

THE INVASION OF THE WESTERN CORN ROOTWORM, *DIABROTICA VERGIFERA VIRGIFERA*, IN EUROPE AND POTENTIAL FOR CLASSICAL BIOLOGICAL CONTROL

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ABSTRACT

The maize-destroying western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) originates from Mexico and is an invasive species in the United States and in Europe. *Diabrotica v. virgifera* was accidentally introduced into Europe near Belgrade in Serbia in 1992. Within 10 years, this invasive alien species spread throughout Central Europe. Recently, several new spots of isolated invasions were reported in Europe showing that *D. v. virgifera* is a very successful invader. Its major success is suggested to result from: (a) suitable pathways for multiple introduction events; (b) the high flight ability of the beetle, which allows a successful initial colonization movement towards maize fields; (c) a high rate of success in pheromone-mediated mate location even at small initial population sizes; and (d) a high potential fecundity. Furthermore, this success of invasion does not seem to be reduced by the high generational mortality of more than 99% or the low realized fecundity, both of which should reduce the probability of establishment and population growth. Conclusively, European maize production is threatened. Classical biological control could be one element of a sustainable management strategy against *D. v. virgifera*. After conducting reviews, surveys and experiments on potential classical biological control agents, the following conclusions were compiled from a detailed step-by-step approach: (1) effective indigenous natural enemies are not attacking any life stage of *D. v. virgifera* in Central Europe; (2) in the area of origin surveyed, *Celatoria compressa* Wulp (Diptera: Tachinidae) was the only parasitoid found on the target species, *D. v. virgifera*, and its host range is considered to be restricted to Diabroticite beetles; (3) prior to its potential importation, the parasitoid's basic and reproductive biology has been clarified; and (4) according to the results of host specificity testing, *C. compressa* would be safe for introduction as direct and indirect impacts on other organisms would be extremely low. Therefore a sustainable integrated management approach is likely to incorporate classical biological control with other control measures such as tolerant maize varieties and crop rotation.

INTRODUCTION

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) as well as its host plant *Zea mays* L. (Poaceae) evolved together in the subtropics of Mexico and Central America, and are non-native species in Europe. *Diabrotica v. virgifera* was accidentally introduced into Europe and was first observed near Belgrade in Serbia in 1992 (Kiss *et al.* 2005). Within 10 years, this invasive alien species spread over 310,000 km² throughout Central Europe and its eradication became impossible (Kiss *et al.* 2005). Recently, several new spots of isolated invasions were reported, such as in Lombardy (Italy), near Paris (France), near Basel (France and Switzerland), Amsterdam (The Netherlands), and London (UK) showing that *D. v. virgifera* is a very successful invader (Kiss *et al.* 2005), and will significantly change European maize production systems. Pathways of introductions of *D. v. virgifera* have never been formally investigated. The theory of introduction into Europe from North America via airplanes is generally accepted (Kiss *et al.* 2005). Possible causes of the successful invasiveness of *D. v. virgifera* theoretically include: (a) suitable pathways for multiple introduction events; (b) a remarkable flight ability of the beetle, which allows successful initial colonization movements towards maize fields; (c) a low viable population size required to build up a population; (d) a high capability of finding the counter sex for copulation at small initial population sizes by using sex pheromones; (e) a low mortality of developmental stages of *D. v. virgifera* in the invaded areas; and (f) a high fecundity.

20 Due to the successful invasion of *D. v. virgifera* in Europe, a sustainable management strategy against this invasive alien pest is needed. Classical biological control may have an important application in such a management policy as it provides an opportunity to partially reconstruct the natural enemy complex of an invading alien pest. *Diabrotica v. virgifera* is considered a prime target for a classical biological control approach. In order to investigate the initial colonization process of this invasive alien pest towards maize fields, mark-release-recapture studies were conducted in southern Hungary in 2003 and 2004. To study the potential use of classical biological control, life table studies were used to find host niches of *D. v. virgifera* that are not presently occupied by indigenous natural enemies in the area of invasion. Based on this information, specific and effective natural enemies from the area of origin were selected and investigated for potential introduction into Europe. Over the last five years we evaluated the potential of classical biological control applying a step-by-step approach: (1) investigating the initial colonization process of introduced *D. v. virgifera*; (2) conducting life table studies for *D. v. virgifera* in the area of invasion and studying the minimum viable population size of *D. v. virgifera*; (3) surveying for natural enemies in the area of invasion and origin; and (4) assessing the suitability and host specificity of candidate biological control agents.

MATERIALS AND METHODS

MARK-RELEASE-RECAPTURES DURING THE INITIAL COLONIZATION PROCESS OF INTRODUCED *D. V. VIRGIFERA*

Mark - release - recapture studies were conducted in southern Hungary in order to investigate the movements of *D. v. virgifera* towards suitable habitats, such as to its host plant maize

(for details refer to Toepfer *et al.* 2004). In 2003 and 2004, nine mark-release-recapture experiments were carried out in a grass steppe-area and alfalfa field, in which two small maize fields had been planted 300 m distant from the release points. After each release of 5,500 to 6,000 *D. v. virgifera*, adult beetles were recorded three times every second day by non-baited yellow sticky traps placed at regular intervals around the release point. The probability of arrival of female beetles in maize field was estimated to assess the risk of establishment of starter populations.

LIFE-TABLE STUDY TO MEASURE MORTALITY FACTORS AND MINIMUM VIABLE POPULATION SIZE

Life-table studies of this univoltine species were conducted in two maize fields in southern Hungary from 2000 to 2003 in order to provide an ecological understanding of mortality factors regulating population dynamics of this invasive pest (for details refer to Toepfer and Kuhlmann 2005). The mortality affecting the egg stage was assessed by exposing several thousand eggs to pre-overwintering, overwintering and post-overwintering conditions and by recovering the surviving eggs. The mortality levels of larval to adult stages were measured by artificially infesting 286 maize plants and subsequently recovering (1) the three larval instars and pupae at six time intervals using soil-root sampling and (2) the adults using emergence cages. Three age-specific life-tables were constructed. Finally, the reproductive rates of small founding populations were calculated to discern the minimum viable population size.

NATURAL ENEMY SURVEY IN THE AREA OF INVASION AND ORIGIN

A three-year field survey was conducted in Hungary, Yugoslavia, and Croatia, which are currently the focal points of invasion, to determine the occurrence of indigenous natural enemies of *D. virgifera* in Europe. A total of 9,900 eggs, 550 larvae, 70 pupae and 33,000 adults were examined for the occurrence of parasitoids, nematodes, and fungal pathogens. Moreover, the above-described life-table study was used to determine host niches of *D. v. virgifera* that are not presently occupied by indigenous natural enemies. In a next step, the structure and function of natural enemies in the area of origin of *D. v. virgifera* was assessed, with a special emphasis placed on parasitoids of *Diabrotica* adults. Surveys were conducted in collaboration with Dr. Astrid Eben (Instituto de Ecologia, Xalapa, Mexico) and Dr. Rebeca Alvarez Zagoya (Instituto Politecnico Nacional, CIIDR-IPN, Durango, Mexico). Adults of *Diabrotica* spp. were collected in agricultural and natural habitats containing a high species diversity including the target species *D. v. virgifera* in northern Mexico. In collaboration with G. Cabrera Walsh, (USDA-ARS South American Biological Control Laboratory, Buenos Aires, Argentina) *Diabrotica* adult natural enemy surveys were carried out in central and northern Argentina as well as southeastern Brazil. *Diabrotica* adults were collected directly from leaves and flowers of maize, beans, squash or wild plants within the fields. In all surveys, every available species of the sub-tribe Diabroticina, e.g. *Diabrotica* spp., *Acalymma* spp., and *Ceratoma* spp., were collected. *Diabrotica* adults were separated based on species, collection site and collection date and colonies were maintained in cages until emergence of parasitoid larvae. Based on the original host ranges of the parasitoids discovered, as well as their respective parasitism rates and overwintering strategies, the most promising parasitoid species was selected for further studies.

SUITABILITY AND HOST SPECIFICITY OF THE CANDIDATE BIOLOGICAL CONTROL AGENT

In order to study in detail the suitability of the selected candidate biological control agent, *Celatoria compressa* Wulp (Diptera: Tachinidae), the following attributes were investigated: (a) behavior of host attacks by *C. compressa* females, (b) the larviposition period, (c) the number of daily larviposition attempts per female, (d) the number of puparia produced daily per female and (e) the cumulative puparia production per female over the entire larviposition period (for details refer to Zhang et al. 2003). The functional response of *C. compressa* was also studied. A randomly chosen density between one and 50 adults of *D. v. virgifera* was offered to an individual 8 to 10 day-old mated female for 24 hours. After exposure, the hosts were kept for 20 days and emerged *C. compressa* larvae were recorded for each host density.

With respect to the safety of biological control, standards and frameworks recently developed for the release of exotic biological control agents were followed (van Lenteren et al. 2003). This study focused first on the selection of potential non-target species at risk in *D. v. virgifera* invaded areas in Europe, and secondly on host specificity testing of *C. compressa*. According to the phylogenetic centrifugal method proposed for weed biological control agents and a practical approach suggested by Kuhlmann and Mason (2003), a simplifying procedure was applied and indigenous Coleopteran species were selected for testing under quarantine laboratory conditions (for details refer to Kuhlmann et al. 2005). Thereafter, the host specificity of the candidate classical biological control agent *C. compressa* was assessed in (1) no choice tests, (2) sequential no choice tests, (3) choice tests, and (4) sequential choice tests.

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RESULTS

INITIAL COLONIZATION PROCESS OF INTRODUCED *D. V. VIRGIFERA*

In five out of 15 recapture periods, released *D. v. virgifera* populations performed a uni-directional movement (38%), in three cases beetles performed a bi-directional movement (20%), and in seven cases no directional movement was found (46%). In ten out of 15 recapture periods, the released populations were moving in a direction that was comparable with the mean wind direction. Averaging over sites and years, *Diabrotica* beetles did not move in the directions of the two small maize fields more frequently than expected if assuming random movement. However, beetles did travel significantly more frequently in the direction of naturally occurring maize fields (within a radius of 1,500 m) than towards other habitats. On average, $2.8\% \pm 3.2$ SD of all recaptured *Diabrotica* beetles arrived in a 300 m distant small maize plot.

MORTALITY FACTORS AND MINIMUM VIABLE POPULATION SIZE

A total mortality of $99.6\% \pm 0.16$ SD was determined during the time period between oviposition and the emergence of adults. Highest losses were generated by mortality during the first instar larval stage (94.2% marginal death rate) and by the inability of adults to realize their potential fecundity (80.4%). The most successful age intervals were pre- and post-dia-pausing eggs, with marginal death rates of only 17.3% and 18.4%, respectively. The third instar larvae and pupae also had a moderately low marginal death rate of 36%. Factors that

varied greatly between years and sites, such as realized fecundity and mortality of second and third instar larvae and overwintering eggs, had the highest potential to change population growth. *Diabrotica v. virgifera* net reproductive rates were generally below 1 (mean $R_0 = 0.62$) indicating declining pest populations. Therefore, the risk is low that newly introduced females of this alien beetle would produce enough eggs, larvae and emerging adults to establish a new generation. However, when considering the maximum potential progeny of females, the capacity of increase could reach growth factors between 2 and 4.5. Luckily, only 19.6% of this potential fecundity was usually realized under field conditions and the mean realized fecundity of starter populations varied substantially between years and between individual females.

NATURAL ENEMY SURVEY IN THE AREA OF INVASION AND ORIGIN

The natural enemy survey as well as the life-table study revealed that effective indigenous natural enemies were generally not attacking any of the life stages of *D. v. virgifera* in Europe. Two exceptions were the fungi *Beauveria bassiana* (Bals.) Vuill. (Mitosporic fungi; formerly Deuteromyces) and *Metarhizium anisopliae* (Metsch.) Sorok (Mitosporic fungi) attacking adults of *D. v. virgifera* on an extremely low level (< 1%). However no other entomopathogenic fungi, entomopathogenic nematodes, or parasitoids were found on eggs, larvae, pupae or adults. Therefore, each life stage of *D. v. virgifera* would be a suitable target for a classical biological control agent, and the natural enemy complex of the invading alien *D. v. virgifera* may be reconstructed in Europe.

Based on survey results and literature records in the area of origin of *Diabrotica* spp. (Cabrera Walsh *et al.* 2003; Eben and Barbercheck 1996; Guimaraes 1977; Heineck-Leonel and Salles 1997), adult parasitoids are probably the most common natural enemies of species in the *virgifera* group of the genus *Diabrotica*, followed by mermithid nematodes of the genus *Hexamermis* (Eben and Barbercheck 1996; Kuhlmann and Burgt 1998). As the focus of this survey was to obtain parasitoids of adult *Diabrotica*, knowledge of natural enemies attacking the soil dwelling larval stages of *Diabrotica* beetles is still incomplete. In the classical biological control agent selection process, five of the six known parasitoid species of adult *Diabrotica* in the area of origin were excluded from consideration based on information gleaned from the literature. The North and Central American tachinid fly, *Celatoria setosa* was not selected as Fisher (1983) indicated that this fly is almost exclusively a parasitoid of *Acalymma* species. Similarly, the North American tachinid fly, *Celatoria diabroticae* Gahan was also not chosen because it appears to be strongly associated with only *Diabrotica undecimpunctata howardi* Barber (Summers and Stafford, 1953), and *D. undecimpunctata undecimpunctata* Mannerheim (Fischer, 1981). The North American braconid *Centistes diabroticae* was not considered as it has been only reared from *Acalymma vittata* (F.) (Gahan, 1922; Fischer, 1981). Experimental data indicated that the South American *Celatoria bosqi* will not accept *D. v. virgifera* adults as hosts, which suggested that this tachinid appears to be specific to the *fucata* group within the genus *Diabrotica*. The South American braconid *Centistes gasseni* Shaw was rejected as a potential biological control agent due to the parasitoid's incompatible overwintering strategy and rearing difficulties. The only parasitoid actually found on the target species, *D. v. virgifera*, was the tachinid *Celatoria compressa* from northern Mexico. It attacked a range of species in four different genera of Diabroticite beetles suggesting a large

number of host species, but nonetheless restricted to Diabroticite beetles. *Celatoria compressa* was ultimately the only parasitoid that was selected as a candidate biological control agent for *D. v. virgifera* in Europe based on its availability in northern Mexico, its known host range including the target host record, and its suitability for rearing under laboratory conditions. Generally, it should be noted that distribution and the efficacy known for *C. compressa* should be considered as provisional due to the fact that these tachinid species have been little studied.

SUITABILITY AND HOST SPECIFICITY OF THE CANDIDATE BIOLOGICAL CONTROL AGENT

The age of *C. compressa* adults was found to be the most crucial factor in achieving mating. Only newly emerged, one hour-old females mated successfully with 2 to 5 day-old males. During the pre-larviposition period, the egg load of females increased steadily from day one (mean = 16.6 ± 1.1 SE) to a maximum egg load on day four (mean = 69.3 ± 0.8 SE). During a female's larviposition period (mean = 22.5 ± 0.6 SE days; $n = 19$), a total of 33.2 ± 0.9 SE first instars were larviposited into hosts. This represents only half of the female's egg load. The cumulative number of larviposition attempts per female reached a mean of 120 ± 2.2 SE, whereas the mean cumulative puparia production per female was only 29.7 ± 5 SE. The number of hosts parasitised by *C. compressa* increased with increasing host density until an upper limit was reached. This functional response of *C. compressa* fit the Holling type II response ($R^2 = 0.239$; $F = 96.40$; $df = 2, 80$; $P < 0.001$).

24 Nine European non-target Coleopteran species potentially at risk of being attacked by *C. compressa* were selected as representative species for testing in the quarantine laboratory: two-spotted lady beetle, *Adalia bipunctata* L. (Coleoptera: Coccinellidae); red pumpkin beetle, *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae: Galerucinae: Luperini); thistle tortoise beetle, *Cassida rubiginosa* Müller (Coleoptera: Chrysomelidae: Cassidinae); golden loosestrife beetle, *Galerucella pusilla* Duft (Coleoptera: Chrysomelidae: Galerucinae: Galerucini); green dock beetle, *Gastrophysa viridula* Deg. (Coleoptera: Chrysomelidae: Chrysomelinae); *Gonioctena fornicata* Bruggemann (Coleoptera: Chrysomelidae: Chrysomelinae); cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae: Criocerinae); elm leaf beetle, *Pyrrhalta luteola* (Müller) (Coleoptera: Chrysomelidae: Galerucinae: Galerucini); and pea and bean weevil, *Sitona lineatus* Linnaeus (Coleoptera: Curculionidae).

In no-choice or choice tests, naïve females of *C. compressa* never parasitised eight of nine non-target species tested. In the absence of *D. v. virgifera* adults, *A. foveicollis* was occasionally accepted (6 larvae in 260 hosts), but complete development by *C. compressa* was not achieved. The acceptance of *A. foveicollis* by *C. compressa* was significantly lower than that of the target host, *D. v. virgifera*, 2.3% versus 28.7%. In the sequence of no-choice tests, *A. foveicollis* was accepted (4 larvae in 260 hosts) but it was again significantly lower than that of the target species compared within the same day of three successive days. From the four *A. foveicollis* adults parasitised, a single *C. compressa* larva completed its development and formed a puparia. In the presence of *D. v. virgifera* in the choice test, *A. foveicollis* was never accepted by *C. compressa* but during the sequence of choice tests *A. foveicollis* was again accepted by

C. compressa in a few cases. However, host acceptance was significantly lower than that observed for *D. v. virgifera* on the first, second and third day. In contrast to the results of the sequence of no-choice tests, host suitability for *A. foveicollis* by *C. compressa* was not found.

It can be predicted that the candidate biological control agent *C. compressa* will have a narrow host range in Europe, being restricted to a few genera on the tribe level of Luperini among the subfamily Galerucinae. These results of the physiological host range of *C. compressa* obtained under quarantine conditions are in agreement with the known field host range from the area of origin in Mexico.

DISCUSSION AND CONCLUSIONS

Accidentally introduced specimens of the invasive alien maize pest, *D. v. virgifera*, must initially migrate to their target habitat, maize, in order to feed, reproduce, establish and then to invade other areas. Mark-release-recapture experiments revealed that small introduced *Diabrotica* beetle populations mainly show non-directional dispersal. However, beetles were moving significantly more frequently towards commercial scale maize fields within a radius of up to 1,500 m than in the direction of other habitats. Adult populations of *D. v. virgifera* were spreading over more than 100 hectares of non-maize areas, and 2.8% of all recaptured beetles arrived in maize plots at a distance of 300 m from the release point. This probability of arrival would increase linearly with the number of maize fields. Next to this number of maize fields, the number of introduced specimens is essential to estimate risk of arrival of *D. v. virgifera* in their target habitat. Literature references suggest that in most cases hardly more than 10 specimens are introduced in a single event of introduction (Wittenberg and Cock 2001). However, in a hypothetical case where 10 maize fields are in close proximity to a point of alien introduction, already one to two females out of the ten *Diabrotica* beetles would reach a maize field, leading to a risk of establishment of a starter population of the introduced species. Fortunately, there would be a less than 1 % chance that those newly arriving female beetles would produce enough progeny to ensure the successful emergence of a new adult generation. The rate of increase from a starter population to the following generation was often found to be less than factor one, which indicates declining populations and would result in the extinction of introduced founder populations. However, when considering the maximum potential progeny of females, the capacity of increase could reach growth factors between 2 and 4.5. Luckily, only 19.6 % of this potential fecundity was usually realized under field conditions and the mean realized fecundity of starter populations varied considerably between years and between individual females. Due to this variability it remains difficult to predict the probability and quarantine risk for establishment of newly introduced starter populations. Still, the high percentage of non-directional flight by adults, the moderate probability of accidentally introduced specimens to arrive in maize, the low realized fecundity of females as well as the extremely low survival probability until the subsequent generation very much decrease the probability of a successful invasion of this alien *D. v. virgifera*. Reasons for the ongoing invasions in Europe must be explained by either optimal oviposition conditions or by an increased frequency of introduction events.

With regard to the use of classical biological control as a sustainable management strategy against *D. v. virgifera* the following conclusions are compiled:

1. Effective indigenous natural enemies are not attacking any life stage of *D. v. virgifera* in Central Europe;
2. In the area of origin surveyed, *Celatoria compressa* was the only parasitoid found on the target species;
3. Prior to its potential importation, the parasitoid's basic and reproductive biology was clarified;
4. According to host specificity testing, *Celatoria compressa* would be safe for introduction as direct and indirect impacts on other organisms would be extremely low (host range is considered to be restricted to Diabroticite beetles).

Regarding the tachinid *C. compressa*, we have studied a promising candidate classical biological control agent that would be safe for introduction against *D. v. virgifera* in Europe. Nonetheless, there are a number of questions that remain to be answered before its potential importation, such as the hibernation strategy of *C. compressa*, its cold tolerance and its impact under more natural conditions than in bioassays in quarantine laboratories. In conclusion, a sustainable integrated approach against *D. v. virgifera* in Europe is likely to incorporate classical biological control with other pest management options, such as tolerant maize varieties, crop rotation, and cultural techniques which have the potential to enhance the conservation of natural control.

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