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Basic biology and small-scale rearing of *Celatoria compressa* (Diptera: Tachinidae), a parasitoid of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae)

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Abstract

The tachinid *Celatoria compressa* Wulp has been evaluated as a candidate biological control agent for the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in Europe, where it is an invasive alien pest of maize. Special emphasis has been placed on understanding aspects of the parasitoid basic biology and on developing a rearing technique for a small-scale production of *C. compressa* puparia. The age of *C. compressa* adults was found to be the most crucial factor in achieving mating. Only newly emerged, 1-h-old females, mated successfully with 2- to 5-day-old males, achieving a success rate of 74%. After mating, a prelarviposition period of 4 days occurred. The 5-day-old *C. compressa* females inserted their eggs containing fully-developed first instars directly into adults of *D. v. virgifera*. Total larval and pupal developmental time, including a pre-larviposition period of 4 days, was 29 days under quarantine laboratory conditions (25°C daytime, 15°C at night, L:D 14:10, 50% ± 10% r.h). Females of *C. compressa* were capable of producing on average 30 puparia throughout a female's mean larviposition period of 15 days. A large number of host attacks by *C. compressa* were unsuccessful, resulting in a mean larviposition success rate of 24% per female. Parasitoid females appear to have difficulties inserting the egg through the intersegmental sutures or membranes around leg openings of the host adults. Although the small-scale rearing technique of *C. compressa* presented is both time and labour intensive, *C. compressa* has been reared successfully for at least 20 successive generations without shifting the 1 male : 1 female sex ratio using a non-diapause strain of *D. v. virgifera.*

Introduction

Tachinidae rank together with the more important families of Hymenoptera as biological control agents (Greathead, 1986) as many tachinid hosts are pests of economic importance (Belshaw, 1994). Therefore tachinids have been involved in many operations of applied biological control worldwide (Grenier, 1988 for a review; Belshaw, 1994). Successes have been relatively rare but some of them have been spectacular (Greathead, 1986), for instance, *Lixophaga diatraeae* (Townsend) has been used against sugarcane borer, *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae), in Barbados (Alam *et al.*, 1971), *Lixophaga sphenophori* (Villeneuve) against sugarcane weevil, *Rhabdoscelus obscurus* (Boisduval) (Coleoptera: Curculionidae) in Hawaii (Rao *et al.*, 1971), *Argyrophylax basifulva* (Bezzi) against coconut spike moth, *Tirathaba rufivena* Walker (Lepidoptera: Pyralidae) in Fiji (O'Connor,

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1950), and *Cyzenis albicans* (Fallén) against winter moth, *Operophtera brumata* (Linnaeus) (Lepidoptera: Geometridae) in Canada (Murdoch *et al.*, 1985). However, several tachinid species considered as control agents have been rejected because of failure to achieve mating or rearing in captivity, such as *Jaynesleskia jaynesei* (Aldrich) and *Miobiopsis diadema* (Wiedemann), two potentially useful parasitoids of *Diatraea* species for the Caribbean (Cock, 1985), or *Ocytata pallipes* (Fallén) considered for control of the European earwig, *Forficula auricularia* Linnaeus (Dermaptera: Forficulidae), in Canada (Kuhlmann *et al.*, 2002). In fact, the primary key to the successful use of Tachinidae is the ability to find techniques for mating and rearing in captivity (Campadelli, 1977; Greathead, 1986).

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is the most destructive pest of maize *Zea mays* L. (Poaceae) in North America (Krysan & Miller, 1986). This species was accidentally introduced into Central Europe in the early 1990s (Baca, 1994; Sivcev *et al.*, 1994) and is spreading steadily throughout the continent. The majority of yield loss attributed to this univoltine pest species is due to larval feeding on the roots of maize, which ultimately results in plant lodging. In addition, the adults can occasionally cause yield losses due to intensive silk feeding (Krysan & Miller, 1986). While several natural enemies in North and Central America are known to attack *Diabrotica* species, it is apparent that indigenous natural enemies in Europe have not adapted to the high population density of this alien invasive species. Classical biological control provides an opportunity to reconstruct the natural enemy complex of an invading alien pest and its use for managing *D. v. virgifera* populations in Europe should be considered (Kuhlmann & van der Burgt, 1998).

In their region of origin, *Diabrotica* species are attacked by a range of pathogens, nematodes, predators and parasitoids, some of which appear to be specifically adapted for parasitizing *D. v. virgifera* (Kuhlmann & van der Burgt, 1998). Regarding insect parasitoids, four tachinids in the genus *Celatoria* are known from literature records: *Celatoria diabroticae* (Shimer), *Celatoria setosa* (Coquillet), *Celatoria bosqi* Blanchard, and *Celatoria compressa* Wulp (Herting, 1973; Guimaraes, 1977; Arnaud, 1978; Elsey, 1988; Cox, 1994; Eben & Barbercheck, 1996; Heineck-Leonel & Salles, 1997). Recently a braconid, *Centistes gasseni* Shaw, was discovered (Shaw, 1995) and its biology described (Schroder & Athanas, 2002; Cabrera Walsh *et al.*, 2003). *Celatoria* species parasitize adults in single or related genera within the Galerucinae or Alticinae (Cox, 1994). However, the efficacy of the *Celatoria* species in controlling *Diabrotica* species in the field remains to be investigated (Levine & Oloumi-Sadeghi, 1991). Although the biology and ecology of these *Celatoria* species is little understood, their reproductive strategy is almost identical. Females lay eggs that contain fully-developed first instar larvae directly into the host, a process termed larviposition (Bussart, 1937; Fischer, 1983).

Celatoria compressa has been selected as a candidate biological control agent for further investigations as it has been obtained from the target species *D. v. virgifera* in North America and also for practical reasons such as availability and rearing. The success of a classical biological control attempt against *D. v. virgifera* in Europe using *C. compressa* will depend on a thorough knowledge of its biology and ecology. Therefore it is necessary to study aspects of its basic biology and to develop a rearing technique. Also this will be instrumental in providing the baseline data required for assessing host specificity of *C. compressa* in the near future. The aim of this paper is to describe aspects of the basic biology such as mating, larviposition, larval and pupal development, and adult emergence as well as a small-scale production technique of *C. compressa*.

Materials and methods

Source of Diabrotica virgifera virgifera *and host rearing*

A non-diapausing strain of *D. v. virgifera* was continuously reared under quarantine laboratory conditions (25°C day, 15°C night, 14L:10D, 50% ± 10% r.h.). Eggs were obtained from the USDA-ARS Northern Grain Insect Research Laboratory at Brookings, South Dakota, United States. *Diabrotica v. virgifera* adults were kept in gauze cages (300 × 300 × 550 mm) with water source, maize leaves and artificial diet (Jackson, 1985). Fine sieved sand (< 0.18 mm diameter) was provided as the oviposition medium and offered in Petri dishes covered with a lid containing holes for females to enter. The Petri dishes were covered with folded aluminium foil. Sand with eggs was sieved and washed with water with the aim to obtain eggs. Eggs were stored for another 14 days in Petri dishes, and then incubated in plastic trays containing soil with 4- or 5-day-old maize plants. *Diabrotica* larvae were reared in plastic trays (30 cm × 20 cm × 5 cm) with an abundant root mass of maize plants for 25 days, and then the plastic trays were placed into adult emergence cages (for rearing details see Jackson, 1985).

Source of Celatoria compressa *and adult rearing*

In order to obtain *C. compressa*, surveys were conducted in collaboration with Dr Astrid Eben (Instituto de Ecologia, Xalapa, Mexico) and 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 *D. v. virgifera* in northern Mexico. Field-collected adults were reared in Mexico and 104 puparia of *C. compressa* were shipped to the quarantine laboratory at the CABI Bioscience Centre in Switzerland. Individuals were sent to Dr Nigel Wyatt (The Natural History Museum, London, UK) to confirm the identification of *C. compressa*.

Adult *C. compressa* were separated by sex and kept in gauze-topped, transparent plastic cylinders (diameter 100 mm, height 100 mm) (see Kuhlmann, 1995). The bottom of the plastic cylinder was covered with a gauze layer, and a single cardboard egg container served as a hiding place. A food source was provided consisting of 1:1:1 mixture of honey, raw sugar and brewers yeast (Fischer, 1983). Two moistened dental cotton wicks provided water from a water-filled Petri dish through the bottom of the cylinders.

Mating and larviposition

To achieve mating of *C. compressa*, a set of different conditions were combined in different observation arenas. The following factors were varied:

1. Sex ratio, i.e. every sequential sex ratio combination starting with 1 female and 1 male up to 5 females and 5 males.

2. Age group, 10-min to 15-day-old females or males.
3. Cage size, i.e. Eppendorf tubes, Petri dishes (diameter 100 mm), transparent plastic urine containers (diameter 60 mm, height 80 mm), gauze-topped transparent plastic cylinders (diameter 120 mm, height 120 mm), gauze-covered wooden cages (290 × 290 × 550 mm), large gauze cages (1500 × 1500 × 1500 mm).
4. Light source and intensity, i.e. twilight bulb (36 W), and sun light lamps (400 W).
5. Temperature conditions, 25°C and 30°C.
6. Observational period during the day, i.e. in the morning, afternoon or evening.

In addition, different habitat/host characteristics were provided in the plastic cylinders and gauze-covered wooden cages, i.e. the host, maize cobs and leaves, and host frass. Mating behaviour was observed for up to 8 h depending on parasitoid female activity, and terminated after half an hour of inactivity. Mating behaviour was described, such as walking, flying, resting, contacts between males and females, and mating attempts. In the case of successful mating, its duration was recorded as well as the age of the males and females, and the number of males and females present in the experimental arena. The number of puparia produced per mated female was recorded to study the relationship between the duration of mating and the number of puparia produced.

The pre-larviposition period for gestation after mating was determined by dissecting mated females held under daytime conditions of 25°C. It was defined as the period of time until the first eggs with fully-developed first instar larvae were visible in different female age classes. Observations of the larviposition behaviour were made with 5- to 6-day-old inexperienced mated females that were exposed to 10 *D. v. virgifera* adults for 30 min in small cages (15 × 15 × 30 cm) to clarify the mode of egg insertion. As soon as *D. v. virgifera* adults were parasitized, adults were dissected to locate the parasitoid egg within the host body.

Larval and pupal development and adult emergence

The impact of temperature on the duration of larval and pupal development was assessed at daytime temperatures of 25°C, 22°C, 20°C and 15°C. Other conditions remained constant such as night-time temperature of 15°C, a photoperiod of 14L:10D, and a relative humidity of 50% ± 10%. After the pre-larviposition period, a mated female was exposed to 10–15 *D. v. virgifera* adults for parasitism over a time period of 30–60 min. After exposure, parasitized *D. v. virgifera* adults were kept in rearing cages consisting of two plastic containers (diameter 8.5 cm, height 12.5 cm), one of which was bottomless, stacked together and separated by cotton gauze. The bottom container of the rearing cage contained moist cellulose paper and the upper, bottom-gauzed container contained *D. v. virgifera* diet on a strip of filter paper. Emerging *C. compressa* larvae were able to pass through the gauze layer and pupated in the cellulose paper. Plastic containers were kept separately under different temperature treatments and individuals of *C. compressa* were followed through their larval and pupal development until adult emergence. Data on larval and pupal development time at different daytime temperatures were analysed using ANOVA and followed by Student-Newman-Keuls test to compare means. Some 30 to 40 adults of *D. v. virgifera*, held at a daytime temperature of 22°C were also dissected every two days to assess the number of parasitoid larval instars, their sizes over time as well as their development time. Tachinid puparia were collected daily and placed on corrugated cardboard in a transparent plastic cylinder as described above for tachinid adult maintenance, and emerged *C. compressa* adults were collected daily. To study the pattern of adult emergence under laboratory conditions at 25°C, 15°C at night, 14L:10D, 50 % ± 10 % r.h., the emergence time of males and females was recorded daily and the sex of adults was determined.

Small-scale rearing

Observations on host attacks by *C. compressa* females were carried out to identify important reproductive characteristics of the tachinid influencing the establishment of a small-scale production of C. *compressa* puparia. Therefore, the larviposition period within *C. compressa* adults' life span, the number of daily larviposition attempts per female, the number of puparia produced daily per female as well as the cumulative puparia production per female within the females' larviposition period were determined. Individual mated females were exposed to ten *D. v. virgifera* adults for 30 min twice every day from parasitoid age of 5 days old until death. In the 30 min period of exposure, parasitized adults of *D. v. virgifera* were removed and replaced with new hosts when a host was attacked. Results of the two daily observational sessions were pooled for further analysis in the results section as there was no significant difference (paired t test, $t = 0.35$, df = 17, $P = 0.73$). The number of successful host attacks was recorded each time per female. After exposure and attack, *D. v. virgifera* adults were kept for 20 days in a plastic cylinder, as described above, and sorted by day and parasitoid female.

Results

Mating and larviposition

Observational experiments of the mating behaviour showed that the age of *C. compressa* adults was the most crucial factor to achieve mating in captivity. A total of 163 females of *C. compressa* with different ages were observed, and of these, 104 females mated, representing 64%. Mating was most successful when females were less than 1 h old (n = 77), representing 74% of the successful matings. Mating was most successful when males were 2–5 days old (74%), and less successful when males were 1 day (3%), 6–10 days old (11%), or over 11 days old (12%). In addition, an increasing number of males relative to a single female in the mating arena increased the probability of mating. The optimal sex ratio to achieve successful mating in captivity was five males to one female (n = 122). In this study, mating of *C. compressa* was obtained in an inverted transparent plastic container (diameter 60 mm, height 80 mm) with moistened filter paper on the bottom, however, the size or type of cage had no influence on achieving mating of *C. compressa* in captivity.

The mating behaviour of *C. compressa* was stereotypical. Males appeared to be excited by the presence of newly emerged virgin females, and they aggressively pursued females by flying or landing directly on the female or by walking after her. While grasping the female, the male

vibrated the wings very rapidly and immediately positioned himself for mating and extended his genitalia to attempt to mate with the female. Some of the females resisted mating attempts in the beginning by kicking their hind legs and running around to escape from the grasp of the male. Once mating began, the male released his grasp of the two anterior pairs of legs and held them clasped to his body, and the female became quiescent. The duration of successful mating varied from 9 to 109 min, with an average of 17.6 ± 1.0 SE min (n = 121). Linear regression analysis demonstrated that there was no relationship between the duration of mating and the number of tachinid puparia produced (ANOVA, F = 0.001, df = 1, 35, $P = 1.0$). From 121 matings observed, only 10.7% of the females were recorded to mate twice whereas males mated several times on the same day or on different days.

After mating a pre-larviposition period of 4 days occurred at a daytime temperature of 25°C before 5-day-old *C. compressa* females started to insert their eggs containing fully-developed first instar larvae directly into the host adults through intersegmental sutures or through membranes around leg openings of *D. v. virgifera* adults. In newly parasitized adults of *D. v. virgifera*, the first instar larva was always found in the cavity of the host's thorax close to the wing bases.

Larval and pupal development and adult emergence

From 520 *D. v. virgifera* adults parasitized by *C. compressa*, 91.5% of solitary parasitoid larvae succeeded in developing within the host and pupating externally. There were three larval instars for *C. compressa*. Under laboratory conditions at 22°C, the duration of the first, second and third larval instars was 3–4 days (n = 35), 3–4 days (n = 39), and 6–7 days (n = 26), respectively. The average length of the first, second and third instar larvae was on average 0.86 ± 0.01 SE mm (n = 35), 1.57 ± 0.06 SE mm (n = 39), and 2.81 ± 0.09 SE mm (n = 26), respectively. The duration of the larval developmental time was significantly influenced by different daytime temperatures as shown in table 1 (ANOVA, F = 150.35, df = 3, 360, $P < 0.01$). The developmental time of the larval stages increased as the temperature decreased. However, no significant difference was found between larval development at daytime temperatures of 22°C and 25°C (table 1).

The mature third instar larva of *C. compressa* exited from its inactive host and pupated within 1 h at emergence. Like larval development, pupal development was significantly influenced by daytime temperature (table 1; ANOVA, F = 227.86, df = 3, 259, $P < 0.01$). Mean pupal development lasted 11.9 ± 0.3 SE days (n = 64) at a daytime temperature of 25°C, and no significant differences were found between males and females (Student's *t* test, $t = 0.608$, df = 62, $P > 0.05$). In contrast, male puparia completed their development significantly faster than females at daytime temperatures of 22°C (Student's *t* test, $t = 2.401$, df = 87, $P < 0.05$), 20°C (Student's *t* test, $t = 5.231$, df = 64, $P < 0.01$), and 15°C (Student's *t* test, $t = 3.034$, df = 42, $P < 0.01$). Total larval and pupal developmental time including the pre-larviposition period of 4 days at a daytime temperature of 25°C accounted for 29.2 days, which represents the time needed to complete one generation of *C. compressa*.

From 456 puparia observed, 69.9% of the *C. compressa* adults emerged successfully, 24.7% of the adults failed to emerge, and an additional mortality of 5.4% occurred as *C. compressa* adults failed to complete emergence from the puparia. Adult emergence of *C. compressa* took place on an average 1 h after changing from darkness to daylight (mean = 63.5 ± 0.6 SE min, n = 64). This emergence peak occurred about 50 min (mean = 48.4 ± 0.6 SE min, n = 64) after the temperature started to increase from 15°C to 25°C in the morning. There were no differences between males and females in diurnal pattern of emergence (Student's *t* test, $t = 0.81$, df = 133, $P = 0.42$). The sex ratio was 1.1:1 (n = 309, male = 162, female = 147).

Small-scale rearing

The mean adult life span of a *C. compressa* female was 18.9 ± 0.6 SE days (min. = 11, max. = 33, n = 18) and the mean larviposition period per female was 14.9 ± 0.6 SE days (min. = 7, max. = 29, n = 18). During the first six days of the larviposition period, *C. compressa* females showed a high activity of larviposition attempts with a mean of 14.3 ± 0.4 SE *D. v. virgifera* adults per day but the number of attempts decreased linearly during the females' life span (linear regression, $y = -0.58x + 15.8$, $R^2 = 0.88$, F = 190.16, df = 1, 25, $P < 0.001$) (fig. 1a). The frequency of daily larviposition attempts was on an average 7.4 ± 0.4 SE per female during its life span with a maximum of 46 daily attempts (n = 18) and the cumulative number of larviposition attempts per female reached a mean of 120 ± 2.2 SE (min. = 22, max. = 323, n =18).

During the first three days of the larviposition period, daily mean puparia production per female increased from 1.8 ± 0.8 SE, to 3.1 ± 0.7 SE and then to 5.3 ± 1.3 SE, respectively, but afterwards the number of puparia produced decreased linearly (linear regression, $y = -0.16x + 3.89$, $R^2 = 0.63$, F = 42.67, df = 1, 25, $P < 0.001$) (fig. 1b). Results demonstrate that the number of puparia produced daily per female was significantly lower compared to the number of daily larviposition attempts observed (paired *t*-

Table 1. Mean developmental time of larvae and puparia of *Celatoria compressa* at different daytime temperatures in *Diabrotica virgifera virgifera* (15°C at night for all treatments).

Temperature (±1°C) 15°C at night	Mean stage development time (days) ± SE				Total time including pre-larviposition of 4 days
	Larvae	Male puparia	Female puparia	Puparia	
25	13.3 ± 0.3 (55) a	11.8 ± 0.4 (39) a	12.2 ± 0.5 (25) a	11.9 ± 0.3 (64) a	25.2
22	13.8 ± 0.2 (68) a	13.3 ± 0.2 (48) b	14.1 ± 0.2 (41) b	13.7 ± 0.2 (89) b	27.5
20	14.7 ± 0.1 (143) b	15.2 ± 0.1 (33) c	15.8 ± 0.1 (33) c	15.5 ± 0.1 (66) c	30.2
15	18.9 ± 0.2 (98) c	19.3 ± 0.2 (19) d	20.4 ± 0.3 (29) d	20.0 ± 0.2 (48) d	38.9

Means given within a column followed by different letters are significantly different ($P < 0.05$, Student-Newman-Keuls Test).

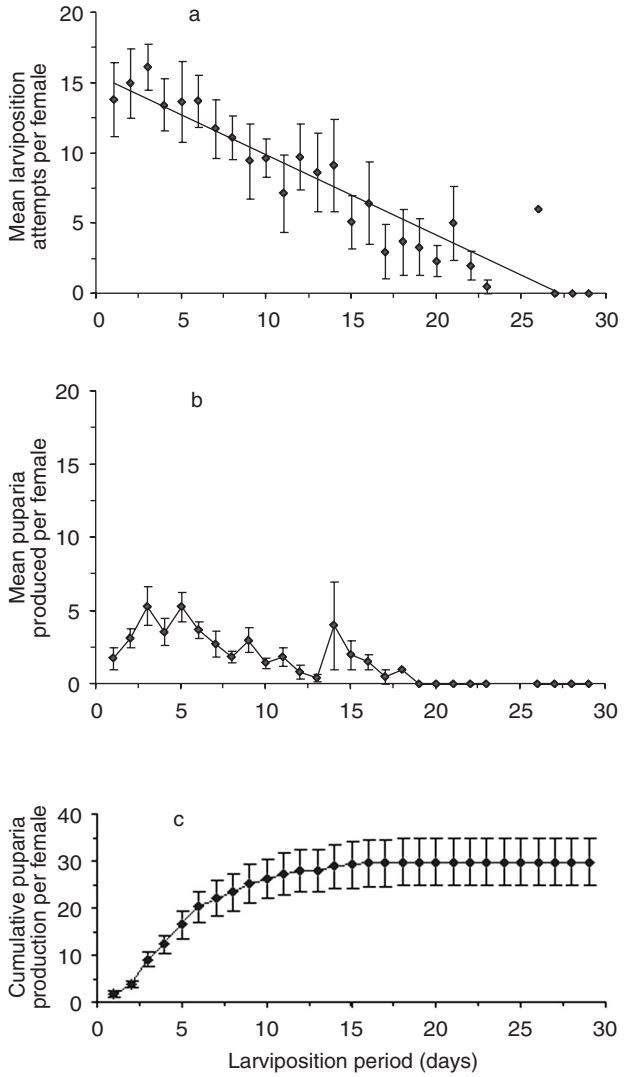

Fig. 1. (a) Mean frequency of daily larviposition attempts of *Celatoria compressa* females in *Diabrotica virgifera virgifera* adults during the parasitoid life span ($y = 20.58x + 15.8$, $R^2 = 0.88$, $P < 0.001$); (b) mean number of puparia produced daily per *C. compressa* female over the entire larviposition period; and (c) mean cumulative puparia production per *C. compressa* female (standard errors are indicated by vertical bars).

test, $t = 7.93$, df = 26, $P < 0.001$). The daily larviposition success rate per female reached on an average 24.1% ± 0.8 SE (min. = 4.6%, max. = 43.8%, n = 18).

The cumulative puparia production per *C. compressa* female varied widely between 5 and 54 (n = 18), and the mean cumulative puparia production per female was 29.7 ± 5 SE (fig. 1c). Fifty percent of the total puparia production was already achieved by the females within the first five days of the females' larviposition period (fig. 1c).

Discussion

In this paper, a laboratory rearing technique for a small-scale production of the tachinid *C. compressa*, a candidate biological control agent for *D. v. virgifera*, was examined. Special emphasis was placed on understanding aspects of the parasitoid's biology, as studies on *Celatoria* species are rare. The limited literature available includes a description of the biology of *C. setosa*, a parasitoid of the striped cucumber beetle, *Diabrotica vittata* Fabricius (Bussart, 1937). Later Fischer (1983) contributed again to the biology of *C. setosa* and made the first biological studies of the closely related tachinid *C. diabroticae*. The general findings on the basic biology of *C. compressa* are comparable with investigations on *C. setosa* and *C. diabroticae* conducted by Bussart (1937) and Fischer (1983). For instance, the pre-larviposition period lasted 4 days in all three *Celatoria* species and also eggs with fully developed first instars were inserted singly into the host adult body.

Despite their important role as primary agents of natural control for many insect pests, the Tachinidae as a group remains underused in biological control programmes (Grenier, 1988) primarily because of failure to achieve mating in captivity (Campadelli, 1977; Greathead, 1986). In most tachinids, very specific conditions are needed to induce mating. Natural sunlight can be important for mating in *C. bosqi* (W. Cabrera Walsh, personal communication, 2002), *Doryphorophaga doryphorae* (Riley) (Tamaki *et al.*, 1982), *Argyrophylax basifulva* Bezzi (Godfray, 1985), and *Lydella jalisco* Woodley (Rodriguez-del-Bosque & Smith, 1996). Some species, such as *Lydella thompsoni* Herting, needed a light intensity of at least 8000–10000 lux in the laboratory (Galichet *et al.*, 1985). In *Ceranthia samarensis* (Villeneuve) for example, mating was stimulated by varying cloudiness of the sky or a change in light conditions in the laboratory (Quednau & Lamontagne, 1998). Godfray (1985) reported that mating activity of *A. basifulva* females was increased by passing air through the cage with an electric fan. In this study, it has been demonstrated that the age of *C. compressa* adults was found to be the most crucial factor in achieving mating. Only newly emerged 1-h-old females mated successfully with 2- to 5-day-old males, achieving a success rate of 74%. There are a number of other studies available showing that mating in captivity by tachinids is strongly age dependent, such as *Plagiprospherysa trinitatis* Thompson (Beg & Bennett, 1974), *Clausicella suturata* Rondani (Kugler & Nitzan, 1977), *C. samarensis* (Quednau, 1993), and *Triarthria setipennis* Fallén (Kuhlmann, 1995). In addition, this age-dependence has been also demonstrated for *C. diabroticae* (Fischer, 1983), and *C. setosa* (Bussart, 1937; Fischer, 1983).

Females of *C. compressa* were capable of producing on average 30 puparia throughout a female's mean larviposition period of 15 days. A maximum of 54 puparia were obtained from a single female during a larviposition period of 16 days. In comparison to the high number of larviposition attempts per female, it is obvious that many host attacks by *C. compressa* were unsuccessful, as a mean larviposition success rate of 24% per female was determined. It appears to be difficult for *C. compressa* females to insert the egg through the intersegmental sutures or membranes around leg openings of *D. v. virgifera* adults. According to Belshaw (1994), oviposition by tachinids on or in the host is always associated with a lower fecundity but, in this case, it remains to be determined if a limited egg load in *C. compressa* females might have contributed to the low larviposition success rate.

Should *C. compressa* be selected as a biological control agent of *D. v. virgifera* in Europe, large-scale rearing of the

tachinid under laboratory conditions must be achieved for a field release programme. However, large-scale multiplication of *C. compressa* remains difficult due to the low larviposition success rate. As the eggs are directly inserted into the host, a manipulation technique is required to allow the artificial implantation of the first instar larvae into the bodies of the *D. v. virgifera* hosts. Manipulation techniques have been already developed for tachinid mass rearings such as the Box-Scaramuzza method for ovolarviparous tachinids (Scaramuzza, 1930). This method initiated the successful use of *L. diatraeae* against *Diatraea saccharalis* (Bennett, 1969). However, this technique cannot be applied to *C. compressa,* as it is based on the manual transfer of tachinid first instar larvae onto the host body. Thus, a small-scale production technique of *C. compressa* has been successfully developed using a non-diapausing strain of *D. v. virgifera*. Although the current rearing approach is time and labour intensive, *C. compressa* has been reared successfully for at least 20 successive generations without shifting the sex ratio (approximately 500 adult *C. compressa* were produced each generation). The following factors were critical:

1. Successful mating using 2- to 5-day-old males and < 1-h-old females (sex ratio 5:1).
2. A 4-day pre-larviposition period for gestation.
3. Daily exposure of females to 15 hosts for 1 h over a period of 15 days to achieve efficient production of puparia.
4. A daytime temperature of 25°C to achieve a full generation of *C. compressa* in 29 days.
5. Continuous rearing of a non-diapausing strain of *D. v. virgifera*.

These results are the very first important steps in assessing the potential of *C. compressa* as a candidate biological control agent for introduction into Europe.

With respect to the safety of biological control and increasing concern of non-target risks by introduced biological control agents, standards and frameworks developed for the release of exotic biological control agents should be followed (i.e. FAO, 1997; van Lenteren *et al*., 2003). Therefore, assessing the host specificity of *C. compressa* is another important step towards the implementation of a classical biological control programme in Europe and this is currently being carried out following the protocol proposed by van Lenteren *et al*. (2003).

Firstly, European non-target coleopteran species potentially at risk of being attacked by *C. compressa* have been selected and exposed to *C. compressa* in no-choice and choice tests. Secondly, the hibernation strategy of *C. compressa* is being investigated, as this is likely to be a major factor affecting the successful establishment of *C. compressa* in the wild in Europe. *Celatoria setosa* was reported to overwinter inside the host body of *D. vittata* (Bussart, 1937), while Herzog (1977) suggested that *C. diabroticae* overwinters as pupae in the soil. Further investigations on *C. compressa* are needed to clarify its overwintering development stage and determine whether it has sufficient cold tolerance for permanent establishment in Europe.

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