia succedana* (Denis and Schiffermüller), to enable the agent's introduction into New Zealand.

Plant Classification	Scientific Name	Common Name
Related plants (same family)		
Tribe Trifoliae	*Medicago arborea* L.	Moon Trefoil
	Ononis campestris Koch & Ziz	
	M. sativa L.	Alfalfa
	Trifolium pratense L	Red Clover
Tribe Trifoliae (continued)	*T. repens* L.	White Clover
	Trifolium sp. "alexandria"	
	Trifolium sp. "zig zag"	
Tribe Vicieae	*Lathyrus odoratus* L.	Sweet Pea
	Lens esculentum Moench	Lentil
	Pisum sativum L. S. lat. cv.1	Garden Pea
	Pisum sativum L. S. lat. cv.1	Garden Pea
	Vicia faba L.	Broad Bean
Sub-family Mimosoideae	*Acacia* sp.	Wattle
2. Unrelated plants (different families)		
Family Pinaceae	*Pinus* sp..	
Family Rosaceae	*Malus* sp.	Apple

3.2 Report of host-specificity testing on Australian species (Landcare Research New Zealand contract report LC0001/095 - A. H. Gourlay)

3.2.1 Summary

Project and Client

The susceptibility of 35 Australian plants (species and cultivars) to the gorse pod moth *Cydia succedana* was determined in laboratory tests carried out by Landcare Research, Lincoln, in 2000/01 for the Tasmanian Institute of Agricultural Research.

Objectives

- To measure the oviposition preferences of adult *C. succedana* and the survival of unfed first-instar larvae on pods of 35 species and cultivars of Australian plants, using laboratory experiments.
- To determine the current host range of *Cydia succedana* at release sites in New Zealand.

Methods

- In 'choice with target' and 'choice without target' oviposition preference tests, adult moths were released into cages with cut shoots of test plants bearing flowers, pods, and leaves.
- Experiments were conducted to measure the survival of unfed first-instar larvae on 35 species and cultivars of Australian plants.
- To determine the current host range of *C. succedana* in the field, seed pods were collected for dissection from three sites where the moth had been released.

Results

- Unfed first-instar larvae were unable to survive and develop to adult in starvation tests, on any plant species other than gorse.
- In oviposition preference experiments, 10 to 200 times more eggs were laid on gorse than on test plants.
- Field surveys confirmed that the predicted host range of *C. succedana* is *Ulex* spp.

Conclusions

Results of tests described here support the view that *Cydia succedana* is specific to *Ulex* spp. and that the Australian plants tested are not at risk from its proposed introduction to that country.

3.2.2 Introduction

The gorse pod moth *Cydia succedana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) is being considered as a potential biological control agent for gorse in Australia. The susceptibility of 35 Australian plants (species and cultivars) to the gorse pod moth was determined in laboratory tests carried out by Landcare Research, Lincoln, for the Tasmanian Institute of Agricultural Research from March 2000 to May 2001.

3.2.3 Background

Gorse (*Ulex europaeus)* is a widespread weed species in New Zealand and seven biocontrol agents have been released within a nationwide programme of gorse biological control. New Zealand is the first country to use most of these agents, and experience gained here is of interest to other countries where gorse is a weedy species.

Gorse can produce seed in both spring and autumn. A study conducted at a site in Canterbury, New Zealand, has shown that the gorse seed weevil (*Apion ulicis*) and the gorse

pod moth (*Cydia succedana*) are both active in spring and are complementary, not competitive, in the spring gorse-seed crop (Partridge submitted). The impact of the two agents has reduced the annual seed crop of gorse at this site by 60%, although in some parts the crop was reduced by 99% and in others 15% depending on flowering phenology (Partridge *et al*. submitted). However, studies of gorse seed dormancy carried out in New Zealand suggest that it is autumn, rather than spring, seed that contributes most to the long-lived gorse seed bank in the soil, and in warmer areas autumn-formed seed makes up the larger part of the annual seed crop (Hill 1990). For these reasons it is important that the agents released for the control of gorse include species that reduce seed fall in autumn. At present the only gorse seed feeder introduced into Australia is the univoltine gorse seed weevil (*A. ulicis*), which attacks seed produced in spring only. In contrast, *C. succedana* is a bivoltine species whose larvae attack gorse pods in spring and autumn, and for this reason is being considered for release in Australia.

3.2.3 Objective

- To measure the oviposition preferences of adult *Cydia succedana* and the survival of unfed first-instar larvae on pods of 35 species and cultivars of Australian plants (Table 1, Section 3.1), using laboratory experiments.
- To determine the current host range of *Cydia succedana* at release sites in New Zealand.

3.2.4 Methods

Test species

Of the 35 plant species selected for testing, some were obtained from the Landcare Research nursery at Lincoln, New Zealand, but others were imported into the Lincoln invertebrate quarantine facility from Australia for the trial. All the plant material from Australia was shipped as cut shoots. Because the test plant species produce flowers and seed pods at different times of the year, it was necessary to obtain nine shipments of cut shoots from Tasmania between March 2000 and May 2001. Gorse (*Ulex europaeus*) shoots from New Zealand were cut from plants at the same time as imported shoots and used as controls for oviposition preference and larval starvation tests.

All experiments were carried out in clear plastic cages set up in an indoor rearing-facility under conditions of controlled daylight, temperature, and humidity. The cut shoots of test plant species plus gorse from Tasmania and New Zealand used in the oviposition experiments bore leaves, flowers, young and old seed pods, and were used in experiments within 5 days of being collected and shipped. Excised seed pods, both young and old, were used in the larval starvation tests. Gorse pod moths were field collected from a site at McLeans Island, Canterbury, and first-instar larvae emerged from eggs laid on gorse shoots in the laboratory.

Oviposition preference experiments

To assess the oviposition preference of adult gorse pod moths, trials were carried out with test plants only (‘choice without target’) and the test plants and gorse together (‘choice with target’). Plants were set up in clear plastic cages (600 × 450 × 300 mm) with a hole in the front (300 × 400 mm) stoppered by a piece of sponge rubber. Cut shoots of each test plant species were randomly arranged in a 3 × 3 Latin square design of eight test species and a space in ‘choice without target’ tests and eight test species plus gorse in ‘choice with target’ tests. Five pairs of adult gorse pod moths were released into each cage and left for 48 h. Control tests on gorse were conducted at the same time as each of the choice tests by placing two to five cut gorse shoots in a cage with five pairs of moths for 48 h. Each test species was included in five different replicates of random host-plant design. Each replicate was not

totally independent in that some moths were used in more than one test, run consecutively in the same cage.

First instar larval starvation experiments
Five randomly selected seed pods were removed from imported and field-collected cut shoots of test plants plus gorse and placed onto damp filter paper in each inverted, ventilated, 9-cm-diameter, plastic Petri dish (five replicates per test plant species). A single larva, less than 1 day old, was transferred by camel hair brush onto a pod surface, one per pod, and left for 5 days. Every 5 days the number of larvae surviving was checked and recorded until all larvae on test pods were dead. Pods were replaced as they rotted or as larvae emerged. The numbers of larvae alive was summarised as percentage larval mortality to the nearest 5 days.

Field surveys
Up to 1500 seed pods of *Ulex europaeus, Lupinus polyphyllus*, *Cytisus scoparius* (Genisteae), *Sophora microphylla, S. tetraptera, S. prostrata* (Sophoreae), *Carmichaelia arborea* (Carmichaelieae), *Lotus corniculatus* (Loteae) and *Trifolium pratense* (Trifolieae), were collected from up to three release-sites, one in Canterbury and two in the Mckenzie Basin, New Zealand, and dissected under a microscope for the presence of *C. succedana* larvae and eggs.

3.2.5 Results
'Choice without target' oviposition preference
Eggs were laid on the leaves of 10 test plant species and on the flowers of five in choice tests without the target gorse plants available (Table 3). Fewer than 10 eggs in total were laid on leaves in only one of the five replications for each of *Aotus ericoides, Bossiaea riparia, Hovea corrickiae, Lotus australis*, *Oxylobium ellipticum, Pultenaea juniperina,* and *Swainsona laxa*. Twelve eggs were laid on the leaves of *Lupinus angustifolius* 'Gungurru' and 21 eggs were laid on the leaves of *L. angustifolius* 'Yandee' in three of the five replicates. Seven eggs were laid on the flowers of *Crotalaria cunninghamii* and three eggs were laid on *Goodia lotifolia*, in two of the five replicates. In only one replicate a single egg was laid on a flower of *Dillwynia glaberrima*.

In only one replicate, 10 eggs were laid on the flowers (4), and leaves (6) of *Platylobium formosum* and 2 eggs on the flowers of *Genista monspessulana*. No eggs were laid on the remaining 20 test plant species (Table 3). The mean number of eggs (6.2 ± 1.7) laid on gorse shoots from Tasmania was similar to the overall mean (10.0 ± 1.3) number of eggs laid per shoot on New Zealand gorse in 'choice without target' controls. A total of 244 eggs were laid on the flowers and 40 eggs on the spines of (New Zealand) gorse.

'Choice with target' oviposition preference
Even in the presence of gorse, where the total number of eggs laid on gorse controls was 318, oviposition occurred on nine different test plant species but only in one of the five replicated experiments (Table 4). Up to three eggs were laid on the leaves of *Aotus ericoides, Bossiaea riparia*, *Dillwynia glaberrima, Eutaxia microphylla,* and *Platylobium formosum.* A single egg was laid in one replicate only on the flowers of *Macroptilium atropurpureum*, *Oxylobium ellipticum*, *Pultenaea juniperina,* and *Swainsona laxa.* The total number of eggs laid on flowers of gorse controls was 269. The mean number of eggs laid on gorse shoots from Tasmania (7.4 ± 2.2) was similar to the overall mean number of eggs laid per shoot on New Zealand gorse (8.1 ± 1.5) in 'choice with target' controls.

First-instar larval development
First-instar larvae transferred onto pods did not complete development on any test species even those closely related to gorse (Table 5). By day 5, 100% larval mortality had occurred on 16 species of test plant pods, by day 10 on 10 more species, and by day 15 on 6 more species, without causing significant damage to pods or seeds. Only on *Genista monspessulana* did larvae survive to day 30, still 10 days fewer than required to complete development on gorse. There was no larval feeding on seeds or pods of any test plants until day 5. Minor pod-wall damage occurred on *Aotus ericoides, Bossiaea riparia, Chamaecytisus palmensis, Daviesia latifolia, Hovea corrickiae, Lotus australis, Lupinus angustifolius* 'Gungurru', Merrit', and 'Yandee', *Lablab purpureus, Phaseolus vulgaris* 'Broker', 'Flo', 'Labrador', and 'Rapier', and *Swainsona laxa,* by approximately 10% of larvae by day 10 and 15. The seed pod wall was attacked by two of five larvae, in one replicate, on *Goodia lotifolia,* and the single larva that survived to day 15, in the same replicate, had died by day 20 without attacking any seeds inside the pod.

Genista monspessulana seed pods were attacked and some seeds were consumed inside the pod in four of the five replicates by 11 of the 25 first-instar larvae in the test. Larval mortality by day 25 was 85% on *G. monspessulana* compared to 50% mortality on gorse controls (Table 5). All larvae were dead in *G. monspessulana* pods by day 30 whereas 40% of larvae survived to day 35 and developed to adult on gorse pods. Larval mortality and development to adult on gorse pods from Tasmania and New Zealand were similar (Table 5).

Field surveys
Dissections of *Ulex europaeus* pods from three sites (one in Canterbury, two in the Mckenzie Basin, South Island, New Zealand) in spring and autumn revealed that *C. succedana* larvae were present in 2.5– 8% of gorse pods at the two Mckenzie Basin sites and 10–60% of gorse pods at the Canterbury site. Seed pods of *Sophora* spp*., S. prostrata, Carmichaelia arborea*, *Lotus corniculatus, Lupinus polyphyllus, Cytisus scoparius*, and *Trifolium pratense* (not *Genista monspessulana*) were collected and dissected from these three sites in spring and autumn. No *C. succedana* larvae or eggs were found on any of these non-target species.

3.2.6 Discussion
Host-range tests carried out in the UK and in New Zealand before the gorse pod moth was introduced into New Zealand showed that *C. succedana* has a narrow host range restricted to the Genisteae (Hill 1990). Larval feeding occurred on seven species other than gorse, and development to adult of one or two larvae was completed on *Pisum sativum*, *Clianthus puniceus*, and *Lens culinaris*. Oviposition occurred on 18 species in the laboratory, but in expanded field-cage tests oviposition occurred on *Genista lydia,* an ornamental and close relative of gorse, and *Ulex europaeus* only (Hill 1990). This current series of host range tests has produced very similar results to those conducted in the UK and New Zealand. Although *C. succedana* did lay 72 eggs on 14 non-target plant species in the 'choice without target' tests and 13 eggs on 9 non-target plant species in 'choice with target' tests, this is insignificant in comparison to the 284 eggs on gorse controls for the 'choice without target' tests and 318 eggs laid on gorse in 'choice with target' tests. Eggs were laid on *Lupinus* spp. in 'choice without target' tests, but in 'choice with target' tests no oviposition occurred on any of the three *Lupinus* spp. In the absence of gorse, eggs were laid on *Lotus australis, Goodia lotifolia, Hovea corrickiae,* and *Crotolaria cunninghamii,* but no eggs were laid on these species in the presence of gorse. Conversely, two species, *Macroptilium atropurpureum* and *Eutaxia microphylla,* onto which eggs were laid in the presence of gorse, did not receive eggs in the absence of gorse. In both 'choice with target' and 'choice without target' tests,

eggs were laid on *Aotus ericoides, Bossiaea riparia, Dillwynia glaberrima, Oxylobium ellipticum, Platylobium formosum, Pultenaea juniperina,* and *Swainsona laxa.* However, all larvae placed onto excised pods of the species that received eggs had died, without feeding, by day 15. Larvae continued to survive to day 20 on *Goodia latifolia* and to day 30 on *Genista monspessulana* but died without consuming any seeds before completing development to adult (Table 5).

Two eggs (1% of those laid on the gorse controls) were laid on *Genista monspessulana* flowers in the 'choice without target' tests and larvae fed on seeds inside excised seed pods in larval development tests, but no oviposition occurred in 'choice with target' tests and all larvae had died by day 30 before completing development to pupa. *Genista monspessulana* is closely related to gorse and has physiologically similar seed pods covered in thick hairs providing cover and protection for young burrowing larvae, and this may explain the oviposition and minor feeding damage on this plant species in this series of host tests. There is a low risk that *G. monspessulana* may receive minor damage in the field in Tasmania.

Our observations, from mass-rearing populations of *C. succedana*, were that adult female moths preferred to lay eggs on the calyx of fertilised gorse flowers. In the oviposition preference tests reported here a number of eggs were laid on the leaves of non-target plant species (Tables 3 & 4) suggesting an indiscriminate 'dumping' of eggs by females. On the gorse controls, however, most eggs (269 'choice with target' and 244 'choice without target') were laid on flowers, while only 49 ('choice with target') and 40 ('choice without target') eggs were laid on gorse spines.

We note from the results in Hill (1990) that larvae, left to hatch from eggs laid on pods of non-target species in 'choice without target' tests, wandered off the pods and died without feeding. Larvae were observed wandering off seed pods in this series of tests also. This suggests that although adults can oviposit on non-target plants, it is highly unlikely that in natural conditions larvae will emerge and feed on the seed pods of any species other than gorse.

The gorse pod moth has become common in some areas of New Zealand, especially Canterbury, since its release in 1990. Field surveys have been carried out in the South Island, New Zealand, to determine whether the host range of *C. succedana,* predicted by host tests conducted in the UK and New Zealand prior to its introduction, were accurate. Adult moths have been found resting on non-target species such as *Rosa* sp., *Sophora* spp., *Acacia* spp., and *Pinus* spp., and have been caught in light traps set up in areas where no gorse was present within a 4-km radius (S.V. Fowler, R.L. Hill, E.G. White, pers. comm.). Field studies carried out to determine the host range of the gorse pod moth at three sites in the South Island, New Zealand, using light traps, pheromone traps, and by dissecting seed pods, have produced no evidence to suggest *C. succedana* attacks or causes any non-target impacts to any species other than *Ulex europaeus*. Seed pods of *G. monspessulana* were not collected or checked.

These results strongly suggest that *C. succedana* is highly specific to *Ulex* spp. in the field, but that there is a low risk of minor damage to *G. monspessulana*, a close relative of *Ulex*. The other plants tested are not at risk from the proposed introduction of *C. succedana* into Australia.

Table 3 - Results of laboratory oviposition preference choice 'without target' tests for *Cydia succedana*

Plant classification	Species	Mean		Test *n* = 5		NZ gorse control *n* = 5		Mean eggs laid		Total eggs
		Pods	Flowers	Mean eggs	± se	Mean eggs	± se	Flowers	Leaves	
Genisteae	***Ulex europaeus* (NZ) (*n*=25) gorse controls**	10	16	10.0	1.3			9.5	1.1	284
	Ulex europaeus (Tasmania)	11	6	6.2	1.7	9.6	3.0	6.0	0.2	31
	Chamaecytisus palmensis	4	25	0	0	12.2	2.0	0	0	0
	Genista monspessulana	32	13	0.4	0.4	9.6	3.0	0.4	0	2
	Lupinus angustifolius 'Gungurru'	5	11	2.4	1.2	12.2	2.0	0	2.4	12
	Lupinus angustifolius 'Merrit'	4	16	0	0	7.4	3.0	0	0	0
	Lupinus angustifolius 'Yandee'	7	9	4.2	2.5	12.2	2.0	0	4.2	21
Phaseoleae	*Centrosema pubescens*	5	1	0	0	9.0	1.8	0	0	0
	Hardenbergia violacea	3	4	0	0	8.0	3.5	0	0	0
	Lablab purpureus	2	3	0	0	9.6	3.0	0	0	0
	Macroptilium atropurpureum	4	4	0	0	7.4	3.0	0	0	0
	Phaseolus vulgaris 'Broker'	4	0.8	0	0	9.6	3.0	0	0	0
	Phaseolus vulgaris 'Flo'	3	2	0	0	8.0	3.5	0	0	0
	Phaseolus vulgaris 'Labrador'	3	1.4	0	0	6.6	1.3	0	0	0
	Phaseolus vulgaris 'Rapier'	3	1.2	0	0	6.6	1.3	0	0	0
	Vigna radiata	0.6	3	0	0	6.6	1.3	0	0	0
Trifolieae	*Trifolium subterraneum*	10	3	0	0	9.6	3.0	0	0	0

Table 3 (continued) - Results of laboratory oviposition preference choice 'without target' tests for *Cydia succedana*

Plant classification	Species	Mean		Test *n* = 5		NZ gorse control *n* = 5		Mean eggs laid		Total eggs
		Pods	Flowers	Mean eggs	± se	Mean eggs	± se	Flowers	Leaves	
Galegeae	*Swainsona laxa*	4	9	0.4	0.4	7.4	3.0	0	0.4	2
Loteae	*Lotus australis*	2	5	0.4	0.4	8.0	3.5	0	0.4	2
Acacieae	*Acacia dealbata*	0.6	8	0	0	6.6	1.3	0	0	0
	Acacia mearnsii	8	5	0	0	6.6	1.3	0	0	0
Mirbelieae	*Aotus ericoides*	5	15	0.2	0.2	7.4	3.0	0	0.2	1
	Daviesia latifolia	2	28	0	0	7.4	3.0	0	0	0
	Dillwynia glaberrima	1	11	0.2	0.2	12.2	2.0	0.2	0	1
	Eutaxia microphylla	8	16	0	0	8.0	3.5	0	0	0
	Gompholobium huegelii	6	4	0	0	14.6	4.0	0	0	0
	Kennedia prostrata	0.6	6	0	0	6.6	1.3	0	0	0
	Oxylobium ellipticum	0	24	0.6	0.6	12.2	2.0	0	0.6	3
	Pultenaea juniperina	0.4	16	0.4	0.4	8.0	3.5	0	0.4	2
Indigofereae	*Indigofera australis*	18	18	0	0	14.6	4.0	0	0	0
Bossiaeeae	*Bossiaea riparia*	0	9	0.2	0.2	8.0	3.5	0	0.2	1
	Goodia lotifolia	3	16	0.6	0.3	14.6	4.0	0.6	0	3
	Hovea corrickiae	0.4	14	1	1	14.6	4.0	0	1	5
	Platylobium formosum	1	31	2	2	8.0	3.5	0.8	1.2	10
Crotalarieae	*Crotalaria cunninghamii*	3	8	1.4	0.7	14.6	4.0	1.4	0	7

Table 3 (continued) - Results of laboratory oviposition preference choice 'without target' tests for *Cydia succedana*

Plant classification	Species	Mean		Test *n* = 5		NZ gorse control *n* = 5		Mean eggs laid		Total eggs
		Pods	Flowers	Mean eggs	± se	Mean eggs	± se	Flowers	Leaves	
Psoraleeae	*Psoralea pinnata*	1	28	0	0	14.6	4.0	0	0	0

Table 4 - Results of laboratory oviposition preference choice 'with target' tests for *Cydia succedana*

Plant classification	Species	Mean		Test *n* = 5		NZ gorse control *n* = 5		Mean eggs laid		Total eggs
		Pods	Flowers	Mean eggs	± se	Mean eggs	± se	Flowers	Leaves	
Genisteae	***Ulex europaeus* (NZ) (*n*=35) gorse controls**	10	11	8.1	1.5			7.6	1.4	318
	Ulex europaeus (Tasmania)	16	8	7.4	2.2	7.2	1.8	4.8	2.6	37
	Chamaecytisus palmensis	6	30	0	0	7.2	1.8	0	0	0
	Genista monspessulana	30	14	0	0	3.4	0.9	0	0	0
	Lupinus angustifolius 'Gungurru'	3	6	0	0	7.2	1.8	0	0	0
	Lupinus angustifolius 'Merrit'	7	14	0	0	7.2	1.8	0	0	0
	Lupinus angustifolius 'Yandee'	2	8	0	0	7.2	1.8	0	0	0
Phaseoleae	*Centrosema pubescens*	4.4	3	0	0	10.2	1.4	0	0	0
	Hardenbergia violacea	2	3	0	0	7.2	1.8	0	0	0
	Lablab purpureus	5	0.8	0	0	17.4	4.0	0	0	0
	Macroptilium atropurpureum	3	5	0.2	0.2	17.4	4.0	0.2	0	1
	Phaseolus vulgaris 'Broker'	4	1	0	0	3.4	0.9	0	0	0
	Phaseolus vulgaris 'Flo'	4	0.8	0	0	7.2	4.7	0	0	0
	Phaseolus vulgaris 'Labrador'	4	1	0	0	3.4	0.9	0	0	0
	Phaseolus vulgaris 'Rapier'	4	2	0	0	7.2	4.7	0	0	0
	Vigna radiata	2	2	0	0	7.2	4.7	0	0	0

Table 4 (continued) - Results of laboratory oviposition preference choice 'with target' tests for *Cydia succedana*

Plant classification	Species	Mean		Test *n* = 5		NZ gorse control *n* = 5		Mean eggs laid		Total eggs
		Pods	Flowers	Mean eggs	± se	Mean eggs	± se	Flowers	Leaves	
Trifolieae	*Trifolium subterraneum*	13	9	0	0	3.4	0.9	0	0	0
Galegeae	*Swainsona laxa*	12	11	0.2	0.2	17.4	4.0	0.2	0	1
Loteae	*Lotus australis*	2	6	0	0	7.2	4.7	0	0	0
Acacieae	*Acacia dealbata*	2	8	0	0	3.4	0.9	0	0	0
	Acacia mearnsii	0.6	6	0	0	3.4	0.9	0	0	0
Mirbelieae	*Aotus ericoides*	0.2	17	0.6	0.6	17.4	4.0	0	0.6	3
	Daviesia latifolia	0.8	25	0	0	4.0	1.7	0	0	0
	Dillwynia glaberrima	0	8	0.2	0.2	7.8	2.7	0	0.2	1
	Eutaxia microphylla	5	12	0.2	0.2	7.8	2.7	0	0.2	1
	Gompholobium huegelii	9	0	0	0	7.8	2.7	0	0	0
	Kennedia prostrata	2	5	0	0	17.4	4.0	0	0	0
	Oxylobium ellipticum	7	13	0.2	0.2	7.8	2.7	0.2	0	1
	Pultenaea juniperina	0.2	13	0.2	0.2	4.0	1.7	0.2	0	1
Indigofereae	*Indigofera australis*	24	18	0	0	4.0	1.7	0	0	0
Bossiaeeae	*Bossiaea riparia*	0.4	9	0.4	0.4	17.4	4.0	0	0.4	2
	Goodia lotifolia	9	21	0	0	4.0	1.7	0	0	0
	Hovea corrickiae	0.6	14	0	0	4.0	1.7	0	0	0
	Platylobium formosum	3	14	0.4	0.4	7.8	2.7	0	0.4	2
Crotalarieae	*Crotalaria cunninghamii*	1	7	0	0	7.2	4.7	0	0	0

Table 4 (continued) - Results of laboratory oviposition preference choice 'with target' tests for *Cydia succedana*

Plant classification	Species	Mean		Test *n* = 5		NZ gorse control *n* = 5		Mean eggs laid		Total eggs
		Pods	Flowers	Mean eggs	± se	Mean eggs	± se	Flowers	Leaves	
Psoraleeae	*Psoralea pinnata*	0	27	0	0	4.0	1.7	0	0	0

Table 5 - Results of first-instar larval starvation tests for *Cydia succedana*

Plant classification	Species	*n*	Percentage mortality at day							
			5	**10**	**15**	**20**	**25**	**30**	**35 pupa**	**40 adult**
Genisteae	***Ulex europaeus* (NZ controls)**	**100**	**15**	**40**	**45**	**45**	**50**	**55**	**60**	**60**
	Ulex europaeus (Tasmania)	25	1	25	35	35	45	50	60	80
	Chamaecytisus palmensis	25	70	100						
	Genista monspessulana	25	50	55	55	60	85	100		
	Lupinus angustifolius 'Gungurru'	25	80	100						
	Lupinus angustifolius 'Merrit'	25	70	100						
	Lupinus angustifolius 'Yandee'	25	90	100						
Phaseoleae	*Centrosema pubescens*	25	100							
	Hardenbergia violacea	25	100							
	Lablab purpureus	25	90	100						
	Macroptilium atropurpureum	25	100							
	Phaseolus vulgaris 'Broker'	25	95	100						
	Phaseolus vulgaris 'Flo'	25	95	100						
	Phaseolus vulgaris 'Labrador'	25	80	100						
	Phaseolus vulgaris 'Rapier'	25	95	100						
	Vigna radiata	25	100							
Trifolieae	*Trifolium subterraneum*	25	80	95	100					
Galegeae	*Swainsona laxa*	25	80	85	100					

Table 5 (continued) - Results of first-instar larval starvation tests for *Cydia succedana*

Plant classification	Species	*n*	Percentage mortality at day							
			5	10	15	20	25	30	35 pupa	40 adult
Loteae	*Lotus australis*	25	80	95	100					
Acacieae	*Acacia dealbata*	25	100							
	Acacia mearnsii	25	100							
Mirbelieae	*Aotus ericoides*	25	90	100						
	Daviesia latifolia	25	90	95	100					
	Dillwynia glaberrima	25	100							
	Eutaxia microphylla	25	100							
	Gompholobium huegelii	25	100							
	Kennedia prostrata	25	100							
	Oxylobium ellipticum	25	100							
	Pultenaea juniperina	25	100							
Indigofereae	*Indigofera australis*	25	100							
Bossiaeeae	*Bossiaea riparia*	25	80	85	100					
	Goodia lotifolia	25	90	90	95	100				
	Hovea corrickiae	25	80	85	100					
	Platylobium formosum	25	100							
Crotalarieae	*Crotalaria cunninghamii*	25	100							
Psoraleeae	*Psoralea pinnata*	25	100							

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APPENDIX 4

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Why did specificity testing fail to predict the field host-range of the gorse pod moth in New Zealand?

Q. Paynter [a,*], A.H. Gourlay [b], P.T. Oboyski [c], S.V. Fowler [b], R.L. Hill [d], T.M. Withers [e], H. Parish [b], S. Hona [a]

[a] *Landcare Research, Private Bag 92170, Auckland, New Zealand*
[b] *Landcare Research, P.O. Box 40, Lincoln, New Zealand*
[c] *University of California, Berkeley, CA 94720-3114, USA*
[d] *Richard Hill & Associates, Private Bag 4704, Christchurch, New Zealand*
[e] *Scion, Private Bag 3020, Rotorua, New Zealand*

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Ulex

ABSTRACT

Contrary to predictions based on host-range testing, the gorse pod moth (GPM) infests pods of several exotic Genisteae and Loteae species, as well as the target weed gorse *Ulex europaeus*, throughout New Zealand. The original host-range tests were conducted on moths collected in southern England; however, the offspring of Portuguese moths were also released in New Zealand. We investigated whether failure to predict non-target attack was because (a) a cryptic species was accidentally introduced; (b) asynchrony between the oviposition period of GPM and gorse flowering results in deprivation, causing less preferred plants to become more acceptable for oviposition and (c) the Portuguese GPM population has a different host-range to the tested English population. Dissections of genitalia and molecular data collected on COI mtDNA indicated that a cryptic species was not introduced. Specificity tests on moths sourced from England concurred with the original tests and indicated that GPM should be unlikely to exploit the non-target species that are attacked in New Zealand. In contrast, GPM sourced from Portugal were able to exploit a broader range of plants, although choice oviposition tests indicated that gorse is, nevertheless, the preferred host of this population. Adult GPM activity was often poorly synchronized with gorse flowering in New Zealand and non-target attack was most prevalent when gorse flowers and pods were absent. We conclude that the release of untested moths sourced from Portugal, coupled with asynchrony between the flight period of GPM and gorse flowering explains the unanticipated non-target attack in New Zealand.

1. Introduction

Sheppard et al. (2006) noted that evaluating the risk of non-target use by potential weed biological control agents is a relatively straightforward procedure because the majority of agents tested are either quickly rejected, or clearly demonstrate sufficient specificity in even the most conservative (no-choice starvation) specificity tests. However, one scenario where problems arise is when the field host-range of an agent is larger than that demonstrated by specificity testing. This is because the usual expectation is that the realized host-range expressed in the field will be smaller, not greater than the fundamental host-range demonstrated in lab tests.

The gorse pod moth (GPM), a tortricid moth that was introduced into New Zealand in 1992 as *Cydia succedana* (Denis and Schiffermüller) (Hill and Gourlay, 2002) is an example of this scenario. As well as gorse *Ulex* spp., there are literature records of GPM feeding on several other members of the Genisteae and on species of *Lotus* (Loteae) in the moth's native range (reviewed in Hill and Gourlay, 2002). However, host-range testing indicated that GPM collected at Chobham Common and Yateley Common UK was highly host-specific (Hill and Gourlay, 2002). Unlike on *U. europaeus* controls, where 56–86% of larvae survived for 10 days and approximately 40% survived to pupation, few larvae survived beyond a few days and none survived to pupation in no-choice tests, when presented with pods of Scotch broom *Cytisus scoparius* L. (Link), *Genista hispanica* L., *Genista* (=*Teline*) *monspessulana* (L.) L.A.S. Johnson[1] and *Lotus pedunculatus* Cav. Furthermore, in no-choice oviposition tests, few eggs were laid on representatives of these genera, while *U. europaeus* plants were heavily oviposited upon (for example, a mean of 0.9 eggs were laid per *L. pedunculatus* shoot versus a mean of 40.4 eggs per gorse shoot). On the basis of

* Corresponding author. Fax: +64 09 574 4101.
E-mail address: PaynterQ@LandcareResearch.co.nz (Q. Paynter).

[1] Legume nomenclature in the current manuscript follows the ILDIS World Database of Legumes http://www.ildis.org/.

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these host-range test results, records of GPM from other hosts were considered to be erroneous or only occasional. Erroneous host plant records occur through misidentification of insect or host plant or errors during transcription of records or due to records of adult feeding on fruit or flowers being confused with larval host records (Robinson et al., 2008). They are often cumulative, where repeated citation gives them a spurious authority, and they are extremely difficult to detect (Robinson et al., 2008). However, despite these test results, subsequent field surveys, performed after the release of GPM in New Zealand (Withers et al., 2008), indicated that the field host-range mirrored host-range records from the native range and included several other species of exotic Genisteae (*C. scoparius*, *G. monspessulana*, and *Lupinus arboreus* Sims), as well as *L. pedunculatus* (Loteae). Subsequent surveys (Gourlay, unpublished data) have shown that GPM also occasionally infests *Spartium junceum* L., *Genista lydia* Boiss., *Lu. polyphyllus* Lindl., *Cytisus proliferus* L.f. (Genisteae) and *Lotus corniculatus* L. (Loteae) in New Zealand.

No adverse environmental or economic impacts of GPM non-target herbivory have been reported in New Zealand. Indeed, none of the non-target host plants utilized by GPM in New Zealand is native to New Zealand and most are invasive weeds listed by Roy et al. (1998), so that GPM non-target attack in New Zealand could be considered as beneficial 'collateral damage'. Nevertheless, non-target attack has been dubbed the "Achilles' heel of biological control" (Louda et al., 2003) and we considered it essential to determine why the host-range of GPM was broader than predicted to improve the reliability and safety of host-range testing of future biological control agents.

Although the original host-range tests were conducted on moths collected at Yateley Common, or nearby Chobham Common, England, the population that was released into New Zealand also contained the progeny of moths collected at Viana do Castello, Portugal. Danilevsky and Kuznetzov (1968) recognized *C. succedana* (Denis and Schiffermüller) and *C. ulicetana* (Haworth) as separate species. However, many authorities (e.g. Bradley et al., 1979; Emmet, 1988) considered *C. ulicetana* to be an inferior synonym of *C. succedana* at the time that GPM was cleared for release in New Zealand. GPM was, therefore, introduced into New Zealand under the name *C. succedana* (Denis and Schiffermüller), following formal identification, prior to release, in accordance with contemporary regulatory procedure. Since then, however, Razowski (2003) reinstated the separation between *C. succedana* and *C. ulicetana*. There is, consequently, uncertainty regarding the distributions and host-ranges of both *C. succedana* and *C. ulicetana* because many literature records do not distinguish between the two species (Danilevsky and Kuznetzov, 1968; Brown et al., 2005). According to this separation, only *C. ulicetana* occurs in the United Kingdom (D. Agassiz, personal communication). It is conceivable that both may occur in Portugal, although only *C. ulicetana* is currently confirmed to be present there, but several similar closely related *Cydia* species are present (J. Baixeras, personal communication), raising the possibility that a cryptic species may have been accidentally introduced along with GPM as a culture contaminant (e.g. Balciunas and Villegas, 2001). Indeed, differences between tortricid species can be extremely cryptic; for example, Foster et al. (1987) revealed that two species of morphologically indistinguishable tortricids could be distinguished by their use of different sex pheromones.

We describe experiments and field surveys designed to test three hypotheses regarding why the original host-range testing failed to predict the host-range of GPM in New Zealand: (1) that, as well as GPM, a cryptic species was accidentally introduced; (2) that asynchrony between the oviposition period of the biocontrol agent and the flowering phenology of the target plant results in deprivation that might cause less preferred plants to become more acceptable for oviposition; and (3) that the population collected at Viana do Castello, Portugal has different host preferences and performed differently on different hosts, compared to the tested population from England.

2. Materials and methods

2.1. Examination of genitalia

A sub-sample of 57 adult moths from 14 localities throughout both main islands of New Zealand that had been reared from *U. europaeus* (37 moths) and five non-target plant species: *C. scoparius* (two moths), *G. monspessulana* (two moths), *Lotus spp.* (nine moths), *Lu. Arboreus* (six moths) was prepared for examination of dissected genitalia as follows: 10% KOH was transferred into a small tube, using a pipette. For each specimen, the abdomen was removed and placed in the tube and immersed in the 10% KOH overnight. Next, the abdomen was transferred to an excavated glass block filled with 70% alcohol and, using a binocular microscope, the abdomen contents of male moths were dissected and compared to the figures of *C. succedana* and *C. ulicetana* in Danilevsky and Kuznetzov (1968).

2.2. Molecular analysis

A sub-sample of four individuals each from six different New Zealand host plants (*C. scoparius*, *G. lydia* Boiss., *G. monspessulana*, *Lo. corniculatus*, *Lu. arboreus*, *and U. europaeus*) was analyzed for mitochondrial DNA sequence variation. Three individuals each from *U. europaeus* from both original collection sites in England and Portugal (see Section 2.4.1 below) were also sampled for sequence comparison. Total genomic DNA was extracted from two legs (adult moths) or a section of abdominal muscle (larvae) using a DNEasy tissue kit (Qiagen Corporation®) following the manufacturer's protocol for animal tissue. The mitochondrial gene region cytochrome oxidase subunit I (COI) was amplified using the polymerase chain reaction (PCR). A 658 basepair (bp) region was obtained for COI using the primer pair LCO1490 (5′-GGTCAAC AAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTG ACCAAAAAATCA-3′) (Folmer et al., 1994). Each 20 μL PCR reaction contained 3 μL 10× sequencing buffer (500 mM KCL, 100 mM Tris–HCL at pH 8.3, 15 mM), 2.5 mM magnesium chloride, 0.4 mM dNTPs, 0.5 μM of each primer, 0.05 U AmpliTaq Gold® polymerase, and 2 μL of DNA extract.

The touchdown PCR thermal profile consisted of 10 min at 95 °C; 15 cycles of 30 s at 95 °C, 45 s at *X* °C (where annealing temperature *X* varied from 60 to 45 °C decrementing 1 °C after each cycle), and 90 s at 72 °C; 25 cycles of 30 s at 95 °C, 45 s at 50 °C and 90 s at 72 °C; and an extension cycle of 10 min at 72 °C. PCR products were purified using ExoSAP-IT® (USB Corporation, Cleveland, Ohio) following manufacturer's specifications. Cycle sequencing of purified PCR products was done in both directions for each specimen using BigDye v3.1 sequencing kit (ABI) following the manufacturer's protocols and subsequently cleaned by EtOH/EDTA precipitation (CITE). Sequencing was performed on an ABI 3730 automated sequencer (Applied BioSystems). Sequence editing and alignment using Sequencher 4.0 (GeneCodes Corporation) was trivial since no gaps were present for the gene sequenced. The resulting sequences have been submitted to GenBank with Accession Nos. EU684241–EU684256.

2.3. Field surveys to investigate synchrony between GPM and its host plants

We located four field sites; two in the South Island and two in the North Island (Table 1) where both *U. europaeus* infested with GPM and potential non-target Fabaceae; either one, or a combina-

Table 1
Details of sites for field surveys of non-target Fabaceae and infested *U. europaeus*

Site	Description	Lat long
Ashley Forest	Mt. Grey Forest area, Canterbury	43°14′37″S, 172°34′47″E
Lincoln	Landcare Research Campus, near Christchurch	43°37′59″S, 172°28′58″E
Lake Rotoiti	Okawa Bay, edge of Tikitere Forest	38°03′21″S, 176°19′54″E
Kaharoa	Roadside Te Matai Rd., Kaharoa, Tauranga	37°57′27″S, 176°09′ 42″E

tion of the following species: *C. scoparius*, *Lu. arboreus*, *G. monspessulana*, and *Lo. pedunculatus* were present. We assessed the phenology of GPM activity (of both adults and larvae) and the plant species attacked by GPM as follows:

We visited the four field sites at approximately monthly intervals from October 2003 to March 2006. Two or three delta traps with sticky bases baited with (E,E)-8,10-dodecadien-1-yl acetate (Suckling et al., 1999) were set up to sample adult male GPM at each site. The sticky bases were replaced at each sample date and the number of moths trapped was counted and divided by the number of traps present and the number of days the traps had been in the field to give an abundance index of moths per trap per day.

At each sampling date we noted whether plants were flowering (early, full bloom or late) and whether pods were present (young and green i.e. suitable for GPM or old and brown i.e. unsuitable for GPM). Samples of mature pods (i.e. fully expanded) were collected, when present, from *U. europaeus* and the non-target plants present at each site as follows: For each plant species, 20 pods per plant were collected from five separate plants (selected arbitrarily), so that 100 pods were collected from each legume species. These pods were placed into sealable plastic bags and returned to the laboratory. A separate bag was used for each plant species, to prevent larvae from transferring between pods of different plant species. At the laboratory, the pods were placed onto dampened tissue paper in rectangular plastic rearing containers (one container for each plant species at each site) and left at ambient temperature, away from direct sunlight until adult moths emerged. After three months, when emergences had ceased we emptied the boxes and counted any emerged moths that were not detected earlier, giving a total number of moths reared per 100 pods in each sample.

To enable moths to be identified, emerged moths were etherized and stored in labeled specimen tubes. Approximately half were stored in 100% alcohol, immediately after etherizing them, for use in subsequent dissections or molecular work and the remaining moths were allowed to dry out so that the external features of moths that attacked *U. europaeus* and non-target plants could be compared.

2.4. Host-range testing of original source populations in the laboratory

To investigate whether the provenance of the moths released into New Zealand might explain the unanticipated non-target attack, we returned to the original collection sites and collected shipments of GPM and imported them into quarantine in New Zealand to repeat the original specificity tests.

Lotus corniculatus normally flowers after *U. europaeus* in New Zealand so, to synchronize flowering for host-range testing, we grew potted *Lo. corniculatus* plants inside a greenhouse, so that they flowered earlier than they would outdoors. This provided sufficient shoots with flowers and pods for the oviposition tests. However, for the larval starvation tests we had insufficient pods to rear larvae through to pupation. Therefore we conducted first-instar starvation tests to determine how many larvae could survive beyond the first instar.

2.4.1. Collection of source populations

Moths were collected from three populations during May 2006: Approximately 80 adult moths were collected at the original collection site at Yateley Common, England, (51°19′N, 0°45′W). Several hundred *Ulex europaeus* pods infested with GPM larvae were collected in the vicinity of the original Portuguese collection site near Viana do Castello, Portugal (41°45′N, 8°51′W). In addition, approximately 50 adult moths were collected from *U. europaeus* growing near to Santiago de Compostela, Spain (42°52′N, 8°32′W). Moths were collected from a 1 ha area at each site. Although Spanish moths were not introduced into New Zealand we included them in host-range testing because, as at Yateley Common, GPM at this inland site was associated with *U. europaeus* L. subsp. *europaeus*. In Portugal, GPM was associated with *U. europaeus* subsp. *latebracteatus* (Mariz) Rothm., which occurs mainly near the coast of northwest Spain and north and central Portugal (Guinea and Webb, 1968). These subspecies of *U. europaeus*, which are not monophyletic (Ainouche et al., 2003), are differentiated by the shape and size of their bracteoles, and they exhibit different chromosome numbers. Populations of subsp. *latebracteatus* have larger bracteoles and are tetraploid ($2n = 4x = 64$), and subsp. *europaeus* have small bracteoles and are hexaploid ($2n = 6x = 96$) (Cubas and Pardo, 1997). Therefore, we hypothesized that moths which feed on *U. europaeus* subsp. *europaeus* may differ in their specificity from moths that feed on *U. europaeus* subsp. *latebracteatus*.

Moths were shipped in a sealed ice box to a secure insect containment facility at Lincoln, New Zealand. Between 80% and 90% of adult moths survived shipment and 51 moths emerged from the pods collected in Portugal. In quarantine, each population was reared separately, according to the protocol developed by Hill and Gourlay (2002) to provide offspring for subsequent host-range testing.

2.4.2. Oviposition preference tests in the laboratory

The ability of female GPM to lay eggs on *U. europaeus* subsp. *europaeus*, *Lo. corniculatus* L. and *C. scoparius* was measured during September 2006, using the F1 offspring of the moths collected in Europe during May 2006, in a secure insect containment facility at Lincoln, New Zealand. No-choice tests were performed because concurrent survey work (see Section 2.3) had indicated that moths were often active when *U. europaeus* flowers and pods were absent, so that female GPM moths often experience "no-choice" situations in the field. Choice tests were also performed for *U. europaeus* and *Lo. Corniculatus* (insufficient moths were available to include *C. scoparius* in choice tests). For each replicate, three moths (one male and two females) were placed into a 30 × 30 cm closed, cylindrical, clear plastic arena, with a dental roll soaked in a dilute solution of honey in water for moths to feed on. For no-choice tests, a fresh shoot that was *c.* 10 cm in length and bore both mature flowers and green pods of either *U. europaeus* or the test plant species was arranged in a 10 × 2.5 cm glass vial of water sealed with Parafilm®. For the choice tests, two shoots were presented to moths; one of *U. europaeus* and one of *Lo. corniculatus*. The containers were arranged randomly on a bench at 18 °C and 16 L: 8D photoperiod for 4 days, after which the number of eggs laid on each shoot was counted. Five replicates were performed for each test plant and each population of GPM.

2.4.3. First-instar larval starvation tests

The ability of larvae to feed and develop on pods of *U. europaeus* subsp. *europaeus*, *Lo. corniculatus*, *C. scoparius* and *G. monspessulana* was measured during October and November 2006 using first-instar starvation tests. As in Hill and Gourlay (2002), two to five young pods of each test plant were picked and placed on damp filter paper in a Petri dish (one species per dish). Young *U. europa-*

eus pods were set up in the same way as controls. Five newly-hatched and unfed larvae were placed on the pods in each Petri dish and the number of larvae surviving beyond first instar was determined after 7 days by dissection of the pods. Fifteen replicates were performed for each test plant and each population of GPM.

2.5. Analysis

2.5.1. Oviposition tests

For no-choice tests, analyses of variance were performed to determine if the number of eggs laid (the numbers of eggs recorded were log (n+1) transformed prior to analysis) varied according to test plant species (a factor with two levels "gorse" and "lotus" for the first analysis and "gorse" and "Scotch broom" for the second analysis) and source population of moths (a factor with three levels; "Spain", "Portugal" and "England"). A similar analysis was performed for the choice test, but with replicate declared as a blocking term because shoots of *U. europaeus* and *Lo. corniculatus* presented simultaneously to moths in the same container were not independent samples. Two replicates where no eggs were laid on either *U. europaeus* or on *Lo. corniculatus* were treated as missing values in the analysis.

2.5.2. Larval starvation tests

Separate analyses of variance were performed for comparisons between *U. europaeus* and each of the three test plants to determine if the number of larvae surviving beyond first instar in the starvation test varied according to test plant species (*U. europaeus*, *Lo. corniculatus*, *C. scoparius*, *G. monspessulana*) and source population of moths (Spain, Portugal and England). Note, due to a shortage of larvae, *U. europaeus* was tested only once, and the same *U. europaeus* dataset was used for each comparison.

3. Results

3.1. Taxonomy

3.1.1. Examination of moth genitalia

Twenty-seven of the 57 moths reared were male. Of these, most (17) were reared from *U. europaeus*, with two each reared from *C. scoparius*, *G. monspessulana*, and *Lu. arboreus* and four from *Lotus* spp. The genitalia of all 27 males resembled *C. ulicetana*, based on comparison of the ventral angle of the cucullus--see plates 368a (*C. succedana*) and 372a (*C. ulicetana*) (Danilevsky and Kuznetzov, 1968)--regardless of the host plant or locality from which they were collected.

3.1.2. Molecular analyses

Mitochondrial DNA sequences of 30 GPM specimens had only 5 polymorphic sites for 658 base pairs (bp) of the COI gene region. Three of these five polymorphic sites were synonymous substitutions, two of which occurred in a single individual from Portugal. The third synonymous substitution occurred in three individuals--one each from *G. lydia*, *G. monspessulana*, and *C. scoparius* host plants from New Zealand. Of the two non-synonymous substitutions, one occurred in a single individual from *G. monspessulana* (different from the sample above), while the other occurred in a single individual from *U. europaeus*, both in New Zealand. All other 24 individuals from New Zealand, England, and Portugal were identical for this gene region.

3.2. Field surveys to investigate synchrony between GPM and gorse flowering and pod formation

The numbers of adult moths captured in pheromone traps was consistently low or zero in mid winter (June–August), rising in spring and peaking between November to January, with a smaller peak, corresponding to the second generation, between February and May (Figs. 1–3). At both South Island sites (Ashley Forest and Lincoln), *U. europaeus* began flowering in late summer and autumn (February–April) and bloomed through winter until spring (October). Consequently, *U. europaeus* plants had no pods or flowers present during summer months (from late November to early February), when moth numbers were at their peak (Figs. 1a and 2a). Following *U. europaeus*, a progression of other Fabaceae came into bloom: *G. monspessulana*, followed by *C. scoparius*, *Lu. arboreus* and finally *Lotus* spp. There was some overlap between the flowering periods of these species and *U. europaeus*, but virtually all non-target attack was recorded when *U. europaeus* was not in bloom. Furthermore, the degree to which non-target species were infested was generally somewhat lower than *U. europaeus*. For example, peaks of 55 and 29 moths were reared from 100 *U. europaeus* pods at Ashley forest in 2004 and 2005, respectively, compared to a peak of six moths reared from 100 *Lu. arboreus* pods in 2004 and nine moths from 100 *C. scoparius* pods in 2005 (Fig. 1a and b). Similarly, at Lincoln a peak of 30 moths was reared from 100 *U. europaeus* pods in April 2004, whereas the maximum recorded number of moths reared from a non-target plant was about half that (Fig. 2a and b; 14 moths reared from 100 *L. pedunculatus* pods in February 2006).

At the North Island sites (Lake Rotoiti and Kaharoa), *U. europaeus* began flowering in late winter (July) and finished flowering in midsummer (December; Fig. 3a and b). Moth numbers were much lower (peaking at *c.* 1.5 moths day^{-1}) than at the South Island sites where there were 4–6 moths day^{-1}. Like the South Island sites, most non-target attack occurred in late summer when *U. europaeus* was no longer flowering (Fig. 3a and b). In early 2004, similar numbers of moths were reared from *U. europaeus* and non-target plants. However, during the following two years the spring peak in moth numbers was better synchronized with *U. europaeus* flowering at both sites and levels of non-target attack were lower (Fig. 3a and b).

3.3. Host-range testing of original source populations in the laboratory

3.3.1. No-choice oviposition tests

For comparisons between *Lo. corniculatus* and *U. europaeus*, there was no significant difference in the number of eggs laid between "Country" treatments ($F_{2,24} = 0.43$, n.s.). There was a significant host plant effect ($F_{1,24} = 12.45$, $P < 0.01$) and a significant interaction between country and host plant ($F_{2,24} = 5.48$, $P < 0.05$). Moths from both England and Spain laid significantly more eggs on *U. europaeus* than on *Lo. corniculatus* whereas Portuguese moths laid similar numbers of eggs on both plant species (Fig. 4a).

For comparisons between *C. scoparius* and *U. europaeus*, again there was no significant difference in the number of eggs laid between "Country" treatments ($F_{2,24} = 0.17$, n.s.). Moths from Yateley Common laid three times more eggs on *U. europaeus* than they did on *C. scoparius*, whereas Portuguese moths laid more eggs on *C. scoparius* than on *U. europaeus* (Fig. 5). However, neither the host plant effect ($F_{1,24} = 0.04$, n.s.) nor the interaction between country and host plant was statistically significant ($F_{2,24} = 1.58$, n.s.).

3.3.2. Choice oviposition tests

Like the no-choice tests there was no significant difference in the number of eggs laid between "Country" treatments ($F_{2,25} = 1.95$, n.s.) and a significant host plant effect ($F_{1,25} = 23.17$, $P < 0.001$), indicating that significantly more eggs were laid on *U. europaeus* versus *Lo. corniculatus* (Fig. 4b). Unlike the no-choice tests, there was no significant interaction between country and host plant ($F_{2,25} = 1.08$, n.s.) indicating that in a choice situation

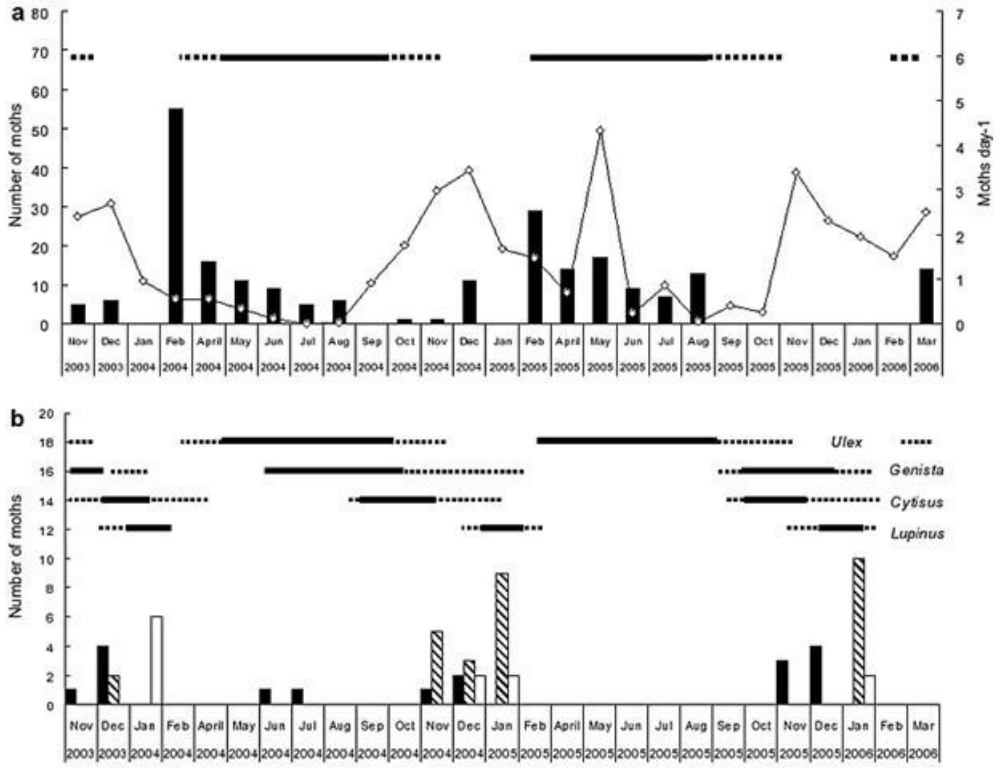

Fig. 1. (a) Monthly number of moths reared per 100 *U. europaeus* pods (vertical bars) and seasonal abundance of adult GPM moths in pheromone traps (open circles; moths trap^{-1} day^{-1}) in relation to the phenology of *U. europaeus* reproduction (horizontal bars; solid = flowers and pods present; dashed = flowers or pods only) at Ashley Forest; (b) Monthly attack rate (number of moths reared per 100 pods) on non-target plants (dark fill = *Genista monspessulana*; diagonal hatch = *Cytisus scoparius*; empty bars = *Lupinus arboreus*) and phenology of Gorse *U. europaeus* and non-target plants at Ashley Forest.

moths from all populations preferentially oviposited on *U. europaeus*.

3.3.3. Larval starvation tests

For comparisons between *Lo. corniculatus* and *U. europaeus*, there was a significant difference in the number of larvae surviving beyond seven days between both "country" ($F_{2,84} = 10.94$, $P < 0.001$) and "host plant" treatments ($F_{1,84} = 50.42$, $P < 0.001$) and there was a significant interaction between "country" and "host plant" treatments ($F_{2,84} = 29.02$, $P < 0.001$). Significantly fewer larvae from both England and Spain survived for 7 days when feeding on *Lo. corniculatus*, compared to *U. europaeus*. In contrast, larvae from the Portuguese population survived well on both host species (Fig. 6a).

Very similar results were obtained for comparisons between *G. monspessulana* and *U. europaeus* (Fig. 6b): there was a significant difference in the number of larvae surviving beyond seven days between both "country" ($F_{2,84} = 5.71$, $P < 0.01$) and "host plant" treatments ($F_{1,84} = 27.15$, $P < 0.001$) and there was a significant interaction between "country" and "host plant" treatments ($F_{2,84} = 20.74$, $P < 0.001$). Significantly fewer larvae from both England and Spain survived for 7 days when feeding on *G. monspessulana*, compared to *U. europaeus*. In contrast, larvae from the Portuguese population survived equally well on both host species.

In contrast, for comparisons between *C. scoparius* and *U. europaeus* (Fig. 6c), there was no significant effect of "country" ($F_{2,84} = 3.00$, n.s.) on the number of larvae surviving to second instar. However, "host plant" treatment ($F_{1,84} = 103.01$, $P < 0.001$) was highly significant. There was no significant interaction between "country" and "host plant" treatments ($F_{2,84} = 2.63$, n.s.), indicating that significantly fewer larvae from all source populations survived for 7 days when feeding on *C. scoparius*, compared to *U. europaeus*.

4. Discussion

Examination of male genitalia indicated that only one *Cydia* species was present in our New Zealand samples. However, according to Brown et al. (2005), *C. conjunctana* (Möschler), which is present in Portugal (J. Baixeras, personal communication) and also has the "long-sweep of cucullus" like *C. succedana* (see Danilevsky and Kuznetzov, 1968, pp. 514–515), has been synonymized with *C. ulicetana*. Therefore, the appearance of male genitalia may not be a reliable identification feature between *C. succedana* and *C. ulicetana*. Crucially, however, sequence analysis of the COI mtDNA gene region provided no evidence for cryptic species associated with non-target host plants. Although sequences varied at five sites within the 658 bp region sequenced, these variations were limited to six individuals (one variant per individual, except one individual with two sequence variations), each from different host plants and different locations. Otherwise, the remaining 25 specimens from New Zealand, England, and Portugal were identical for this gene region. Sequence variation in the COI gene of at least 1% is typical even for a complex of cryptic species (e.g. Hebert et al., 2004),

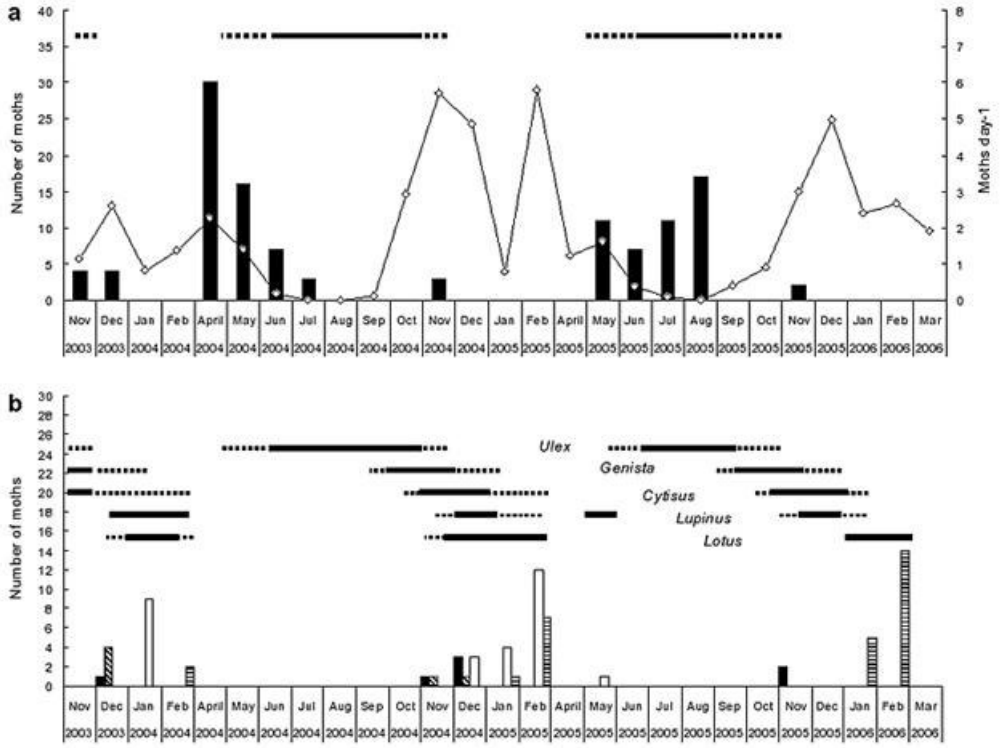

Fig. 2. (a) Monthly number of moths reared per 100 *Ulex europaeus* pods (vertical bars) and seasonal abundance of adult GPM moths in pheromone traps (open circles; moths $trap^{-1}$ day^{-1}) in relation to the phenology of *U. europaeus* reproduction (horizontal bars; solid = flowers and pods present; dashed = flowers or pods only) at Lincoln; (b) monthly attack rate (number of moths reared per 100 pods) on non-target plants (vertical bars: dark fill = *Genista monspessulana*; diagonal hatch = *Cytisus scoparius*; No fill = *Lupinus arboreus*; horizontal bars = *Lotus pedunculatus*) and phenology of gorse *U. europaeus* and non-target plants at Lincoln.

whereas the <0.5% sequence divergence among individuals in this study is concordant with natural variation within a single species (Moore, 1995).

COI is a relatively rapid-evolving gene commonly used to detect cryptic species of insects (e.g. Brunner et al., 2004; Hebert et al., 2004; Simmons and Scheffer, 2004; de Leon et al., 2006). Had a second cryptic species of *Cydia* that attacks non-target hosts been accidentally released it would likely have been evident by sequence variation within COI. However, recently diverged species or intraspecific "host races" (e.g. Dres and Mallet, 2002) may not necessarily exhibit considerable variation in the COI region (Meyer and Paulay, 2005). To uncover such recently evolved population structure, governed either by host plant preference or isolation by distance, it may be more appropriate to analyze data using multiple loci with a high degree of polymorphism, such as microsatellites or AFLPs (e.g. Voetdijk et al., 2007). Although microsatellite loci have been developed for *C. pomonella* (L.) (Franck et al., 2005; Zhou et al., 2005), attempts to amplify these loci in GPM specimens have thus far been unsuccessful. However, our host-range testing provided evidence that intraspecific host races of GPM may exist.

For moths sourced from Yateley Common UK, our no-choice oviposition tests produced similar results to the original host-range testing reported by Hill and Gourlay (2002) in that moths displayed a preference for *U. europaeus* over the other test plant species. Furthermore, first-instar larval survival of moths sourced from Yateley Common was significantly lower on *Lo. corniculatus*, *C. scoparius* and *G. monspessulana*, compared to *U. europaeus*. Therefore, our repeated test results do not contradict the findings of Hill and Gourlay (2002), who concluded that there was only a low probability that *Lotus*, *Cytisus* and *Genista* spp. should be suitable host plants for GPM in New Zealand.

In contrast, moths sourced from Portugal laid similar numbers of eggs on *U. europaeus*, *C. scoparius* and *Lo. corniculatus* during our no-choice oviposition testing and our larval starvation tests indicated that first-instar larvae sourced from the Portuguese population survived equally well or better on *Lo. corniculatus*, and *G. monspessulana* compared to *U. europaeus*. Overall, these contrasting results for the two source populations provide strong evidence that provenance may be an important factor explaining the unanticipated non-target attack in New Zealand because the Portuguese population of GPM appears capable of exploiting a broader range of plants than the originally tested Yateley Common population.

Nevertheless, when both *U. europaeus* and *Lo. corniculatus* flowers and pods were presented together in a choice test, the Portuguese moths laid significantly more eggs on *U. europaeus* than on *Lo. corniculatus*. Therefore, one might predict that if GPM was active only when *U. europaeus* was in bloom in New Zealand, then preferential oviposition on *U. europaeus* should reduce the incidence of non-target attack. Indeed, while there was some overlap between the flowering periods of non-target hosts and *U. europaeus*, virtually all non-target attack was recorded when *U. europaeus* was not in bloom (Figs. 1–3). Nevertheless, preference may not provide complete protection from non-target attack (for example, patches of non-target plants growing in isolation of *U. europaeus*, may be akin to a no-choice scenario). Nonetheless, the asynchrony

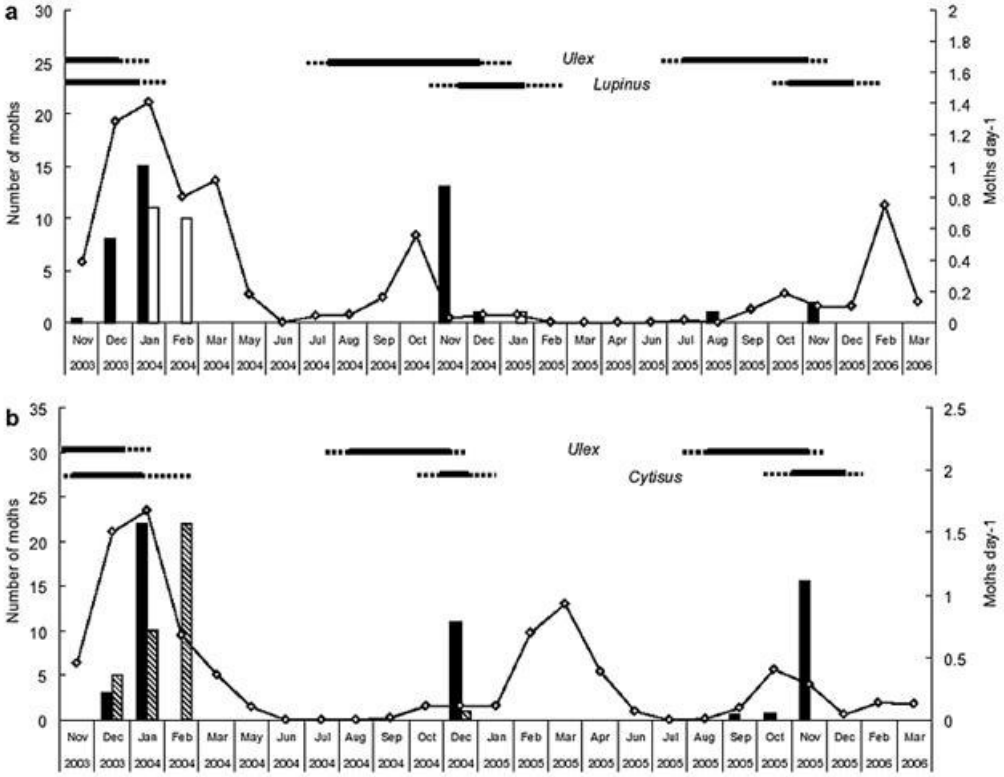

Fig. 3. (a) Monthly number of moths reared per 100 pods of *Ulex europaeus* (vertical bars, dark fill) and *Lupinus arboreus* (vertical bars, no fill) and seasonal abundance of adult GPM moths in pheromone traps (open circles; moths trap^{-1} day^{-1}) in relation to the phenology of *U. europaeus* reproduction (horizontal bars; solid = flowers and pods present; dashed = flowers or pods only) at Lake Rotoiti; (b) monthly attack rate (number of moths reared per 100 pods) of *U. europaeus* (vertical bars, dark fill) and *Cytisus scoparius* (vertical bars, diagonal hatch) and phenology of *U. europaeus* and *C. scoparius* at Kaharoa.

between the flight period of GPM and *U. europaeus* flowering appears to contribute to the incidence of non-target attack.

Tarayare et al. (2007) examined the flowering phenology of *U. europaeus* in France. As we have found in the current study, they reported that the flowering phenology of individual *U. europaeus* plants was highly variable, with some flowering from winter to spring and some flowering in spring only. Tarayare et al. (2007) postulated that the opposing selection pressures that promote coexistence of these two flowering types are (1) seed predation, which occurs in spring only and, therefore, selects for winter-flowering and (2) cold winter temperatures that may cause pods to freeze or abort, thereby selecting for spring flowering.

In New Zealand, the seed weevil *Exapion ulicis* (Forst.) was the first biological control agent to be released (in 1931) against *U. europaeus*, where it can destroy up to 90% of the spring seed production although seed produced during the rest of the year escapes predation (Hill and Gourlay, 2002). Therefore, there should have been strong selection pressure for winter-flowering in New Zealand for over 75 years. This does not, however, explain the asynchrony between the flight period of GPM and *U. europaeus* flowering: At both the North Island sites that were dominated by spring-flowering plants and the South Island sites, that were predominantly winter-flowering, asynchrony between the flight period of GPM and *U. europaeus* flowering occurred during summer.

Zwölfer (1963) noted that GPM is bivoltine in Europe, with the spring generation feeding on *U. europaeus* pods and a late summer and autumn generation feeding on the pods of the related gorse species *Ulex minor* Roth and *U. gallii* Planch. Barat et al. (2007) conducted a detailed study of the phenology of these *Ulex* species in Brittany, France and showed that *U. minor* and *U. gallii* flowered and began producing green pods during summer and autumn, before autumn/winter-flowering *U. europaeus* began to bloom. In New Zealand, *U. gallii* is absent and *U. minor* is rare and highly localized (Webb et al., 1988). If the second generation of GPM is adapted to be synchronized with these *Ulex* species, this would explain the poor synchrony with *U. europaeus* flowering and subsequent unanticipated non-target attack on other related plants. However, it should be noted that in Brittany, Barat et al. (2007) found that GPM attacked c. 25% of *U. europaeus* pods in spring, but did not observe GPM attacking *U. minor* or *U. gallii* pods in summer, indicating the GPM may have been univoltine at those sites. Nevertheless, based on Zwölfer's (1963) observations, the potential for asynchrony between the summer flight period of GPM and *U. europaeus* flowering was anticipated, before the release of GPM in New Zealand. It was assumed that, in the absence of acceptable alternative hosts, there would be a strong selection pressure for the moths emerging late in summer to build up exploitation of autumn flowers. However, the unanticipated alternative host use in summer is likely to have reduced that selection pressure and maintained the emergence patterns observed in Europe.

Despite the closer geographical proximity to Portugal, moths sourced from Spain displayed similar specificity test results to

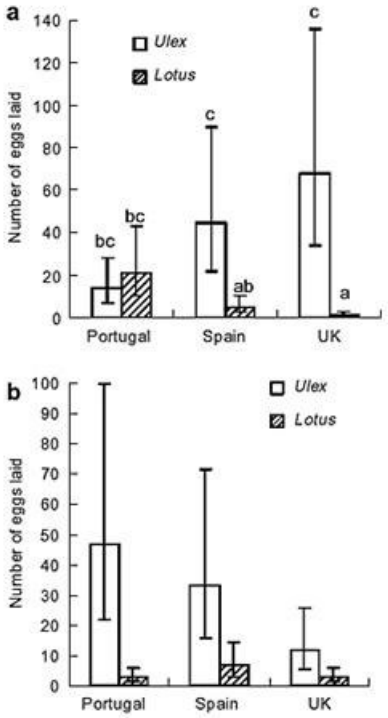

Fig. 4. Mean ± SEM number of eggs laid on *Ulex europaeus* shoots (no fill) versus *Lotus corniculatus* shoots (diagonal hatch) for the three populations of GPM sourced from Viana do Castello (Portugal), Santiago de Compostella (Spain) and Yateley Common (UK) during (a) no-choice oviposition tests and (b) choice oviposition tests. All data presented are the back-transformed parameter estimates from the analysis performed on Log(n + 1) transformed data. For (a) columns with the same letter are not significantly different (LSD); for (b) overall, significantly more eggs were laid on *U. europaeus* versus *L. corniculatus* but there were no significant differences between countries (see text for details).

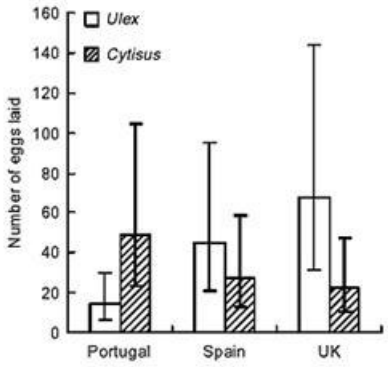

Fig. 5. Mean ± SEM number of eggs laid on *Ulex europaeus* (no fill) shoots versus *Cytisus scoparius* (diagonal hatch) shoots for no choice tests conducted on the three populations of GPM sourced from Viana do Castello (Portugal), Santiago de Compostella (Spain) and Yateley Common (UK). All data presented are the back-transformed parameter estimates from the analysis performed on Log(n + 1) transformed data. There were no significant differences between plant species or countries (see text for details).

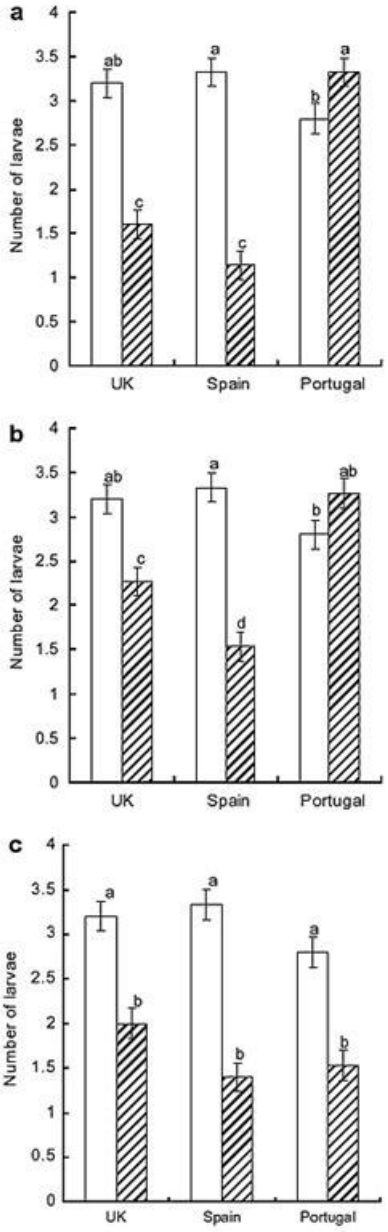

Fig. 6. Mean ± SEM number of larvae (out of five) surviving for 7 days on *Ulex europaeus* pods (no fill) versus pods of (a) *Lotus corniculatus* (diagonal hatch); (b) *Genista monspessulana* (diagonal bars) and (c) *Cytisus scoparius* (diagonal bars) for the three populations of GPM sourced from Viana do Castello (Portugal), Santiago de Compostella, Spain and Yateley Common (UK). Columns with the same letter are not significantly different (LSD).

moths sourced in England. The differences between each population's responses to the specificity tests could be related to the host plant subspecies to which they are adapted: For example, the two gorse subspecies may exhibit subtle differences in host plant chemistry to which the different moth populations may be adapted (e.g. Zangerl and Berenbaum, 1993). However, the habitats in

which each host plant subspecies occurs may also be important. For example, *U. minor*, grows in close association with *U. europaeus europaeus* at Yateley Common (Paynter, personal observations), and *U. europaeus europaeus*, *U. minor* and *U. gallii* all grow together in the vicinity of Santiago de Compostela (Sheppard, 2004), so that specialization on *Ulex* hosts may be selected for in both generations at these localities. In contrast, in sand dune habitats where *U. europaeus latebracteatus* is dominant in northern Portugal, *U. gallii* is absent and *U. minor* is rare (Honrado et al., 2006), so that there may be a selection pressure for GPM to utilize a broader range of hosts.

Sheppard et al. (2006) reported on the non-target attack due to the broom seed beetle *Bruchidius villosus* (Fabricius) in New Zealand and there are some close parallels between their findings and the findings of the current study: Original host-range testing of a UK population of *B. villosus* indicated that it is specific to *C. scoparius*, even though populations from elsewhere in Europe exploit other related plant species, such as *S. junceum* L. and *G. monspessulana*. Following release, *B. villosus* displayed a broader host-range than would be expected from the species' host-range tests, attacking *Cytisus proliferus* L.f as well as the target weed.

Sheppard et al. (2006) argued that to assume a stable specific host race has been found, just because host-range testing indicates a narrower host-range than for the species as a whole, without demonstrating genetic divergence or comparing host-specificity across different populations of a species would be imprudent—see Jaenike (1981) for criteria for ascertaining the existence of host races. They went on to predict that the risk of shifts in the field host-specificity of released biological control agents might be high if there is genetic variability in agent phenology and, or, if the new environment presents conditions that change the synchrony of interactions between agents and potential hosts.

On the basis of host-range testing, one might predict that if only the population of moths collected at Yateley Common had been released in New Zealand, then GPM may have failed to establish due to asynchronies between *U. europaeus* flowering and moth activity and the inability of this population to exploit other hosts. Nevertheless, although the tested UK population does appear to be highly specific it may not fulfill the criteria of a host race if it interbreeds with populations that exploit other hosts (Marohasy 1996, cited in Haines et al., 2004). As Sheppard et al. (2006) suggest for *B. villosus*, it may be that discrete 'host races' of GPM do not occur in Europe. Different populations may maintain a capacity to exploit whatever suitable host is available by outcrossing between populations and, or, through relative host attractiveness varying through time as a result of changing apparency and availability. However, although the original host-range tests performed on *B. villosus* also indicated that it was unlikely to attack other hosts (Haines et al., 2004), *B. villosus* was only subjected to choice oviposition specificity tests. No-choice testing may have indicated which species would be acceptable for oviposition if beetles were active when *C. scoparius* was not in bloom.

We cannot rule out the possibility that a very small proportion of the UK GPM population could exploit other hosts, but there was insufficient replication of host-range tests to detect this. However, unlike *B. villosus*, both no-choice oviposition (small cage in New Zealand and field cage in the UK) and larval starvation tests were performed on the UK population of GPM, both in the UK and in quarantine in New Zealand (Hill and Gourlay, 2002); replicated for several species of Genisteae and *Lo. pedunculatus*. These tests, together with our repeated testing, indicated little risk to a range of species that had been recorded as hosts in the field in Europe, including *Lotus* spp., *C. scoparius* and *G. monspessulana*. We believe that the different abilities of the two populations of GPM released in New Zealand to exploit different hosts, therefore, demonstrates the risk of releasing an untested population of a biological control agent.

We conclude that for species such as flower- and seed-feeders, which exploit seasonally ephemeral resources, it would be prudent to investigate the potential influence of asynchronies between target plant flowering and agent activity before deciding whether the release of an apparently specific population is safe. Unless the mechanisms that promote synchrony between agent and target plant are well understood and predictable, well-replicated no-choice testing should be relied upon to assess risk. This would be particularly important for populations of species which apparently have a narrower host-range than the species as a whole.

One recommendation for improving success of biological control introductions is to maximize the genetic diversity of the insects released into the new country (DeBach, 1964). To achieve this aim, releasing biological control agents sourced from different geographic locations was common practice in the early 1990s. However since this time, our understanding has increased about just how common host races that differ in their host utilization are (Wink and Legal, 2001). Future biological control releases should be made up only of the same geographic populations as those agents that were thoroughly host tested. This is now best practice in New Zealand.

Acknowledgments

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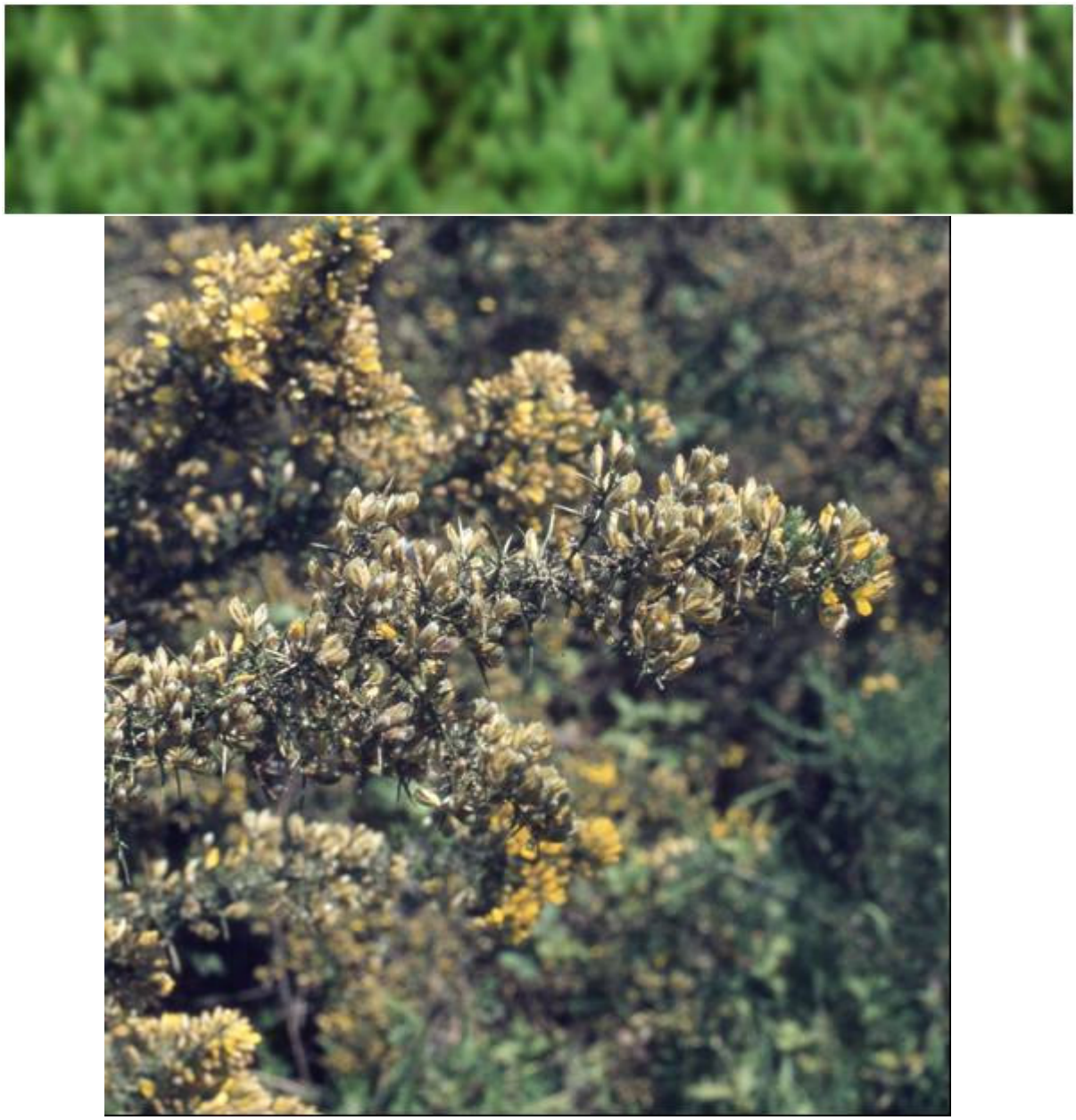
APPENDIX 5

Assessing gorse pod moth (*Cydia succedana*) infestation levels in *Lupinus* spp. at Tikitere Forest, New Zealand



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REPORT INFORMATION SHEET

REPORT TITLE	ASSESSING GORSE POD MOTH (CYDIA SUCCEDANA) INFESTATION LEVELS IN LUPINUS SPP. AT TIKITERE FOREST, NEW ZEALAND
AUTHORS	TONI M WITHERS, MICHELLE WATSON, BELINDA GRESHAM SCION
CLIENT	JOHN IRESON
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EXECUTIVE SUMMARY

The problem

The gorse pod moth, *Cydia succedana* (Lepidoptera:Tortricidae) was introduced into New Zealand as a biological control agent against gorse *Ulex europaeus*. It is also being considered for introduction into Australia for the same purpose. However, in the last decade post-release impact studies revealed its host range in the field in New Zealand was broader than that predicted by original host range testing.

This project

To assist with the risk assessment for Australia we investigated the degree of infestation that potted flowering lupin plants of commercial seed cultivars would receive within a dense gorse infestation that is well populated by *C. succedana* in New Zealand (Tikitere Forest skid site, Rotorua, growing on volcanic pumice soil).

Key Results

When gorse is flowering in spring infestation by *C. succedana* of the lupin cultivars in the field trial was virtually non existent. However during the months when gorse was not flowering, particularly during February and March, all four cultivars of lupins were infested to some level by *C. succedana* larvae.

Implications of Results for Client

These data confirm that the first generation of *C. succedana* is well synchronised with spring flowering gorse in New Zealand. The problem of non-target attack generally occurs with the second generation of moths, because they emerge as adults and oviposit before gorse starts to flower again in early autumn. This is when the majority of non-target attack on some closely related Fabaceae has been recorded previously. If commercial seed crops of lupins growing in Australia have already been harvested from the field before February then the risk from *C. succedana* to lupin crops is probably low.

Further Work

Depending on feedback from Australia, additional field trials could be conducted in even more dense infestations of *C. succedana*, such as in Canterbury, to provide a more reliable measure of risk.

Assessing gorse pod moth (*Cydia succedana*) infestation levels in *Lupinus* spp. at Tikitere Forest, New Zealand

Toni M Withers, Michelle Watson, Belinda Gresham[1]
Forest Protection, Scion, PB 3020, Rotorua 3046, New Zealand[1]

4 April 2012

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Introduction

The gorse pod moth, *Cydia succedana* (Lepidoptera:Tortricidae) was introduced into New Zealand as a biological control agent against gorse *Ulex europaeus*. It is also being considered for introduction into Australia for the same purpose. However, in the last decade post-release impact studies revealed its host range in the field in New Zealand was broader than that predicted by original host range testing (Withers, Hill, Paynter, Fowler, & Gourlay, 2008). Several species of exotic Genisteae, including Scotch broom *Cytisus scoparius*, Montpellier broom *Genista monspessulana*, and tree lupin *Lupinus arboreus*, as well as lotus *Lotus pedunculatus* (Loteae) growing in the vicinity of infested *U. europaeus* plants, were shown to be non-target hosts of *C. succedana* in both the North and South Islands of New Zealand (Paynter, et al., 2008).

In Australia, lupins are grown commercially to produce seed and for forage crops. The largest lupin producing state is Western Australia, followed by South Australia, New South Wales and Victoria. There are three commercial species of lupins, *L. angustifolius* (narrow leafed lupin), *L. albus* (white lupin) and *L. luteus* (yellow lupin). Production is dominated by *L. angustifolius* which accounts for 95% of all tonnage. Both *L. albus* and *L. luteus* make up the remainder of the lupin species grown (Ireson, Relf, Sagliocco, Kwong *et al*., 2011). No choice laboratory tests on single cultivars of each of these three lupin species were conducted in Australia using an English population of *C. succedana*. Although the results showed that *C. succedana* could complete development on all three cultivars, survival levels were significantly lower than on gorse (Ireson, Relf, Sagliocco, Kwong *et al*., 2011). Oviposition assays subsequently revealed was no significant difference between numbers of eggs laid on gorse, *L. albus* and *L. luteus* in choice oviposition tests (Ireson, Relf, Sagliocco, Kwong, Holloway, et al., 2011). Because of this the risk posed by *C. succedana* to the commercial lupin cultivars in Australia remains questionable. To assist with the risk assessment for Australia we investigated the degree of infestation that potted flowering lupin plants would receive within a dense gorse infestation that is well populated by *C. succedana* in New Zealand (Tikitere Forest, Rotorua, volcanic pumice soil, 300m asl).

Materials and Methods

Potted specimens were prepared by importing seed of the following species and cultivars from Australia:
Lupinus luteus L. cv. Pootalong
Lupinus luteus L. cv. Wodjil
Lupinus angustifolius L. cv. Wonga
Lupinus albus L. cv. Kiev

Seeds were germinated into individual pots, with staggered timing of sowing. Seedlings were potted on into mixed media in 20cm diameter pots, and hardened off first in a shade house then full sun, until flowering. All plants were watered once during hardening off with a mix of *Lupinus arboreus* duff obtained from the field (Tikitere) to ensure plants had been inoculated with suitable mycorrhizae.

When flowering was occurring simultaneously in all the lupin species and cultivars, seven plants of each were transported to the Tikitere field site. Each potted plant was sunk into holes dug into the ground and watered with a drip watering spike connected to an inverted 2L water bottle to prevent the plants from wilting. Water bottles were re-filled every two weeks throughout the trial. All plants and seeds were destroyed at the completion of the trial to ensure no cultivars new to New Zealand were accidentally introduced as a result of this trial.

The field trial site was established within a small grassy clearing at Tikitere Forest, Rotorua, and was surrounded by a 1 m high pest-proof fence in year one, increased to 2m in year two following mammalian browsing. The field trial site was completely surrounded by mature gorse that had never been controlled by aerial herbicide application because of the proximity to high voltage power lines. The trial was set out in a 7 x 4 latin square design (7 plants per lupin cultivar) each plant one metre apart.

December to February has been shown to be the most likely timing for infestation of non-target plants by *C. succedana* (Paynter, et al., 2008) so the first field trial was initiated in December 2010 through until the plants senesced in March 2011. Then additional information was obtained that lupins are sown in Australia from mid-April until early June, with susceptible green pods and young seeds present mainly from September through to November, so the second seasons' field trial was initiated in October 2011 and run until March 2012. Every 2 weeks pods were harvested and returned to the lab in labelled paper bags, examined externally for signs of eggs, and left undisturbed for 2 more weeks to allow larvae to feed, before pods being dissected in the lab and any lepidopteran larvae transferred to Hiltons diet containing 4% dried gorse seed that was obtained from Plant and Food Research, Auckland, for rearing to adult. Moths were identified to species. Data are reported as the proportion of pods infested with gorse pod moth, and includes those having shown clear signs of *C. succedana* infestation such as frass, seeds being eaten out, or the pod containing silk, head capsules or exit holes identical to those caused by *C. succedana*.

In December and January when gorse is setting seed, samples of gorse pods were returned to the laboratory and dissected to obtain percent infestation by *C. succedana* . In addition, to obtain the seasonal activity of moths, sticky bases from within red delta traps containing *C. succedana* sex pheromone (Suckling, Hill, Gourlay, & Witzgall, 1999) were collected (n = 3 traps) from the Tikitere field site every month for the duration of the study, and counts made.

Photo 1: Lupin field trial site in 2011 prior to erection of the fence to prevent mammalian browsing

Photo 2: Dissected lupin pod showing damage typical of *C. succedana* feeding (note frass and silk and a missing seed from the apex of the seed pod)

Results and Discussion

Cydia succedana phenology

Gorse pod moths were abundant at the site throughout the period of the study as indicated by the pheromone trapping of male moths (Figure 1A), with the expected two generations per year observed. The first generation flight appears from late spring and the second from late summer. The level of gorse pod infestation was usually less than 10%, though the last sample taken in January showed an unusually high 24 % infestation level (Figure 1B).

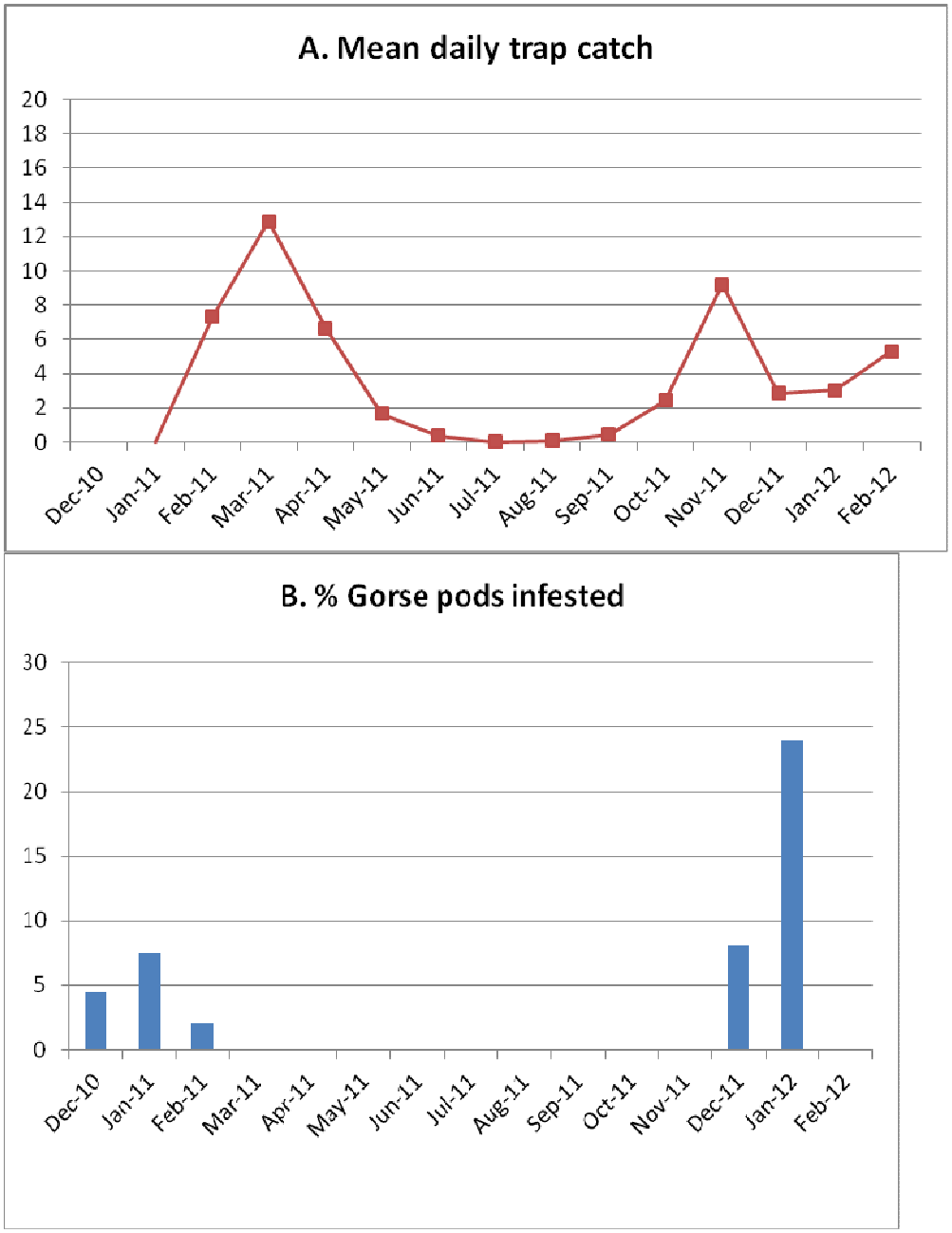

Figure 1: Mean daily trap catch and percent gorse pod infestation levels of *C. succedana* during the field trial

Infestation levels recorded in *Lupinus* species

All the cultivars and species of lupin in the field trial had green seed pods infested by *C. succedana* at some stage during the trial. The average *C. succedana* infestation levels across all dates is within the range of 4 to 10% of pods (Table 2). Despite our best efforts, some plants failed to produce pods in the field in Tikitere for longer than a few weeks. All plants were susceptible to fungal leaf pathogens, and only *L. angustifolius* cv. Wonga thrived throughout both seasons of the field trial. This is why the number of pods harvested from this plant is so much higher. Because of this high variability in pod harvest we have not undertaken any further statistical analysis and don't feel confident the data would reveal which cultivar was the most susceptible to *C. succedana* infestation in the field. The raw data can be obtained from the senior author should further analysis be considered to be important.

Table 2: Average proportion of *Lupinus* pods harvested from the field trial infested by *C. succedana* summed across all dates

Species	Sum of Pods picked	Average of Proportion infested	Variance p of Proportion infested
L. albus	126	0.047	0.036
L. angustifolius	902	0.065	0.024
L. luteus Pootalong	370	0.102	0.041
L. luteus Wodjil	362	0.055	0.019
Total	**1760**	**0.069**	**0.029**

Timing of infestation in *Lupinus* species

The most important result that can be gleaned from this field trial is that during the months when gorse is flowering (May through to October) or when gorse pods are present (November to December) infestation of lupins in the field site by *C. succedana* was either not possible as plants had senesced, or was low to non-existent. However non-target attack by *C. succedana* was recorded in both seasons of the field trial during the months when gorse was not flowering, particularly during all collections made in February and March (Figure 2).

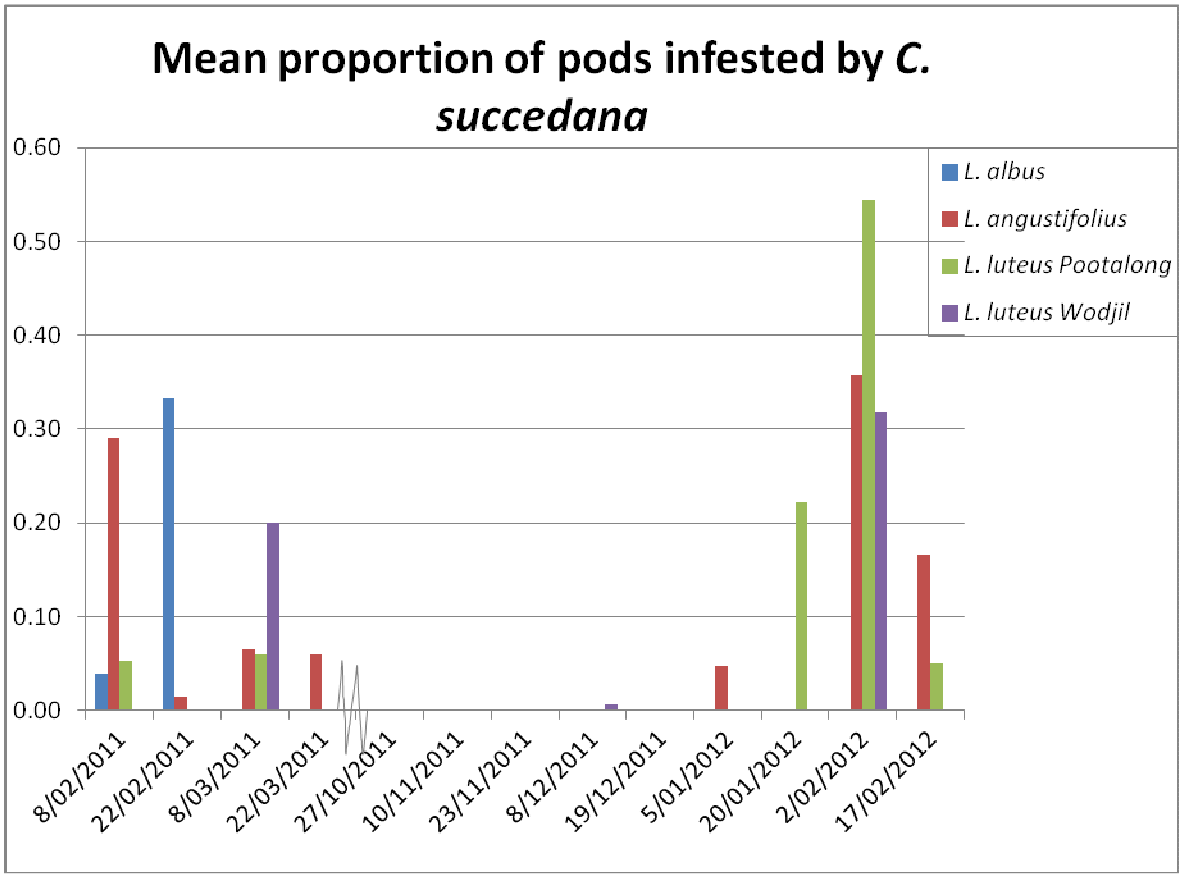

Figure 2: Mean proportion of *Lupinus* species pods at each collection date infested with *C. succedana* during the field trial

Recommendations and Conclusions

In Australia, the phenology of commercial cultivars of *L. angustifolius*, *L. albus* and *L. luteus* varies depending on where they are being grown. However, because they are usually planted over a period extending from mid-April until early June, periods of flowering and immature pod and seed development in lupins are overlapped by flowering and immature pod and seed development in gorse which occurs over longer periods from early winter to late spring. Cultivars of the commercial lupin species are harvested for their mature seed from early December and this can extend into January and early February (Ireson, Relf, Sagliocco, Kwong, Holloway, et al., 2011). Only immature, soft, green pods and seed of non-target plants are able to be attacked by larvae of *C. succedana*, even if the adult female does oviposit on more mature plants bearing mature pods.

In this trial lupin pods of commercial Australian cultivars were not attacked by *C. succedana* to a significant level during spring when gorse was flowering at its peak. As expected, the infestations were recorded more consistently when gorse was not flowering, particularly during February and March. Therefore, in Australia (should the Australian government approve its introduction there as biological control against gorse), commercial cultivars of lupins growing during winter and spring will probably be protected to some degree from non-target attack by the greater oviposition preference that *C. succedana* shows for the target weed, gorse.

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Appendix A

Data from *Lupinus* species pod collections:

	Values		
Dates and Species bearing pods:	**Sum of Pods picked**	**Average of Prop infested**	**Variance p of Prop infested**
8-Feb-11	**127**	**0.130**	**0.037**
albus	69	0.040	0.004
angustifolius	34	0.291	0.053
luteus Pootalong	19	0.053	0.000
luteus Wodjil	5	0.000	0.000
22-Feb-11	**115**	**0.079**	**0.066**
albus	10	0.333	0.222
angustifolius	95	0.014	0.001
luteus Pootalong	2	0.000	0.000
luteus Wodjil	8	0.000	0.000
8-Mar-11	**133**	**0.072**	**0.007**
angustifolius	95	0.065	0.003
luteus Pootalong	33	0.060	0.009
luteus Wodjil	5	0.200	0.000
22-Mar-11	**113**	**0.046**	**0.005**
albus	1	0.000	0.000
angustifolius	110	0.060	0.005
luteus Pootalong	2	0.000	0.000
27-Oct-11	**86**	**0.000**	**0.000**
albus	18	0.000	0.000
angustifolius	28	0.000	0.000
luteus Pootalong	14	0.000	0.000
luteus Wodjil	26	0.000	0.000
10-Nov-11	**158**	**0.000**	**0.000**
albus	4	0.000	0.000
angustifolius	61	0.000	0.000
luteus Pootalong	41	0.000	0.000
luteus Wodjil	52	0.000	0.000
23-Nov-11	**202**	**0.000**	**0.000**
albus	5	0.000	0.000
angustifolius	105	0.000	0.000
luteus Pootalong	37	0.000	0.000
luteus Wodjil	55	0.000	0.000
8-Dec-11	**426**	**0.002**	**0.000**
albus	5	0.000	0.000
angustifolius	200	0.000	0.000
luteus Pootalong	91	0.000	0.000

luteus Wodjil	130	0.006	0.000
19-Dec-11	**36**	**0.000**	**0.000**
angustifolius	24	0.000	0.000
luteus Pootalong	5	0.000	0.000
luteus Wodjil	7	0.000	0.000
5-Jan-12	**63**	**0.029**	**0.003**
angustifolius	55	0.048	0.005
luteus Pootalong	2	0.000	0.000
luteus Wodjil	6	0.000	0.000
20-Jan-12	**46**	**0.028**	**0.005**
angustifolius	37	0.000	0.000
luteus Pootalong	9	0.222	0.000
2-Feb-12	**189**	**0.357**	**0.059**
albus	9	0.000	0.000
angustifolius	12	0.357	0.065
luteus Pootalong	100	0.544	0.012
luteus wodjil	68	0.318	0.033
17-Feb-12	**66**	**0.092**	**0.042**
albus	5	0.000	0.000
angustifolius	46	0.167	0.071
luteus Pootalong	15	0.050	0.003
Grand Total	**1760**	**0.069**	**0.029**