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

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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

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[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

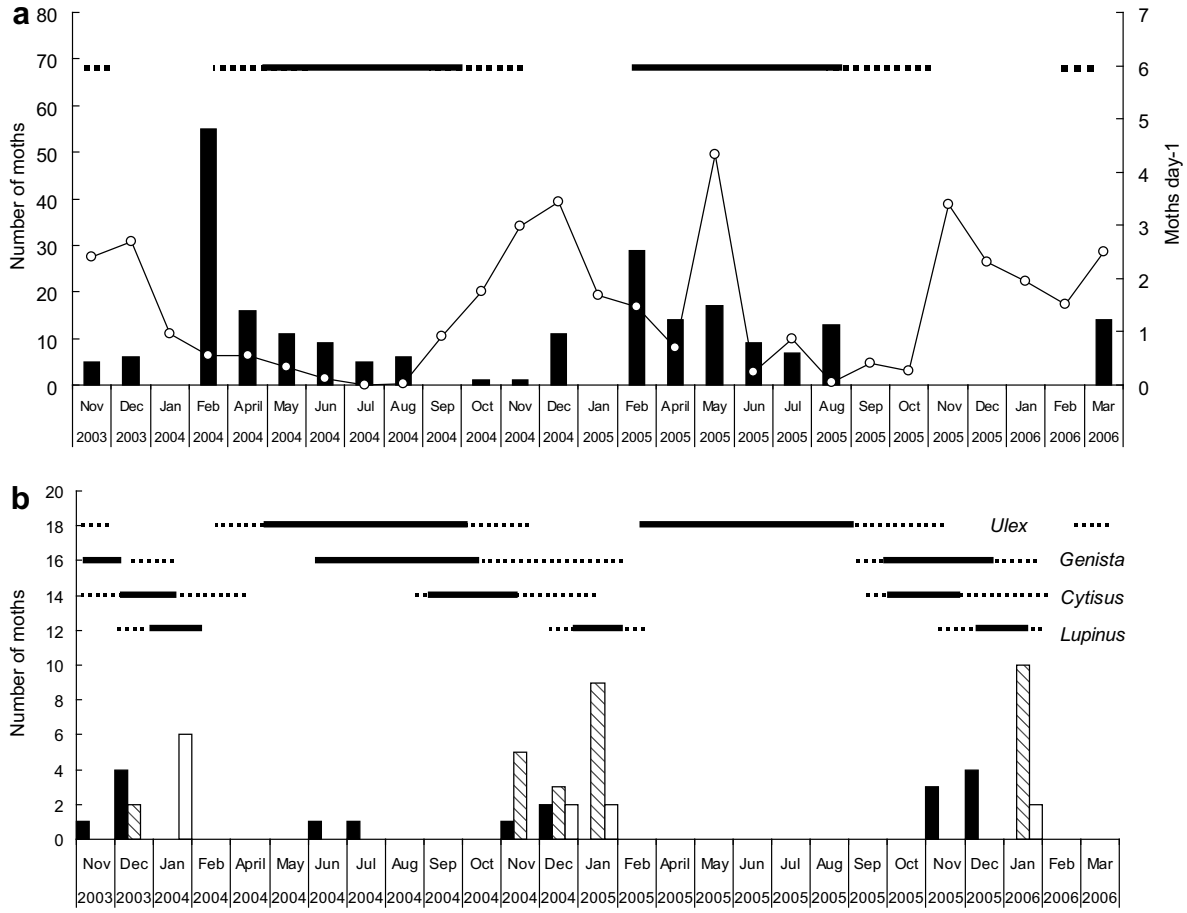

Fig. 1. (a) Monthly number of moths reared per 100 U. *europaeus* pods (vertical bars) and seasonal abundance of adult GPM moths in pheromone traps (open circles; moths trap^{-1} day^{-1}) in relation to the phenology of *U. europaeus* reproduction (horizontal bars; solid = flowers and pods present; 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),

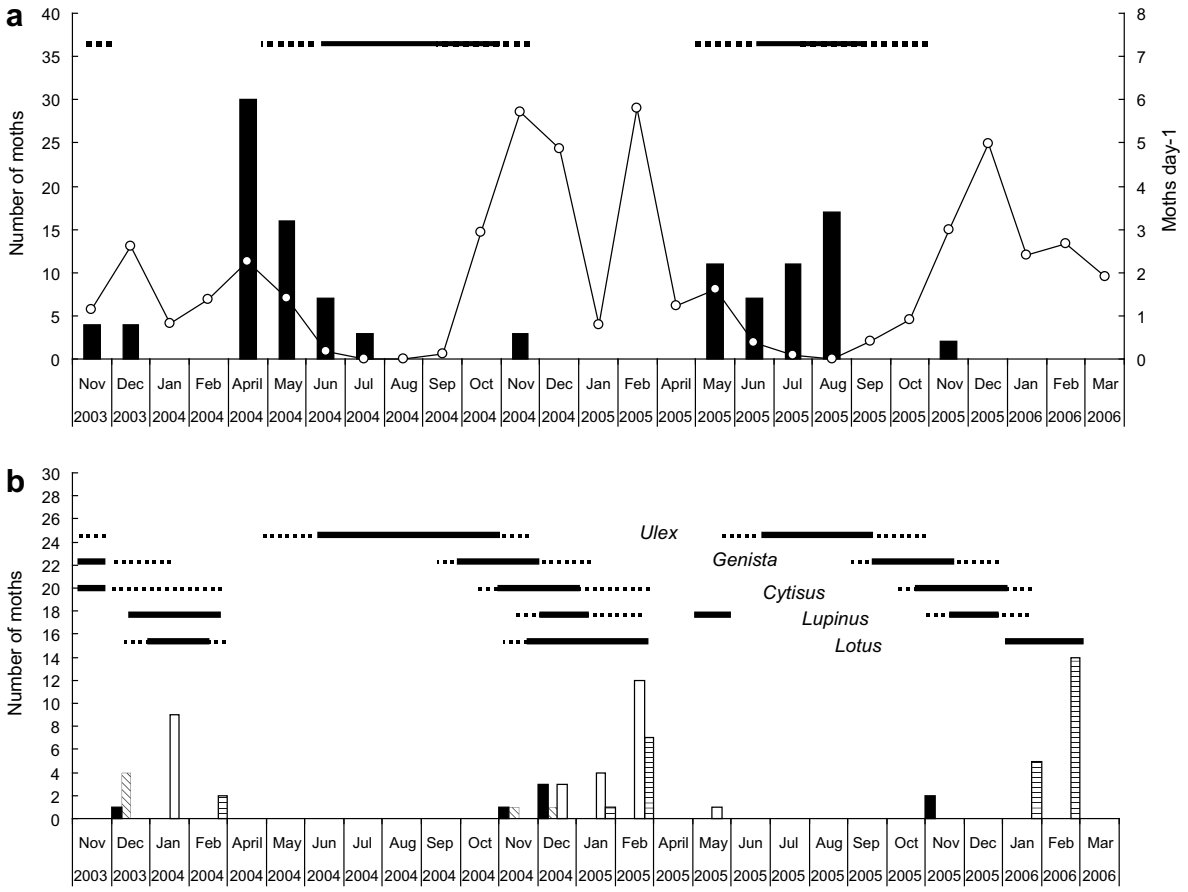

Fig. 2. (a) Monthly number of moths reared per 100 *Ulex europaeus* pods (vertical bars) and seasonal abundance of adult GPM moths in pheromone traps (open circles; moths trap^{-1} day^{-1}) in relation to the phenology of *U. europaeus* reproduction (horizontal bars; solid = flowers and pods present; 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

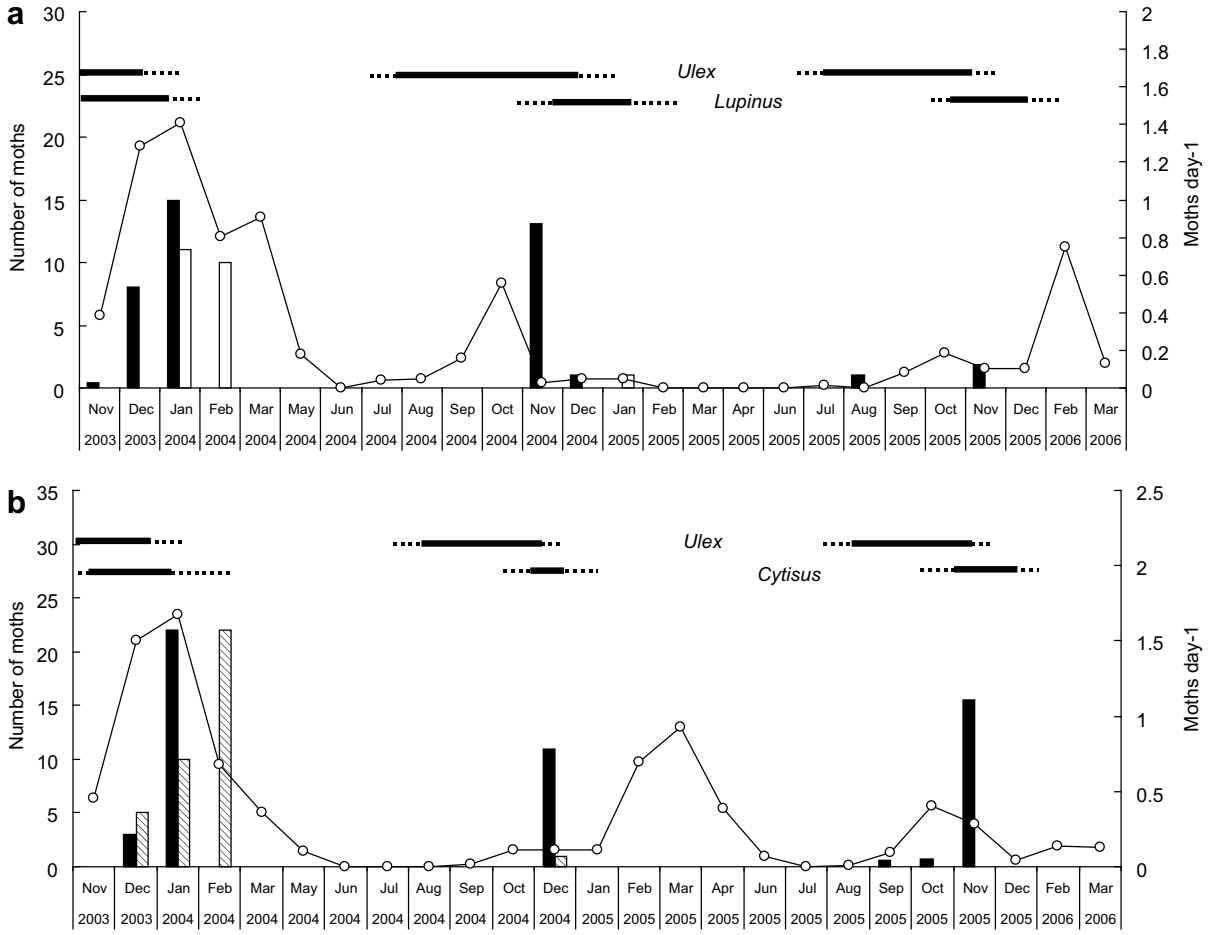

Fig. 3. (a) Monthly number of moths reared per 100 pods of *Ulex europaeus* (vertical bars, dark fill) and *Lupinus arboreus* (vertical bars, no fill) and seasonal abundance of adult GPM moths in pheromone traps (open circles; moths trap^{-1} day^{-1}) in relation to the phenology of *U. europaeus* reproduction (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

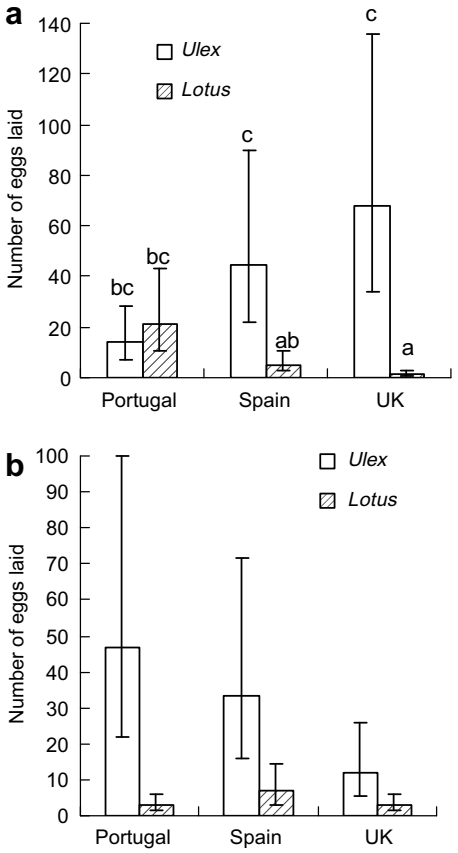

Fig. 4. Mean ± SEM number of eggs laid on *Ulex europaeus* shoots (no fill) versus *Lotus corniculatus* shoots (diagonal hatch) for the three populations of GPM sourced from Viana do Castello (Portugal), Santiago de Compostella (Spain) and Yateley Common (UK) during (a) no-choice oviposition tests and (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).

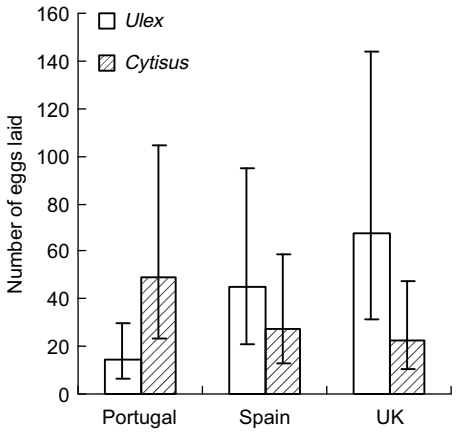

Fig. 5. Mean ± SEM number of eggs laid on *Ulex europaeus* (no fill) shoots versus *Cytisus scoparius* (diagonal hatch) shoots for no choice tests conducted on the three populations of GPM sourced from Viana do Castello (Portugal), Santiago de Compostella (Spain) and Yateley Common (UK). All data presented 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).

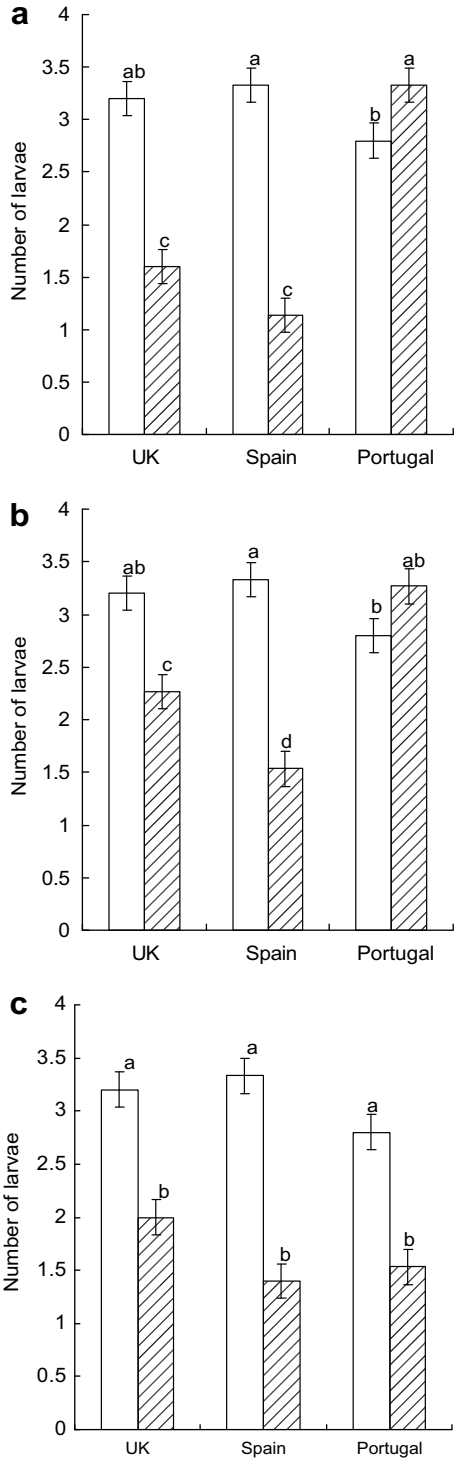

Fig. 6. Mean ± SEM number of larvae (out of five) surviving for 7 days on *Ulex europaeus* pods (no fill) versus pods of (a) *Lotus corniculatus* (diagonal hatch); (b) *Genista monspessulana* (diagonal bars) and (c) *Cytisus scoparius* (diagonal bars) for the three populations of GPM sourced from Viana 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.

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