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Assessing host specificity of a classical biological control agent against western corn rootworm with a recently developed testing protocol

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ABSTRACT

Celatoria compressa (Wulp) (Diptera: Tachinidae), a parasitoid of adult chrysomelid beetles in the subtribe Diabroticina in North America, has been selected as a candidate for classical biological control of the alien invader, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae: Galerucinae), into Europe. We conducted host specificity testing to evaluate the fundamental host range of *C. compressa* and potential risks to native European coleopteran species. Nine potential non-target beetles were tested for host selection with *D. v. virgifera* in no-choice tests, sequential no-choice tests, choice tests and sequential choice tests in small experimental arenas in a quarantine laboratory. The nine representative non-target species were selected for experimentation based on (1) ecological host range information of *C. compressa*, (2) ecological similarities to *D. v. virgifera*, (3) close phylogenetic/taxonomic relationships, (4) safeguard considerations, (5) morphological similarities, geographical distributions, overlap of temporal occurrences with *D. v. virgifera* and *C. compressa*, and (6) accessibility and availability. Of the potential nine non-target hosts tested, gravid *C. compressa* only parasitized a few red pumpkin beetles, *Aulacophora foveicollis* (Chrysomelidae: Galerucinae), regardless of the presence or absence of *D. v. virgifera.* However, *C. compressa* significantly preferred *D. v. virgifera* (44.6% parasitized) over *A. foveicollis* (2.7%) in choice tests. Of the 1110 *A. foveicollis* tested among all experiment types, only 23 were parasitized and only one *C. compressa* successfully developed from the parasitism, demonstrating that *A. foveicollis* is a poor host. In conclusion, *C. compressa* has a fundamental host range restricted to the subtribes Diabroticina and Aulacophorina, and would therefore be unlikely to have a direct impact on indigenous species in Europe.

1. Introduction

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae: Galerucinae: tribe Luperini, subtribe Diabroticina), is one of the most serious pest of maize (*Zea mays* L.) in North America (Krysan and Miller, 1986). It is hypothesized to have originated in Mexico, spreading with maize cultivation to the USA and Canada (Krysan and Smith, 1987). *D. v. virgifera* was accidentally introduced several times from North America into Europe in the late 1980s to the early 2000s (Miller et al., 2005).

Classical biological control provides an opportunity to partially reconstruct the natural enemy complex of an invading alien pest (Mills, 1994), and its application has been highly recommended to control established alien pest populations (Wittenberg and Cock, 2001). *D. v. virgifera* has been identified as one such target for classical biological control. While classical biological control can be a cost effective alternative to pesticides for managing invasive alien pests, there are growing concerns regarding non-target impacts of introduced natural enemies (Follett and Duan, 2000; Wajnberg et al., 2001). While the ability of introduced biological control agents to persist, to reproduce and to spread in the environment are advantageous for pest control (Waage, 2001), these same traits increase the risk of unexpected ecological effects agents may cause to native species both within and beyond the targeted agro-ecosystems (Louda et al., 2003).

International standards for regulations of biological control agents have recently been developed incorporating methods of risk assessment studies (Bigler et al., 2006; Hunt et al., 2008; REBECA, 2007) and act both to protect biodiversity and optimize biological control (Mason and Kuhlmann, 2002). In order to predict potential non-target effects of biological control introductions, the fundamental (synonym potential) host range of a biological control candidate needs to be investigated (van Driesche and Hoddle, 1997; Hopper, 2001). This fundamental host range is the genetically delimited range or host specificity of a biological control candidate including species that were found to support the development of a candidate under laboratory conditions (Onstad and McManus, 1996; Haye et al., 2005, 2006). Non-target field parasitism can be predicted if native species which may be suitable hosts for the biological control candidate are included in such host specificity testing (Barratt et al., 1997; Fuester et al., 2001; Louda et al., 2003).

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However, compared with weed biological control agents (Blossey, 1995), the development and standardization of robust methods both for selecting native non-target species and for conducting the host specificity tests of arthropod biological control agents are lagging (Sands, 1997; van Driesche and Hoddle, 1997). In 2006, van Lenteren et al. proposed a framework for step-wise host range testing including how to test the host specificity of exotic biological control agents based on earlier studies from Sands (1997), van Driesche and Hoddle (1997), van Lenteren et al. (2003). van Lenteren et al. (2006) provided for the first time a detailed protocol for evaluating the environmental risk of introducing an alien organism for biological control. Few studies have used comparable protocols (Babendreier et al., 2005), such as for the biological control of *Sitona* weevils in lucerne in New Zealand (Barratt et al., 1997), *Lygus* plant bugs in Canada (Haye et al., 2005, 2006) and the cabbage maggot *Delia radicum* (L.) in Canada (Andreassen et al., 2008).

The parasitoid *Celatoria compressa* (Wulp) (Diptera: Tachinidae) is being evaluated as a candidate biological control agent to manage *D. v. virgifera* (Kuhlmann et al., 2005). This adult parasitoid inserts an egg containing a fully developed first-instar larvae into the hosts using its elaborately modified piercing ovipositor (Zhang et al., 2003). After 13–14 days, a third-instar larva emerges and pupates within an hour. About 1 week later, an adult tachinid emerges which will, after 5–6 more days, start to parasitize adult hosts. In the field, this parasitoid has been found to parasitize beetles in the genera *Acalymma*, *Cerotoma*, *Gynandrobrotica* and *Diabrotica* of the subtribe Diabroticina in Mexico, including *D. v. virgifera* (Eben and Barbercheck, 1996; Gámez-Virués and Eben, 2005). Usually 0.5–10% of *Diabrotica* adults are parasitized in the field. Occasionally parasitism rates can reach up to 16% (Toepfer et al., 2008). Our study follows previous studies on the basic biology and small-scale rearing (Zhang et al., 2003) and on the reproductive biology of *C. compressa* (Zhang et al., 2004).

In our study, potential non-target species were identified/selected and used in *C. compressa* host specificity testing in order to predict non-target risks prior to its importation into Europe. Indigenous coleopteran species were selected for testing under quarantine laboratory conditions according to a recently developed protocol proposed by Kuhlmann et al. (2006). Thereafter, no-choice and choice tests for determining the fundamental host range of inundative biological control agents, outlined by the framework of van Lenteren et al. (2006), were applied and slightly modified (see Section 2 and 4) to assess the host specificity of the candidate classical biological control agent *C. compressa*.

2. Materials and methods

2.1. Sources and rearing of parasitoid and target host

In order to obtain the parasitoid *C. compressa*, surveys were conducted in Mexico in collaboration with Astrid Eben (Instituto de Ecologia, Xalapa, Mexico) and Rebeca Alvarez – Zagoya (Instituto Politecnico Nacional, CIIDR-IPN, Durango, Mexico). *Diabrotica* spp. adults including *D. v. virgifera* were collected in maize, pumpkin and bean fields in the states of Durango, Oaxaca, Puebla and Veracruz. Field-collected adults were reared in Mexico and 104 puparia of *C. compressa* were shipped to a quarantine laboratory at CABI Europe – Switzerland. Individuals were sent to Nigel Wyatt (Natural History Museum, London, UK) to confirm the identification of *C. compressa*. A small-scale rearing method of *C. compressa* as described by Zhang et al. (2003) was implemented to produce sufficient females for host specificity testing. Naïve mated females (Monteith, 1963) were held individually in one-liter plastic containers (Zhang et al., 2003) and used in the non-target testing after a pre-oviposition period of 6 days to ensure availability of mature eggs.

A non-diapausing laboratory strain of *D. v. virgifera* was continuously reared under quarantine conditions (for rearing details see Branson et al., 1975; Jackson, 1985). Eggs were obtained from the USDA-ARS Northern Grain Insect Research Laboratory at Brookings, South Dakota, USA. *D. v. virgif*era adults were kept in gauzed wooden cages (300 × 300 × 550 mm) with water source, maize leaves and artificial diet (Branson et al., 1975; Jackson, 1985). Additionally, adults of a wild diapausing strain from the Central South Eastern European *D. v. virgifera* population (Miller et al., 2005) were collected from maize fields in Csongrad County in southern Hungary, and kept in the same way as described above.

2.2. Selection of non-target species

The following guideline was applied step-by-step in order to select indigenous non-target species for host specificity testing of *C. compressa* under quarantine conditions (Kuhlmann et al., 2006):

2.2.1. Ecological host range information

Information has been compiled about the ecological (synonym realized) host ranges of the known *Celatoria* species: *C. compressa*, *Celatoria bosqi* Blanchard, *Celatoria diabroticae* (Shimer), and *Celatoria setosa* (Coquillett), based on published host parasitoid records from North, Central and South America (Toepfer et al., 2008). Such field data from the area of origin are considered to be an indication of the host range of *C. compressa* in the area of introduction (Hopper, 2001).

2.2.2. Ecological similarities

Literature records have been scanned in the CAB Abstracts database (Anonymous, 1913–2007) to compile a non-target list of the coleopteran species which occur in European agricultural habitats within and surrounding the areas where *D. v. virgifera* has invaded, including: maize (*Z. mays* L.), lucerne (*Medicago sativa* L.), pumpkin (*Cucurbita maxima* Duch.), wheat (*Triticum aestivum* L.), and sunflower (*Helianthus annuus* L.), as well as field margin habitats.

2.2.3. Phylogenetic/taxonomic affinities

Relationships of the non-target species to the target have been checked to ensure that European species of phylogenetically related genera, tribes or subfamilies of the target are added to the non-target list (Wilcox, 1972; Gillespie et al., 2008). This approach is similar to the phylogenetic centrifugal method successfully applied for evaluating the safety of organisms for the biological control of weeds (Wapshere, 1974, 1989; Blossey, 1995) and recently also for the control of arthropods (Haye et al., 2006).

2.2.4. Safeguard considerations

Representatives of beneficial insect families including weed biological control agents have been included as well as representatives from other coleopteran families which are abundant in the above mentioned agricultural habitats.

2.2.5. Spatial, temporal, morphological attributes

The non-target list was progressively reduced, due to the fact that non-target species at risk must have similar affinities overlapping with those of the target, such as (a) the geographical distribution (i.e., European continent excluding Scandinavia), (b) temporal pattern of adult occurrence in the field (i.e., June until October for *D. v. virgifera* (Hemerik et al., 2004; Toepfer and Kuhlmann, 2006)) and (c) morphological similarity in size [i.e., 3–12 mm required for parasitoid development within the host (Eben and Barbercheck, 1996)].