

USE OF FIELD HOST RANGE SURVEYS FOR SELECTING CANDIDATE NON-TARGET SPECIES FOR PHYSIOLOGICAL HOST SPECIFICITY TESTING OF ENTOMOPHAGOUS BIOLOGICAL CONTROL AGENTS

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INTRODUCTION

In classical arthropod biological control, a high level of host specificity in natural enemies is desirable and should be sought during foreign exploration (Nechols *et al.*, 1992). Potential environmental risks or evaluation of the ecological host range of arthropod biological control agents have usually seemed negligible, but recent concern about the impact on biodiversity and natural ecosystems of alien species has changed this opinion (Howarth, 1991, 2000; Waage, 2001). Additionally, regulatory agencies are responding to concerns of non-target impacts by requiring more rigorous testing and a high degree of host specificity of candidate arthropod biological control agents before granting permission for release (Mason and Kuhlmann, 2002). As a result of the earlier lack of concern for non-target arthropods, host specificity screening of arthropod natural enemies was nonexistent or perfunctory until the 1990s. Consequently, recent publications are suggesting procedures to estimate the likelihood of the impact of parasitoids of arthropod pests on non-target hosts (Sands, 1997, 1998; Van Driesche and Hoddle, 1997; Hopper, 1998; Thomas and Willis, 1998; Barratt *et al.*, 2000; Kuhlmann *et al.*, 2000; Sands and Van Driesche, 2000; Van Lenteren *et al.* 2002). Hopper (2001) stated that field studies to evaluate the parasitoid-host complexes in the area of origin provide the necessary information for correctly interpreting host range estimation made via laboratory testing.

We propose that ecological information obtained by field surveys in the area of origin will also aid in selecting non-target species for physiological host specificity testing. Using this practical approach, the number of non-target species that need to be tested in the laboratory can be reduced, avoiding the need to maintain cultures of a large number of non-target host species, which often makes testing programs cumbersome. Two ongoing arthropod biological control programs will be discussed, illustrating how field surveys in the parasitoid's area of origin can be the basis to assess the host range of a candidate biological control agent. These projects were directed at *Lygus* plant bugs and *Ceutorhynchus obstrictus* Marsham and were intended to define non-target species for physiological host range testing. For further information on these programs refer to Broadbent *et al.* (2002) and Kuhlmann *et al.* (2002).

MATERIALS AND METHODS

Field Surveys to Assess Host Range of *Lygus* Parasitoids

Before starting field host range surveys for *Lygus* parasitoids, host-parasitoid associations were determined by a literature analysis of reliable published information (Kuhlmann *et al.*, 2000). According to the literature reviewed, the host ranges of the parasitoid species belonging to the genus *Peristenus* (Hymenoptera; Braconidae) seem to be restricted to the family Miridae. Previously, Loan (1980) concluded that all of the *Peristenus* spp. for which host records exist have restricted host ranges, parasitizing only one or a few species of plant bugs. Therefore, field surveys to assess target and non-

target hosts of *Peristenus* spp. were restricted to the family Miridae. Cultivated and non-cultivated habitats were chosen for field surveys based on their ecological characteristics (e.g., field crops, mountain meadow, weedy fallow) and the dominant flowering plants in each habitat were identified. At each field site, sampling was carried out weekly between mid May and mid September, from 1998 to 2000 (White, 2002). Eight sweep net samples, each consisting of 20 sweeps, were taken, beginning at the field edge and moving to the center of the site along two transects, with four samples taken per transect. Samples of all mirid nymphs and adults found were sorted to morphologically similar types, each species authoritatively identified, parasitoid cocoons obtained by rearing host nymphs, and the parasitoid cocoons reared to obtain adults for identification.

Field Surveys to Assess Host Range of *Ceutorhynchus* Parasitoids

Host-parasitoid associations for cabbage seedpod weevil, *C. obstrictus*, in the area of origin were first analyzed using literature and museum records, focusing on the larval ectoparasitoids *Trichomalus perfectus* (Walker) and *Mesopolobus morys* L. (both Hymenoptera; Pteromalidae). Based on this review it was concluded that these two species are probably restricted to the genus *Ceutorhynchus*, but certainly are restricted at least to the subfamily Ceutorhynchinae.

Before conducting field host range surveys, we compiled a list of nontarget host species under the assumption that parasitoids of *C. obstrictus* might parasitize other ceutorhynchine hosts in the same feeding niche (seeds) and in other above-ground feeding niches (stem and leaf), as well as in a feeding niche below ground (roots). In Europe, the subfamily Ceutorhynchinae includes three tribes, Orobitini, Coryssomerini, and Ceutorhynchini, which is the largest and has 28 genera and 151 known species (Dieckmann, 1972). From these 151 species, a selection was made of non-target Ceutorhynchinae that feed on (1) *Brassica napus* L. ("cultivated agricultural habitat"), (2) major weed species in the cultivated agricultural habitat, (3) major weed species near the cultivated agricultural habitat, and (4) wild crucifers in natural habitats. In order to select weed species, we used information in Schroeder *et al.* (1993), who determined the major weed species in or near *B. napus* fields known to be hosts of Ceutorhynchinae (*Capsella bursa-pastoris* [L.] Medicus, *Thlaspi arvense* L., and *Cirsium arvense* [L.] Scopoli). Additional Ceutorhynchinae species feeding on wild crucifers (i.e. *Alliaria officinalis* (Bieberstein) Cavara and Grande, and *Cardaria draba* [L.] Desv.) in natural habitats were also considered as potential hosts for entomophagous biological control agents of *C. obstrictus*. Further, *Ceutorhynchus* spp. introduced as weed biological control agents in Canada and in the United States (based on Julien and Griffiths, 1998) were added to the list.

Based on these considerations a list consisting of 27 species target and non-target Ceutorhynchinae species on specific host plants were sampled from March to July in 1999 through 2001 in the canola-growing region of northern Germany. Sites were sampled by removing host plants of Ceutorhynchinae at almost weekly intervals and dissecting stems or seed pods. We recorded the number of weevil larvae per infested plant tissue, their ectoparasitoids, and the exit holes of weevils (Klander, 2001).

RESULTS

Field Surveys to Assess Host Range of *Lygus* Parasitoids

Field surveys were made in alfalfa, red clover, and mustard (cultivated habitats), as well as Swiss Mountain meadows (up to 1,000 m), and weedy fallow fields (noncultivated habitats). In total, 27 mirid species were collected and studied for *Peristenus* parasitoid occurrence. Mirids collected belonged to 21 genera in five subfamilies: *Lygus*, *Adelphocoris*, *Polymerus*, *Leptopterna*, *Megaloceraea*, *Stenodema*, *Notostira*, *Calocoris*, *Closterotomus*, *Stenotus*, *Megalocoleus*, *Capsus*, *Lygocoris*, *Pithanus* (Mirinae),

Lepidargyrus, *Amblytylus*, *Plagiognathus* (Phylinae), *Heterotoma*, *Orthocephalus* (Orthotylinae), *Dicyphus* (Dicyphinae), and *Deraeocoris* (Deraeocorinae). Mirid species richness was high, with 21 mirid species in the Swiss mountain meadow, 16 in alfalfa, 13 in red clover, and 12 in the weedy fallow field. Only eight mirid species were found in the mustard crop habitat. Interestingly, the number of *Peristenus* species reared from host mirids was not related to habitat or to the mirid species richness per habitat.

Field survey results demonstrated that some mirid species are common and present in all habitats sampled, i.e., *Lygus pratensis* (L.), *Lygus rugulipennis* Poppius, and *Adelphocoris lineolatus* (Goeze). All *Peristenus* species reared from mirid collections were identified by Dr. Henri Goulet, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada. The host-parasitoid associations for the most common *Peristenus* spp., *Peristenus digoneutis* Loan, *Peristenus stygicus* Loan and *Peristenus rubricollis* (Thomson), are summarized in Table 1, and it is clear that the realized host ranges appear to be limited in habitats investigated in Switzerland and Germany. For example, *P. digoneutis* is present in several different European habitats, but only parasitizes *L. pratensis*, *L. rugulipennis*, and *A. lineolatus* in the alfalfa, red clover, mustard and weedy fallow habitat. Low numbers of *Leptopterna dolobrata* (Linnaeus) were parasitized by *P. stygicus* and *P. digoneutis* in the mountain meadow habitat. Several new *Peristenus* and *Leiophron* species were discovered and new mirid host-*Peristenus* associations in a variety of habitats were recorded during this survey (Table 2).

Table 1. Host range of *Peristenus* species in selected cultivated and natural habitats in Germany and Switzerland (adapted from White, 2002).

<i>Peristenus</i> species ^a	Hosts ^b in various habitats			
	Alfalfa and red clover	Mustard	Weedy fallow	Mountain meadow
<i>P. digoneutis</i>	<i>A. lineolatus</i> <i>L. rugulipennis</i> <i>L. pratensis</i>	<i>A. lineolatus</i> <i>L. rugulipennis</i> <i>L. pratensis</i>	<i>L. rugulipennis</i> <i>L. pratensis</i>	<i>L. dolobrata</i>
<i>P. rubricollis</i>	<i>A. lineolatus</i> <i>L. rugulipennis</i> <i>L. pratensis</i> <i>L. dolobrata</i> ^c		<i>L. dolobrata</i>	
<i>P. stygicus</i>	<i>A. lineolatus</i> <i>L. rugulipennis</i> <i>L. pratensis</i>	<i>A. lineolatus</i> <i>L. rugulipennis</i> <i>L. pratensis</i>	<i>L. rugulipennis</i> <i>L. pratensis</i>	<i>L. dolobrata</i>
Number of Mirid Species	16/13	8	12	21

^a*Peristenus digoneutis* Loan, *Peristenus rubricollis* (Thomson), *Peristenus stygicus* Loan

^b*A. lineolatus* = *Adelphocoris lineolatus* (Goeze); *L. rugulipennis* = *Lygus rugulipennis* Poppius; *L. pratensis* = *Lygus pratensis* (L.); *L. dolobrata* = *Leptopterna dolobrata* (Linnaeus)

^c*Leptopterna dolobrata* was found only in alfalfa (not red clover)

Table 2. Miridae-parasitoid associations in Germany and Switzerland.

Parasitoid species	Host species
<i>Peristenus adelphocoridis</i> Loan	<i>Adelphocoris lineolatus</i> (Goeze); <i>Closterotomus norvegicus</i> (Gmelin)
<i>Peristenus pallipes</i> (Curtis) (spring)	<i>Adelphocoris lineolatus</i> (Goeze); <i>Lygus rugulipennis</i> Poppius; <i>Lygus pratensis</i> (L.)
<i>Peristenus pallipes</i> (Curtis) (summer)	<i>Stenodema calcarta</i> Fallèn; <i>Stenodema virens</i> L.
<i>Peristenus</i> n. sp. near <i>pallipes</i>	<i>Closterotomus norvegicus</i> (Gmelin); <i>Leptopterna dolobrata</i> (L.)
<i>Peristenus</i> n. sp. near <i>digoneutis</i>	<i>Leptopterna dolobrata</i> (L.)
<i>Leiophron</i> n. sp. near <i>defeciens</i>	<i>Adelphocoris lineolatus</i> (Goeze); <i>Lygus rugulipennis</i> Poppius; <i>Lygus pratensis</i> (L.)

Field Surveys to Assess Host Range of *Ceutorhynchus* Parasitoids

Field host range surveys to determine parasitoid-host associations for Ceutorhynchinae species on specific host plants were conducted in northern Germany. The host plants surveyed included canola (*B. napus*), Shepherd's purse (*C. bursa-pastoris*), garlic mustard (*A. petiolata*), scentless chamomile (*T. perforatum*), Canada thistle (*C. arvensis*), and cabbage (*Brassica oleraceae* L.). Ectoparasitoids reared from Ceutorhynchinae survey collections in 2000 and 2001 were identified by Dr. Hannes Baur, Natural History Museum, Berne, Switzerland. Based on these identifications the host plant-Ceutorhynchinae-ectoparasitoid associations were established (Table 3). Although several new parasitoid host records were found, particular attention was given to host associations for *T. perfectus* and *M. morys*, as both species are potential biological control agents of *C. obstrictus* in Canada. Results showed that *C. obstrictus* was found only in canola seeds and *T. perfectus* was a common parasitoid. Interestingly, *T. perfectus* was reared for the first time from *C. constrictus* (Hinz and Gerber, 2000; Klander, 2001), which feeds in the seeds of garlic mustard. More surprisingly, *T. perfectus* was also found in low numbers parasitizing *Ceutorhynchus alliariae* Brisout and *Ceutorhynchus roberti* Gyllenhal larvae in stems of garlic mustard (Hinz and Gerber, 2000; Klander, 2001). *Mesopolobus morys* was reared for the first time from the host *Ceutorhynchus floralis* (Paykull) (Klander, 2001), which feeds on *C. bursa-pastoris* present in the canola habitat.

DISCUSSION

The necessity for detailed host-specificity testing of all agents before field release has been an accepted doctrine in weed biological control since the biological control of prickly pear, *Opuntia lindheimeri* Engelm (McFadyen, 1998). This approach is quite different from that generally used in the biological control of arthropods, in which host range testing of parasitoids or predators before release only began in Australia in the 1980s, and its wider implementation is still an ongoing process. When selecting non-target and native species for host specificity testing with exotic agents, Wapshere's centrifugal (phylogenetic) approach (Wapshere, 1974) has been generally thought to be equally valid for weeds and arthropod biological control programs (Sands, 1997; Sands and Van Driesche, 2000). How-

Table 3. Host plant-Ceutorhynchinae-ectoparasitoid associations in Germany (adapted from Klander, 2001)

Host Plant <i>Ceutorhynchus</i> species	Feeding Niche	Parasitoids Reared
<i>Brassica napus</i> <i>Ceutorhynchus obstrictus</i> Marsham	Seed	<i>Trichomalus perfectus</i> (Walker) <i>Trichomalus lucidus</i> Walker <i>Mesopolobus morys</i> L. <i>Stenomalina gracilis</i> (Walker) <i>Chlorocytus diversus</i> (Walker)
<i>Brassica oleracea</i> <i>Ceutorhynchus pallidactyllus</i> (Marsham)	Stem	<i>Trichomalus lucidus</i> <i>Trichomalus</i> sp. (new) <i>Stenomalina gracilis</i>
<i>Capsella bursa-pastoris</i> <i>Ceutorhynchus floralis</i> (Paykull)	Seed	<i>Mesopolobus morys</i> <i>Mesopolobus</i> cf. <i>incultus</i> (Walker) <i>Necremnus tidius</i> (Walker) <i>Baryscapus</i> sp.
<i>Tripleurospermum perforatum</i> <i>Microplontus edentulus</i> (Schulz) <i>Microplontus rugulosus</i> (Herbst)	Stem	<i>Stenomalina gracilis</i> <i>Eurytoma</i> sp.
<i>Alliaria petiolata</i> <i>Ceutorhynchus constrictus</i> Marsham	Seed	<i>Trichomalus perfectus</i>
<i>Alliaria petiolata</i> <i>Ceutorhynchus alliariae</i> Brisout <i>Ceutorhynchus roberti</i> Gyllenhal	Stem	<i>Trichomalus lucidus</i> <i>Trichomalus perfectus</i> <i>Stenomalina gracilis</i>
<i>Cirsium arvense</i> <i>Hadroplontus litura</i> (Fabricius)	Stem	<i>Stenomalina gracilis</i>

ever, there are practical problems in applying Wapshere's (1974) methods for selection of appropriate non-target species for physiological host range testing of entomophagous biological control agents. Systematic difficulties often exist and the phylogenetic relationships are frequently not well understood, even if recent revisions have been done (Kuhlmann *et al.*, 1998). Nonetheless, if this information is available, phylogenetic hypotheses for host and parasitoid groups can provide valuable insights when interpreting the significance of host range data.

In biological control of weed projects, testing the hosts of species closely related to the candidate agent is recommended to confirm specificity (Wapshere, 1974). However, for arthropod pests, there is no such basis for selecting non-target taxa since the relationships between the hosts of closely related agents differ extensively, even when the agents belong to the same genus (Sands, 1998). This has been shown in the *Lygus* biological control project, where *Peristenus* parasitoids attacked only a few mirid species, which are not closely related even though they occur in the same habitat (see Schwartz and Footitt, 1998 for the proposed phylogeny of *Lygus* plant bugs). In such cases, the life history, plant hosts, or habitats of the target may be more important in influencing the foraging and selection behavior of a parasitoid than the hypothesized taxonomic relatedness among potential hosts (Van Driesche and Hoddle, 1997). Phylogenetic information about the Ceutorhynchinae is not available. Therefore, there are practical problems to apply the Wapshere method.

The collection and maintenance of native and target arthropod species for physiological host specificity testing with exotic agents poses additional difficulties (Sands, 1997; Kuhlmann *et al.*, 1998). Further, for potential non-target test arthropods, there is a lack of biological and ecological information that often prevents culture of appropriate stages required for testing. In weed biological control programs it is not uncommon to test 50 to 100 non-target plant species with candidate herbivorous agents. This extensive testing approach would make arthropod biological control programs impossible due to these practical problems. Therefore, alternative approaches are needed.

Use of ecological information about the habitat-host-parasitoid relationships obtained in the area of origin is an appropriate approach for determining species to be tested in entomophagous biological control because it more accurately reflects the real host range and aids in reducing the number of non-target species that need to be tested in the area of importation. In both studies presented above, it has been shown clearly, that sampling non-target Miridae and Ceutorhynchinae in the area of origin provides an opportunity to obtain information about the diversity of host species that live in different habitats or feed on different host plants and to obtain information on the host ranges of the associated parasitoids. Both studies provide a good indication of the host specificity of particular parasitoids. Therefore it is suggested that the non-target species list for the physiological host range testing can be limited to the subfamily Mirinae for *P. digoneutis* and to the subfamily Ceutorhynchinae for *T. perfectus*. Additional field surveys in the area of introduction for indigenous non-target species belonging to the Mirinae and Ceutorhynchinae are also necessary to select appropriate non-target species for testing exotic entomophagous biological control agents in the quarantine laboratory.

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