

BIOLOGICAL CONTROL OF ARTHROPODS OF CONSERVATION IMPORTANCE

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SESSION 2 INTRODUCTION

Invasive species, and the associated development of a relatively new biological discipline broadly known as invasion biology, are a mainstream and widely recognized scientific endeavor. Parties interested in invasive species and their management, modes of infiltration, and impact represent widely disparate groups including: political officials, conservationists, ecologists, agricultural, silvicultural, horticultural, and aquacultural producers, the lay public, and entomologists, including biological control scientists (Wittenberg and Cock 2001). Invasive species and their management currently enjoy a high public profile. Books, T.V. shows, and magazine and newspaper articles regularly appear on this subject, particularly in North America, often including discussion of biological control as a means of environmentally-friendly suppression.

Biological control practitioners are interested in invasion events from a variety of angles, but manipulating two dynamic processes form the pragmatic basis for applied biological control: (1) reducing biotic drivers that promote invasion by pest organisms (i.e., reducing population growth rates, spread, and resulting economic and ecological damage), and (2) promoting biotic drivers of invasive upper trophic level organisms deliberately released for the suppression of unwanted invaders (i.e., carefully increasing the likelihood of population growth, spread, and impact of natural enemies by selecting species with close biological and ecological links to the target pest).

Biological control has been applied widely and often with some level of success against a variety of insect, weed, and vertebrate pests. Most biological control applications have targeted pests of agricultural and forestry importance. This is particularly true for arthropod pests (i.e., insects and mites), and until relatively recently this was mostly the situation for weeds too. However, most weeds are multi-sectoral in their impact, and earlier weed biological control programs yielded environmental benefits as well as the traditional economic benefits. There is now an increasing trend for the environmental impact of alien invasive plants to be recognized, so that there is demand and resources for the biological control of weeds of conservation importance (terrestrial and aquatic). A similar trend is emerging for arthropod

pests of conservation importance as the ecological impact of these incursive species in infiltrated ecosystems is widely appreciated and the need for remedial action recognized. Indeed, biological control of arthropods of conservation importance has gone a step further. New projects are assessing the feasibility of controlling arthropod pests in aquatic situations, most remarkably the marine environment. Aquatic arthropod pests, most notably an invasive crab, the European green crab, *Carcinus maenas* (L.) (Brachyura: Cancridae) are being assessed as targets for classical biological control and this novel area is providing unique challenges in determining and assessing host specificity, threats to non-target organisms, and the complicated theoretical issues arising from open vs. closed systems for pest and natural enemy recruitment which are being investigated with mathematical models.

One of the first steps in a biological control program is to assess the area of origin of the target pest. When the target is an economic pest, there is usually information available on the status of the pest in the economic literature from around the world, which provides a starting point for projects. The exact origin may still be obscured by subsequent spread, and taxonomic problems related to correct identification (e.g., coffee mealy bug, *Planococcus kenyae* Le Pelley [Greathead 2003]), and sometimes the pest has not been recognized as such in its area of origin (e.g., cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero [Neuenschwander 2003] and Levuana moth, *Levuana iridescens* Bethune-Baker [Tothill *et al.* 1930; Kuris 2003]). When dealing with pests that affect indigenous plants of conservation importance, it may well be that the origin of a newly introduced species is unknown, since the species attacks no plants of economic importance in its area of origin. *A priori*, it seems likely that this may happen more frequently in the case of environmental pests, and we include one such example here: the horse chestnut leaf miner (Kenis *et al.*). Another example from the field of insects of conservation importance is the web spinning pamphilid sawfly, *Cephalcia tannourinensis* Chevan, of unknown origin, which appeared in Lebanon more than 15 years ago and started to cause substantial damage to the few remaining stands of cedar of Lebanon (*Cedrus libani*). At present, it is being managed with an IPM program (Nemer and Naser 2004), as no classical biological control options have been identified.

Many problems associated with determining true species identity and exact area of origin may be overcome through the use of molecular tools. "DNA fingerprints" such as microsatellites can theoretically be used to identify haplotypes within populations that are naturally spread over vast areas within the home range. It should be possible to use a molecular approach to circumscribe within an area of origin a more exact range for the invading population and focus foreign exploration efforts here. One potential benefit arising from such a highly focused search would be the selection of natural enemies from within the pest's home range that are most adapted to the particular haplotype that is the target of the biological control project. A highly focused search within the pest's home range using a molecular-based rationale is feasible when an invasion has resulted from a limited number of founders sourced from a highly localized area and a low number of successful invasion events resulting in establishment occurred. Focused searches based on haplotype matching may increase the success of classical biological control of arthropod pests beyond the somewhat static rate of 10-12% (Gurr *et al.* 2000). Conversely, there is the possibility of rapid evolution through a bottleneck on arrival in a new habitat, so that there is no exact match between the introduced population and any source populations. Similarly this approach can be used to demonstrate

multiple introductions, and the introduction of a new biotype with different, more damaging, characteristics.

Despite the recognized potential for natural enemies to suppress pests of economic and conservation importance in a variety of different environments, there has been a very vocal outcry from concerned ecologists and conservationists that biological control is not always a safe and risk free method of pest suppression (e.g., Louda and Stiling 2004). These criticisms have been challenged and arguments have been forwarded that biological control has the potential to be highly effective and safe when conducted according the recognized scientific protocols regardless of whether the target is of economic or conservation concern (Hoddle 2004a,b,c). We reaffirm that, in many instances, once prevention, containment, and eradication options have been exhausted or deemed infeasible, carefully orchestrated biological control programs against well selected targets may be the only feasible option for bringing invasive species of conservation importance under permanent control. The goals of these conservation-oriented biological control projects are to restore population balance and ecosystem health to similar levels seen prior to the invasion without radically disrupting pre-existing food webs (it should be noted that food webs are almost always disrupted in native systems by invasive alien species that reach extremely high densities) or perturbing natural ecosystems in unforeseen ways. Conservation land managers are increasingly recognizing that the options for sustainable management of alien invasive plants are frequently very limited, and that biological control is often the best option. It is becoming ever more apparent that the same also applies to arthropod pests having environmental impact.

50

The purpose of session 2 in the Second International Symposium on the Biological Control of Arthropods is simple – to showcase examples of the application of classical biological control for suppressing invasive pests of conservation importance. The five selected speakers will present projects that have either been completed (projects by Causton and Fowler) or are underway (projects by Kenis *et al.* and Frank and Cave) against insects causing the demise of native plant plants in either island (cottony cushion scale on the Galapagos Islands [Causton] and orthezia scale on St. Helena [Fowler]) or mainland habitats (bromeliad weevils in Florida, U.S.A. [Frank and Cave] and horse chestnut leaf miner in Europe [Kenis *et al.*]). Kuris *et al.*, review green crab biological control and the potential use of castrating barnacles for the suppression of this globally aggressive marine invader. Additionally, the five presentations in session 2 will be complimented by other presentations on biological control of arthropod pests of conservation importance that are being discussed in different contexts in other sessions, fire ants [Porter and Gilbert session 13], and woolly hemlock adelgid [Zilahi-Balogh session 13]).

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THE SUCCESSFUL CONTROL OF *ORTHEZIA INSIGNIS* ON ST. HELENA ISLAND SAVES NATURAL POPULATIONS OF ENDEMIC GUMWOOD TREES, *COMMIDENDRUM ROBUSTUM*

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ABSTRACT

52 The small South Atlantic island of St. Helena has a highly degraded but internationally significant terrestrial flora, now covering only 1% of its land area. The 2500 gumwood trees, *Commidendrum robustum*, in the last two natural stands, are an important part of this remnant flora. In 1991, a scale insect infesting the gumwoods was identified as *Orthezia insignis*. This South American pest is widespread in the tropics, but this was the first record from St. Helena. By 1993, there were severe patches of infestation of the scale, and over 100 gumwood trees were dead. If the exponential increase in the number of dead trees had continued, all 2500 trees would have been killed by 1995. This was a likely outcome given the lack of natural enemies, and abundance of alternative host plant species for the scale. Fortunately, *O. insignis* had a history of successful biological control in Hawaii, and several African countries, through the introduction between 1908 and 1959 of the predatory South American coccinellid beetle, *Hyperaspis pantherina*. The life history and environmental safety of the predator were studied in quarantine in the U.K., and in 1993 the St. Helena government gave permission for its introduction onto the island. In May 1993, 80 *H. pantherina* survived the 6-day journey to St. Helena, and were used to establish a laboratory colony, from which over 5000 beetles were released from June 1993 to February 1994. Monitoring was undertaken using visual counts of *O. insignis* and *H. pantherina* on 300 labelled branchlets on the gumwood trees. Although the cause of tree death was visually obvious, monitoring demonstrated significant correlations between the levels of attack by the scale and tree mortality. *H. pantherina* was detected on the labelled shoots in February 1994, and numbers then increased, coinciding with a 30× decrease in mean scale numbers. This measured reduction is conservative, because the number of live scales tended to be underestimated when debris from recent feeding by the coccinellid was present. There have been no further problems reported with the scale on St. Helena since 1995. Laboratory rearing of *H. pantherina* was discontinued in July 1995 because insufficient *O. insignis* could be found anywhere on the island. Biological control of *O. insignis* was successful, but the extensive blackening from sooty moulds on all surviving gumwood trees in February 1995, suggested that the predator was effective only just in time to prevent most of

the trees being killed. Experimental transfers of *O. insignis* showed that the other three members of the endemic genus *Commidendrum* could also be at risk from the scale. The deliberate introduction of *H. pantherina* into St. Helena is an early example of biological control being initiated solely for conservation of indigenous biodiversity. It appears that this successful programme has saved the field population of a rare endemic plant from extinction.

INTRODUCTION

The 122-km² island of St. Helena is situated in the South Atlantic Ocean (15° 56' S, 5° 42' W). Despite widespread environmental degradation since the 16th century, the extant biota of the island is of international significance (Pearce-Kelly and Cronk 1990). St. Helena's flowering plants, for example, include 30 endemic species in 23 genera, and 10 of these genera are also endemic (Pearce-Kelly and Cronk 1990). Native vegetation covers less than 1% of the land area, and many of the indigenous plant species exist in only very small numbers (Cronk 1989). On the positive side, plant species thought to be extinct for over 100 years have been rediscovered, and ambitious restoration programs have been started (Cronk 1989). The endemic genus *Commidendrum* contains four species, including *C. robustum* (Roxb.) DC. (St. Helena gumwood) (Asteraceae), the island's national tree. The once extensive forests of gumwoods are now represented by 2500 trees in two small stands.

In 1991, an insect was noticed attacking gumwood trees at Peak Dale (G. Benjamin, pers. comm.) (Fig. 1). This was identified as the South American scale, *Orthezia insignis* Browne (Homoptera: Ortheziidae), a polyphagous pest that has been accidentally introduced into many tropical countries on imported plants. The first gumwood deaths attributed to *O. insignis* occurred in 1992. Control of *O. insignis* using insecticides was not an option because of the steep terrain, strong winds and risk to indigenous insects. CAB International suggested biological control as an option.



Figure 2. Adult *Hyperaspis pantherina*.
UGA1390006

A predatory beetle, now known as *Hyperaspis pantherina* Fürsch (Coleoptera: Coccinellidae) (Fig. 2), was a recognised biological control agent for *O. insignis*, but because of poor past monitoring its success record was uncertain, and little was known about its biology. Consequently, the first steps were to review the past record of the agent, and study its life history with an emphasis on assessing its host specificity and optimising methods for rearing and transportation.



Figure 1. Gumwood branch heavily infested with *Orthezia insignis*, Peak Dale, St. Helena, May 1993. Note the blackening from sooty molds. UGA1390005

PAST USE OF *HYPERASPIS PANTHERINA* FOR BIOLOGICAL CONTROL OF *ORTHEZIA INSIGNIS*

Attempts to control *O. insignis* biologically using *H. pantherina* began with the first introduction of just five individuals from its native Mexico to Hawaii in 1908 (Clausen 1978). Since then *O. insignis* has reportedly been under effective control by *H. pantherina* (Zimmerman 1948). In 1948, *H. pantherina* from Hawaii were introduced into Kenya: *O. insignis* is no longer considered a major pest in Kenya, which again was attributed to *H. pantherina* (Greathead 1971). From Kenya, *H. pantherina* was distributed to Tanzania, Uganda and Malawi, where reports indicated that control of *O. insignis* was generally successful (Greathead 1971), although the outcome was disputed in Malawi. *H. pantherina* is the only biological control agent for *O. insignis* that has definitely established and achieved substantial control of the pest, although the evidence for this is non-quantitative and often anecdotal (Booth *et al.* 1995).

BIOLOGY AND CULTURING OF *ORTHEZIA INSIGNIS* AND *HYPERASPIS PANTHERINA*

Orthezia insignis is a mobile scale insect, which as an adult female has a large wax ovisac (Fig. 3). The species is parthenogenetic. The eggs hatch inside the ovisac and the 1st instar nymphs then move out to feed. *Orthezia insignis* was reared in large cages on various plant species as described by Booth *et al.* (1995).

Hyperaspis pantherina is difficult to rear successfully in large cages because the supply of prey can easily become exhausted, resulting in cannibalism by the predator, and collapse of the culture. Rearing methods were developed that used large numbers of small containers, with fresh *H. pantherina* eggs (normally attached to an adult scale) transferred into fresh containers every 2-3 days (Booth *et al.* 1995). Regular transfers of eggs, and provision of prey, reduced cannibalism because there were only small numbers of *H. pantherina* at similar growth stages in each container. After hatching, the first instar larvae of *H. pantherina* usually enter the ovisac of the female scale, where they consume scale eggs and hatching nymphs. *Hyperaspis pantherina* larvae normally became visible in the containers in the third instar when they leave the ovisac, in the process they normally kill and consume the adult scale.

When four female *H. pantherina* were closely observed for their adult life, over 90% of the total of 657 eggs produced were laid on adult female *O. insignis*. Almost all of the eggs laid on female *O. insignis* were either on the dorsal surface of the abdomen (Fig. 3) or on the dorsal surface of the ovisac. The few eggs laid on the substrate include those laid on the exuviae of *O. insignis*, on other fragments of the prey, nearby on the host plant, and on other suitable surfaces such as filter paper. In the complete absence of *O. insignis* as live individuals, exuviae or other remains, only one egg was laid in nearly 2 years of culturing. That *H. pantherina* almost never laid eggs in the absence of *O. insignis* (insects, exuviae or debris after predation), and that over 90% of eggs were laid on the adult female scales, suggest a very close predator-prey relationship. However, when deprived of *O. insignis*, caged adult beetles did attack *Planococcus* and *Pseudococcus* species (Homoptera: Pseudococcidae) (Booth *et al.* 1995). A risk of attack on other mealybug or scale species (Homoptera: Coccoidea) on St. Helena

was not considered important, as all such insects recorded on the island are accidental introductions, and most are pests (Booth *et al.* 1995; Fowler 1993).



Figure 3. Adult *Orthezia insignis* with the 0.7mm oval, grey egg of *Hyperaspis pantherina* on its abdomen. UGA1390007

SHIPMENT TO ST. HELENA, REARING, AND RELEASE

St. Helena is remote, with the fastest access route being a military flight from the U.K. to Ascension Island, followed by a sea voyage to the island itself. No *O. insignis* were allowed in the shipment of *H. pantherina*, so the predator had to survive on water and honey agar (details in Booth *et al.* 1995). In May 1993, 80 larvae, pupae and adults of *H. pantherina* survived the 6-day journey to St. Helena, and were used to initiate a rearing programme using the methods previously developed. The first releases of *H. pantherina*, from June to November 1993, used a total of approximately 50 surplus ovipositing females from the laboratory culture. Each female was placed into a small 1mm mesh sleeve enclosing a scale-infested gumwood branchlet at Peak Dale. At weekly intervals, each sleeve was moved to a new branchlet, until the female died. The first three individual *H. pantherina* released into sleeves were checked after 4 days: all three females had survived, and eggs could be seen on the dorsal surface of several adult scales. No further assessment of this release method was made, because detecting *H. pantherina* eggs or young larvae in the field was difficult. In February 1994, 5000 beetles were released onto the gumwoods without sleeving. The rearing facility operated for 2 years until July 1995, when insufficient numbers of *O. insignis* could be found on St. Helena to maintain the culture of the predator. After the mass release onto the gumwoods, various other releases were made onto exotic ornamental plants at the request of private landowners. No parasitoids or other natural enemies of *O. insignis* were noticed in any of the field collections of the scale used in the rearing facility.

DISTRIBUTION OF ORTHEZIA INSIGNIS ON ST. HELENA

Monitoring was undertaken in stands of *L. camara* at 27 additional sites across the island in 1993 (Fowler 2003). At all these sites, *O. insignis* infestations on *Lantana camara* L. (Verbenaceae) were visually categorised as abundant, present (but not abundant), or absent. *Lantana camara* was selected because it is a common host plant for *O. insignis* found all over the island at all altitudes. In contrast, gumwood trees are only common at Peak Dale and at the new restoration plantings at Horse Point (Fig. 4).

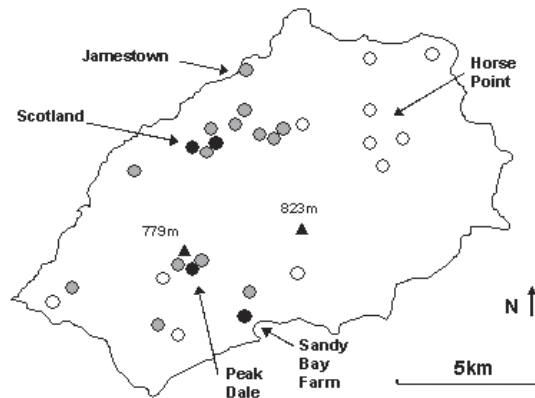


Figure 4. The island of St Helena. Circles indicate areas where the weed, *L. camara*, was checked for *O. insignis* infestations in 1993 (black = abundant scale; gray = scale present, but not abundant; open = scale not detected).

During May/June 1993, *O. insignis* was only abundant at Scotland (near to the main urban centre and only port at Jamestown), at Sandy Bay Farm and Peak Dale. This pattern of occurrence was consistent with *O. insignis* being a recent introduction onto the island, followed by accidental translocation to Sandy Bay Farm on cultivated plants, and then dispersal in the prevailing SE trade winds up to Peak Dale. *Orthezia insignis* was absent in the north-east part of the island and from many of the steep coastal cliffs in the south (Fig. 4). By February 1995, *O. insignis* had become abundant on *L. camara* at Horse Point, suggesting continued dispersal. Eggs and larvae of *H. pantherina* were seen in February 1995 on lantana about 1 km from a release site, demonstrating that the predator was also dispersing. The limited distribution of *O. insignis* in 1993 suggested that other *Commidendum* spp., particularly the large recovering areas of scrubwoods (*C. rugosum* [Ait.] DC.) in the steep, dry coastal zone, might not have been exposed to the pest. Consequently, some simple investigations of the potential acceptability of several plant species endemic to St. Helena, including scrubwood, were undertaken in 1993 (see next section).

THREATS TO OTHER ENDEMIC PLANT SPECIES

Single cut shoots (10–15cm long) were taken from 2–9 plants each of *Lantana camara* and of 8 species of endemic trees and shrubs; gumwood, *C. robustum*; scrubwood, *C. rugosum*; bastard gumwood, *C. rotundifolium* (Roxb.) DC.; false gumwood, *C. spurium* (Forst.f.) DC.; he-cabbage tree, *Pladaroxylon leucadendron* (Forst.f.) Hook.f.; she-cabbage tree, *Lachanodes arborea* (Roxb.) B. Nord (all Asteraceae); St. Helena ebony, *Trochetiopsis melanoxyton* (Ait.f.) Marais; ebony/redwood hybrid, *T. erythroxyton* (Forst.f.) Marais x *T. melanoxyton* (Sterculiaceae). Fifty *O. insignis* nymphs were allowed to transfer onto each cut shoot from small pieces of infested lantana that were placed onto the cut shoots. The shoots, in vials of water, were positioned so that the foliage of each shoot was not touching anything. Remaining nymphs were counted after 3–4 days. The results are shown in Table 1. Mean survival of

O. insignis nymphs on the genus *Commidendrum* (gumwoods and scrubwoods) (Asteraceae) ranged from 34% for *C. robustum* to 70% for *C. spurium*. This was markedly higher than the 2.5% nymphal survival on the *Trochetiopsis* species (ebony and ebony/redwood hybrids).

Only the comparison of *Trochetiopsis* species/hybrid with *C. rugosum* and *C. spurium* was statistically significant. Given the suitability of *C. robustum* to *O. insignis* in the field, this result suggests that all 4 *Commidendrum* species were likely to be suitable hosts, but that the highly endangered *Trochetiopsis* species/hybrid in the family Sterculiaceae might be relatively much less suitable. The cut shoots probably deteriorated in the time required for scale nymphs to transfer from the drying pieces of *L. camara*, so even transfers to cut shoots of *L. camara* only had a 40% survival rate. Given the low number of replicates and high variability, little can be concluded about the suitability of the two species of cabbage trees (Asteraceae), although mean nymphal survival was 40% on the two replicates of he-cabbage trees, *P. leucadendron* (Table 1).

Table 1. Summarized results of the laboratory host range test, exposing cut shoots of selected endemic plants species in St. Helena to 50 nymphs of *O. insignis* for 3–4 days. The introduced weed *L. camara* was used as a control. Means followed by the same lower case letters are not significantly different. (Overall ANOVA on arcsin transformed data, $F_{8,29}=4.66$, $P<0.01$, comparison of means used Tukey HSD, $P<0.05$, SYSTAT [SPSS 1997]). Data from Fowler (2003).

Plant species	Replicates	Mean % survival (\pm SE)
<i>Lantana camara</i>	4	40 (± 7.4) ^{ab}
Gumwood, <i>C. robustum</i>	9	34 (± 3.4) ^{ab}
Scrubwood, <i>C. rugosum</i>	9	50 (± 4.3) ^a
Bastard gumwood, <i>C. rotundifolium</i>	2	50 (± 15) ^{ab}
False gumwood, <i>C. spurium</i>	2	70 (± 5.0) ^a
He-cabbage tree, <i>L. leucadendron</i>	2	40 (± 10.0) ^{ab}
She-cabbage tree, <i>L. arborea</i>	2	10 (± 5.0) ^{ab}
St Helena ebony, <i>T. melanoxylon</i>	4	2.5 (± 0.8) ^b
Ebony/redwood hybrid, <i>T. erythroxyton</i> x <i>T. melanoxylon</i>	4	2.5 (± 1.3) ^b

MONITORING PREDATOR/PREY ABUNDANCE AND IMPACT

Prior to 1993, the numbers of trees dying after infestation by *O. insignis* were counted, but infestation levels of the scale were not quantified. Nevertheless, the infestation levels were clearly very high as shown in Fig 1. In 1993 a monitoring program was started to relate scale infestation levels to damage or death of trees, and determine whether these measurements changed in response to increasing levels of the introduced predator. The two relict stands of

gumwood trees at Peak Dale were designated sites A and B: site A being where infestations of *O. insignis* were first noticed in 1991, and site B being the stand approximately 0.5 km further to the south-west. At both sites, 15 trees were selected and labelled, with 5 trees in each of 3 visually assessed damage categories: severely infested (>50% of canopy affected); moderately infested (<50% of canopy affected); and uninfested. The selection of these 30 trees was as random as possible, although heavily shaded trees, and those with most of their canopy out of easy reach, were avoided. Gumwood trees have a simple, sparse canopy that was divided conveniently into approximately 20 cm long branchlets, comprising a group of 1–3 growing points, each with 10–20 leaves. Ten branchlets per tree were selected randomly and labelled for the non-destructive sampling program. At 1–3 month intervals, the numbers of adult and nymphs of *O. insignis* on each branchlet were visually estimated, and numbers of *H. pantherina* larvae and adults counted. The presence, or approximate percentage damage, due to other herbivores or predators was also assessed by eye. A visual estimate was made of the percentage of canopy of each tree that was heavily infested with scale.

The total numbers of dead gumwood trees at Peak Dale increased exponentially from 1991 to 1993–4 (Fig. 5). By 1995 mortality of gumwoods had reached an asymptote, with only 12% dead from a total of 2500 trees. If the exponential rate of loss of gumwoods from 1991 to 1994 had continued, all trees in the two relict stands at Peak Dale would have been killed by 1995.

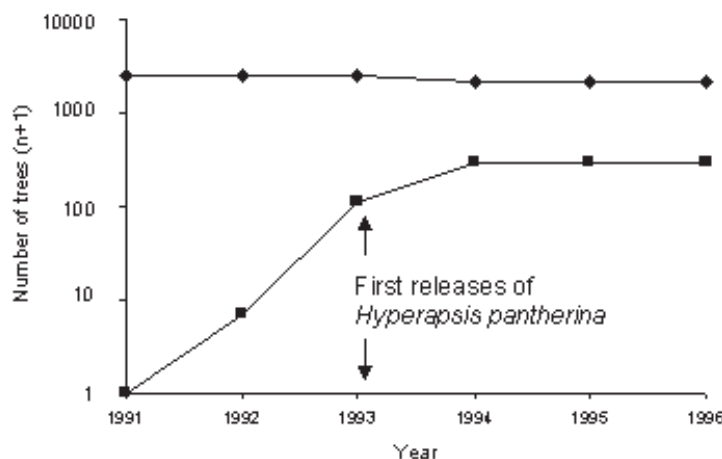


Figure 5. The numbers of live (◆) and dead (■) gumwood trees at Peak Dale, during the outbreak and subsequent biological control of *Orthezia insignis*. The exponential increase of the scale numbers from 1991 to 1993–4 was significant ($F_{(1,2)}=66.3$, $P<0.05$, $r^2=0.97$, $\log(y+1)=0.77x-1532$, SYSTAT [SPSS 1997]). Data from Fowler (2003).

Mortality among labelled trees was highest at site A, and higher among trees that were severely damaged at the start of the release program (Table 2). However, all labelled trees became infested during the monitoring period, and mortality increased rapidly between 1993 and 1994 (Table 2) showing a similar pattern of tree death as the overall stand (Fig. 6). Mean numbers of *O. insignis* per 20 cm branchlet on severely infested trees peaked at over 3000.

The causative link between infestation by *O. insignis* and death of gumwood trees was visually obvious at the site, and is supported by data: the mean percentage of canopy infested with *O. insignis* on trees that were killed was significantly higher than on surviving trees (Fig. 7).

Data from trees in plots A and B, that were initially severely or moderately infested, were combined in Fig. 7. Data from the initially uninfested trees were excluded because *O. insignis* numbers mostly remained low. However, the numbers of *O. insignis* on the initially uninfested trees rose during the monitoring period, and then fell towards the end (as in initially severely and moderately infested trees).

Table 2. Cumulative mortality of gumwood trees at sites A and B in Peak Dale. There were 30 labelled trees, with five in each initial infestation category at each site. Dates were simplified to show numbers of trees dead at the end of 6-month periods, plus the final sampling date in February 1995. Data from Fowler (2003).

Initial Infestation Category	Site	June 1993	December 1993	June 1994	December 1994	February 1995	Total % Mortality
Severe	A	0	4	4	4	4	80%
	B	0	0	2	2	3	60%
Moderate	A	0	0	3	3	3	60%
	B	0	0	0	0	0	0%
Uninfested	A	0	0	0	1	1	20%
	B	0	0	0	0	0	0%
Total dead trees		0	4	9	10	11	37%



Figure 6. Mean (+/- SE) percentage of the tree canopy severely infested by *O. insignis* from 1993 to 1995. Trees that died had higher mean % canopy infestations by *O. insignis* than trees that survived ($t = 4.62$, $df = 18$, $P < 0.001$, SYSTAT [SPSS 1997]). Initially uninfested trees were excluded as only one died. Data from Fowler (2003).

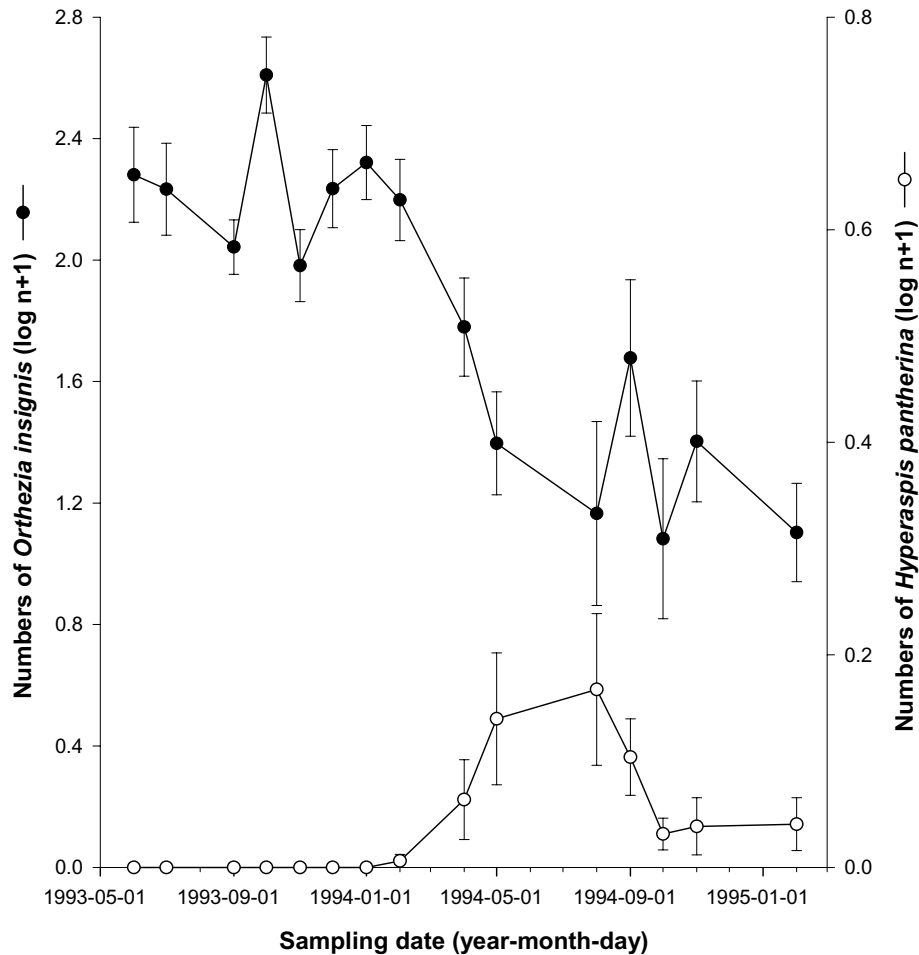


Figure 7. The mean numbers of *O. insignis* and *H. pantherina* on the labelled shoots of initially severely and moderately infested gumwood trees at Peak Dale. Error bars show the standard error for each mean, calculated on log-transformed data.

Data in Fig. 7 show an approximately 30× reduction in mean scale numbers per 20 cm branchlet, from >400 adults and nymphs (in September 1993) to <15 (in February 1995) when sampling ceased. The reduction in *O. insignis* populations was probably greater than this because monitoring staff overestimated scale numbers as the predator became common; in the field it was difficult to distinguish live scales from dead and partially consumed scales.

Numbers of *H. pantherina* counted on the labelled shoots were highly variable and usually low. High mean *H. pantherina* numbers were recorded occasionally on individual trees, in one case peaking at 1.3 adults and 3.4 larvae per 20 cm branchlet. The mean numbers of *H. pantherina* increased from January to August 1994, coinciding with the only consistent decrease in the mean numbers of *O. insignis* (Fig. 7). Earlier decreases in scale numbers might also have coincided with increases in predator numbers, but at this stage predator numbers were too low to be detected in the monitoring program.

Other herbivores detected in the monitoring program were limited to occasional lepidopteran larvae and mealybugs (*Pseudococcus* spp.). Few predatory arthropods, other than *H. pantherina*, were recorded: eggs of *Chrysoperla* species (Neuroptera: Chrysopidae) were

found several times on gumwood shoots infested with *O. insignis*, but there was no evidence that the larvae were attacking the scale; one adult *Cheilomenes lunata* (F.) (Coleoptera: Coccinellidae) was collected from gumwoods infested with *O. insignis*, but did not attack *O. insignis* in confinement.

The relict natural population of St. Helena gumwoods, and probably related endemic species, were under severe threat from the alien scale insect, *O. insignis*. The number of dead gumwood trees increased exponentially from 1991 to 1993–4, and if this trend had continued, all the gumwoods in the two main stands would have been killed by 1995 (Fig. 5). Though speculative, this prediction is supported by the appearance of the gumwoods in February 1995: the foliage of most surviving trees in both sites showed very substantial blackening from sooty molds indicating that scale populations had been high and extensive (Fig. 8). There was also direct evidence from the abundance of exuviae and partly-predated scales that the densities of *O. insignis* had been high. It appeared that biological control had been successful only just in time to save most of the trees. The gumwoods were showing signs of recovery from the outbreak of *O. insignis*, with new growth appearing that was uninfested by the scale.

A number of factors contributed to the severity of the threat to the gumwoods from *O. insignis*. There were large numbers of alternative host plants for *O. insignis*, such as *L. camara*, around both sites. Hence, a decline in the numbers and/or health of the gumwoods was un-

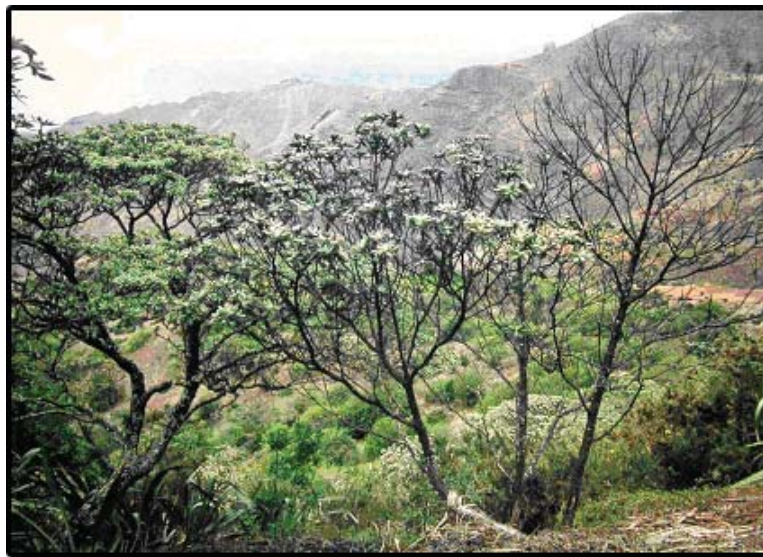


Figure 8. Surviving and dead gumwoods at Peak Dale in 1995. The central tree has uninfested regrowth emerging from leaves that are blackened by sooty molds. UGA1390011

likely to have limited scale numbers sufficiently to save any of the gumwoods at Peak Dale. It was also unlikely that existing predators or parasitoids on St. Helena would have suppressed *O. insignis*, as there was no sign of any mortality from natural enemies other than *H. pantherina* during the 2-year study. Finally, the gradual spread of *O. insignis* onto all of the initially uninfested gumwood trees, and the blackened appearance of most trees in 1995, gave little indication that any trees were less susceptible to the pest, and hence might have survived the outbreak.

Although the data in this study are only correlative, the success of *H. pantherina* as a biological control agent for *O. insignis* on St. Helena is consistent with its past record in Hawaii and Africa (Booth *et al.* 1995). Since 1995 there have been no further problems with *O. insignis* reported from St. Helena. Restoration projects, to encourage natural gumwood regeneration by controlling weeds in and around the two relict stands, can now proceed. These projects were considered pointless unless biological control of *O. insignis* was achieved (T. Upson, pers. comm.). A program to establish a millennium forest of gumwoods on a previously wooded site on the island began in 2000. Molluscs and lepidopteran larvae cause minor pest problems on these young trees, but *O. insignis* has not been noticed (I. Peters, pers. comm.), providing further evidence that the scale is under satisfactory biological control. The introduction of *H. pantherina* to St. Helena provides a particularly clear, quantitative study where the field population of a rare endemic plant was likely saved from extinction by biological control of an alien insect pest. Conservation benefits from biological control need to be considered in the current debate on the harm that introduced biological control agents can do to indigenous species (Howarth 1983; Louda *et al.* 1997; Simberloff and Stiling 1996)

ACKNOWLEDGEMENTS

G. Forrester provided statistical advice, and K. Alders helped sort the raw data. The Agriculture and Forestry Department (A & F), St. Helena, helped with many aspects of the project. A. Hill (A & F) managed the monitoring program. A. Cross, R. Mitchell and R. Shaw (CAB International) developed rearing methods in the UK, under license from the Ministry of Agriculture, Fisheries and Food (licence PHF 873A/667/73). A. Cross took the photographs for Figs. 3 and 4. P. Markham and I. Bedford (John Innes Institute) provided the culture of *O. insignis*. Funding was provided by the UK Government (ODA project OMC 92/94 349/053/001A).

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EVALUATING RISKS OF INTRODUCING A PREDATOR TO AN AREA OF CONSERVATION VALUE: *RODOLIA CARDINALIS* IN GALÁPAGOS

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ABSTRACT

Conservation areas can be highly susceptible to alien species introductions, and therefore extra care should be taken to assess potential interactions between a biological control agent and its new environment. In the Galápagos Islands, biological control was first considered to mitigate the threats posed to flora and specialist phytophagous Lepidoptera by the invasive scale *Icerya purchasi* Maskell (Hemiptera: Margarodidae). *Rodolia cardinalis* Mulsant (Coleoptera: Coccinellidae) was selected as a candidate agent, but a risk assessment that included prey range studies was considered necessary to evaluate the threats to Galápagos biota. In this paper important considerations for assessing the potential impacts of introducing an insect predator into a vulnerable ecosystem and for defining test procedures are discussed. These include the assessment of potential ecological impacts at different trophic levels, criteria for identifying and ranking non-target species, and the use of the precautionary principle for assessing acceptable levels of risk. Limitations encountered in conducting tests are also discussed, such as test species availability and the influence of host volatiles.

INTRODUCTION

The implementation of a biological control program of a pest of conservation importance will often involve liberating agents into areas of high conservation value. These may be habitats of threatened populations that are highly susceptible to the introduction of alien species. Island ecosystems, because of their isolation and late colonization, are especially sensitive to invasion because of an underrepresented, disharmonic and genetically impoverished biota that has developed few strategies to deal with alien species (Elton 1958; Loope *et al.* 1988). Biological control in areas of conservation importance therefore requires careful deliberation to evaluate potential ecological impacts of introducing a new exotic species. Release of a biological control agent should proceed only if non-target effects are shown to be minimal or highly improbable in comparison to the damage inflicted on native species by the target pest.

The Galápagos archipelago, a UNESCO world heritage site and biosphere reserve, is under threat from introduced species. A recent inventory reported 463 introduced insects (Causton *et al.*, unpubl. data). One of the most serious of these species is the invasive scale *Icerya purchasi* Maskell (Hemiptera: Margarodidae) which threatens both the endemic flora

and specialist phytophagous Lepidoptera that feed on them (Causton 2001, 2003a; Roque-Albelo 2003). To mitigate its impacts, biological control was considered for the first time in Galápagos. The much-used coccinellid predator, *Rodolia cardinalis* Mulsant (Coleoptera: Coccinellidae), was selected as a potential control agent. Although renowned for its narrow prey range elsewhere, this was unconfirmed and a risk assessment that included prey range studies was carried out to determine if its introduction would harm the Archipelago's indigenous biota (Causton 2004; Causton *et al.* 2004).

Using *R. cardinalis* as an example, this paper discusses the procedures used to evaluate the risk of introducing a predator into a vulnerable ecosystem and includes important considerations for: 1) assessing potential ecological impacts; 2) identifying criteria for selecting non-target species; 3) conducting tests; and 4) assessing acceptable levels of risk for introducing a biological control agent.

POTENTIAL ECOLOGICAL INTERACTIONS

Areas of conservation value are often ecologically complex compared with human-modified environments and the introduction of a biological control agent and removal of the prey species in such a system is likely to have consequences (Louda *et al.* 2003; Strong and Pemberton 2001). Predators such as *R. cardinalis* are high in the food chain and the outcome of their feeding and related population explosion and declines could impact the food web widely. Furthermore, both larvae and adults may be voracious feeders and may have high dispersal and searching abilities increasing potential encounters with non-target species. Because of this, risk assessments should consider not only the direct impacts of feeding on non-target species, but also other interactions that might occur, and, in response to these, appropriate research methods should be designed to quantify risk. Interactions that might take place with the introduction of a predator such as *R. cardinalis* are shown in Fig. 1 and are discussed below.

INTERACTIONS WITH LOWER TROPHIC LEVELS

A predator may feed on and cause the decline of species other than the target prey in the proposed region of introduction, particularly when the target prey is scarce or when the predator disperses to areas outside the distribution of the target prey. Species at highest risk will be those closely related to the preferred prey of the control agent or species that overlap ecologically. Non-target species may be at greater risk of exposure if the agent is not efficient at controlling the target prey but is maintained in high numbers (Holt and Hochberg 2001). On the other hand, some interactions might be beneficial to both the biological control agent and a non-target species; such as an insect defended from natural enemies and/or transported to new locations, or a plant pollinated by the control agent. The consequences of such synergistic interactions for indigenous biota could be negative if they increase the fitness of another introduced species (Simberloff and Von Holle 1999).

For example, predators that feed on pollen or nectar may compete with insect pollinators. Although not considered a risk group in Galápagos (Causton 2003a), pollinating species in other conservation areas may be impacted if they specialize on threatened plant species

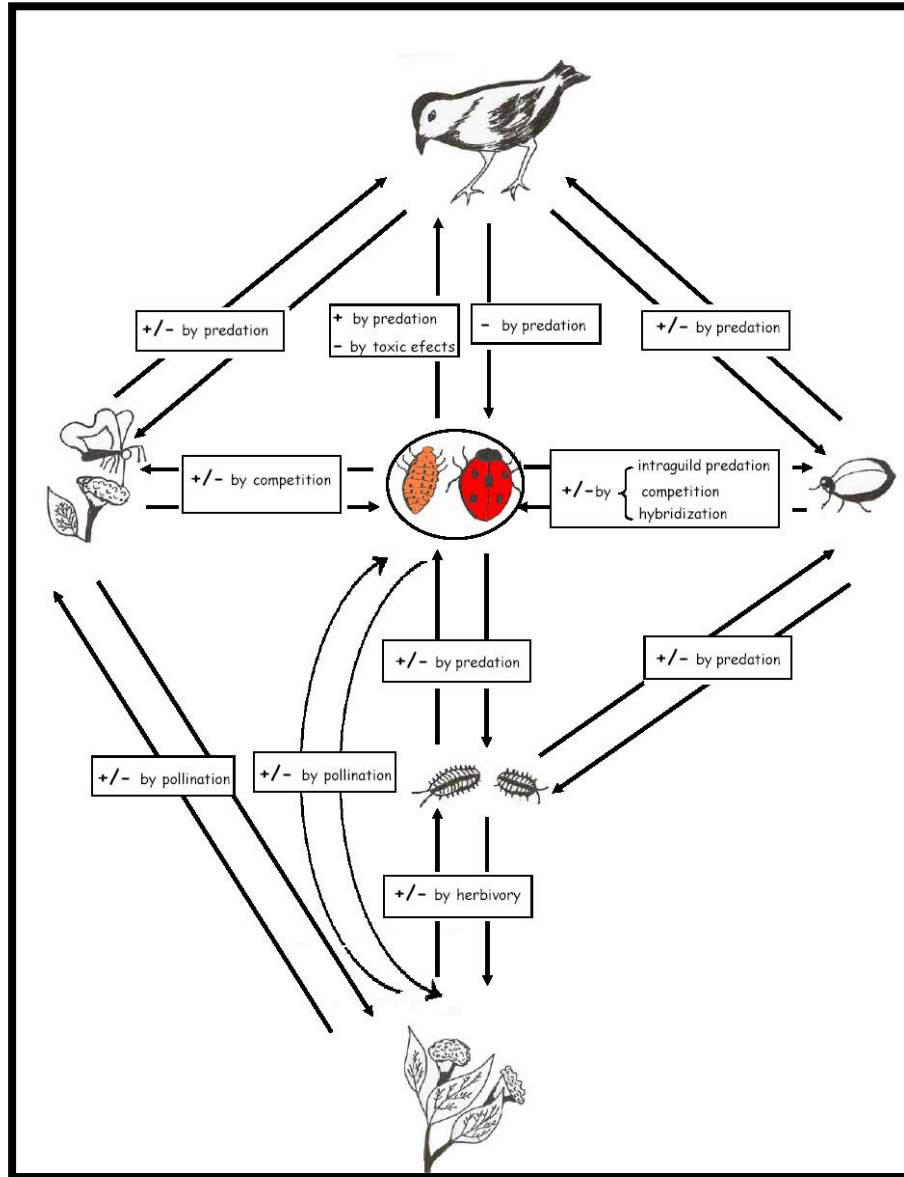


Figure 1. Overview of potential interactions between an introduced predator and its new environment. Introduced predators may remove or reduce resources such as food and nesting sites from other invertebrate species (e.g., Dixon 2000; Obrycki *et al.* 2000).

with small populations. Some coccinellids have been shown to deter parasitoids from ovipositing purely by their presence near the prey (Dixon 2000). On the other hand, the presence of a predator may benefit other predators by making a prey species more accessible (Charnov *et al.* 1976). Another potential negative result of a species introduction is hybridization with a species of conservation value or another biological control agent (causing extinction or a decline in fitness through the production of sterile hybrids), or with an introduced species (causing increased fitness which in turn may affect indigenous species).

INTERACTIONS WITHIN THE SAME TROPHIC LEVEL

Species at the same trophic level can also be directly impacted by feeding (intraguild predation). For example, coccinellids can feed on conspecific and heterospecific ladybirds, other predators and parasitoids (see Dixon 2000). In the laboratory, larvae of *R. cardinalis* have been observed to kill and or displace larvae of *Rodolia iceryae* Janson (Coleoptera: Coccinellidae), even when target prey were available (Mendel and Blumberg 1991). Ultimately, the outcome of the interaction between predators will depend on their size, aggressiveness, defense mechanisms, and whether the species is a top or primary predator (Dixon 2000). In Galápagos, the only native predator of *I. purchasi* is the lacewing, *Ceraeochrysa cincta* Schneider, (Neuroptera: Chrysopidae), which was not considered at risk because it was found to attack both larvae and adults of *R. cardinalis* in captivity (Causton *et al.* 2004). On the contrary, the foraging behavior of *R. cardinalis* in Galápagos could be affected by the presence of this lacewing as well as by introduced species of ant that defend *I. purchasi* in return for honeydew.

The consequences to biota of removing the target prey should also be considered. In Galápagos, negative effects on indigenous species were improbable because there were few species (one lacewing and some bird species) that fed on *I. purchasi*, and all of these were generalist predators that did not rely exclusively on the target prey. In contrast, a positive response was expected because the removal of the target prey would eliminate an important food source for two species of invasive wasp and the invasive fire ant, *Wasmannia auropunctata* Roger (Hymenoptera: Formicidae).

INTERACTIONS WITH HIGHER TROPHIC LEVELS

Insectivores at higher trophic levels may benefit directly from the abundance of the predator (as a food source in its own right) producing either positive or negative spillover effects on other species down the food chain (e.g., Holt and Hochberg 2001; Pearson and Callaway 2003). Hoddle (2004) refers to this as natural enemy subsidization of food webs. Alternatively, a decline or a change in behavior in species at higher trophic levels could occur as a direct result of feeding. Because some coccinellids produce toxins as a means of defense (Dixon 2000; Marples *et al.* 1989), insectivorous vertebrates were considered at risk from the introduction of *R. cardinalis*. Vertebrates may also be affected if the introduced biological control agent competes for the same food.

In conclusion, detrimental impacts are normally perceived as effects that negatively change the status of indigenous biota. However, interactions that increase the fitness of an introduced species should also be of concern because they may also have an impact on the ecosystem. In the long-term, any of these interactions could lead to species displacement, alteration of community structure and dynamics, and disruption of natural evolutionary patterns. Although it will not be possible to test all the potential interactions, prioritization of potential impacts according to their likelihood should be carried out to identify non-target species at risk and assess the safety of the biological control agent.

CRITERIA FOR IDENTIFYING NON-TARGET SPECIES POTENTIALLY AT RISK

To best evaluate non-target species at risk, a thorough understanding of the biology of the predator and target prey is essential and may involve studies in their native range (budget permitting) or introduced range, as well as in the laboratory. Research topics should include: 1) phylogeny, 2) feeding range, 3) stimuli that prompt foraging and host location (such as specific plant and insect chemicals and morphological features), 4) habitats occupied, 5) geographical range and climatic tolerance, 6) phenology, and 7) place in the food web.

It should not be assumed that the potential non-target impacts of a predator are only restricted to the geographical extent of the target prey. Strong flying species and/or species that are wind or water dispersed may reach areas outside that range. Furthermore, humans may assist spread. For example, *R. cardinalis* is adapted to a wide range of climatic regimes and adults are strong fliers suggesting that it would be able to reach and adapt to most parts of Galápagos if food were available.

Criteria that we used for *R. cardinalis* (Causton 2004; Causton *et al.* 2004) and that might apply for other predators are listed below:

- **Species closely related to *I. purchasi* or the Margarodidae.** Centrifugal testing (Wapshere 1974), used for weed and arthropod biological control agents, assumes that the closer the species is taxonomically to the target pest, the more likely it is to be attacked.
- **Species previously reported as prey for any *Rodolia* species.** Because coccinellids that prey on scales are known to exhibit restricted feeding ranges (Dixon, 2000), the feeding habits of congeners were also considered to be a useful indicator of the potential feeding range of *R. cardinalis*.
- **Species morphologically or physiologically similar to *I. purchasi*.** Olfactory and visual cues such as wax filaments produced by scale insects are often necessary to prompt coccinellid foraging and oviposition (Dixon, 2000; Merlin *et al.* 1996). We assumed that such prey characteristics would influence prey selection by *R. cardinalis* in Galápagos.
- **Species that live in close proximity to the prey of *R. cardinalis*.** The greater the host plant range of the target prey the greater the number of species interactions. Species of insects, in particular, Homoptera or endangered insects, were considered to be at risk if they occupied niches close to *I. purchasi*. Furthermore, natural enemies that fed either on the pest *I. purchasi*, or on other taxa identified as potential prey of *R. cardinalis* were also considered to be at risk due to competition or intraguild predation.
- **Species of conservation value that might interact with *R. cardinalis* in other ways.** For example, insectivorous vertebrates that feed on coccinellidae.

RANKING NON-TARGET SPECIES

Prioritization is an important tool when a large number of non-target species have been identified, or as in our case, when information about the non-targets was sparse and field collections were limited by budget constraints. Species had to be ranked according to conservation priority and/or importance as an indicator of the prey range of *R. cardinalis*. For our purposes, host plant distribution was often used as an indicator of the distribution and abundance of phytophagous non-target species because more information is available on the Galápagos flora. Species of highest priority were the species endemic to a single island and specialized feeders with a small host range, especially those that are closely related to *I. purchasi* or in genera reported as prey, or those that feed on rare plant species that are attacked by *I. purchasi*. An endemic, but subterranean margarodid, *Margarodes similis* Morrison (Hemiptera: Margarodidae) was also considered a priority for testing because it is the closest species phylogenetically to *I. purchasi*.

IMPORTANT CONSIDERATIONS FOR CONDUCTING TESTS

Prey range tests form the bulk of a risk assessment allowing one to assess some of the direct and indirect impacts that could occur with the introduction of a biological control agent. This is the traditional focus of testing. However, other interactions such as competition may also require testing experimentally, which will depend on the potential ecological interactions identified. The efficacy of the agent in controlling the target pest should also be studied to reduce the risks of spillover and other indirect effects (see Hoddle 2004; Holt and Hochberg 2001).

The order in which studies are conducted and species tested will also influence the type of tests that need to be carried out. By defining the prey range of the predator first, one can better identify the species that might be affected (by niche overlap, intraguild predation, or competition) and thus reduce the number of species that need to be tested.

Important considerations for designing tests for *R. cardinalis* and other predators are listed by Causton (2004). Some of the salient points for ensuring the success and accuracy of the tests are listed below.

TESTING CORRECT BIOTYPE

The prey range and behavior of geographically distinct predator populations may vary (Phillips *et al.* 2002; Sands and Van Driesche 2004), and as a precaution, tests should be conducted on the biotype that will be introduced and future introductions restricted to the same biotype.

SELECTION OF PREDATOR STAGES FOR TESTING

Predator stages that need to be tested will depend on the biology of the predator. For example, both *R. cardinalis* adults and larvae are vagile entomophages and required separate tests to determine feeding on non-targets. On the other hand, neonate larvae were tested to determine life cycle development because adult oviposition was shown to be an unreliable parameter (Causton 2004).

CONDITION OF PREDATOR STAGES USED IN TESTS

Several factors may influence the test outcome and should be considered:

1. *Prior feeding experience.* Naïve, unfed individuals may feed on a test species that is rejected by individuals that have fed previously on the target prey. Our studies did not show any behavioral differences but other studies suggest that some predators may be conditioned to feeding on a preferred prey (e.g., Rayor and Munson 2002).
2. *Hunger levels.* Satiated individuals often do not respond quickly to prey, while naïve (unfed) individuals may become weak and therefore uninterested in feeding if not tested immediately. Mature individuals are less likely to feed.
3. *Reproductive status.* Mated individuals may behave differently to non-mated individuals.
4. *Fitness of test individual.* Crowded rearing conditions produce smaller and less fecund individuals that are less likely to eat and develop normally (e.g., Booth *et al.* 1995).

CHOICE OF PREY LIFE STAGES AND HOST PLANT

The prey life stage that is tested should reflect the life stage of the predator. For example, neonate predator larvae may be unable to feed on tougher later instars.

The host plants of the non-target test species should also be considered carefully because they may affect the predator's choice of prey. Phytophagous insects can sequester alkaloids from some host plants that deter predation or predator development (e.g., Mendel and Blumberg 1991; Mendel *et al.* 1992). Host plant volatiles and plant defenses, and the condition of the plant may also influence behavior by attracting or deterring predators (e.g., de Boer and Dicke, 2003; Eisner *et al.* 1998; Palmer 1999).

To reduce these effects we recommend:

1. preliminary tests be run to evaluate predator feeding on target prey reared from as wide a range of plants as possible,
2. where possible, more than one food plant be used for testing a non-target species, and
3. plants with alkaloids, trichomes or pronounced pubescence be avoided in tests.

TEST ENVIRONMENT AND TEST TYPE

The test environment should not be too small to disrupt host location cues or too big making host location impossible.

Test designs should accurately reflect the physiology and behavior of both the control agent and the test species, be standardized and well replicated. Experimental controls should be used in all trials. Both positive controls (target prey) and negative controls (no food) are recommended to understand better the response of the agent to a test species. No-choice tests were considered to be the most appropriate for testing *R. cardinalis* because they allowed us to quickly determine whether a test species was an acceptable prey, and avoided the risk of

contamination with target prey semiochemicals. Other options for testing predators are summarized by Van Driesche and Murray (2004).

TEST SPECIES AVAILABILITY

- 1. Substituting near relatives to confirm general patterns.** Because it was hard to locate some priority species for testing, our field collections were sometimes opportunistic using a find and test approach. Testing alternative species (including introduced species) in the same genus or family as those non-target species that could not be located allowed us to test a greater number of species. The rationale used here was that as long as we could define the prey range of *R. cardinalis*, it did not matter if we could not find all the non-target species desired for testing. This approach has been used for testing the host range of other candidate agents and is considered a solution for analyzing the risks of the agent to rare or endangered species (Barratt 2004; Coombs 2004).
- 2. Acceptability of using field-collected versus laboratory-reared test species.** Rearing non-target species in the laboratory was not feasible in Galápagos because: 1) little information was available about the biology/phenology of the non-target species; 2) techniques for growing host plants of phytophagous insects were few, and 3) there was insufficient infrastructure to rear species from other islands under quarantine conditions. By using field-collected specimens we were able to test a wide range of species. Testing field-collected material was deemed acceptable because few Galápagos Homoptera seemed to have parasitoids or pathogens (except for aphids), which could alter the feeding behavior of the biological control agent. Rearing non-target species in organandy sleeves in the field has been suggested as a possible alternative but would only be feasible in areas within easy reach (Van Driesche, 2004). We also found that this ad hoc method of prey collection made coordinating test species collection with predator rearing difficult. In addition to this, the repetition of experiments was not guaranteed unless the test species was available year round. In retrospect, it would have been better to attempt to rear at least the high priority test species in the laboratory to simulate field conditions better.

71

WHAT IS AN ACCEPTABLE LEVEL OF RISK?

In any risk assessment for biological control two questions will arise: 1) what types of interaction between a biological control agent and the ecosystem are likely and what is acceptable? 2) How much research is necessary before a conclusion can be reached?

There is a general consensus that a biological control agent should not be released if it can complete its life cycle on non-target species of ecological or economic significance, but levels of risk of short-term feeding are not well defined. Moreover, the consequences of interactions other than feeding are rarely considered. Temporary foraging on non-target species is in some cases considered acceptable and necessary for sustaining population numbers of a biological control agent when its target prey population is low (e.g., Sands 1997; Sands and Van Driesche 2000). However, if species of conservation importance are implicated it is pref-

erable to apply the precautionary principle (Cooney 2003). In ecosystems such as Galápagos, short-term feeding and some of the other interactions listed earlier may have considerable impact on non-target species, especially on already threatened endemic species. Even common or non-endemic indigenous species all have some role in ecosystem dynamics and should be protected. Moreover, interactions that cause an increase in an introduced species that is already or could be damaging should be avoided at all cost. In a conservation context, short-term feeding should therefore only be acceptable if it involves a species that is of no value (i.e., introduced species), and this, only if the consequences of this interaction would have no indirect impact on the ecosystem.

Accurate prediction of all potential interactions between a biological control agent and species in the proposed area of introduction is difficult because of a lack of understanding of ecosystem structure and dynamics. Furthermore, even when risk species are identified it may be hard to find or rear them. Nevertheless, the goal of a biological control program should be to understand these interactions as much as possible and to introduce an agent that will interact least with the environment. It is clear that the more restricted the feeding and the ecological habitats of the biological control agent, the fewer the interactions that are likely to occur (Hoddle, 2004).

Determining an acceptable level of risk for introducing a biological control agent will ultimately depend on the immediate and long-term impacts of the target pest and the urgency of mitigating that threat. In Galápagos, evidence about the lack of risk of immature *R. cardinalis* to Galápagos fauna was conclusive (Causton 2003; Causton *et al.* 2004), but the potential impacts of adults could not be tested to the desired level because trials were limited by test species availability. The decision of whether research should continue in order to better understand the potential interactions of adult *R. cardinalis* with indigenous biota was guided by the precautionary principle and the perceived cost-benefit of releasing the biological control agent. Because key habitats and endangered plant species were being seriously affected by *I. purchasi*, the Galápagos National Park concluded that compared to the high costs of not controlling *I. purchasi* immediately, the potential negative effects of releasing *R. cardinalis* into the Galápagos environment would be minimal. The results of the risk assessment indicated that:

- *R. cardinalis* would be effective in controlling the target pest.
- Immature *R. cardinalis* were highly prey specific (demonstrated through tests on a wide range of species).
- A narrow prey range of adult *R. cardinalis* was suggested by feeding trials.
- *R. cardinalis* was distasteful to some vertebrates and should not affect insectivorous predators.
- Damaging interactions with other indigenous biota would be minimal. because of a restricted feeding range resulting in little niche overlap with other species.

Rodolia cardinalis was consequently released in 2002 and has since been liberated on eight islands (Causton 2003b). Monitoring results to date show a decline in *I. purchasi* num-

bers, recuperation in some plant species, and no interactions with non-targets. Longer term studies will reveal whether this species has had any negative impacts on this fragile ecosystem.

ACKNOWLEDGEMENTS

My thanks to Chris Buddenhagen, Heinke Jäger and Alan Tye for constructive comments on the manuscript. I would particularly like to thank Piedad Lincango for help with the figure. This project would not have been possible if not for the help of a long list of people the names of whom are available in Causton (2004).

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THE HORSE-CHESTNUT LEAF MINER IN EUROPE – PROSPECTS AND CONSTRAINTS FOR BIOLOGICAL CONTROL

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77

ABSTRACT

The horse-chestnut leaf miner, *Cameraria ohridella* Deschka and Dimic (Lepidoptera: Gracillariidae), is a moth of unknown origin that was first observed in Macedonia in the late 1970s. Since then, it has spread over most of Europe, causing permanent outbreaks on the European horse-chestnut, *Aesculus hippocastanum* L. (Sapindales: Sapindaceae), a major urban tree in Europe. The horse-chestnut is endemic to the Balkans where the few remaining

natural stands are also severely attacked, causing concern for the survival of this rare tree species. Classical biological control is considered as the only long-term control option, but shows two major constraints i.e. the fact that the origin of the moth is unknown, and the low number of specific species among the natural enemy complex of Gracillariidae. A collaborative programme has been set up to locate the area of origin of the moth, including the following methods: (1) studies on the parasitoid communities of *C. obridella* in the Balkans; (2) host tree screening tests; (3) surveys in the potential areas of origin, i.e. the Balkans, Asia and North America, with the help of pheromone traps; (4) molecular studies on *C. obridella* and congeneric species. If the area of origin is not found, the introduction of parasitoids of congeneric species from Asia or North America could be considered, provided parasitoids specific at genus level are found.

INTRODUCTION

The horse-chestnut leaf miner, *Cameraria obridella* is a moth of unknown origin that was first observed attacking the European horse-chestnut, *Aesculus hippocastanum* L. in Macedonia in the 1970's, and described as a new species in 1986 (Deschka and Dimic 1986; Simova-Tosic and Filev 1985). In 1989, it was found in Austria, from where it spread to most of Europe. Thanks to two to four generations per year, a high fecundity and the low impact of indigenous natural enemies (Freise et al. 2004; Girardoz and Kenis unpublished data; Grabenweger 2003; Grabenweger et al. 2005), *C. obridella* reaches outbreak densities a few years only after its arrival (Gilbert et al. 2004). Despite yearly fluctuations in population levels, no outbreak has ever collapsed, even in Macedonia after 30 years of permanent defoliation. Horse-chestnut is one of the most commonly planted shaded trees in European towns and cities. In regions where the pest occurs, the trees are defoliated before the end of the summer, year after years. Studies in Italy have shown that the pest does not affect the survival of the tree (Salleo et al. 2003). However, in more northern climates, *C. obridella* is suspected to cause the decline of horse-chestnut because defoliation induces a second flowering, decreasing frost hardness (Balder et al. 2004). Despite a low risk for the survival of the trees in urban areas, the aesthetic damage is so severe that some municipalities are already replacing this highly valuable tree by other species. It has been estimated that the replacement of 80% of the horse-chestnut trees in Berlin alone would cost about 300 million Euros (Balder and Jäckel 2003). The environmental impact of the pest may be even more serious. The horse-chestnut is endemic to the Balkans. The few natural stands remaining in Greece, Macedonia, Albania and Bulgaria are also severely attacked (Avtzis and Avtzis 2003; Grabenweger et al. 2005, Tomov and Kenis unpublished data). Studies have shown that, in these areas, the permanent outbreaks may hamper the regeneration process, causing concern for the survival of this rare tree species (Thalmann 2003). In addition, *C. obridella* is occasionally found attacking and developing on maple trees (*Acer pseudoplatanus* and *A. platanoides*), in which case damage levels may be as high as on horse-chestnut (Freise et al. 2003a; Hellrigl 2001). It cannot be ruled out that the damage on maple will increase with time, considering the constant pressure on the moth to find new suitable host trees when horse-chestnut trees are totally defoliated. The reasons for seeking control measures for this pest therefore relate to minimising the impact on planted ornamentals, to the conservation of the remaining natural stands and to minimising the chance of developing strains that may become serious pests of maples in Europe.

Control measures include the aerial spraying of diflubenzuron, the injection of systemic insecticides and the removal of dead leaves, in which pupae overwinter (Heitland *et al.* 2003; Kehrli and Bacher 2003). However, these methods are not sustainable. They are expensive, have to be repeated every year, and chemical treatments are not well perceived by the public. Therefore, biological control methods have been considered. Invasive leaf miners are notorious for being recruited by indigenous parasitoids in the region of introduction, which may result in the control of the pest (e.g., Digweed *et al.* 2003; Urbaneja *et al.* 2000). As expected, *C. ohridella* has been adopted by a whole complex of polyphagous parasitoid species (e.g., Freise *et al.* 2002; Grabenweger 2003; Grabenweger *et al.* 2005; Hellrigl 2001). Nevertheless, parasitism remains unusually low for a leaf miner, even at the type location 30 years after its arrival, and apparently of low incidence on moth populations (Grabenweger *et al.* 2005). Kehrli *et al.* (2005) have developed an ingenious system to augment parasitism at local scale. They stored dead leaves with overwintering moths in containers that, at emergence, allow the parasitoids to escape without their host. They observed increased parasitism rates at the experimental plots, but no effect on moth populations. This system is presently being improved and may, ultimately, provide an interesting method to be used in specific urban environments. Nevertheless, it will never provide a solution for natural horse-chestnut stands. In the long run, unless a native European natural enemy suddenly improves its capability of controlling the moth, the only sustainable solution to the *C. ohridella* problem will be the introduction of an exotic natural enemy, most likely a parasitoid. Classical biological control against *C. ohridella* shows potential, but also major constraints, the two main ones being the fact that the origin of the moth remains unknown, and the low number of specific species among the natural enemy complex of Gracillariidae. This paper reviews these two major constraints and describes collaborative efforts to resolve them.

REGION OF ORIGIN OF *C. OHRIDELLA*

Since the discovery of *C. ohridella* in Macedonia in 1984, the origin of the moth has been a matter of debate. It was first suggested to be a relict species that has survived the Ice Age with its host in southeastern Europe (Deschka and Dimic 1986; Grabenweger and Grill 2000). However, there are several arguments against this theory (Holzschuh 1997; Kenis 1997). Firstly, *C. ohridella* has shown high dispersal capacities in recent years (e.g., Gilbert *et al.* 2004). Thus, if the moth was native to the Balkans, why would it spread only now? Secondly, *Cameraria* is not a European genus. The 74 described, and many undescribed species all occur in North and South America and East and Central Asia (Grabenweger and Grill 2000). Thirdly, parasitism is unusually low for a leaf miner, and composed only of polyphagous species. Finally, since its first observation in Macedonia about thirty years ago, outbreaks in the region have continued unabated and recent surveys in natural stands of *A. hippocastanum* in Greece, Macedonia and Bulgaria have shown that these natural stands are as heavily attacked as planted trees in urban areas (Avtzis and Avtzis 2003; Grabenweger *et al.* 2005, Tomov and Kenis unpublished data). This situation characterizes an exotic rather than a native herbivorous insect. Hellrigl (2001) proposes another hypothesis that needs consideration and may explain some of the anomalies mentioned above. He suggests that the moth may have shifted from another host tree (e.g., an *Acer* species) in the Balkans or the Near East but does not provide conclusive evidence for this hypothesis.

In recent years, various activities have been carried out to help locating the area of origin of the moth. These include (1) studies on the parasitoid complex of the moth in the Balkans; (2) screening tests to assess the host range of *C. obridella*; (3) surveys in potential regions of origin of the moth; (4) molecular studies on *C. obridella* and other *Cameraria* spp. The present state-of-the-art of these studies is briefly described herein.

STUDY OF THE PARASITOID COMPLEX OF THE MOTH IN THE BALKANS

Parasitism of *C. obridella* in Europe has been abundantly studied (e.g., Freise *et al.* 2002; Grabenweger 2003; Hellrigl 2001) and the low parasitism rates and lack of specific parasitoids have since long been used as an argument in favor of a non-European origin of the moth (e.g., Holzschuh 1997; Kenis 1997; Pschorn-Walcher 1994). However, until recently, all these studies on parasitism had been made in invaded areas whereas no data existed on the parasitoid complex in natural stands in the Balkans, where the moth is supposed to have originated. Thus, we conducted surveys for parasitism in these habitats and compared them with data from plantations in the Balkans and Central Europe (Grabenweger *et al.* 2005). The parasitoid complexes are summarized in Table 1. We found marked differences neither in the parasitoid complexes nor in the parasitism rates between natural and artificial stands. Furthermore, all species found during this study were polyphagous parasitoids of various leaf miners. The major difference between the Balkans and the rest of Europe is the prevalence of the eulophid pupal parasitoid *Pediobius saulius*, the dominant species in artificial and natural stand in the Balkans. In Central and Western Europe, it is a common parasitoid of other leaf miners but rarely attacks *C. obridella*.

80

HOST RANGE SCREENING TESTS

Screening tests were carried out to assess the present or potential host range of *C. obridella*. No-choice tests in field and laboratory cages and open-field tests were carried on *Aesculus* spp. and *Acer* spp. Oviposition rates and development successes were observed (see Freise *et al.* 2003a; 2003b for methods). Eleven out of the 13 world *Aesculus* species and many hybrids and *A. hippocastanum* cultivars were screened by Freise *et al.* (2003a,b) (Table 2). The two most suitable hosts were *A. hippocastanum* and the Japanese horse-chestnut *A. turbinata*, whereas successful development also occurred on the American species *A. glabra*, *A. sylvatica* and *A. flava* (= *A. octandra*). In contrast, it did not develop successfully on the Asian *A. chinensis*, *A. assamica* and *A. indica* and on the American *A. pavia*, *A. californica* and *A. parviflora*. *A. X carnea*, a hybrid of *A. hippocastanum* and *A. pavia*, often planted in European cities, is also unsuitable for development (Freise *et al.* 2003a,b).

Screening tests on *Acer* spp. are still on-going. Twenty-six European, Asian and North American species have been preliminary screened in non-choice tests in field and laboratory cages. *C. obridella* oviposited on most of the species but, in general, larvae died in the early stages. Larvae developed successfully in the North American *A. circinatum* and, occasionally, in the European *A. pseudoplatanus*, *A. tataricum* and *A. heldreichii*, and the Asian *A. japonicum* (Heitland and Schlinsog, unpublished data). For unknown reasons, larvae usually failed to pupate. Strong intra-specific variations were observed with several *Acer* species, i.e. larvae developing well in some individual trees and not at all in others. Similar observations

Table 1. Parasitoids of *C. ohridella* found in the Balkans in natural horse-chestnut stands and plantations (Grabenweger *et al.* 2005) and in Austria and Switzerland by the same investigators (Girardo, Kenis and Quicke, submitted; Grabenweger 2003; Grabenweger and Lethmayer 1999). X = rare; XX = common; XXX = dominant.

	Balkans- Natural stands	Balkans- Plantations	Austria- Switzerland
Eulophidae			
<i>Baryscapus nigroviolaceus</i> (Nees)	XX	XX	X
<i>Chrysocharis nephereus</i> (Walker)	XX	XX	XX
<i>C. pentheus</i> (Walker)		X	X
<i>C. phryne</i> (Walker)	XX		
<i>Cirrospilus elegantissimus</i> Westwood		X	
<i>C. pictus</i> (Nees)			X
<i>C. variegates</i> (Masi)		X	
<i>C. viticola</i> (Rondani)	XX	XX	X
<i>C. vittatus</i> (Walker)		X	X
<i>C. talitzkii</i> (Boucek)	X	X	
<i>Closterocerus lyonetae</i> (Ferriere)	X	X	
<i>C. trifasciatus</i> Westwood	XX	XX	XX
<i>Elachertus inunctus</i> Nees	X	X	
<i>Minotetrastichus frontalis</i> (Nees)	XX	XX	XXX
<i>Neochrysocharis chlorogaster</i> (Erdos)	X	X	
<i>Pediobius saulius</i> (Walker)	XXX	XXX	X
<i>Pnigalio agraulis</i> (Walker)	X	XX	XX
<i>P. pectinicornis</i> (L.)	X	X	X
<i>Sympiesis sericeicornis</i> (Nees)		X	X
Eupelmidae			
<i>Eupelmus urozonus</i> (Dalman)	X	X	X
Pteromalidae			
<i>Pteromalus semotus</i> (Walker)	X	X	X
Braconidae			
<i>Colastes braconius</i> (Haliday)	X		X
Ichneumonidae			
<i>Itoplectis alternans</i> (Gravenhorst)			X
<i>Scambus annulatus</i> (Kiss)	X	X	X

are made in the field in Europe. Most maple trees are apparently resistant to *C. obridella*, but fully-grown mines are occasionally found on *A. pseudoplatanus* and, rarely, on *A. platanoides*, in which case the tree is usually heavily attacked. Further screening tests are presently being continued with the same and other *Acer* species.

SURVEYS

Surveys have been carried out in potential regions of origin. Two methods have been used: visual inspections of potential host trees and set up of pheromone traps. Surveys have been primarily focused on *Aesculus* spp., but other trees have been inspected to collect *Cameraria* spp. for molecular studies (see below). The regions and *Aesculus* spp. that have been investigated (as in April 2005) are listed in Table 2. At least 13 *Aesculus* spp. are clearly identified in the world, five in Asia, seven in North America and one in Europe. Six additional species have been described from China, but they are nearly indistinguishable from previously described species and their recognition remains tentative (Xiang *et al.* 1998). Most of the 13 *Aesculus* spp. have already been inspected, albeit not all extensively. *C. obridella* was not found, but other *Cameraria* spp. were collected on *A. turbinata* in Japan, *A. flava* and *A. parviflora* in U.S.A. (M. Kenis, G. Grabenweger and C. Lopez Vaamonde, unpublished data).

Surveys were also made in Greece and Bulgaria, in regions where *A. hippocastaneum* is endemic, to check for the presence of *C. obridella* on *Acer* spp. The following *Acer* species were inspected: *A. heldreichii*, *A. hyrcanum*, *A. monspessulanum*, *A. obtusatum*, *A. platanoides*, *A. pseudoplatanus* and *A. tataricum*. No mines were found on any *Acer* species. A network of pheromone traps placed in Greece on *Acer* spp., far from infested horse-chestnut trees, did not catch any *C. obridella* (N. Avtzis, unpublished data).

MOLECULAR STUDIES

Molecular analyses may help locating the area of origin of *C. obridella* in various ways. Firstly, the genetic variability of European populations can be assessed to describe the phylogeography of the moth in Europe and, especially, to evaluate if European populations come from a single introduction. In other words, a genetic homogeneity in European populations would suggest a non-European origin. Preliminary analyses using isozymes (Perny, 1997) and RAPD-PCR (Kovács *et al.* 2000) showed very little variability among central European populations. However, investigations using additional genetic methods (i.e., AFLPs) and including populations from natural horse-chestnut populations in the Balkans are needed before drawing firm conclusions regarding the genetic variability of European populations.

Molecular techniques can also be used to reconstruct the phylogeny of the genus *Cameraria*. Knowing where, and on which host tree the sister species of *C. obridella* occurs would be of tremendous help in the search for the area, and tree of origin of the pest. Lopez Vaamonde *et al.* (2003) studied the evolution of host plant use of *Phyllonorycter*, the genus most closely related to *Cameraria*. They used the molecular phylogeny of the moths to reconstruct the ancestral host use and different host switches. The main result was that closely related *Phyllonorycter* species often feed on closely related host plant species, regardless of their geographic distribution. This is interpreted as due to host switching (colonization followed by speciation) being more likely to happen amongst phylogenetically related plants. A

Table 2. Visual surveys and pheromone trapping for *Cameraria* spp. on *Aesculus* spp. (M. Kenis, G. Grabenweger and C. Lopez Vaamonde, unpublished data) and host suitability for *C. ohridella* in screening tests (Freise *et al.* 2003a,b). Only the 13 traditional *Aesculus* spp. of Hardin (in Xiang, 1998) are mentioned here.

<i>Aesculus</i> sp.	Region	Level of Surveys	Pheromone Trapping	Host Suitability ³	<i>Cameraria</i> sp. Found ¹
<i>A. turbinata</i>	Japan	high	yes	yes	yes
<i>A. wilsonii</i>	Central China	medium	yes	not tested	no
<i>A. chinensis</i>	China	medium	yes	no	no
<i>A. indica</i>	Himalaya	high	yes	no	no
<i>A. assamica</i>	South-East Asia	none	no	no	-
<i>A. flava</i>	Eastern USA	medium	yes	(yes)	yes ²
<i>A. glabra</i>	Eastern & Central USA	low	no	yes	no
<i>A. parviflora</i>	Eastern USA	high	no	no	yes ²
<i>A. sylvatica</i>	Eastern USA	none	no	(yes)	-
<i>A. pavia</i>	Eastern USA	medium	no	no	no
<i>A. parryi</i>	Baja California	medium	no	not tested	no
<i>A. californica</i>	California	high	no	no	no
<i>A. hippocastanum</i>	South-East Europe	high	yes	yes	yes

¹*Cameraria* sp. found during visual surveys. No *Cameraria* sp. has been caught with pheromone traps.

²It is not yet clear whether the same *Cameraria* sp. was found on *A. flava* and *A. parviflora*. *Cameraria aesculisella* is known from *A. flava* and *A. glabra*.

³Host suitability: yes = at least 20% of *C. ohridella* reaching the adult stage; (yes) 5-20% reaching the adults stage; no = less than 1% of the mines reaching the adult stage.

similar phylogenetic study is presently being carried out with the genus *Cameraria*. The nuclear gene 28S rDNA (D1-D3 expansion regions) is used to reconstruct a combined molecular phylogeny at species level. Over 30 species have already been collected both in North America (U.S.A. and Mexico) and Asia (Japan and China) on several tree and shrub species, including *Aesculus* spp. and *Acer* spp. If *Cameraria*'s host range shows a similar phylogenetic conservatism as *Phyllonorycter*, we can expect a molecular phylogeny where *Cameraria* species cluster according to their host plant group. Thus, if *A. hippocastanum* is the original host plant of the latter moth species and not the result of a recent colonization event, we would expect to obtain a clade of *Aesculus*-feeding *Cameraria* spp. including *Cameraria ohridella*. The analyses are presently on-going, but preliminary results show that *C. ohridella* is genetically very distinct from two Asian and North American *Cameraria* spp. collected on *Aesculus* spp. In contrast, it clusters with another species, probably *Cameraria nipponica* Kumata, feeding on *Acer palmatum* and *A. japonicum* in Japan and *A. pseudosieboldianum* in China.

DISCUSSION

LOCATING AREA AND HOST OF ORIGIN

Finding the area and host of origin of *C. obridella* remains a challenge and no option can yet be discarded. The moth could originate (1) from *Aesculus hippocastanum* in the Balkans; (2) from another host genus in the Balkans; (3) from another *Aesculus* sp. in Asia or North America; (4) from another host genus in a non-European region. However, our investigations described above allow us to better evaluate these possibilities and to narrow our future surveys.

FROM *A. HIPPOCASTANUM* IN THE BALKANS

Although this solution cannot be totally ruled out, it is probably the least likely. The dramatic increase in population densities of a previously undetected insect still needs to be explained. Furthermore, our surveys confirmed that natural, endemic horse-chestnut stands in the Balkans are also seriously affected and that parasitism in these stands is as low as elsewhere in Europe. This situation strongly suggests an “unnatural host-herbivore association”. The best way to verify whether *A. hippocastanum* is the original host tree of *C. obridella* would be to perform molecular analyses to measure the genetic heterogeneity of moth populations in natural horse-chestnut stands in the Balkans.

FROM ANOTHER HOST PLANT IN THE BALKANS

A host-shift from another host plant could partly explain the persistent outbreak populations, limited parasitism and sudden spread in Europe. However, the original host remains to be found. The lepidopteran fauna of the Balkans is relatively well known, and the moth has never been observed on any plant species before its first record on horse-chestnut in the 1980s. The only other plant genus which is suitable for the development of *C. obridella* is *Acer*, which is also the closest relative to *Aesculus* in Europe. All *Acer* species occurring in the Balkans have been checked for *C. obridella*, without success. Dramatic host-shifts have been reported from many insects, including gracillariid leaf miners. For example, *Phyllonorycter messaniella* (Zeller), a polyphagous European leaf miner largely increased its host range to other families when introduced in New Zealand (Wise 1953). Similarly, the Californian species *Marmara gulosa* Guillén and Davis apparently expanded its host range from native willows to various introduced plants such as citrus, avocado, cotton and oleander (Guillén *et al.* 2001). However, host-shift in insects usually occurs when an insect encounters a new plant, i.e. when either the plant or the insect is exotic. Since *A. hippocastanum* is endemic to the Balkans, a host-shift would be more conceivable if *C. obridella* was exotic. Nevertheless, to verify the hypothesis of another original host plant in the Balkans, networks of pheromone traps could easily be set up in various environments.

FROM ANOTHER *AESCULUS* SP. IN ASIA OR NORTH AMERICA

This option remains plausible although host screening tests and surveys have seriously limited the possible host trees. *Aesculus turbinata*, the sister-species of *A. hippocastanum* (Xiang 1998) was also the most suitable alternative host for *C. obridella* in our screening tests. However, despite extensive surveys in Japan, *C. obridella* was not found. Other Asian *Aesculus*

spp. are apparently unsuitable for the development of the moth, and surveys in China and Pakistan also remained unsuccessful. Among the North American species, three species are suitable hosts for *C. ohridella*: *A. sylvatica*, *A. glabra* and *A. flava*. *A. sylvatica* has a very limited distribution in south-eastern U.S.A. and has never been checked for *C. ohridella*. *A. glabra* has not yet been surveyed extensively and has a large geographic range. *Aesculus* spp. are rather insignificant tree and shrub species in North America and their fauna has rarely been studied. Therefore, a rare, specific leaf miner may have remained undetected. Additional surveys should focus on these three species.

FROM ANOTHER HOST GENUS IN A NON-EUROPEAN REGION

The low probability that *C. ohridella* originates from the Balkans and the limited number of *Aesculus* spp. that remain plausible hosts of origin have made this hypothesis more likely. *Acer* has been sometimes cited as potential host genus of origin (e.g., Hellrigl, 2001) and it is indeed the most likely. *Acer pseudoplatanus* and *A. platanoides* are, until now, the only two non-*Aesculus* species that are occasionally attacked in Europe. Screening tests showed that *C. ohridella* oviposits on most tested *Acer* spp. and develops at least to mature larvae on a few of them. Furthermore, preliminary results of the phylogenetic study of the genus *Cameraria* suggest that the sister species of *C. ohridella* feeds on *Acer* spp. in China and Japan. Based on recent phylogenetic studies, *Acer* and *Aesculus* are now both placed in the family Sapindaceae (APG 1998) a family which comprises mainly tropical and sub-tropical genera. Considering that overwintering *C. ohridella* pupae are easily able to survive low winter temperatures in eastern and northern Europe, it can reasonably be expected that *C. ohridella* originates from a temperate region. Thus, most other members of the Sapindaceae family can be discarded. Interestingly, during our surveys in Japan, another *Cameraria* sp. has been found mining both *Aesculus turbinata* and *Acer mono*, suggesting that the switch from *Aesculus* to *Acer* and vice-versa is possible. Nevertheless, host-shifts such as those of *Phyllonorycter messaniella* and *Marmara gulosa* show that phylogenetically unrelated hosts can sometimes be adopted. Thus, other host genera in other families cannot be excluded for *C. ohridella*. Surveys should be carried out in temperate regions in the distribution range of *Cameraria* spp., i.e. North America and East or Central Asia. Considering that the Lepidopteran fauna, particularly that of *Acer* spp., is quite well known in North America and Japan, surveys should focus primarily on China and other rather unexplored countries.

PROSPECTS FOR FINDING SUITABLE BIOLOGICAL CONTROL AGENTS

If the area of origin of *C. ohridella* is found outside Europe, studies on its natural enemies should be carried out in this region to select biological control agents, probably parasitoids, to be introduced into Europe. If it appears that the moth originates from the Balkans, or if the area of origin remains undiscovered, an alternative would be to look for parasitoids of other *Cameraria* spp. (Kenis 1997). Studies should focus either on other *Cameraria* spp. on *Aesculus* spp. or on the sister species, *C. ? nipponica* feeding on *Acer* spp. in China and Japan. However, whatever the source of parasitoids, it is not certain that suitable species will be found for introduction into Europe. Nowadays, natural enemies used in classical biological control have to show a high degree of host specificity, to avoid non-target effects in the region of introduction (Kuhlmann and Mason 2003). Gracillariid leaf miners are known to be attacked

mainly by polyphagous parasitoids (Askew 1994). No other *Cameraria* species occurs in Europe and, thus, a parasitoid specific at genus level would be acceptable. Similarly, since no native leaf miner occurs on horse-chestnut in Europe, a parasitoid that would be specific to leaf miners on *Aesculus* spp. would be suitable as well. Parasitoid complexes of *Cameraria* spp. are poorly known. Literature data are lacking for Asian species and scarce for North American species, usually with little or no information on parasitism rates or host specificity. Host-parasitoid data bases such as those of Krombein *et al.* (1979), Yu (1999) or Noyes (2002) provide useful reviews, but are poor information sources for parasitoid host range because they include unchecked literature records which are full of errors in parasitoid or host identification and wrong host-parasitoid associations. Among the 46 parasitoid species recorded from *Cameraria* spp. in these reviews, only seven are not recorded from another host genus (Table 3). Many genera, however, are typical koinobiont endoparasitoids: *Mirax*, *Phanomeris*, *Orgilus* (Braconidae), *Ageniaspis*, *Copidosoma* (Encyrtidae), *Achrysocharoides* (Eulophidae). Most of these are surely more host specific than the usual idiobiont leaf miner parasitoids. These genera are not represented in the parasitoid complex of *C. obridella* in Europe.

Besides *C. obridella*, the only other *Cameraria* species that has been the target of a specific study on its parasitoid complex is *C. caryaefoliella* (Clemens), a minor pest of pecan in North America. Heyerdahl and Dutcher (1985) listed 22 parasitoid species attacking *C. caryaefoliella*. More interestingly, they collected, with *C. caryaefoliella*, three other pecan leaf mining moths, the nepticulid *Stigmella juglandifoliella* (Clemens), the heliozelid *Coptodisca lucifluella* Clemens and the gracillariid *Phyllonorycter caryaealbella* (Chambers). Seven of the 22 parasitoids found on *C. caryaefoliella* were found only on this host, among which three were qualified as “common”: the encyrtid *Copidosoma* sp., and the eulophids *Achrysocharoides bipunctatus* (Girault) and *Pnigalio* sp. nr. *pallipes* (Heyerdahl and Dutcher 1985). This suggests that parasitoids of *Cameraria* spp. may be more specific than indicated in the parasitoid lists as those presented in Table 3.

Few publications mention parasitism rates data on the impact of natural enemies on the control of *Cameraria* spp. Heyerdahl and Dutcher (1990) measured mean parasitism rates of 24-34% and 14-42% in larvae and pupae of *C. caryaefoliella*, respectively. Faeth (1980) found a parasitism rate of 48% in an undescribed *Cameraria* sp. on oak and Connor (1991) measured 22 to 55% apparent parasitism in *C. hamadryadella* (Clemens) on four different oak species. These parasitism rates are higher than those observed on *C. obridella*. Furthermore, they probably underestimated the impact of parasitism in the *Cameraria* populations. Faeth (1980) and Connor (1991) noted that the total larval and pupal mortality was 94-100%, predation and “unknown” mortality being mentioned as other main mortality factors. As noted by Connor (1991), high predation rates mask or reduce the estimated rate of parasitism because predators prey as well on parasitized mines. Furthermore, unknown mortality probably includes host feeding and pseudoparasitism, as observed by Heyerdahl and Dutcher (1990).

Hardly anything is known of parasitism of *Cameraria* spp. in Asia. In our recent surveys, we made opportunistic observations on parasitism in *C. ? nipponica* on *Acer* spp. in China and Japan and a *Cameraria* sp. on *Aesculus turbinata* and *Acer mono* in Japan. Based on emergence data only, parasitism of *C. ? nipponica* was 92% (n=26) in China and 62 %

(n=52) in Japan, whereas parasitism of *Cameraria* sp. in Japan was 76% (n=21) (Kenis, unpublished data). Parasitism was equally shared between braconids and eulophids. These numbers have to be taken with great caution because mortality in rearing was high and probably affected more the hosts than the parasitoids. Nevertheless, these data and the previously cited American publications clearly show that parasitism is more important on native *Cameraria* spp. than on *C. obridella* in Europe. The exact role of parasitism on the population dynamics of *Cameraria* spp. in their native range is not known but we believe that parasitoids are at least partly responsible for the lower population levels compared to *C. obridella*. Life table studies have been carried out for *C. obridella* in Switzerland and Bulgaria, which include assessments of the respective mortality factors on the different developmental stages (Girardoz, Tomov and Kenis, in preparation). Similar studies should be made on native *Cameraria* spp. to better understand the factors causing the long-lasting outbreaks observed in Europe.

ACKNOWLEDGEMENTS

This project is a collaborative work between several teams. In addition to the authors of the paper, many more colleagues and students participated surveys, collections, laboratory work, placement of pheromone traps in various regions of the world, and parasitoid identification. They are too numerous to be cited here but are all warmly thanked. We also thank Mathew Cock for his comments on the manuscript. This study was funded by the European Commission and the Swiss Federal Office for Education and Science, as part of the FP5 project CONTROCAM, QLK5-CT-2000-01684.

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METAMASIOUS CALLIZONA IS DESTROYING FLORIDA'S NATIVE BROMELIADS

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ABSTRACT

Bromeliads (Bromeliaceae) are a family of about 2,500 species native to the Neotropics, with 16 native to Florida. For decades, enthusiasts have imported into Florida numerous species from Neotropical countries for their attractive foliage and colorful flowers. The impression of enthusiasts, still fostered by growers' manuals, was that bromeliads have no serious pest insects – only a few easily controllable scale insects and mealybugs (Coccoidea). In 1989, an unidentified weevil was detected on ornamental bromeliads at a nursery in Ft. Lauderdale. The nursery was treated with chemicals to eradicate the population, but too late – surveys showed that this weevil was established on native Florida bromeliads in nearby county parks. It was *Metamasius callizona* (Chevrolat) (Coleoptera: Dryophthoridae, formerly Curculionidae). It had been shipped from infested shadehouses in the state of Veracruz, Mexico. USDA-APHIS records showed interceptions year after year of this and other *Metamasius* weevils on bromeliads imported to Florida. There was little knowledge of it in Mexico except as an occasional pest of cultivated pineapples, which was unpublished information at the time. A chemical eradication attempt was not supportable because (1) the weevil was already in county parks that may not be treated with chemicals, and (2) there were no funds except for eradication of major agricultural pests. Bromeliad enthusiasts called for research but could raise little money. Their immediate needs were met by the discovery that carbaryl (Sevin®) would control bromeliad weevil in plant collections. As the multivoltine weevil population spread from county to county destroying native bromeliad populations and invading state parks, botanists of Florida's Endangered Plant Advisory Council became alarmed and declared two more of Florida's native bromeliad species to be endangered (Florida Administrative Code). Brief searches for potential biological control agents were carried out in Mexico, Panama, and Honduras. In Honduras, an undescribed fly (Diptera: Tachinidae, cf. *Lixophaga* sp.) was found as a parasitoid of the closely related *M. quadrilineatus* Champion, a species that inhabits remnant cloud forests on hilltops. Climatic conditions could not be duplicated in Florida's unsuitable main quarantine facility, and attempts to rear this fly failed. Recently, Florida Park Service personnel saw the devastation to native bromeliad populations and became alarmed. Increased funding led to further exploration in Guatemala, Belize, Mexico

(again) and even Paraguay for candidate biological control agents. None, other than the Honduran tachinid fly (also found in Guatemala), was detected. Research efforts were then concentrated in Honduras where adequate stock of the fly could be obtained and maintained at temperatures cooler than obtainable in Florida's Gainesville quarantine facility. Stock of *M. callizona* and *M. mosieri* Barber (a native Florida non-pest species) has been supplied to Honduras for tests. A new Biological Control Research and Containment Laboratory at Ft. Pierce has just become operational. At time of writing, the weevil's population was outside the northern edge of Everglades National Park.

INTRODUCTION

Bromeliads (Bromeliaceae) are a family of about 2,500 species native to the Neotropics, with 16 native to Florida. For decades, enthusiasts have imported to Florida numerous species from Neotropical countries for their attractive foliage and colorful flowers. From these have been created numerous hybrids. The impression of enthusiasts was that bromeliads have no serious pest insects – only a few controllable scale insects and mealybugs – a false idea that is perpetuated in horticultural advice to growers (e.g., Black and Dehgan 1993; Wall 1988). *Ananas comosus* (L.), pineapple, is the only major agricultural bromeliad crop, but its extent is trivial in Florida.

In 1989, an unfamiliar weevil was detected on ornamental bromeliads at a nursery in Ft. Lauderdale. The nursery was treated with chemicals to eradicate the population, but too late – surveys showed that this weevil was established on native epiphytic Florida bromeliads in nearby county parks. It was found to be *Metamasius callizona* (Chevrolat), native to Mexico and Guatemala, and believed at the time also to occur in other Central American countries (O'Brien and Thomas 1990). It was the realization that the larvae do not merely feed on native bromeliads, but kill them (Frank and Thomas 1991a), that made this pest serious. The probable origin was traced to infested shadehouses in the state of Veracruz, Mexico (Frank and Thomas 1994). USDA-APHIS records showed interception year after year of this and other *Metamasius* weevils on bromeliads imported to Florida. Exporters (in Latin America) and importers (in Florida and other parts of the U.S.A.) had been careless. USDA-APHIS inspection of imported plants at U.S. ports and airports examines fewer than 2% of shipments (Frank and Thomas 1994). This had been a potential disaster waiting for years to manifest itself. If the infested plants had been shipped to a nursery in most states of the U.S.A., the weevils might have harmed only the stock of that nursery. But in southern Florida, susceptible native bromeliads grow in trees in close proximity to nurseries, easing establishment barriers for weevils outside of cultivation.

A chemical eradication attempt in Florida was not supportable because (1) the weevil was already in county parks which may not be treated with chemicals, (2) the situation was unprecedented and could not be evaluated confidently, (3) there were no funds for eradication except of major new pests of major agricultural crops (e.g., citrus), and (4) there are no low density monitoring tools to accurately measure the efficacy of eradication attempts.

There was little knowledge of *M. callizona* in Mexico except as an occasional pest of cultivated pineapples, unpublished until Rebolledo *et al.* (1998). *M. callizona* invades pine-

apple fields in newly-cleared land close to forest edges and has been controlled by use of broad-spectrum chemicals. Bromeliad enthusiasts in Florida called for research for control but could raise little money. Their immediate needs were met by the discovery that carbaryl (Sevin®) would control *M. callizona* in their collections. However, all of Florida's native bromeliads are epiphytic, and chemical control of weevils in bromeliads growing high above ground over large areas of land is an infinitely more difficult proposition technically, environmentally, legally, and financially.

Lack of feasibility of chemical eradication led to initiation of a biological control project. The project is far from complete. This paper reviews its progress to date.

M. CALLIZONA DEVASTATES NATIVE FLORIDA BROMELIAD POPULATIONS

As the *M. callizona* population spread from county to county in native bromeliad populations, botanists of Florida's Endangered Plant Advisory Council became concerned. They declared two more of Florida's native bromeliad species to be endangered. This brought to 10 the number of native species listed as threatened or endangered under the Florida Administrative Code (1998) (Table 1). Florida law is independent of federal U.S. law in this respect, for none of these species is so listed under the federal Endangered Species Act. Suffice it to say that all the native Florida bromeliads but one are known at the **species** level to occur also in the West Indies. So of course is the West Indian manatee, a 'charismatic' species whose Florida populations have been declared to be a distinct subspecies, and it is the Florida **subspecies** which is 'an endangered species' under federal law. One Florida bromeliad species occurs only in Florida (is precinctive) and has now been found to be attacked by *M. callizona*. Twenty years ago this might have resulted in its federal listing as an endangered species, but now the Environmental Protection Agency is under pressure to prevent expansion of the list of endangered species. That species, *Tillandsia simulata*, was only belatedly found to be attacked because its range is in central Florida, north of the counties where weevil-caused damage was at first concentrated.

Monitoring of the spread of *M. callizona* in Florida has been intermittent since 1989 (Frank 2005). The spread of the weevil now seems likely to continue until it has overrun all parts of Florida having those 12 native bromeliad species with individuals capable of growing to a considerable size (Table 1, Fig. 1). Spread is not only by flight of adults but also caused by people carelessly moving infested ornamental bromeliads from place to place. There is one curious anomaly: by 1991 the weevil was found in a county park in southern Miami-Dade County (Frank and Thomas 1991b), but was not found in that park after hurricane Andrew (August 1992), and has not been detected elsewhere in that county despite relatively frequent surveys.

Invasion of Broward County parks in 1989-1991 left drifts of fallen large bromeliads (mostly *T. utriculata*, Fig. 2). Visits to those same parks in 1999 showed very sparse *T. utriculata* populations, with *M. callizona* infesting some of the few remaining larger plants. The slow growth of the plants to flowering size (upward of 10 years), and apparent preference of *M. callizona* for attacking large plants strongly suggested a powerful negative effect on *T. utriculata*

Table 1. Florida bromeliad species, their abundance and status under Florida law, and susceptibility to attack by *M. callizona* larvae.

Bromeliad Species	Florida Status	Attacked by <i>M. callizona</i> Larvae?
<i>Catopsis berteroniana</i> Schult. (f.) Mez	Rare, endangered	probably ¹
<i>Catopsis floribunda</i> L.B. Sm.	Rare, endangered	probably ¹
<i>Catopsis nutans</i> (Sw.) Griseb.	Very rare, endangered	probably ¹
<i>Guzmania monostachia</i> (L.) Rusby ex Mez	Rare, endangered	yes ²
<i>Tillandsia balbisiana</i> Schult. and Schult. f.	Occasional, threatened	yes
<i>Tillandsia fasciculata</i> Sw.	Frequent, endangered ³	yes
<i>Tillandsia flexuosa</i> Sw.	Infrequent, threatened	yes
<i>Tillandsia paucifolia</i> Baker	Occasional	yes
<i>Tillandsia pruinosa</i> Sw.	Rare, endangered	probably ¹
<i>Tillandsia simulata</i> Small	Frequent ⁴	yes
<i>Tillandsia utriculata</i> L.	Frequent, endangered	yes
<i>Tillandsia variabilis</i> Schltdl. ⁵	Occasional, threatened	yes
<i>Tillandsia bartramii</i> Elliott	Frequent	no, too small
<i>Tillandsia recurvata</i> (L.) L.	Common	no, too small
<i>Tillandsia setacea</i> Sw.	Common	no, too small
<i>Tillandsia usneoides</i> (L.) L.	Common	no, too small

¹The three *Catopsis* spp., *G. monostachia*, and *T. pruinosa* have not yet been observed to be attacked in nature, probably because of their rarity.

²Cultivated specimens of *G. monostachia* and *Catopsis* spp. have been attacked.

³*T. fasciculata* is much less susceptible than *T. utriculata* perhaps because of its high fibrosity. Perhaps it was listed as endangered because of its high phenotypic variability. It is still illogical that it should have been listed as endangered, but *T. paucifolia*, which is readily attacked and much less common, is not.

⁴Precinctive; native to and occurring only in central Florida.

⁵Listed as *T. valenzuelana* Richard in Florida Administrative Code (1998).

population sustainability. The research project of graduate student Teresa Cooper (Cooper 2005) is an evaluation of the dynamics of the bromeliad populations attacked by *M. callizona*, especially in the Myakka River State Park.

Most of the larger native bromeliads (Table 1), led by *T. utriculata* and *T. fasciculata*, the species of *Catopsis* and *Guzmania*, down to *T. flexuosa*, *T. simulata* and *T. variabilis*, impound water in their leaf axils, forming phytotelmata. The phytotelmata house an aquatic invertebrate fauna causing no harm to the plants (Frank 1983). An estimated 15 of these invertebrate species, several of which have not yet been described by taxonomists, seem to be specialists, living only in bromeliad phytotelmata (Frank 1983 and unpublished). Destruction of their host plants by *M. callizona* necessarily destroys populations of these invertebrates. A

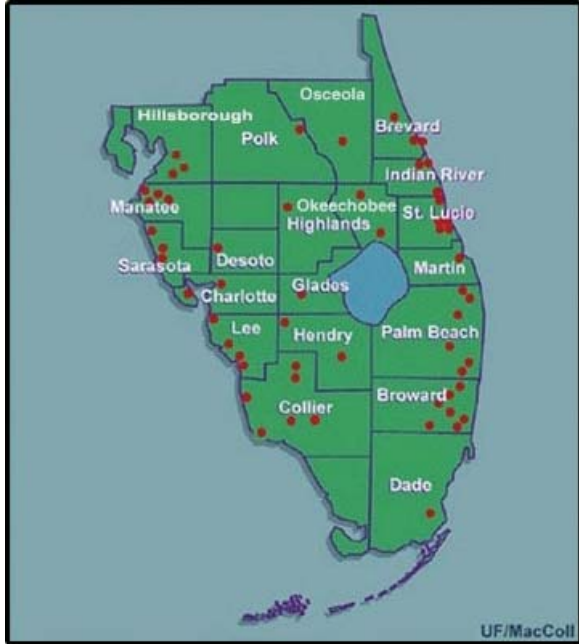


Figure 1. Recorded distribution of *Metamasius callizona* in central and southern Florida and showing county boundaries and their names, as of early 2005.



Figure 2. A fallen *Tillandsia utriculata* showing *Metamasius callizona* cocoons in mined stem. Photo: J. L. Castner. UGA1390013



Figure 3. An adult *Metamasius callizona*. Length 11-16 mm. Photo: J. L. Castner. UGA1390014

few of these invertebrate species, especially the mosquitoes among them, were found to have adapted their existence in phytotelmata of nonnative ornamental bromeliads cultivated in urban habitats (Frank *et al.* 1988), and some of these ornamental bromeliads seem not to be attacked by *M. callizona*. Nevertheless, dependence upon cultivated bromeliads in urban habitats in southern Florida makes for a tenuous existence. The two most abundant mosquito species in native bromeliads are *Wyeomyia mitchellii* (Theobald) and *W. vanduzeei* Dyar and Knab. Females of both species take blood from humans and are pests. However, they are specialists to the bromeliad habitat and, in bromeliads in Florida, their larvae outcompete and exclude invasive mosquitoes whose females are known to be able to transmit viral diseases to humans (Lounibos *et al.* 2003).

If *M. callizona* (Fig. 3) eradicates 12 native bromeliad species in Florida, it also is likely to eradicate up to 15 invertebrate species. A loss of 27 species from a single cause may be the worst ecological disaster to befall Florida by an invasive terrestrial arthropod.

BEHAVIOR OF SOME *METAMASIVUS* WEEVILS

Thirty-two bromeliad-eating weevil species are known in the Neotropics (Frank 1999). Three of them, and one other *Metamasius* species, are important to this project.

***METAMASIVUS MOSIERI* BARBER**

This weevil was described from Florida and Cuba in 1920, and later was reported from the Dominican Republic. For lack of other evidence, it has been assumed to be native to all three land masses. Little was known about *M. mosieri* (Fig. 4) until it was encountered sometimes in searches for *M. callizona*. Adults and larvae are considerably smaller than those of *M. callizona*. Larvae develop in small *Tillandsia* bromeliads, including *T. balbisiana*, *T. paucifolia*, *T. setacea*, *T. simulata*, *T. utriculata*, *T. variabilis* (Larson *et al.* 2001) and occasionally small nonnative *Tillandsia* in growers' collections (Frank 1999). When *T. utriculata* is attacked, it is only small specimens that are attacked. When *T. balbisiana* is attacked, it is often the flower spike that is mined. Why larger plants are not attacked is unknown. Adults are distinctively colored. Larvae are very difficult to distinguish from small *M. callizona* larvae. The species has been detected only in a few counties in the southwest and the southeast. Why it is not more widely distributed, and why its populations are not abundant are unknown. A few score larvae have been collected in the field and reared, but none has produced adult parasitoids. Larvae are very sensitive to ambient conditions and are harder than those of *M. callizona* to rear. In our biological control attempt against *M. callizona* in Florida, *M. mosieri* will be treated as a nontarget native species.

***METAMASIVUS QUADRILINEATUS* CHAMPION**

This weevil attacks bromeliads in Mexico, Guatemala, El Salvador, and Honduras. At least in Honduras and Guatemala, it is host to the only parasitoid of bromeliad-eating weevils that we have yet discovered (see below). Females are believed to oviposit only in fallen epiphytic bromeliads that have tipped, draining the water impounded in their leaf axils (Alvarez del



Figure 4. An adult *Metamasius mosieri*. Length 6-9 mm.
Photo P. M. Choate. UGA139000515

Hierro and Cave 1999). Thus, the weevil is of little or no threat to bromeliad populations. Indeed at the high altitudes in Honduras where this weevil has been found, native bromeliad populations seem to thrive in the presence of a thriving weevil population and seasonally abundant parasitoids. The bromeliads, the weevil and its parasitoid may exist at high elevations (in remnant cloud forest above 1600 m) by climatic restriction, or they may have existed at lower elevations before clearing of vast tracts of forest for agriculture; we do not know which. In the former case, none of them (including the parasitoid) may be able to exist at Florida's low elevations because of higher summer temperatures.

METAMASIVS HEMIPTERUS (L.)

This weevil was detected in Florida in 1984 as an invasive species from the Neotropics (O'Brien and Thomas 1990). It attacks banana, some ornamental palm trees and, rarely, ripe pineapple fruits. Its larvae have not been found to attack native Florida bromeliads. It is to be treated as a nontarget species in our project. Attack on it by any biological control agent that we establish against *M. callizona* could only be beneficial, but this would indicate a wider host range and might require testing of the susceptibility of other weevil genera.

METAMASIVS CALLIZONA

The objective of our project is a substantial reduction in population densities of this weevil. Adult weevils nibble on leaves of a wide range of bromeliads. Females oviposit in a narrower taxonomic range of bromeliads. Eggs are laid in slits that are cut in leaf bases and egg-laying is restricted to bromeliads of a size that will allow development of at least one larva mining in the meristematic tissue. The minimal size of *T. utriculata* plants in which *M. callizona* will oviposit is larger than 11.9 cm diam. (length of longest leaf 9.8 cm) (Sidoti and Frank 2002). The egg takes 7-10 d at 26°C to incubate (Salas and Frank 2001). Hatchling larvae tunnel into the meristematic tissue and begin to mine. When larvae were reared on pineapple stems at

26°C, five instars were detected before larvae pupated, the pupal stage lasted 9-15 days, and the total developmental time from egg to adult was about 8 wk (Salas and Frank 2001). Prepupae construct cocoons of plant fiber. Longevity of adults, preovipositional period of females, and total fecundity are under investigation. All life stages may be found throughout the year in natural areas of Florida. Generations are not discrete and the mean generational time of eggs to adults is speculated to be 13-17 wk (Salas and Frank 2001).

COMPONENTS OF A PROJECT AGAINST *M. CALLIZONA*

The initial components included (a) a literature search, (b) monitoring spread of *M. callizona* in Florida, (c) recording native host plants attacked, (d) collecting living specimens from nature in Florida to determine whether any contained parasitoids, (e) studying the life cycle of *M. callizona*, (f) collecting and studying the nontarget species *M. mosieri*, (g) testing and recording effects of *M. callizona* on ornamental (non-native) species, (h) answering grower questions about control methods, including feasibility of chemical control, and (i) preparing and delivering information to people concerned with the native bromeliads in various parts of Florida, including construction of websites (<http://BromeliadBiota.ifas.ufl.edu/wvbrom.htm> and <http://SaveBromeliads.ifas.ufl.edu>).

All of this led to the need to obtain funds to hire personnel and for foreign exploration to study *M. callizona* in its native habitats, and to detect potential biological control agents. The first funding agency strongly suggested it might contemplate funding the project if a seed-collecting project were begun. The idea was widespread collection of viable seed of the species at risk, their germination and growing out to replace the seedlings in nature once the weevil population had begun to decline. In desperation for funds, we undertook this. We were greatly helped by two professional growers who handled the seeds and their germination once these were supplied, but we needed state permits for these growers to grow the seed. Volunteers helped to collect the seed, but we needed state and county permits for them to do so as well. Further, a computerized database had to be constructed to document and catalogue the seed collections.

THE SEARCH FOR A BIOLOGICAL CONTROL SOLUTION

Lack of feasibility of chemical eradication of *M. callizona* stimulated interest in biological control. Nothing was known about parasitoids of any of the bromeliad-attacking *Metamasius* spp. Brief searches for potential biological control agents were carried out in Mexico and Panama. In Veracruz and Oaxaca, Mexico (1992), *M. callizona* was found abundantly only in the infested shadehouses of the grower whose carelessness caused its invasion of Florida. Over 100 larvae brought to a containment facility in Florida produced healthy adults, with no evidence of parasitoids. In Chiriquí, Panama (1994) *M. callizona* was not found, but a close relative, *M. cincinnatus* Champion with the same behavior was found abundantly only at one locality in nature. Again, over 100 larvae brought to Florida produced only healthy adults. In Honduras, an undescribed fly (Diptera: Tachinidae, cf. *Lixophaga*, Fig. 5) was found as a

parasitoid of the closely related *M. quadrilineatus* Champion, a species that inhabits remnant cloud forests on hilltops. The fly and its host became the subject of a Honduran student project, and were reared successfully at 20°C (Alvarez del Hierro and Cave 1999). Climatic conditions for rearing could not be duplicated in Florida's unsuitable main quarantine facility. A few fly larvae were transferred successfully to *M. callizona* larvae in 1998-1999 and developed, but attempts to establish a colony failed.

More recently, Florida Park Service personnel saw the devastation to native bromeliad populations and became alarmed. Increased funding led to further exploration in Guatemala, Belize and Mexico without discovery of additional parasitoids (Cave *et al.* 2004). Even Paraguay in 2004, and Peru in 2005 were explored for candidate biological control agents of any bromeliad-attacking *Metamasius* sp. None, other than the Honduran tachinid fly (also found in Guatemala in *M. quadrilineatus*), was detected. Research efforts were concentrated in Honduras where adequate stock of the fly could be obtained and maintained at temperatures cooler than obtainable in Florida's Gainesville quarantine facility. Larvae of *M. callizona* and *M. mosieri*, grown in Florida, have been supplied month after month to Honduras for tests. Studies there have confirmed that the tachinid cf. *Lixophaga* will attack *M. callizona*, and does so at least as readily as it will attack *M. quadrilineatus* under experimental comparison. *Metamasius mosieri* has been demonstrated to be an appropriate host for the tachinid cf. *Lixophaga*, but *M. callizona* appears to be a preferred host. Many other questions are being investigated by postdoctoral researcher Alonso Suazo. A new Biological Control Research and Containment Laboratory became operational in February 2005 in Ft. Pierce, Florida, and it is to that facility that a stock population of the tachinid will be shipped as soon as possible. The weevil's population is now at the edge of Everglades National Park (Fig. 6), and the control situation is critical.



Figure 5. An adult male tachinid cf. *Lixophaga*. Photo: B. C. Larson. UGA1390016



Figure 6. A scene from the Everglades showing the color form (yellow bracts) of *Tillandsia fasciculata* that exists there and, above and to the left, *Tillandsia paucifolia*. Both species are under attack by *Metamasius callizona*. Photo: J. H. Frank. UGA1390017

CONCLUSION

This is an unusual biological control project in that it attempts to control an invasive species that endangers native flora. As such, it has little easily-documented economic effect. Adequate funding for the necessary components of the project has been especially difficult to obtain and there is no certainty of its continuation. Progress has been made, but we do not yet know whether the only detected potential biological control agent will survive in Florida's climate, what effect it will have on non-target species, nor whether a release permit will be issued. We do not know whether it will be able to reduce populations of *M. callizona*. We do not know to what extent *M. callizona* will eradicate bromeliad species from Florida if left uncontrolled. It would take little phytophagy to completely eradicate the rarest species. The current measured rate of disappearance of *T. utriculata*, still widespread but becoming rarer, if it were continued, suggests that total eradication would be possible for that bromeliad. Most of the other bromeliads now under attack or likely to be attacked in nature may meet the same fate. Expect publications by Teresa Cooper on the dynamics of weevil-attacked bromeliads in Florida, and by Alonso Suazo on rearing conditions and some host-range testing of the tachinid cf. *Lixophaga* in Honduras. Also, we expect to publish a taxonomic description of the tachinid fly by Monty Wood (Ottawa, Canada). Wish us luck in Florida in successful importation of the fly, laboratory colonization, nontarget testing, release permitting, establishment in the field, and control of *M. callizona*.

ACKNOWLEDGEMENTS

The inspiration and early support for a biological control project against *M. callizona* came from the Florida Council of Bromeliad Societies. The Florida Department of Agriculture and Consumer Services supported the project for two years. Dr. Barbra Larson, as postdoctoral associate with the project, developed the website <http://SaveBromeliads.ifas.ufl.edu>, other educational materials, and grant applications. The Park Service of the Florida Department of Environmental Protection supported it for three years. The U.S. Environmental Protection Agency provided a grant to allow production of materials for public education. Drs. Frank Slansky Jr. and Oscar Liburd kindly reviewed a manuscript draft and made critical comments. Useful editorial suggestions were made by Dr. Mark Hoddle.

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BIOLOGICAL CONTROL OF THE EUROPEAN GREEN CRAB, *CARCINUS MAENAS*: NATURAL ENEMY EVALUATION AND ANALYSIS OF HOST SPECIFICITY

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ABSTRACT

Many introduced marine organisms are ecological and economic pests. Nevertheless, no management approach is available to mitigate their impacts. Now, a theoretical perspective borrows principles from classical biological control, as widely applied to terrestrial and fresh water systems, to control the abundance of introduced marine pests.

A banner example of a marine pest is the European green crab, *Carcinus maenas* (L.) (Brachyura: Cancridae). This crab invaded San Francisco Bay in the late 1980s and has expanded its geographic range at a record rate. Studies on the Pacific coast of the U.S.A. and elsewhere strongly indicate that it is a worst-case introduction, affecting native organisms and potentially harming fisheries and aquaculture. Extensive studies show that a significant element of its success, where introduced, has been release from its natural enemies, notably parasites.

Natural enemies are infectious agents with potential to contribute to green crab biological control include two parasitic castrators: *Sacculina carcini* Thompson (Rhizocephala: Sacculinidae) and, *Portunion maenadis* Giard (Isopoda: Entoniscidae), the parasitoid flatworm, *Fecampia erythrocephala* Giard (Fecampiida: Fecampiidae), and the symbiotic nemertean egg predator, *Carcinonemertes carcinophila* (Kolliker) (Hoplonemertea: Carcinonemertidae). Evidence for their potential efficacy derives from quantitative natural history studies and ecological modeling. Their safety with respect to non-target organisms is of great concern. Relevant information is available from qualitative field studies, host specificity experimentation, evolutionary theory, and the ability to detect and estimate the frequency of unsuccessful parasite attack rates in natural populations.

We interpret host specificity in the context of the double filter paradigm of Combes (2001). For a host to be suitable, two filters must be partially open. When the encounter filter is open, the infective stage can locate and enter the host because it shares the appropriate habitat and has suitable host location and attack behaviors. For the parasite to be successful, the compatibility filter must also be partially open. This occurs if the host provides appropriate nutrition and its defensive mechanisms cannot kill nor block reproduction of the infectious agent. For both rhizocephalan barnacles and entoniscid isopods in native (coevolved) regions, potential alternative hosts appear to be protected via inability to encounter the host, or due to incompatibility, or both filters are closed. Naïve hosts from an introduced area are located under permissive encounter experimental conditions, but the compatibility filter is closed.

INTRODUCTION

We now recognize the ubiquitous importance of introduced marine organisms as pests (Carlton 1989). Several international conferences have been organized in the past eight years and research funding has been substantially expanded. Studies of impacts of some of the most successful invaders such as the *Caulerpa taxifolia* alga in the Mediterranean (Meinesz 2004), *Musculista senhousia* mussels in southern California (Crooks and Khim 1999), and *Carcinus maenas* green crabs in Tasmania and on both coasts of North America (Grosholz *et al.* 2000) demonstrate that marine invaders can deplete native species through competition, predation, and alteration of habitat. Secondary facilitation of other exotics and other tri-trophic consequences also significantly and substantially alter natural communities in ways deemed undesirable. Most invasion problems are tackled at two levels: prevention and mitigation. For agricultural insect and weed pests both elements are actively developed. For marine pests, a major international effort has been mounted to prevent further introductions with increased regulation of major vectors such as ballast water, hull fouling and oyster mariculture (Cangelosi 2002). Some of these options are very costly (mid-ocean ballast exchange is estimated to add \$112-362 million per annum to the cost of shipping just to the U.S.A. (Hayes 2001). Amelioration of the impacts of the marine invaders that are already here receives little attention. The cause for this defeatist attitude is unclear. The vastness of the marine habitat and the pelagic larval dispersal strategies of many marine organisms do make it seem that an invader is unstoppable once it arrives (Kuris and Lafferty 2001). It could also relate to the training of most marine ecologists, which in accordance with the general ecological literature, extrapolates nonscientific biological control horror stories to an *a priori* suspicion of scientific classical biological control.

For marine exotics, Thresher and Kuris (2004) showed that for control options there is an inverse relationship between likelihood of success and the perceived willingness of the approach to be supported by investigators and regulators. One might conclude that, at present, the problems caused by marine exotics are not sufficiently severe to risk costs associated with mitigation. Of course, that begs the question: why then impose the substantial costs to prevent this problem?

Our perspective is that if costs imposed by introduced marine species are great, control strategies are worth developing. This has paid off with the first successful eradications of established marine pests (Culver and Kuris 2000; Kuris 2003a; Myers *et al.* 2000). For use of natural enemies, we showed that a theoretical window for biological control of marine pests was available (Kuris and Lafferty 1992). By extending models of the impact of parasitic castrators and symbiotic egg predators on crustacean fisheries, we proposed that at least these types of natural enemies could act as classical biological control agents against marine exotics and devised a general protocol for development of this approach. This was also extended for the use of marine parasitoids (Kuris *et al.* 2002). Efficacy models were generally related to those developed for insect pest control by parasitoid natural enemies (Lafferty and Kuris 1996) and modified by experiences controlling infectious diseases impacting crustacean fisheries (Kuris and Lafferty 1992). Safety concerns could be evaluated experimentally following protocols for weed pest biological control (Kuris and Lafferty 2001; Lafferty and Kuris 1996).

With the discovery of a population of the European green crab, *Carcinus maenas*, (L.) (Decapoda, Portunidae) in South San Francisco Bay in 1991 (Cohen *et al.* 1995), its rapid spread via larval dispersal to bays and estuaries as far north as Nootka Sound, British Columbia in less than 10 years (Behrens-Yamada 2001) was a record linear range expansion rate for a marine animal. Its eurytopic, euryhaline, and generalist feeding habits suggest European green crab is a worst case pestiferous invader. Experimental and empirical studies demonstrate its strong negative impact on other species of crabs, other invertebrates, and perhaps as a competitor with shorebirds for food (Cohen *et al.* 1995; Grosholz and Ruiz 1996; Grosholz *et al.* 2000). It has now also been shown to facilitate the adverse impact of an earlier introduction, the small clam, *Gemma gemma* (Totten) (Grosholz 2005). Hence, a control campaign against the introduced green crab appears warranted. These studies in California are supported by similar studies of other introduced green crab populations from the east coast of North America, South Africa, and Victoria and Tasmania in Australia (Glude 1955; Le Roux *et al.* 1990; MacPhail *et al.* 1955). Its sibling species, the Mediterranean *C. estuarii* Nardo has also been introduced in Japan.

Here, we will summarize the biology of the natural enemies of European green crab, evaluate their potential with respect to efficacy and safety, note technological difficulties impeding further work, and expand on the evaluation of the safety of parasitic castrators using experiments and field observations in the context of the host specificity encounter-compatibility paradigm of Combes (2001). This will focus on the potential safety of the most promising agent (in terms of efficacy), *Sacculina carcini* Thompson (Rhizocephala: Sacculinidae).

EVALUATION OF GREEN CRAB NATURAL ENEMIES

The green crab, *Carcinus maenas*, is the most common crab along the shores of Europe, and as an introduced species in New England and the Canadian Maritimes. Thus, it is the most studied crab with respect to its growth, reproduction, physiology, role in community ecology, and its parasitofauna. Based on the considerable available literature, and our extensive search for natural enemies in Europe, we provide an evaluation of natural enemies consistent in the context of scientific classical biological control. We briefly considered and re-

jected the use as predators as control agents. Although many species of birds and fishes eat green crabs, none are specialist green crab predators. The use of a generalist predator is unlikely to be an effective control agent and it is likely to have considerable unwanted consequences for non-target species.

MATERIALS AND METHODS

In Table 1, we summarize the published information of the infective agents of green crab. Although extensive and offering considerable detail for a few species of parasites, it does not include even a single study across a variety of taxa. Hence, our first task was to survey native populations of green crab over its entire range in Europe. We conducted several surveys of European green crab populations (20 sites, ~3000 crabs dissected) that extended from Tromsø, Norway to Gibraltar. These data were compared with our surveys of introduced green crab populations along the coasts of North America, in South Africa, Victoria and Tasmania, Australia, and of the closely related *C. estuarii* population in Japan (N=~2000) (Torchin *et al.* 2003). For metazoan parasites, we recovered all but one of the agents reported in the literature and discovered two that had not been previously reported. Table 1 summarizes our findings and provides a few details about their abundance and pathogenicity. Because certain types of host-parasite interactions have general implications for their use as natural enemies we have grouped these by trophic adaptive syndromes according to Lafferty and Kuris (2002).

RESULTS

105

Crabs in introduced populations are infrequently parasitized and parasite species richness in all invaded regions falls well below the European total (California: one species, east coast of North America: 3 species, Victoria: four species, Tasmania: 2 species, South Africa and Japan: no parasites) (Kuris and Gurney 1997; Kuris *et al.* 2002; Torchin *et al.* 1996). Further, no parasitic castrators, nor parasitoids, have been recovered from any introduced green crab population.

EVALUATION OF NATURAL ENEMIES

The parasites listed in Table 1 vary with respect to their likely efficacy and safety as biological control agents for *C. maenas*.

Pathogens. Pathogens are intensity-independent infectious agents whose abundance increases in the host through reproduction. Their populations are generally limited by host defensive responses. They are suitable for modeling as microparasites (Anderson and May 1979). The microbial pathogens reported from the green crab are rarely seen in natural populations. These diseases, particularly the ciliates, appear to be associated with stressed, high density, captive host populations. We conclude that they are inefficiently transmitted, requiring high host densities and cofactors. Stressors including pollution, high temperature, low oxygen, or confinement are some of the needed cofactors to produce disease. Their host specificity has not been experimentally examined, but evidence from related agents in other crustaceans suggests that they may not be host specific (except perhaps *Microspora*) for non-target crabs.

Table 1. The abundance of infective agents of the green crab in Europe from the literature (Lit.) and our surveys (Surv.), + is reported, 0 is not found, – is not investigated. For Type of agent, Path is microbial pathogens (microparasite), TTPF is trophically transmitted parasite in its final (predator) host (macroparasite), TTPI is trophically transmitted parasite in its intermediate (prey) host, PC is parasitic castrator, Ptoid is parasitoid, SEP is symbiotic egg predator (terminology from Lafferty 2002); for references see (Provenzano 1983; Behrens-Yamada 2001; Torchin *et al.* 2001, Stentiford and Feist 2005).

Infective Agent	Type	Lit.	Surv.	Abundance	Remarks
<i>Viruses</i> (6 spp.)	Path	+	-	?	Sporadic, often in captive populations. Several species sometimes lethal.
<i>Bacteria</i> (3 spp.)	Path	+	+	?	Sporadic, sometimes lethal.
<i>Anophrys</i> (histophagic ciliates)	Path	+	0	rare	Only captive populations?
<i>Haematodinium perezii</i> (dinoflagellate)	Path	+	-	?	Can cause mortality in natural populations.
<i>Thelohania maenadis</i> , <i>Abelspora portulacensis</i> (microsporans)	Path	+	0	?	Sporadic, several species, sometimes lethal.
<i>Nematopsis</i> sp. (gregarine)	TTPF	+	+	common	Avirulent
<i>Fecampia erythrocephala</i> (Fecampiida)	Ptoid	+	+	to 20%	Lethal, geographically localized, habitat specialist.
<i>Microphallus lasmob</i> , <i>M. primas</i> , <i>Spelotrema excellens</i> (microphallid trematodes)	TTPI	+	+	often abundant (0-100%)	Metacercaria, birds are final hosts.
<i>Trypanorhynch</i> and <i>tetraphyllid cestodes</i>	TTPI	0	+	rare	Plerocercoid larvae, lasmobranchs are final hosts.
<i>Profillicolis botulus</i> (Acanthocephala)	TTPI	+	+	common	Acanthella and cystacanth larvae, geographically localized, birds are final hosts.
<i>Pararcuaria tridentata</i> , <i>Cosmocephalus obvelatus</i> (larval nematodes)	TTPI	+	0	rare	Found once, birds are final hosts.
<i>Carcinonemertes carcinophila</i> (Nemertean)	SEP	+	+	common (0-100%)	High intensities at some locations.
<i>Lecithomyzon maenadis</i> (nicothoid copepod)	SEP	+	-	common at one location	Sporadic and localized? eats eggs.
<i>Sacculina carcini</i> (rhizocephalan barnacle)	PC	+	+	common (0- 70%)	Stunts crab growth, feminizes males, blocks reproduction, varies with habitat.
<i>Portunion maenadis</i> (entoniscid isopod)	PC	+	+	Can be common (0- 15%)	Blocks reproduction.

Typical parasites and trophically transmitted parasites in final (predator) hosts. These infectious agents induce pathology in an intensity-dependent manner and are effectively modeled using the macroparasite models of May and Anderson (1979). The only such parasites in the green crab are gregarines. These appear to be avirulent and as such have no potential as biological control agents.

Parasitoids. Parasitoids are so closely associated with the study of insect natural enemies that some consider the term taxonomic (applying only to insects). “Parasitoid” is now applied to all intensity-independent infectious agents that require the death of their hosts to complete their life cycles. Examples include viruses (bacteriophage) mermitids, monstilloid copepods and hyperiid amphipods (Kuris 1974; Kuris and Lafferty 2000; Lafferty and Kuris 2002). One of the most unusual parasitoids is the fecampiid flatworm, *Fecampia erythrocephala* Giard (Kuris *et al.* 2002). As with most parasitoids, it infects very early instars (crabs less than 12 mm carapace width [CW]). It is only known from the coasts of England, Ireland and Atlantic France (a related undescribed species may be present in the Mediterranean Sea [Brun 1967]). It is also a habitat specialist, occurring only in rocky or cobble habitats in the middle intertidal zone of semi-protected shores (Kuris *et al.* 2002). Estimates of its growth rate and its prevalence suggest that it is a major mortality factor of young green crabs in these habitats. It can infect other species of crabs (especially *Cancer pagurus* Linnaeus [Decapoda, Cancridae]), although, in its preferred habitat, only *C. maenas* is commonly encountered. As a habitat specialist, *F. erythrocephala* may be a useful biological control agent if those habitats are deemed worthy of protection, or serve as sources for green crab populations on a regional scale. Its host specificity remains to be experimentally investigated. In certain regions, presumably appropriate habitats are common and few other native crab species are found in the habitats of introduced green crabs (e.g., east coast of North America). It is likely that its life cycle can be maintained in the laboratory as the adults are free-living and its larvae are non-feeding.

Trophically transmitted parasites in their intermediate (prey) hosts (TTPIs). Several trophically transmitted parasites (TTPs) use *C. maenas* as their prey (intermediate) host. Predatory final hosts include birds for the nematodes, theacanthocephalan, *Profilicollis botulus*, in the northern range of the green crab in Europe, the trematodes throughout its range, and elasmobranchs for trypanorhynch and tetraphyllid larval tapeworms in the southern part of its range. Accumulating evidence suggests that these TTPIs may reduce host abundance through behavioral modifications leading to increased rates of predation by final hosts on infected intermediate hosts (Kuris 1997). There is some evidence that these parasites may play a role in green crab population control in Victoria, the only region where introduced green crabs are not considered pests. At sites sampled in Victoria, green crabs are frequently parasitized by large trypanorhynch plerocercoids (*Trimacanthus aetobatidis* [Robinson]: Trypanorhyncha: Eutetrarhynchidae). The site of infection of these large encysted parasites overlies the large nerves leading anteriorly from the thoracic ganglion. This site is likely to promote behavioral modifications contributing to higher rates of predation on infected hosts by the abundant final host, the fiddler ray, *Trigonorhinus fasciata* Muller and Henle (Rhinobatidae) (Kuris and Gurney 1997). While TTPIs may markedly reduce the fitness of their prey hosts, they generally have low pathogenicity in their vertebrate predator hosts (Kuris

2003b; Lafferty 1992), some (notably acanthocephalans) are certainly pathogenic in their final hosts. Since there are evident safety issues with respect to vertebrates and since the ability of these TTPIs to regulate prey populations may be modest and ephemeral (only when infected prey hosts are abundant – a condition only met for the microphallids), the available TTPIs do not seem to merit continued investigation. Native TTPIs that use exotic *C. maenas* may be suitable as augmentative natural enemies.

Symbiotic egg predators (SEPs). This distinctive type of natural enemy offers the infestation dynamics of a parasite, but its trophic impact is solely on the developing embryos brooded by the ovigerous crabs. These can have very strong effects on host populations. They have been associated with the collapse of a major fishery (red king crab in Alaska) and the non-recovery of others (Dungeness crab in Central California (Hobbs and Botsford 1989; Kuris and Lafferty 1992; Kuris et al. 1991). *Carcinonemertes carcinophila* (Kollicker) (Hoploneuridae, Carcinonemertidae) can occur at high infestation rates, causing catastrophic brood mortality (Plymouth, England, Mira River estuary, Portugal, our personal observations). There is strong circumstantial evidence for host specificity of nemertean SEPs. The introduced populations of the green crab in the Atlantic coast of North America have never acquired *C. carcinophila* (= *C. c. immunita*, a closely related undescribed species, Kuris and Sadeghian, unpublished information) found at high prevalences on two portunid crabs, *Callinectes sapidus* Rathbun and *Ovalipes ocellatus* (Herbst) despite their considerable habitat overlap with the confamilial green crab (Coe 1902; Torchin et al. 1996). However, there is incontrovertible evidence that other SEP nemerteans have transferred to the green crab (Torchin et al. 1996). Hence, nemertean SEP natural enemies merit further investigation because they may be efficacious control agents. No safety tests have been conducted but will obviously be required. Experimental studies of host specificity remain elusive for these nemerteans, as larval transfer to new hosts has not been achieved. Brood mortality remains to be quantified.

Nicothoid copepod SEPs have been reported from both native and introduced populations of green crabs. However, these have only twice been observed (Gallien and Bloch 1936; Johnson 1957). These natural enemies appear to be geographically patchy and sporadically abundant. The relatively brief brooding period for the green crab and trap aversion by ovigerous females impedes detection and evaluation of SEPs as potential control agents.

Parasitic castrators. There is considerable theoretical and empirical evidence that parasitic castrators can control host populations (Blower and Roughgarden 1987; Kuris and Lafferty 1992; Lafferty 1993). *Sacculina carcini* appears to have the most dramatic effects on green crab growth and abundance (Lafferty and Kuris 1996; Torchin et al. 2001; 2002), and thus on the ecological impact of *C. maenas* on native organisms. Our analysis of crab size and crab population biomass indicated that prevalence of the two parasitic castrators (*S. carcini*, and *P. maenadis*) are inversely correlated with mean and maximum crab size and crab biomass (trapped catch per unit effort). Parasitic castrator prevalence accounts for 60-65% of the variance in these indicators of crab performance. Green crabs in Europe rarely exceed 70 mm carapace width (CW), while in California and Tasmania crabs exceeding 90 mm CW are common. *Sacculina carcini* is the most studied parasite of the green crab. Its life cycle can be completed in the laboratory. Its effects on the host, and its host specificity have been investigated.

Parasitic castrator entoniscid isopods have been less studied. *Portunion maenadis* is not common on the Atlantic coast of Europe. However, it is often highly prevalent in Mediterranean populations of the closely related *C. estuarii* (Veillet 1945). This contrast may reflect differences in abundance or infection rates of the intermediate planktonic copepod host in the Mediterranean versus in the Atlantic.

HOST SPECIFICITY OF CRUSTACEAN PARASITIC CASTRATORS AND THE SAFETY OF *SACCOLINA CARCINI*

Evidence from host use patterns of *Saccolina carcini* and other rhizocephalans indicates that most species have narrow host specificity (one host or a few closely related hosts). We have been able to develop the life cycle of *S. carcini* as a reliable laboratory system, and have conducted host specificity experiments on green crabs and four native California crab species that were ecological analogs or economically important (*Cancer magister* Dana).

We interpret host specificity in the context of the double filter paradigm of Combes (2001). For a host to be suitable, two filters must be partially open. When the encounter filter is open, the infective stage can locate and enter the host because it shares the appropriate habitat and has suitable host location and attack behaviors. For the parasite to be successful, the compatibility filter must also be partially open. The host can provide appropriate nutrition and its defensive mechanisms cannot kill nor block reproduction of the infectious agent. Our experiments were designed to bypass the encounter filter (host location, behavior) and examine the compatibility filter (nutritional suitability, host defenses) (Goddard *et al.* in press). We readily infected all four native California species. However, in the naïve hosts, it never completed its development. Rather, pathology was markedly neurotropic and infected crabs became paralyzed and died. Hence, *S. carcini* can only pose a threat to these non-target species if it is well established in its natural host. In other words, its safety as a biological control agent is directly related to its efficacy. It would have to attain a high prevalence in green crab populations to provide sufficient excess larvae to be available to attack native crabs.

Additional experimental studies showed that infective *S. carcini* larvae could also attack a European crab, *Pachygrapsus marmoratus* (Kuris *et al.*, submitted). However, this crab mounted a fully successful defense by melanizing early internal stages in the thoracic ganglion. We were able to use the presence of these characteristic melanized lesions to estimate the effectiveness of the encounter filter for *S. carcini*. At the Mira River estuary, Portugal, green crabs are heavily infected with *S. carcini* (~ 50% prevalence), and *C. maenas* and *P. marmoratus* exhibit considerable habitat overlap. Here, we examined *P. marmoratus* for the melanized lesions in the thoracic ganglion and compared their presence with crabs from a site on the outer coast, remote from *C. maenas*. No lesions on *P. marmoratus* were associated with *S. carcini*. Consequently, for this species, both the encounter and the compatibility filters are closed. Processes governing the encounter filter have been difficult to investigate. Our use of melanized parasite early infective stages provides a demonstration of the importance of the encounter filter for the maintenance of host specificity in Rhizocephala and entoniscid isopods (Table 2).

Most recently, we were able to use the lesion-detection squash technique to evaluate encounter and compatibility for another parasitic castrator, the entoniscid isopod, *Portunion conformis* (Kuris et al. in prep.). This internal parasite is widespread and often highly prevalent (>90%) in *Hemigrapsus oregonensis* and *H. nudus* (where the latter co-occurs with *H. oregonensis*) (Kuris et al. 1980). At Elkhorn Slough in Central California, both *H. oregonensis* and *Pachygrapsus crassipes* are common and syntopic. The infective stage of the parasite is the cryptoniscus larva. In squashes of crab internal organs (gonads, digestive glands, thoracic ganglia), this stage is readily apparent because it retains its isopodan features as an exuvia even after it molts to the apodous juvenile. The latter becomes encased in a sheath of host blood cells and continues to grow and develop to the adult in a natural host (Kuris et al. 1980). By examining its host, *H. oregonensis*, and its potential host, *P. crassipes*, we were able to show that the encounter filter is as open for *P. crassipes* as it is for *H. oregonensis* (both crabs had similar prevalences and intensities of *Portunion conformis* larvae and juveniles, Kuris et al. in prep.). However, for *P. crassipes*, the compatibility filter was completely closed. All *P. conformis* larvae and juveniles were dead and had elicited a melanization defensive response. Most were still in the cryptoniscus stage; some had successfully molted. These were ensheathed by host blood cells, but had died and were thus melanized (Kuris et al. 1980). Thus, for this native parasite against a native non-host crab, the encounter filter was fully open while the compatibility filter was closed (Table 2); it could not evade the host's defensive response.

Table 2. Experiments and observations evaluating the encounter and compatibility filters for some parasitic castrators of crabs. Crabs categorized by their evolved relationship (E.R.) with the parasite and to whether encounter is possible (E.P.) and compatibility is possible (C.P.) *not evaluated, experimentally wedged open.

Parasite	Natural Host	Non-host	E.R.	E.P	C.P	Reason
<i>Sacculina carcini</i>	<i>Carcinus maenas</i>	<i>Cancer magister</i>	No	*	No	Parasite could not regulate its growth. A variable, partially successful cellular defensive response elicited
		<i>Hemigrapsus oregonensis</i>	No	*	No	same
		<i>H. nudus</i>	No	*	No	same
		<i>Pachygrapsus crassipes</i>	No	*	No	same
		<i>P. marmoratus</i>	Yes	No	No	Elicits a powerful and fully effective cellular defensive response
<i>Portunion conformis</i>	<i>H. oregonensis</i>	<i>H. nudus</i>	Yes	Yes	Yes	Lower prevalence suggests encounter filter may be less open (Kuris et al. 1980). Both hosts mount successful defensive responses against dead parasites.
		<i>P. crassipes</i>	Yes	Yes	No	Elicits powerful cellular defensive response.

FUTURE WORK

Further studies of host specificity of *Sacculina carcini* are needed to evaluate the encounter filter as a potential barrier to infection of non-target hosts. Models investigating the joint effect of *C. maenas* as a competitor/intraguild predator on native crabs, and the lethal parasitization of native crabs from larvae produced by infected green crabs, are needed to bound the costs and benefits of *S. carcini* as a control agent.

The technical ability to conduct experimental infections of both *Fecampia erythrocephala* and *Carcinonemertes carcinophila* should be developed. This will permit experimental investigations of the safety of these agents. A model for a parasitoid of a marine host with open recruitment should be developed to examine the potential of *F. erythrocephala* as a control agent in its specific habitat. Studies of dispersal and recruitment of *C. maenas* larvae to adult habitats will aid the recognition of the importance of different habitats as sources or sinks for larval production. Further field studies to detect Microspora and viruses will improve our understanding of the epidemiology of these potential natural enemies. For symbiotic egg predators (nemertean and nicothoid copepods), we need to estimate their impact on crab natality. The habitat use of *Portunion maenadis* Giard and Bonnier (Peracarida, Entoniscidae) should be evaluated to see if this parasite might be more common under environmental conditions available in regions where green crabs have been introduced. In addition, the regulatory potential of a parasitic castrator with a two-host life cycle (such as *P. maenadis*) should be modeled. Finally, continued investigations of the impact of green crabs on native species are needed to aid management agencies' decision-making processes. They must have a reliable estimation of costs of this pest, the predicted benefit of a control approach and the risk associated with control approaches. The public can then make a more rational decision to institute policies for mitigation of introduced marine pests such as the green crab.

ACKNOWLEDGEMENTS

We thank Jeffrey Goddard, Patricia Sadeghian, Nadia Talhouk, Rob Gurney, Nicole Murphy, Nic Bax, Ron Thresher and James Carlton for their collaborative efforts with aspects of this study, and for their thoughtful contributions towards the development of these ideas. This research was funded by a grant from the National Sea Grant College Program, National Oceanographic and Atmospheric Administration (NOAA), US Department of Commerce under grant number NA06RG0142, project number R/CZ-162 through the California Sea Grant college system, and in part, by the California State Resources Agency as well as by NSF through the NIH/NSF Ecology of Infectious Diseases Program (DEB-024565). The views expressed herein are those of the author and do not necessarily represent the views of NOAA or any of its sub-agencies. The US government is authorized to reproduce and distribute this paper for governmental purposes.

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