Avoiding conflicts between insect and weed biological control: selection of non-target species to assess host specificity of cabbage seedpod weevil parasitoids


1CABI Bioscience Switzerland Centre, Dele ´mont, Switzerland; 2Agriculture and Agri-Food Canada, ECORC, Ottawa, ON, Canada; 3Department of Natural Resources, Cornell University, Ithaca, NY, USA; 4Agriculture and Agri-Food Canada, LRC, Lethbridge, AB, Canada; 5Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada; 6Department of Plant, Soil, & Entomological Science, College of Agriculture, University of Idaho, Moscow, ID, USA; 7Agriculture and Agri-Food Canada, Saskatoon, SK, Canada; 8Institut de Recherche en Biologie Végétale, Université de Montréal, Quebec, Canada; 9McClay Ecoscience, Sherwood Park, AB, Canada; 10Department of Entomology, University of Arkansas, Fayetteville, AR, USA

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Abstract: Classical biological control of insect pests and weeds may lead to potential conflicts, where insect pests are closely related to weed biological control agents. Such a conflict may occur in the classical biological control of the cabbage seedpod weevil, Ceutorhynchus obstrictus (Marsham) in North America, which belongs to the same subfamily, Ceutorhynchinae, as a number of agents introduced or proposed for introduction against non-indigenous invasive weed species. We propose a step-by-step procedure to select non-target species and thereby to develop a non-target species test list for screening candidate entomophagous biological control agents of a herbivore pest insect in a way that would simultaneously evaluate non-target potential on weed biological control agents and other non-target species. Using these recommendations, we developed a non-target test list for host specificity evaluations in the area of origin (Europe) and the area of introduction (North America) for cabbage seedpod weevil parasitoids. Scientifically based predictions on expected host–parasitoid interactions and ecological information about the ecological host range in the area of origin can help avoid conflicts, while still allowing the introduction of safe and effective agents against both insect pests and weeds.

Key words: cabbage seedpod weevil, Ceutorhynchinae, classical biological control, host range, host specificity, non-target species test list

1 Introduction

Classical biological control provides an opportunity to partially reconstruct the natural enemy complex of an invading non-indigenous insect pest or weed (Mills 1994), and its application has been highly recommended to control established non-indigenous invasive insect pest or weed populations (Wittenberg and Cock 2001). The ability of introduced natural enemies to persist in the environment, to reproduce, and to spread gives biological control a unique advantage as a pest control method (Greathed 1986). At the same time, once agents are established, biological control cannot be discontinued and great care in agent selection is required to avoid or minimize potential non-target effects.

Despite many proven benefits (Greathed 1995), classical biological control has recently come under scrutiny because introduced natural enemies may adversely affect native species, including rare and endangered species (Howarth 1991, 2000; Simberloff 1992; Simberloff and Stiling 1996; Louda et al. 1997; Boettner et al. 2000; Elkington and Boettner 2004). Ecological theory has provided some assistance in understanding success and failure in biological control, and can also be applied to identify risks of introduced natural enemies on native communities and non-target species (Wagge 2001; Hoddle 2004). However, little attention has been given to potential conflicts between insect biological control and weed biological control using invertebrate agents. Potential negative impacts of candidate entomophagous biological control agents on weed biological control agents have never been considered before initiating a
biological control programme and were not even defined as a potential conflict of interest amongst practitioners and researchers of weed biological control 20 years ago (Turner 1985). This is surprising as predation and parasitism was suspected as an important factor in limiting the success of biological weed control agents (Crawley 1989; Lawton 1990). To avoid jeopardizing the success of herbivores, great care is taken to eliminate natural enemies of weed biological control agents during host specificity screening and before introduction.

Non-target or unintended impacts of arthropod biological control agents have been documented (e.g. Hawkins and Marino 1997; Boettner et al. 2000; Follett and Duan 2000). Documented cases of where agents released for biological control of arthropods have attacked weed biological control agents are few. Examples include that of an adult endoparasitoid Microtonus aethiopoides Loan released to control the introduced curculionid forage pest, Sitona discoideus Gyllenhal, in New Zealand and endo and ectoparasitoids introduced into Hawaii to control the pepper weevil Anthonomus eugenii Cano, the Mediterranean fruit fly Ceratitis capitata (Wiedemann) and the oriental fruit fly Dacus dorsalis Hendel (Duan and Messing 2000 and references therein). Barratt et al. (1997) discovered that the weed biological control agent Rhyncoclycus conicus Froel was attacked by M. aethiopoides. In eastern North America, M. aethiopoides was released against the alfalfa weevil, Hypera postica (Gyllenhal) but no surveys have yet been conducted to determine if this endoparasitoid attacks R. conicus populations released in western North America to control Carduus nutans L., and Cardus acanthoides L. In Hawaii, the larval-pupal endoparasitoids Diachasmimorpha tryoni (Cameron) and Diachasmimorpha longicaudata (Ashmead), introduced to control the Mediterranean fruit fly, C. capitata and the oriental fruit fly, D. dorsalis, respectively, were also found attacking Procecidochares utilis Stone an introduced biological control agent of pamakani Eupatorium perfoliatum (Regel) K. & R. (Hymenoptera: Braconidae), and the larval ectoparasitoids Eupelmus cushmani (Crawford) and Euvyrola tephritis Fullaway introduced to control the pepper weevil A. eugenii were found attacking P. utilis as well as Procecidochares alani Steyskal, introduced to control Ageratina riparia (Regel) K. & R. (Duan and Messing 2000).

Review of classical biological control agents released or being evaluated in Canada and the United States against weeds indicated that more than 50% of the agents belong to three insect families, the Curculionidae, Chrysomelidae (both Coleoptera), and Tephritidae (Diptera) (Mason and Huber 2002; Coombs et al. 2004). Thus, potential conflicts are most probable when target species for entomophagous agents belong to these families.

Until recently, host specificity testing of entomophagous biological control agents was non-existent or perfunctory because non-target impacts were of little concern (Nechols et al. 1992), Waage (2001) stressed that despite the need to understand non-target effects of classical biological control retrospectively, it is more important to develop sound, ecologically-based methodologies for predicting and assessing the impacts of agents on target and non-target species. Recently a number of procedures or guidelines to select test species and to evaluate the impact of entomophagous biological control agents on non-target hosts have been proposed (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. 2003; Van Driesche and Reardon 2004; Kuhlmann et al. 2005; Bigler et al. 2006). These recent contributions illustrate that an array of criteria such as phylogenetic considerations, ecological features, biological characteristics and socio-economic factors have been used to compile non-target test list.

An important target for classical biological control is the cabbage seedpod weevil, Ceutorhynchus obstrictus (Marsham) (= C. assimilis (Paykull); see Collonelli (1990, 1993)). This invasive species, of European origin, is a serious pest of canola and rapeseed (Brassica napus L. and Brassica rapa L.) in North America (McCaffrey 1992; Buntin et al. 1995; Cárcamo et al. 2001; Dosdall et al. 2002; Mason et al. 2003). Adults feed on flower buds causing their destruction (bud-blasting) and larvae feed within seedpods, causing economic losses (McCaffrey et al. 1986; Buntin and Raymer 1994). The cabbage seedpod weevil was first recorded in North America in 1931 at the port city of Vancouver (McLeod 1962), and has since spread to other parts of western and eastern North America (Baker 1936; Hagen 1946; Crowell 1952; Walz 1957; Anonymous 1977; Dolinski 1979; Boyd and Lentz 1994; Buntin et al. 1995; Brodeur et al. 2001; Cárcamo et al. 2001; Dosdall et al. 2002; Mason et al. 2003). Dosdall et al. (2002) determined that in Canada C. obstrictus is dispersing at a rate of approximately 55 km per year and they predicted that it will eventually spread throughout the entire canola-growing region of western Canada.

Candidate biological control agents for C. obstrictus in North America include the adult endoparasitoid Microtonus melanopus Ruthe (Hymenoptera: Braconidae), and the larval ectoparasitoids Trichomus perfectus (Walker) and Mespodolus morys L. (Hymenoptera: Pteromalidae) (Kuhlmann et al. 2002). In Europe, impact by these species on C. obstrictus populations has been documented in several studies (e.g. Crowell 1952; Bonnemainson 1957; Jourdehuil 1960; Laborius 1972; Lerin 1987; Büchli 1991, 1993; Murcie 1996; Williams 2003). The most important parasitoid is T. perfectus and estimates of its parasitism level are in the range of 10–43.5% (Laborius 1972), 20–40% (Crowell 1952), 37.5–80% (Büchli 1991), and up to 95% (Lerin 1987).

In North America, several chalcid parasitoids were reared from C. obstrictus soon after its introduction

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(McLeod 1953), however, review of the taxonomic status of parasitoids associated with *C. obstrictus* in North America has shown that the principal larval ectoparasitoids *T. perfectus* and *M. morys* are not present (Gibson et al. 2005). The adult endoparasitoid *M. melanopus*, although present in North America, will not probably play a significant role in controlling populations of *C. obstrictus* (Harmon and McCaffrey 1997; Fox et al. 2004). Thus, introduction of *T. perfectus* and/or *M. morys* into North America as classical biological control agents could be considered. However, some Ceutorhynchinae have also been released in North America for biological control of weeds, including *Mogulones crucifer* (Pallas) [=*M. crucifer* (Herbst)] (De Clerck-Floate and Schwartzlaender 2002), *Hadroplontus litura* (Fabricius) (McCay et al. 2002a), and *Microplontus edentulus* (Schultze) (McCay et al. 2002b). Populations of these species possibly could be negatively affected if agents released for biological control of *C. obstrictus* are not specific to the target host.

To address the need to evaluate potential impacts on non-target species and avoid conflicts with weed biological control agents, we describe here a step-by-step procedure for selecting non-target species and for compiling a non-target species test list to assess the host specificity of candidate entomophagous biological control agents. To test the practicality of the proposed step-by-step procedure, we apply them to the classical biological control programme of *C. obstrictus* in Canada. As a result, this paper will present a non-target species test list for host specificity testing of cabbage seedpod weevils in the area of origin (Europe) as well as a test list for assessing the fundamental host range in the area of introduction (North America).

### 2 Materials and Methods

Laboratory methods used to assess host specificity of herbivorous insects proposed for classical weed biological control (see Wapshere 1974) are often suggested as a model for assessing host specificity of entomophagous agents. While the phylogenetic relationship (taxonomic relatedness) of target to non-target species is considered as a useful starting point, the taxonomy and phylogenetic relationships of insects are poorly known compared with plants. For example, worldwide, most of the 23 000 species of the plant family Asteraceae have been described and the evolutionary relationships within the family are well enough known that phylogenetic patterns have been proposed (see Bremer 1994). In contrast, there are more than 60 000 described species in the beetle family Curculionidae (Anderson 2002), but evolutionary relationships have been proposed for only a few groups (Anderson 1993). Thus, the selection of non-target curculionid species for testing is challenging. In North America, the Asteraceae contains 2695 species in 390 genera (Kartesz 1994) while the Curculionidae contains 2388 species in 239 genera (Anderson 2002). Consequently, testing of entomophagous species must rely on a subsample of species in related groups as has been done in weed biological control. In addition, entomophagous insects, as their herbivorous hosts, are less likely to behave normally under laboratory or field cage conditions and many false-positive results may be produced (Blossey et al. 1994; Greathead 1995). Testing of entomophagous species may be even more challenging because host finding and acceptance by insect parasitoids is often more complex than that of plant feeding species and because habitat selection frequently constrains host choice (see Godfray 1994; Quicke 1997). Finally, lack of information about the biology and rearing methods for many insects makes it difficult to assemble sets of test species for laboratory testing in the manner used to test herbivory on plants, which can usually be collected and stored as seeds until needed.

To address these constraints, we propose that field host range surveys in the area of origin provide a practical estimation of the host specificity of entomophagous biological control agents. These studies can be designed to gain insights into important issues such as the interactions between habitat type and parasitism levels by determining the range of habitats a candidate entomophagous insect might forage in if released and the parasitism level it might achieve on various hosts in different habitats. This information would provide an indication of whether taxa closely related to the target are present and if these are attacked.

To achieve this goal, the following step-by-step procedure to select non-target host species should provide a non-target species test list that contains test species of greatest relevance for field sampling in order to determine the ecological host range of entomophagous insects in the area of origin.

#### 2.1 Step 1. Study of biological control agent host range

To provide: (1) information that indicates if the biological control agent is a generalist or specialist natural enemy; (2) a list of known hosts; (3) available habitat–host plant–host information, suggesting habitats in which the natural enemy occurs; and (4) verification of taxonomic names and additional host and natural enemy information through collaboration with taxonomists.

**Outcome** – determination of the potential host specificity and potential limits for the range and number of species to be tested.

#### 2.2 Step 2. Taxonomy and phylogenetic relationships of target and non-target species

To provide: (1) verification that the taxonomic names are correct; (2) an indication of the reliability of the classification at the genus, subfamily, family levels (i.e. how well known); information about host–plant associations; and (3) information on the number of non-target species related to the target species and other known host species. To ensure host specificity potential outgroup and beneficial species must be studied.

**Outcome** – a preliminary list of species that could be tested [using the method described by Wapshere (1974)].

#### 2.3 Step 3. Potential for sympatry of target and non-target species

To provide an indication of overlap in: (1) geographical distribution; (2) time of occurrence; and (3) host plants of non-target species that occur in the same or similar habitats as host plants of the target species.

**Outcome** – determination of potential vulnerability of related and outgroup species defined by taxonomic and phylogenetic relationship studies; a refined non-target species test list. (Representatives are selected for each overlap type. Any established and candidate weed biological control agents must be included.)
2.4 Step 4. Accessibility of non-target species
To identify species on the refined host test list, which can be readily obtained for replicated experiments. For rare and endangered species, it should be acceptable to test closely related congeners as surrogates.

Outcome – reduction in the number of species on the non-target test list to be studied to a manageable level [this does not necessarily exclude rare/endangered species from testing (surrogate species)].

The proposed steps could then be used to develop a similar non-target species test list in the area where the candidate entomophagous biological control agent is planned to be released. The information gained from developing the test list for assessing the ecological host range in the area of origin would guide development of a test list for the area of introduction and allow the latter to focus on native species most likely to be affected.

3 Results
3.1 Selecting non-target species for host specificity testing in the area of origin (Europe)
3.1.1 Step 1. Study of biological control agent host range
The known host ranges of M. melanopus, T. perfectus and M. morys are presented in Table 1. These C. obstrictus parasitoids belong to different guilds (i.e. adult parasitoid guild and larval ectoparasitoid guild), but all appear to be host specific within the genus Ceutorhynchus based on literature records.

Outcome – The results from the application of step 1 indicate that all three parasitoids are probably restricted to the genus Ceutorhynchus but at least to species within the subfamily Ceutorhynchinae. Although M. morys was reported to parasitize the brassica pod midge, Dasineura brassicae Winnertz, this record was based on only a single reference and this association needs to be verified.

3.1.2 Step 2. Taxonomy and phylogenetic relationships of target and non-target species
The higher classification of the Curculionidae is becoming stabilized, yet even at the subfamily level agreement is not unanimous. For example, McNamara (in Bousquet 1991) recognized 26 subfamilies while Lawrence and Newton (1995) recognized only six. The classification used in this paper is that of Anderson (2002) who recognized 18 subfamilies including Ceutorhynchinae worldwide. Reconstructed phylogenies are available for relatively few groups in the Curculionidae and those that are available are often insufficiently resolved (Anderson 1993). At the genus level, taxonomic studies have been recently conducted and problems of nomenclature and species complexes have been clarified. For example, Colonelli (1993) determined that C. obstrictus was the correct name for cabbage seedpod weevil not C. assimilis. The classification of the Ceutorhynchinae was updated by Colonelli (2004), who determined that the subfamily Ceutorhynchinae contains 1316 species worldwide, and is divided into 11 tribes, of which the largest, Ceutorhynchini, has 80 genera and 863 known species. In Europe, the subfamily Ceutorhynchinae includes three tribes, Orobitini, Coryssomerini, and the largest Ceutorhynchini, which has 28 genera and 151 known species (Dieckmann 1972).

To address the requirements for outgroup and beneficial species comparisons, at least one representative beetle other than Ceutorhynchinae that also feeds on Brassicaceae should be considered for host specificity testing, and a predacious or parasitic beetle

<table>
<thead>
<tr>
<th>Parasitoid family, species and host species</th>
<th>Country</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>Braconidae</strong></td>
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<tr>
<td>Microctonus melanopus Ruthe</td>
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<tr>
<td>Ceutorhynchus obstrictus (Marsham)</td>
<td>France, Germany</td>
<td>Sprey 1925a, Risbec 1953, Jourdheuil 1960</td>
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<tr>
<td>Ceutorhynchus pallidicatus (Marsham)</td>
<td>France, Germany, Switzerland</td>
<td>Günthart 1949, Jourdheuil 1960, Lehmann 1965</td>
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<tr>
<td>Ceutorhynchus pleurostigma Marshall</td>
<td>France</td>
<td>Jourdheuil 1960</td>
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<tr>
<td>Ceutorhynchus sudecollis Paykull</td>
<td>France</td>
<td>Jourdheuil 1960</td>
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<tr>
<td>Ceutorhynchus napi Gyllenhal</td>
<td>France</td>
<td>Jourdheuil 1960</td>
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<tr>
<td>Ceutorhynchus picitaris Gyllenhal</td>
<td>France</td>
<td>Jourdheuil 1960</td>
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<td><strong>Pteromalidae</strong></td>
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<tr>
<td>Dasineura brassicae Winnertz (Diptera: Cecidomyiidae)</td>
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<tr>
<td>Trichonomas perfectus (Walker)</td>
<td>France, Switzerland</td>
<td>Secrétariat du service d’identification des entomophages, 1963</td>
</tr>
</tbody>
</table>
species should also be evaluated. The weevil subfamily Baridinae contains at least one species, *Baris coerulescens* Scopoli that feeds on roots of oilseed rape (Alford et al. 2003), thus a representative of this subfamily should be included as an outgroup representative. Several genera of the beetle family Chrysomelidae are Brassicaceae-feeding herbivores, including *Chaetocnema*, *Disonycha*, *Phyllotreta*, *Psylliodes* (Aliticae), *Colaphellus*, *Entomoscelis*, *Phaedon* and *Microtheca* (Chrysomelinae). The polyphagous genera *Galeruca*, *Diabrotica*, *Monoxia* and *Sistema* are also known to have species that are occasional Brassicaceae feeders (Jolivet and Hawkeswood 1995; Jolivet and Verma 2002). Among Chrysomelidae oligophagous species on Brassicaceae, such as *Phyllotreta* and *Psylliodes* spp., larvae feed on the roots and are similar in size to *C. obstrictus*; therefore, a representative species should be included as an outgroup representative. Beneficial Coleoptera that have been recorded from canola habitats in Europe include Carabidae (>20 spp.), Staphylinidae (~30 spp.), several Histeridae, Silphidae and Scarabeidae and two species of Coccinellidae, *Coccinella septempunctata* L. and *Propylea quatuordecimpunctata* (L.) (Bu¨chs and Alford 2003). However, there are no records of *C. obstrictus* parasitoids associated with any of these species (see table 1). Despite this fact, a representative species, such as *C. septempunctata* should be selected for comparisons.

Outcome – The results of the application of step 2 suggest that because phylogenetic relationships of Ceutorhynchinae are poorly known, all 151 species known from Europe are potential candidates for non-target testing in addition to outgroup and beneficial representatives. Therefore, the test list would include at least 153 species.

3.1.3 Step 3. Potential for sympatry of target and non-target species

Anderson (1993) attempted to determine the phylogenetic and ecological relationships of host plant associations in the Curculionidae and found that very few host plant associations are known with certainty. However, he determined that larvae of genera or species groups, and individual species, in the Ceutorhynchinae and related genera feed on a number of plant families (e.g. Brassicaceae, Urticaceae, Umbelliferae, Papaveraceae, Labiatae, Boraginaceae, Resedaceae, Asteraceae and the monocot Liliaceae) and many species of the genus *Ceutorhynchus* feed on a large number of plant species within a given family. Thus, parasitoids of *C. obstrictus* are likely to attack Ceutorhynchinae species feeding on a variety of host plant species. In order to prove this hypothesis, additional habitat–host plant–Ceutorhynchinae associations should be considered to select potential non-target host species for testing. From the 153 species identified in step 2, we selected representative non-target Ceutorhynchinae that feed: (1) on *B. napus* (cultivated agricultural habitat), (2) on major weed species in the cultivated agricultural habitat, (3) on major weed species near the cultivated agricultural habitat, and (4) on wild crucifers in natural habitats.

The list was further refined using information from Schroeder et al. (1993), who determined the major weed species in or near *B. napus* fields known to be hosts of Ceutorhynchinae (such as *Capsella bursa-pastoris* (L.) Medicus, *Thlaspi arvense* L., *Cirsium arvense* (L.) Scopoli). Additional Ceutorhynchinae species feeding on wild crucifers (i.e. *Allaria petiolata* (Bieberstein) Cavara and Grande, *Lepidium draba* L., etc.) in natural habitats must also be considered as potential hosts for parasitoids of *C. obstrictus*. Furthermore, the non-target species test list includes all Ceutorhynchinae weed biological control agents that have been released and established in Canada and in the United States (table 2, Julien and Griffiths 1998). Additionally, candidate weed biological control agents in the Ceutorhynchinae are included in the host specificity studies. Although selection of *M. crucifer* for testing might not be obvious because the larvae feed below ground, it should be studied because *M. melanopus* may parasitize adult *M. crucifer*.

The level of risk for Ceutorhynchinae either established or candidate for release in North America will also depend on the habitat type and distribution of the target weeds relative to where *C. obstrictus* will be targeted for biological control. For instance, the houndstongue infestations targeted for control by *M. crucifer* occur on mountainous and foothill rangelands in British Columbia and Alberta (De Clerck-Floate and Schwarzlaender 2002). These areas are not only in a different habitat type, but are generally remote from the cultivated areas where *B. napus* is grown in these provinces, although *M. crucifer* may eventually be attacked if host range testing has determined it to be a potential host.

However, those Ceutorhynchinae released to control weeds in cultivated crops, such as *H. litura* for Canada thistle or *M. edentulus* for scentless chamomile (table 2), will be at far greater risk of potential attack by parasitoids introduced against *C. obstrictus*. Both weeds are widespread in the Canadian prairie provinces of Manitoba, Saskatchewan and Alberta and where canola is grown (Donald 1990; Woo et al. 1991). Species such as *Trichosirocalus horridus* (Panzer) and *Phrydium ichus tasi* Warner, although established in North America for biological control of thistle and Mediterranean sage (Julien and Griffiths 1998) have been disregarded because they belong to the weevil tribe Ceutorhynchini which is well represented in the proposed European non-target test list (table 3).

Outcome – Based on the application of the criteria outlined above, step 3 results in a list of 26 Ceutorhynchinae species plus outgroup and beneficial representatives. The total number of species is 28 on the test list.

3.1.4 Step 4. Accessibility of non-target species

Before valid field host range surveys can be carried out it is essential to understand the life history of the Ceutorhynchinae test species. As mentioned earlier, lack of biological and ecological information often prevents correct timing of test host collections and the
development of rearing methods to await emergence of parasitoid species. Fortunately this expertise has been developed through studies on numerous Ceutorhynchinae studied as classical weed biological control agents (e.g. *M. edentulus* (Schultz) for scentless chamoimile). Weed biological control projects include studies on biology and ecology of Ceutorhynchinae species as well as development of rearing methods and assessment of potential host plant expansion. This knowledge is of great value and can be adapted to develop collection methods and rearing procedures for studying the ecological host range of cabbage seedpod weevil parasitoids. Determining the ecological interactions of the parasitoids of these other Ceutorhynchinae species is also essential to understanding how they may compete with and possibly regulate parasitoids of *C. obstrictus*. In general, the accessibility and availability of non-target species is an important point to provide enough test replicates ensuring meaningful results.

Outcome – Based on these considerations, a test list of non-target Ceutorhynchinae species was compiled for which parasitoid complexes and ecological host ranges will be determined in the area of origin facilitating the overall aim to assess the host specificity of cabbage seedpod weevil parasitoids in Europe. The list consists of 16 species, which includes beneficial and outgroup representatives (table 3).

### 3.2 Selecting non-target species for host specificity in the area of introduction (North America)

#### 3.2.1 Step 1. Study of biological control agent host range

In North America, the parasitoid *M. melanopus* is known only from *C. obstrictus* (Harmon and McCaffrey 1997; Fox et al. 2004). Records of *T. perfectus* and *M. morys* in the Catalogue of Hymenoptera of American North of Mexico (Krombein et al. 1979) are most likely all misidentifications of *Trichomalus lucidus* (Walker) and *Mesopolobus moryoides* (Gibson et al. 2005). Taxonomic studies (and thus information on host–parasitoid associations) on *Microctonus, Trichomalus* and *Mesopolobus* appear to be very limited. For example, Shaw (1997) stated that there are 26 described and many undescribed Nearctic *Microctonus* species, and that members of this genus parasitize adult Curculionidae, Chrysomelidae and Carabidae. Similarly, Bouček and Heydon (1997) indicated that for both *Trichomalus* and *Mesopolobus* ‘there are several dozens of species’. Although they note that *Trichomalus* spp. parasitize small Curculionidae in herbaceous plants, *Mesopolobus* are parasites of Cynipidae, various Lepidoptera, Symphyla and Coleoptera. Because of insufficient information on host associations in North America, to be safe a non-target species test list should include species from each family that members of the genus are likely to parasitize.

<table>
<thead>
<tr>
<th>Ceutorhynchinae spp.</th>
<th>Target weed</th>
<th>Feeding niche</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hadroplontus litura</em> (Fabricius)</td>
<td>Canada thistle, <em>Cirsium arvense</em> (L.) Scopoli [Asteraceae]</td>
<td>Stem</td>
<td>Established Canada: BC, AB, SK, ON, NS; USA: ID, MD, MT, ND, NE, OR, SD, UT, VA, WY</td>
</tr>
<tr>
<td><em>Trichosirocalus horridus</em> (Panzer)</td>
<td>Plumeless Thistle, <em>Carduus acanthoides</em> L. [Asteraceae]</td>
<td>Rosette</td>
<td>Established Canada: BC, SK, ON; USA: CO, KS, MD, MO, MT, OR, VA, WA, WY; Established Canada: BC, AB, SK, MB, ON; USA: ID, MD, MT, OR, WA, WY; Established Canada: NS</td>
</tr>
<tr>
<td><em>Ceutorhynchus alliariae</em> H. Brisout</td>
<td>Garlic mustard, <em>Aliaria petiolata</em> (Bieberstein) Cavana</td>
<td>Stem</td>
<td>Candidate</td>
</tr>
<tr>
<td><em>Ceutorhynchus roberi</em> Gyllenhal</td>
<td>and <em>Grandel</em> [Brassicaceae]</td>
<td>Stem</td>
<td>Candidate</td>
</tr>
<tr>
<td><em>Ceutorhynchus constrictus</em> (Marshall)</td>
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<td><em>Ceutorhynchus scrobicollis</em> Neresheimer &amp; Wagner</td>
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<tr>
<td><em>Ceutorhynchus turbinus</em> Schultze</td>
<td>Hoary Cress (Whitetop), <em>Lepidium draba</em> L. [Brassicaceae]</td>
<td>Seed</td>
<td>Candidate</td>
</tr>
<tr>
<td><em>Mogulones horraginis</em> (Fabricius)</td>
<td>Houndstongue, <em>Cynoglossum officinale</em> (L.) [Boraginaceae]</td>
<td>Seed</td>
<td>Candidate</td>
</tr>
</tbody>
</table>

Canada: BC, British Columbia; AR, Alberta; SK, Saskatchewan; ON, Ontario; NS, Nova Scotia; MB, Manitoba; USA: ID, Idaho; MD, Maryland; MT, Montana; NE, Nebraska; ND, North Dakota; SD, South Dakota; OR; Oregon; UT, Utah; CO, Colorado; VA, Virginia; WY, Wyoming; WA, Washington; MO, Missouri; KS, Kansas; CA, California.

*Not recently verified taxonomically*

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Table 3. Proposed non-target species test list in Europe for candidate biological control agents of Ceutorhynchus obstrictus (Marsham)

<table>
<thead>
<tr>
<th>Test species</th>
<th>Feeding niche</th>
<th>Host plant(s)</th>
<th>Selection criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curculionidae, Ceutorhynchinae, Ceutorhynchini</td>
<td>Seeds</td>
<td><em>Cynoglossum officinale</em></td>
<td>Same feeding niche, candidate weed biological control agent</td>
</tr>
<tr>
<td><em>Mogulones borraginis</em> (Fabricius)</td>
<td>Seeds</td>
<td><em>Capsella bursa-pastoralis</em> L.</td>
<td>Congener, adventive in NA</td>
</tr>
<tr>
<td><em>Ceutorhynchus typae</em> (Herbst) [=C. floralis (Paykull)]</td>
<td>Seeds</td>
<td><em>Alliaria petiolata</em> (Bieberstein) Cavana and Grande</td>
<td>Same feeding niche, candidate weed biological control agent</td>
</tr>
<tr>
<td><em>Ceutorhynchus constrictus</em> (Marsham)</td>
<td>Seeds</td>
<td><em>Lepidium draba</em> L.</td>
<td>Same feeding niche, candidate weed biological control agent</td>
</tr>
<tr>
<td><em>Ceutorhynchus turbatus</em> Schultze</td>
<td>Seeds</td>
<td><em>Lactuca sativa</em> L.</td>
<td>Same feeding niche, candidate weed biological control agent</td>
</tr>
<tr>
<td><em>Glocianus punctiger</em> (Sahlberg)</td>
<td>Seeds</td>
<td><em>Taraxacum officinale</em> Weber</td>
<td>Same feeding niche, adventive in NA, beneficial</td>
</tr>
<tr>
<td><em>Ceutorhynchus rapae</em> Gyllenhal</td>
<td>Leaves, stem</td>
<td><em>Brassica oleracea</em> L., <em>B. rapa</em></td>
<td>Congener, adventive in NA</td>
</tr>
<tr>
<td><em>Ceutorhynchus pallydactalus</em> (Marsham)</td>
<td>Stem</td>
<td><em>Brassica napus</em> L., <em>B. rapa</em></td>
<td>Same host plant, different feeding niche</td>
</tr>
<tr>
<td><em>Ceutorhynchus roberti</em> Gyllenhal</td>
<td>Stem</td>
<td><em>Alliaria petiolata</em> [Brassicaceae]</td>
<td>Different feeding niche, candidate weed biological control agent</td>
</tr>
<tr>
<td><em>Ceutorhynchus alliaiae</em> H. Brisout</td>
<td>Stem</td>
<td><em>Alliaria petiolata</em> (Bieberstein) Cavana and Grande</td>
<td>Different feeding niche, candidate weed biological control agent</td>
</tr>
<tr>
<td><em>Hydropontus littus</em> (Fabricius)</td>
<td>Stem &amp; crown</td>
<td><em>Carduus, Cirsium</em> [Asteraceae]</td>
<td>Weed biological control agent</td>
</tr>
<tr>
<td><em>Microplontus edentulus</em> (Schultze)</td>
<td>Stem</td>
<td><em>Matricaria perforata</em> Mérat [Asteraceae]</td>
<td>Weed biological control agent</td>
</tr>
<tr>
<td><em>Mogulones crucifer</em> (Pallas)</td>
<td>Shoot, root</td>
<td><em>Cynoglossum officinale</em> L.</td>
<td>Weed biological control agent</td>
</tr>
<tr>
<td><em>Ceutorhynchus scrobicollis</em> Neresheimer &amp; Wagner</td>
<td>Root</td>
<td><em>Alliaria petiolata</em> (Bieberstein) Cavana and Grande [Brassicaceae]</td>
<td>Different feeding niche, candidate weed biological control agent</td>
</tr>
<tr>
<td><strong>Baridinae</strong></td>
<td>Root</td>
<td><em>Brassica napus</em> L., <em>B. rapa</em></td>
<td>Different feeding niche, similar size, different subfamily, outgroup representative</td>
</tr>
<tr>
<td><em>Baris coerulescens</em> Scopoli</td>
<td>Root</td>
<td><em>Brassica napus</em> L., <em>B. rapa</em></td>
<td>Same host plant, similar size, different family, outgroup representative</td>
</tr>
<tr>
<td><strong>Chrysomelidae Alticinae</strong></td>
<td>Root</td>
<td><em>Brassica napus</em> L., <em>B. rapa</em></td>
<td>Same host plant, similar size, different family, outgroup representative</td>
</tr>
<tr>
<td><em>Psylliodes chrysocephala</em> (L.)</td>
<td>Root</td>
<td><em>Brassica napus</em> L., <em>B. rapa</em></td>
<td>Same host plant, similar size, different family, outgroup representative</td>
</tr>
<tr>
<td><strong>Coccinellidae, Coccinellinae, Coccinellini</strong></td>
<td>Root</td>
<td><em>Brassica napus</em> L., <em>B. rapa</em></td>
<td>Same host plant, similar size, different family, outgroup representative</td>
</tr>
</tbody>
</table>

Outcome – The result of the application of step 1 indicates that in North America little is known about the host ranges of *M. melanopus, Trichomalus* spp. and *Mesopolobus* spp.; therefore, a non-target test list would potentially be very broad. However, ecological host range information from the area of origin provides a good indication about potential host specificity.

3.2.2 Step 2. Taxonomy and phylogenetic relationships of target and non-target species

Similar to the European situation, phylogenetic information for North American Ceutorhynchinae is not available. The classification of the Ceutorhynchinae appears to be relatively up to date, with the subfamily containing 164 species divided into six tribes. Ceutorhynchini is the largest tribe with 15 genera and 90 known North American species (Anderson 2002). Although taxonomic studies are limited, Bousquet (1991) and Anderson (2002) listed the species present in Canada. The genus *Ceutorhynchus* containing 68 species is in need of revision, with the unpublished work of Scheibner (1963) providing the most recent key to species (Anderson 2002). As explained in the section on non-target test species for Europe, several genera of the beetle family Chrysomelidae are Brassicaceae-feeding herbivores (Jolivet and Hawkeswood 1995; Jolivet and Verma 2002). *Phyllotreta* spp. and *Psylliodes* spp. are oligophagous on Brassicaceae and...
occur in canola in North America. The native *Psyllodes punctulatus* Melsheimer (hop flea beetle), which is found on canola (L. Burgess and P.G. Mason, unpublished data) is similar in size to *C. obstrictus* and would be a good outgroup representative. Surveys conducted in Saskatchewan indicate that numerous beneficial Coleoptera occur in canola (Burgess and P.G. Mason, unpublished data). Many of these species, e.g. Carabidae (39 spp.), Histeridae (3 spp.), Staphylinidae (4 spp.), Silphidae (2 spp.) and Tenebrionidae (5 spp.), are ground-dwelling predators that are not associated with the niche occupied by *C. obstrictus*. However, adults and larvae of Coccinellidae (5 spp.) are found in close association with canola leaves and inflorescences. Thus, a representative species, e.g. *Hippodamia convergens* (Guérin), should be included in the non-target species test list.

Outcome – The result of the application of step 2 suggests that because phylogenetic relationships of Ceutorhynchinae are not known, all 164 species are potential candidates for non-target testing in addition to outgroup and beneficial representatives.

3.2.3 Step 3. Potential for sympatry of target and non-target species

As explained previously, the host plant range for Ceutorhynchinae and related groups is broad. Additionally, information on host plant ranges for many North American Ceutorhynchinae species is very limited, but, as mentioned in step 1, the European information provides guidance on factors that influence host range. From the 164 species, representative non-target Ceutorhynchinae were selected that feed: (1) on *B. napus* (cultivated agricultural habitat), (2) on major weed species in the cultivated agricultural habitat, (3) on major weed species near the cultivated agricultural habitat, and (4) on wild crucifers in natural habitats. The major weed species in or near *B. napus* and *B. rapa* fields known to be hosts of Ceutorhynchinae include *Sinapis arvensis* L., *C. bursapastoris* (L.) Medicus, *T. arvense* L., *Descaria sophia* (L.) Webb, and *Sisymbrium altissimum* L. (L.M. Dosdall and P.G. Mason, unpublished data). Additional Ceutorhynchinae species that feed on wild crucifers (such as the native *Lepidium virginicum* L., *Neoboeckia aquatica* (Ea.t.) Greene) found in natural habitats must also be considered as potential hosts of herbivores and parasitoids of *C. obstrictus*. However, a number of these species are not native to North America and are already included in the proposed European test list. Beetle species that occur in the same microhabitat as *C. obstrictus* must be considered as potential non-target hosts. Species that feed on the flowers or leaves of canola may potentially serve as hosts. Examples of these include *Anthiscus flavicans* LeConte (Anthicidae), *Pyraclonema dispersa* Green (Lampyridae), and *Collops vittatus* Say (Melyridae) (L. Burgess and P.G. Mason, unpublished data).

Outcome – The result of applying step 3 is a reduction in the number of potential species to include only those native Ceutorhynchinae that occur in or near agricultural habitats and/or that feed on Brassicaceae. The number of species is 33, plus one potential non-target host species. Table 4. Proposed non-target species test list in North America for candidate biological control agents of Ceutorhynchus obstrictus (Marsham)

<table>
<thead>
<tr>
<th>Test species</th>
<th>Larval feeding niche</th>
<th>Host plant(s)</th>
<th>Selection criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceutorhynchus neglectus Blatchley</td>
<td>Seed</td>
<td><em>Iris versicolor</em> L. [Iridaceae]</td>
<td>Same feeding niche, same subfamily</td>
</tr>
<tr>
<td>Mononychini</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mononychus vulpeculus (Fabricius) Phytophili</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhinoncus triangularis (Say)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthicidae</td>
<td>Flowers, foliage</td>
<td><em>Brassica napus</em> L., <em>B. rapa</em> L. [Brassicaceae]</td>
<td>Same host plant, associated feeding niche, different family</td>
</tr>
<tr>
<td>Anthicus flavicans LeConte</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysomelidae, Alticinae</td>
<td>Root</td>
<td><em>Brassica napus</em> L., <em>B. rapa</em> L. [Brassicaceae]</td>
<td>Same host plant, similar size, different family</td>
</tr>
<tr>
<td>Psyllodes punctulata Melsheimer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coccinellidae, Scymninae, Hyperaspini</td>
<td>Predator</td>
<td>Beneficial species, same habitat, widespread in N.A., different family</td>
<td></td>
</tr>
<tr>
<td>Hippodamia convergens Guérin-Ménéville</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melyridae</td>
<td>Flowers</td>
<td><em>Brassica napus</em> L., <em>B. rapa</em> L. [Brassicaceae]</td>
<td>Same host plant, associated feeding niche, different family</td>
</tr>
</tbody>
</table>
beneficial and three outgroup species for a total of 37.

3.2.4 Step 4. Accessibility of non-target species

Before valid field host range surveys can be carried out it is essential to understand the life history of the Ceutorhynchinae test species. For North American species there is a general lack of biological and ecological information. Such information must first be collected to ensure correct timing of test host collections and the development of rearing methods for host range studies. Of the 33 Ceutorhynchinae species mentioned above, the ecology of 20 species including their host plants is unknown. In addition, many of the species have limited distributions, thus, it is only feasible to study species that are sufficiently abundant.

Outcome – Based on these considerations, a list of eight non-target species was compiled which could be used in quarantine host specificity testing of the cabbage seedpod weevil parasitoids with the aim to determine the fundamental host range prior to release in the area of introduction (table 4).

4 Discussion

With increased globalization an increasing number of invasive non-indigenous insects and plants will invade agricultural and natural ecosystems creating an associated demand for classical biological control. This may lead to increased conflicts over introductions between herbivorous and entomophagous biological control agents. As a result, strategies need to be developed to minimize these conflicts while allowing effective biological control for plant and insect pests. Recent studies have used a variety of strategies to select species for non/target host tests (Babendreier et al. 2005; Kuhlmann et al. 2005); however, none have considered the potential conflicts that may arise between the need to introduce an effective biological control agent for an insect pest and introduced or candidate biological control agents for weeds that may serve as host for an entomophagous biological control agent. The step-by-step procedure proposed here attempts to address this potential conflict by incorporating weed biological control agents into the methods that have been proposed for selecting species for host range tests (e.g. Sands and Van Driesche, 2004; Kuhlmann et al. 2005, 2006). The emerging cabbage seedpod weevil problem in North America provided for the first time an opportunity to test the proposed strategy for its practicality, although its reliability needs to be examined through post-release evaluation and long-term monitoring of non-target impacts of any released agent.

Each step in the selection process generated a list of potential species for non-target testing. The study of the host range of candidate biological control agents (step 1) generated an initial list of 151 (subfamily Ceutorhynchinae) for Europe (Dieckmann 1972), and between 67 (Ceutorhynchus) and 159 (subfamily Ceutorhynchinae) potential test species in North America (Anderson 2002). Taxonomic relatedness of non-target species to the target (step 2) requires that unrelated species (i.e. outgroup) and beneficial species (i.e. weed biological control agents and predators) should be included. In this example, representatives of the Carabidae, Chrysomelidae, Coccinellidae, Histeridae, Scarabidae, Silphidae and Staphylinidae would be considered, and the list would be expanded to include up to 100 additional species.

However, the potential for sympathy of the non-target species with the target species (step 3) allows the list to be narrowed down considerably by selecting representatives of various non-target groups, those species most likely to be encountered by the biological control agent. The list is narrowed to 28 species for Europe and 37 for North America. Finally, accessibility of non-target species populations (step 4) reduces the list to the number of species that can actually be tested and results in a total of 17 species for Europe, which will facilitate the assessment of the ecological host range of cabbage seedpod weevil parasitoids in the area of origin (table 3). For Canada, a test list of eight non-target species was compiled for use in quarantine to assess the fundamental host range prior to release (table 4). In both cases the non-target species test lists enable selection of those species which allow prediction of the host range of the candidate entomophagous agent without undue expansion of the test list.

Sands (1997) suggested that testing more than 10 species of non-target species may be impractical, and in those cases where the non-target species test list is long, often the number of species could be reduced to a more manageable size. A review of 22 studies reported by Kuhlmann et al. (2005) found that the number of non-target species tested in the laboratory ranged from 1 to 23. Availability of test individuals is a key restriction; therefore, different sources including commercial or laboratory cultures, field collections, and progeny of field collected individuals could be considered.

Sampling non-target Ceutorhynchinae and their parasitoids in the area of origin provided information about the diversity and occurrence of Ceutorhynchinae that live in different habitats and the host ranges of their associated parasitoids. These studies provided a good indication about the host specificity of the principal parasitoids of C. obstrictus and thus provided strong evidence that the non-target species list for use in quarantine can be restricted to the subfamily Ceutorhynchinae.

By narrowing the non-target species test list we can avoid the need to maintain a large number of non-target host species that often makes testing programmes cumbersome or even impossible. It also resolves practical problems related to test species such as uncertainty about their identity, lack of information about their biology, or difficulty in rearing.

In North America, field surveys for indigenous Ceutorhynchinae species and introduced weed biological control agents are still necessary to fully assess the diversity of Ceutorhynchinae in the proposed areas of introduction. Information obtained from such studies can be used to refine the non-target test species list as recommended by Kuhlmann et al. (2006). This,
when combined with the baseline ecological host range data in Europe, would ensure that only appropriate non-target species are studied.

We hope that our recommendations will help to improve the host specificity testing of entomophagous biological control agents. The compilation of a non-target host test list is a valuable step in the pre-release assessment as it provides a mechanism for assembling and synthesizing relevant information and knowledge. Furthermore, these recommendations may be useful for resolving other weed–insect pest biological control agent conflicts on a case-by-case basis, most likely when insect pest targets belong to the Curculionidae, Chrysomelidae and Tephritidae. While our stepwise protocol is a first step towards a sophisticated screening procedure, we need post-release field evaluations and verifications through long-term monitoring to assess whether our procedures can predict the ecological host range of candidate entomophagous biological control agents.

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Author’s address: Dr Ulrich Kuhlmann (corresponding author), CABI Bioscience Switzerland Centre, Rue des Grillons 1, 2800 Delémont, Switzerland. E-mail: u.kuhlmann@cabi.org

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