OVIPOSITION OF VIBURNUM LEAF BEETLE [*PYRRHALTA VIBURNI* (PAYKULL)]: FROM ECOLOGY TO BIOLOGICAL CONTROL OF AN EMERGING LANDSCAPE PEST

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OVIPOSITION OF VIBURNUM LEAF BEETLE [*PYRRHALTA VIBURNI* (PAYKULL)]: FROM ECOLOGY TO BIOLOGICAL CONTROL OF AN EMERGING LANDSCAPE PEST

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Cornell University 2009

Pyrrhalta viburni (Coleoptera: Chrysomelidae), an invasive chrysomelid native to Eurasia, is a major pest of viburnums in the northeastern U.S. and southern Canada. *P. viburni* oviposition behavior was observed under laboratory and field conditions. The time cost of producing an egg mass was 135.3 $\pm$ 6 min, chewing the egg cavity being the most time consuming part of the process. Choice-tests showed that females laid more eggs in twigs already infested by conspecifics than in non-infested twigs, and positioned their egg masses adjacent to existing ones. *P. viburni* oviposition behavior is therefore aggregative. An observational study showed that wound response, the main plant defense against *P. viburni* oviposition, decreased with increasing levels of egg infestation, suggesting a potential benefit of aggregative oviposition. A field study confirmed that there is a realized fitness benefit of aggregative oviposition on three host species: *V. dentatum*, *V. opulus*, and *V. x bodnantense*. Egg survivorship was higher on twigs with high and very high levels of infestation than on lightly or moderately infested twigs and wound response decreased with increasing levels of infestation. Twig mortality was positively correlated with level of infestation, and egg survivorship was higher on dead twigs than on living twigs. *P. viburni* oviposition on twigs that had died naturally occurred more frequently if the twigs had been infested the previous year, and the probability of new infestation was correlated with the

number of old egg masses present in the twig. A study was conducted under quarantine conditions to determine the longevity and host specificity of *Aprostocetus* sp. (Hymenoptera: Eulophidae), a Eurasian egg parasitoid of *P. viburni*. *Aprostocetus* sp. females lived 53.8 days on average, the maximum longevity being 133 days. *Aprostocetus* sp. successfully parasitized the following non-target species: *Galerucella nymphaea*, *G. pusilla*/*G. calmariensis*, and *Plagiometriona clavata* (Coleoptera: Chrysomelidae). Due to its lack of host specificity, I think that the egg parasitoid *Aprostocetus* sp. should not be considered for introduction as a biological control agent against *P. viburni* in North America. The implications of this research for management of *P. viburni* in North America are discussed.

BIOGRAPHICAL SKETCH

Gaylord Alexandre Desurmont was born in Lomme, France in 1981. Probably driven by an early vocation and the frustration of not having a pet cat or dog, he spent most of his childhood in the family garden looking for six and eight-legged creatures, plus the occasional centipede. There was a war between black and red ants on the patio outside his room and he would sometimes drop honey between the two nests to watch the battle for food ensue, all whilst contemplating how great it would be to record those events. Once Gaylord reached the age of growing a decent beard, he got an undergraduate degree in Sciences and Biology in Douai, France, and a M.S. degree in Agronomy and Agricultural Techniques in Dijon, France. During his time in Dijon, Gaylord had the opportunity to do an internship abroad. He chose the U.S. and was sent to Cornell University to work in Dr. Paul Weston's laboratory, where he first ventured into Entomological research. His immediate love for the work and the location drove him to follow Dr. Weston's advice to apply for a graduate position at Cornell University's Department of Entomology one year later. Gaylord was then paired to work with Dr. John Sanderson to earn a M.Sc. degree on the potential use of a generalist predator, *Podisus maculiventris* (Say), as a biological control agent against viburnum leaf beetle, *Pyrrhalta viburni* (Paykull). It is Gaylord's intention to continue working in the future on insect behavior and ecology. More importantly, he hopes his path will someday traverse another war between black and red ants. This time, he will be ready to record the warfare and present it to the scientific world.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

CHAPTER 1 OVIPOSITION OF VIBURNUM LEAF BEETLE [*PYRRHALTA VIBURNI* (PAYKULL)]: BEHAVIOR DESCRIPTION AND IMPORTANCE OF GRAVITY AND PREVIOUS INFESTATION AS STIMULI

Abstract

Pyrrhalta viburni, an invasive chrysomelid native to Eurasia, is a major pest of viburnums in the northeastern U.S. and southern Canada. *P. viburni* females deposit small groups of eggs in cavities they chew in twigs and cover with a frass-like secretion. Egg masses are often found in aligned clusters on the undersides of terminal twigs of susceptible shrubs. During laboratory trials, we determined that the time cost of producing an egg mass, from pre-oviposition searching to making the egg cap, was 135.3 $\pm$ 6 min, chewing the egg cavity being the most time consuming part of the process (103.9 $\pm$ 5.6 min). When given twigs already infested with egg masses of conspecifics, *P. viburni* females focused most of their searching in the infested area, and positioned their own egg mass adjacent to the existing one(s) in 80.9% of the cases. When given twigs with egg masses present on the upper sides of twigs, females positioned their egg mass in the infested area, on the opposite side in 79.2% of the cases. These results show that both gravity and presence of egg masses laid by conspecifics act as positional stimuli for *P. viburni* and suggest that *P. viburni* oviposition behavior might be aggregative.

Introduction

Viburnum leaf beetle [*Pyrrhalta viburni* (Paykull)] is a chrysomelid that feeds exclusively on plant species belonging to the genus *Viburnum* (Adoxaceae). Viburnums are woody plants that grow as shrubs or, for a few species, as small trees. In North America, they are valued as ornamentals in managed landscapes, and several native species are widespread in natural habitats such as forest understories, old fields, and wetlands. Both larvae and adults of *P. viburni* are leaf-feeders: larvae skeletonize leaves, leaving only the midrib and major veins intact, whereas adults chew irregular circular holes in the leaves. Heavy infestations of both life stages can lead to complete defoliation of susceptible plants.

Native to Eurasia where it is considered an occasional pest of *Viburnum opulus* (L.) and *V. tinus* (L.), *P. viburni* was accidentally introduced to North America where it has become a major landscape pest in the northeastern U.S. and southern Canada. Although early records indicate that the beetle was observed in Canada as early as 1924 (Majka and LeSage 2007), it did not cause serious damage to the local *Viburnum* flora until 1978 when it was found in Ottawa, Ontario and Hull, Quebec (Canada), defoliating *V. opulus* shrubs (Becker 1979). Observed in the U.S.A in Maine in 1994, *P. viburni* has since spread to all New England states, New York, Pennsylvania, and Ohio (Weston and Hoebeke 2003). The beetle has also been reported in 2001 in British Columbia (Canada), and the state of Washington (U.S.A), probably as the result of a separate introduction on the west coast of North America (Weston et al. 2008).

In North America, *P. viburni* is highly destructive and kills infested susceptible shrubs after 2-4 years of repeated defoliations, whereas it is rarely observed causing shrub mortality in its native range. In addition to herbivory damage, infested shrubs can also suffer twig mortality due to the unusual oviposition behavior of this beetle.

A univoltine species overwintering as eggs, *P. viburni* protects its eggs during winter months from both desiccation and natural enemies by laying them into round cavities (ca. 1 mm in diameter and 1 mm deep) excavated in the tissue of terminal twigs of susceptible shrubs. The female chews away the bark and the pith to hollow out the egg cavity (Hilker 1992) before laying an average of 8 eggs per cavity (Weston et al. 2008). The cavity is then closed by a "cap" made of a frass-like material produced by the female (2-4 mm in length). The cap, whose brown-black coloration often fades during winter months, is thought to store moisture and provide humidity to the eggs in addition to protecting them from possible predators and harsh weather conditions. *P. viburni* oviposition can disrupt the vascular tissues of the twigs attacked, causing them to die; in case of a heavy infestation, twig mortality alone can cause serious harm to the plant. The main defense of the plant to oviposition is the production of wound tissue that either overgrows the egg mass or squeezes the eggs out of the egg cavity. Different *Viburnum* species show varying levels of tolerance and/or resistance to *P. viburni* oviposition (unpublished data). Females can lay eggs for most of their lifetimes, producing on average 75 egg masses under laboratory conditions (Weston et al., 2008). Oviposition seems to be more frequent at night, although it has also been observed during daytime (P. Weston, unpublished data).

In the field, *P. viburni* egg masses are rarely found isolated but more often in groups, forming clusters of aligned egg masses along terminal twigs of infested plants. In addition, *P. viburni* egg masses are almost always found on the underside (shaded side) of twigs, the only exception occurring when the twig is perfectly vertical, which is rare under natural conditions. In that particular case, egg masses can sometimes be seen on both sides of the twig (personal observation).

Despite a recent rise in interest due to its pest status in its introduced range, many questions concerning *P. viburni* ecology and behavior remain unanswered. The

present study was conducted to fill some of the gaps in knowledge about *P. viburni* oviposition. Our objectives were to: (1) describe in detail and measure the time for various stages of the oviposition process, on non infested twigs or twigs containing egg masses laid by conspecifics; and (2) to test the relative value of gravity and presence of other egg masses as positional stimuli for *P. viburni* oviposition.

Material and Methods

Insects and plant material. All *P. viburni* adults used for the experiments in this study were collected in the Ithaca area (NY, USA) on naturally infested plants during July-August 2007. Adults were transferred to *V. dentatum* (L.) and *V. opulus* shoots in rectangular plastic containers (30 x 22 x 10 cm) with vented screen lids and kept at 22°C under a 15:9 (L:D) light regime until they were needed for experiments. Each adult was used only once for experimental purposes. Shoots provided both food and oviposition sites for females. All the plant material used for the experiment came from unmanaged *Viburnum* shrubs located in the Ithaca area. Twigs used for experimental purposes were cut in the 72 hours preceding the trials and kept with cut ends in water-filled floral tubes. All shrubs serving as a source of twigs were 8-10 years old, had never been treated with systemic insecticides, and had not been treated with foliar pesticides for the previous two years.

Descriptive study. Females used for the descriptive study were taken from the laboratory colony immediately prior to the experiment. At the beginning of each trial, a mated female was placed on the leaves of a *V. opulus* twig in a cylindrical container (21 cm height x 11 cm diameter) with a vented screen lid. Twigs were homogeneous in size (12 cm) and were 2.5-3 mm diameter, which corresponds to the diameter of most *V. opulus* terminal twigs on full-grown shrubs under field conditions (personal observation). The twigs were placed leaning against one side of the container to

present a clear upper side and underside. Two leaves were left on each twig as a food supply for the female during the trial. Female behavior was then video recorded under red light for 24 hours, on successive 8-hour or 16-hour tapes, depending on availability of tapes. If no egg mass were laid after 24 hours, the trial was considered a failure and stopped. If a female was in the process of ovipositing at the end of the recording period, the trial was extended by 8 additional hours. Tapes of successful trials were then monitored and the behavior of each female analyzed. For quantification, the oviposition process was divided into 3 main steps, as follows.

Searching: All movements made by a female along a twig prior to oviposition were considered searching. These movements were divided into two categories: runs (continuous straight walking movement of a female along a twig) and turns (female executing a 180º turn on a twig). If a female stopped for more than 5 seconds during a run, the run was considered over. Distance of each run, total number of runs, and total searching duration were recorded. *Chewing*: This step was defined as the time spent by a female while chewing the egg mass cavity. Female position during chewing and total chewing duration were recorded. *Egg-laying/Cap making* (EC): This step was defined as the time spent by the female to lay eggs in the cavity and produce the protective cap. The respective durations of these two activities could not be distinguished on the recordings. Female position during EC and total EC duration were recorded.

Two infestation treatments were used for the twigs in our study: infested and non-infested. Infested twigs had 1-5 egg mass(es) present on the underside of the twig at the beginning of the trial. These egg masses had been made by conspecifics in the laboratory in the 24 h preceding the trial. Non-infested twigs did not bear any egg masses at the beginning of the trial. The same twigs were sometimes (17 trials out of 60) used for successive trials. In total, 60 individual oviposition trials were recorded

between July 10 and September 11 2007, using 60 *P. viburni* females and 32 *V. opulus* twigs. Because only one video camera was available, multiple trials could not be conducted simultaneously.

Influence of infestation on the variables measured (searching duration, chewing duration, EC duration, number of runs during searching, and average length of runs during searching) were analyzed by one-way analysis of variance (ANOVA) (Statistix 9.0, Analytical software 2008). Data were transformed using square root transformations to meet the assumptions of the model. Means were compared with the least significant difference (LSD) all-pairwise comparison procedure (Statistix 9.0, Analytical software 2008).

Relative importance of gravity and presence of other egg masses. Females used for this experiment were taken from the colony immediately prior to the experiment. Each female was placed in a cylindrical container (13 cm height x 13 cm diameter) with a vented screen lid. One *V. opulus* twig with two leaves was placed in each container. Twigs were homogeneous in size (12 cm) and diameter (3 mm), and were placed leaning against one side of the container to present an upper side and underside. Each twig was infested with a cluster of 1-5 egg masses (average: 3) made by conspecifics in the laboratory in the 24 hours preceding the trial, and placed in the cylinder so the cluster would be on the upper side of the twig. We defined the infested portion as the portion of the twig bearing the initial egg masses. Numbers of egg masses laid and position of the new egg masses relative to gravity (upper side or underside of the twig) and portion of the twig initially infested (within or outside the infested portion) were recorded after 48 hours. We conducted 10 trials for this experiment.

To determine if females preferred to lay eggs on the upper side or the underside of the twigs, and within or outside the infested twig portion, multinomial

tests (Statistix 9.0, Analytical software 2008) were performed, testing the hypothesis that the observed frequencies of positions of egg masses were not different from the expected frequencies if egg-mass positioning was random. We considered that the expected frequency of laying eggs on one side of the twig was 0.5, and that the expected frequency of laying egg masses within the infested twig portion was 0.21 (=total length of the infested portion divided by total length of the twig).

Results

Descriptive study. Of the 60 trials conducted, 37 were successful (61.7%): 21 on non-infested twigs and 16 on infested twigs. Most of the successful females (81.1%) produced one single egg mass over the trial duration, 10.8% produced 2, 5.4% produced 3, and 2.7% (one female) produced 4 egg masses. The oviposition process was very consistent across trials: before choosing the oviposition location where the egg mass would be laid, females engaged in a searching behavior characterized by successive back and forth runs along the twig in all but one trial. In the remaining trial, on a non-infested twig, the female started chewing the egg mass cavity directly after a single run.

The duration of pre-oviposition searching behavior was 17.6 $\pm$ 1.9 min (mean $\pm$ SE) on average, and ranged from 1.0 to 53.0 min. Females made 14.4 + 2.2 runs on average during searching (range: 1-51), and covered a total distance of 61.1 + 10.9 cm (range: 2.7-335.3). Once the oviposition location was chosen, females always placed their bodies parallel to the twig, head facing down, and started chewing the egg cavity with their mandibles. Once they started chewing, females usually did not interrupt the process or move their bodies until engaging in egg-laying. In one instance, a female took a 5-minute break from chewing (mandibles not touching the twig) before starting again. It is possible that these "resting periods" during chewing were more frequent

than observed: the resolution of our video recordings did not always allow us to see clearly the movements of the mandibles and their contact with the twig. Chewing duration was 103.9 $\pm$ 5.6 min on average, ranging from 45.0 to 210.0 min.

After chewing, females rotated by 180° to start the process of egg-laying and cap making with their heads facing away from gravity. During this process, females kept contact between the twig and their terminal abdominal segments, but in a few instances, females rotated again immediately after positioning their abdomen to inspect the egg cavity with their mouthparts, and then rotated again to resume egg-laying. During the cap making, repeated back and forth movements of the end of the abdomen were observed, producing and smearing the protective secretion over the egg mass cavity. Once the egg cap was finished, 32.4% of the females rotated again to inspect the fresh egg cap, touching it with their mouthparts and antennae for 1-10 min before walking away. Females that did not inspect their egg mass (67.6%) just walked away once they were done making the cap. The duration of the egg-laying/cap making behavior was 15.6 $\pm$ 0.8 min on average, ranging from 7.0 to 27.0 min. The duration of the total oviposition process, from pre-oviposition searching to cap making was 135.3 $\pm$ 6 min on average and ranged from 82.0 to 258.0 min.

There was no significant effect of previous infestation on searching duration (F = 0.15, P = 0.7), chewing duration (F = 2.06, P = 0.15), EC duration (F = 0.1, P = 0.76), or the total number of runs made by a female during searching (F = 1.76, P = 0.19; overall average 14.3 $\pm$ 2.2 runs). However, infestation had an effect on the length of runs: the average length of each run was shorter on infested twigs (3.0 cm/run) than on non-infested twigs (3.8 cm/run) (F = 4.54, P = 0.04). Females engaged in searching on non-infested twigs usually inspected the entire length of the twig, repeatedly making extensive back and forth runs before choosing an oviposition location, whereas females searching on infested twigs usually concentrated most of

their searching in the area of the twig already infested.

Females ovipositing on an already infested twig consistently aggregated their egg masses with the ones already present: 80.9% of the females deposited new egg masses immediately adjacent (i.e. < 0.5 cm) to the existing mass(es) on the distal portion of the twig, 14.3% less than 2.5 cm adjacent the existing egg mass(es) on the distal portion of the twig, and 5.0% less than 2.5 cm adjacent to the existing egg mass on the basal portion of the twig. Females never deposited a new egg mass in a portion of the twig distant by more than 2.5 cm from the existing egg mass(es).

Figure 1.1 shows the searching patterns of three *P. viburni* females (A, B, and C) successively ovipositing on the same twig during three consecutive trials. During the first trial, female A oviposited three times: her first pre-oviposition searching pattern was relatively long (32 min) and included 48 runs covering the entire length of the twig, whereas her second and third pre-oviposition searching patterns were shorter (3 and 5 min, respectively) and consisted in only a few runs of short length. During the second trial, Female B oviposited twice: her first and second pre-oviposition searching patterns were very similar in duration (16 and 15 min, respectively), consisting of a few initial runs covering the entire length of the twig, followed by runs of shorter lengths focused on the infested area of the twig. Female C oviposited only once on the twig: her searching pattern was short (7 min) and consisted of a few turns rapidly converging on the infested area. Overall, Fig. 1.1 shows the formation of a cluster of

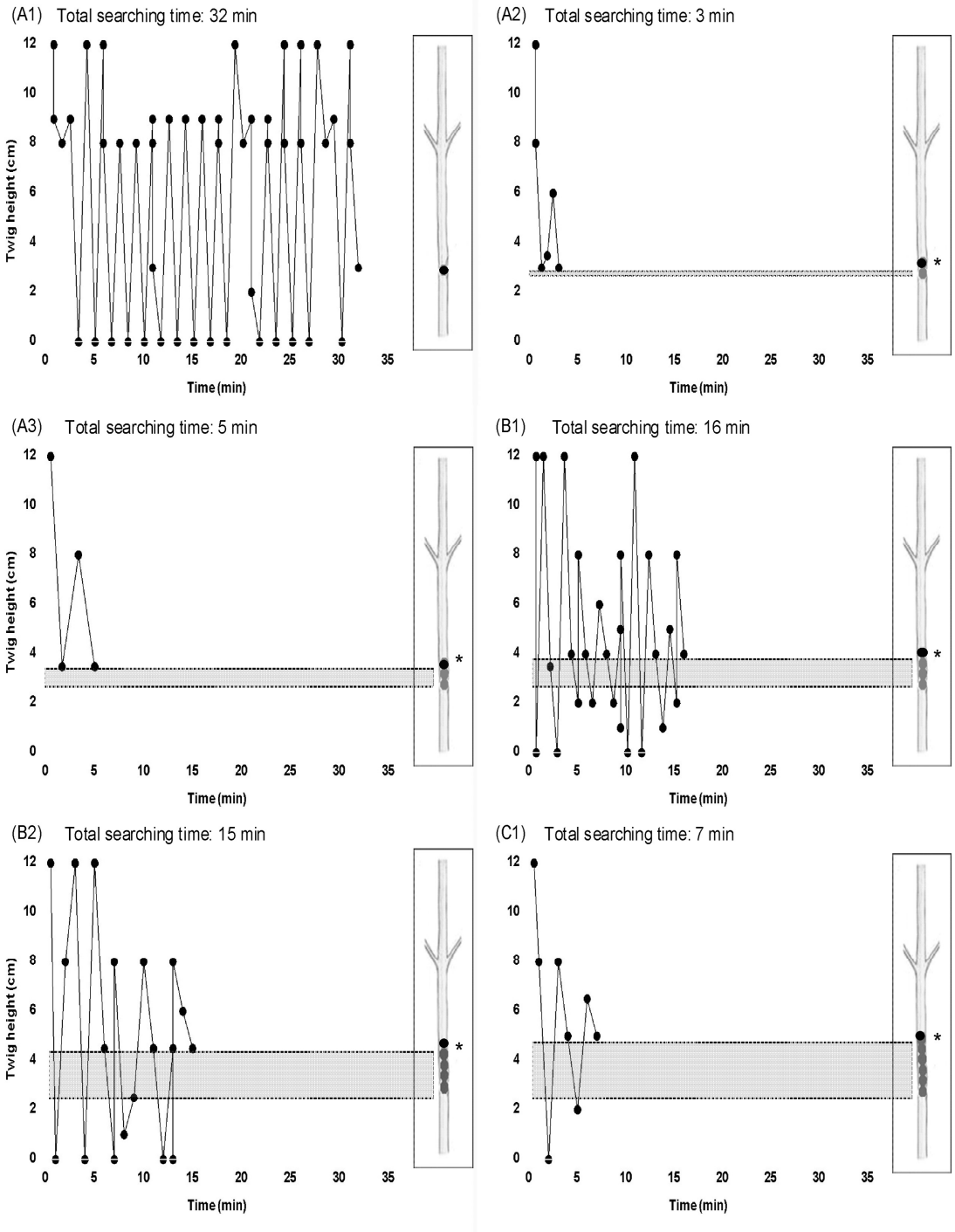

Figure 1.1. Searching patterns of females A, B, and C during six consecutive ovipositions on the same twig. Each point indicates a turn or a stop between two runs. The gray band indicates the infested portion of the twig at the beginning of the searching pattern, if any. The black egg mass on the twig (marked by a star if several egg masses are represented) represents the position of the egg mass laid at the end of each searching pattern.

egg masses perfectly aligned along a twig as the result of three females successively aggregating their egg masses immediately above the initial egg mass.

Relative importance of gravity and presence of other egg masses. The 10 females used for this experiment laid a total of 24 egg masses on the infested twigs at the end of the 48 h, ranging from 1 to 4 egg masses per female. Of these 24 egg masses, 79.2% were laid on the underside of the twig, within the portion of the twig initially infested; 16.7% were laid on the underside of the twig outside the infested portion, and 4.2% were laid on the upper side of the twig, within the infested portion (=adjacent to the existing egg mass(es)) (Fig. 1.2). Statistically, there was a significant positioning preference for the underside of the twig ($\chi^2 = 20.17$, $P < 0.0001$) and the infested portion of the twig ($\chi^2 = 56.84$, $P < 0.0001$).

Discussion

Our descriptive study sheds light on some previously undocumented aspects of *P. viburni* oviposition behavior. First of all, the time cost of oviposition for *P. viburni* females, from pre-oviposition searching to the production of the egg cap, was quantified and estimated to be 135.3 $\pm$ 6 min, chewing the egg cavity being by far the most time consuming part of the process (103.9 $\pm$ 5.6 min). Given the amount of time invested and the energy cost of searching, chewing the egg cavity, and producing the egg cap, it is interesting to note that 18.9% of the females tested still produced more than 1 egg mass, with an impressive maximum of 4, in 24 hours.

When placed on a twig that was already infested, *P. viburni* females focused most of their searching around the infested portion of the twig, and positioned their egg masses adjacent to the existing egg masses in 80.9% of the cases. This is strong

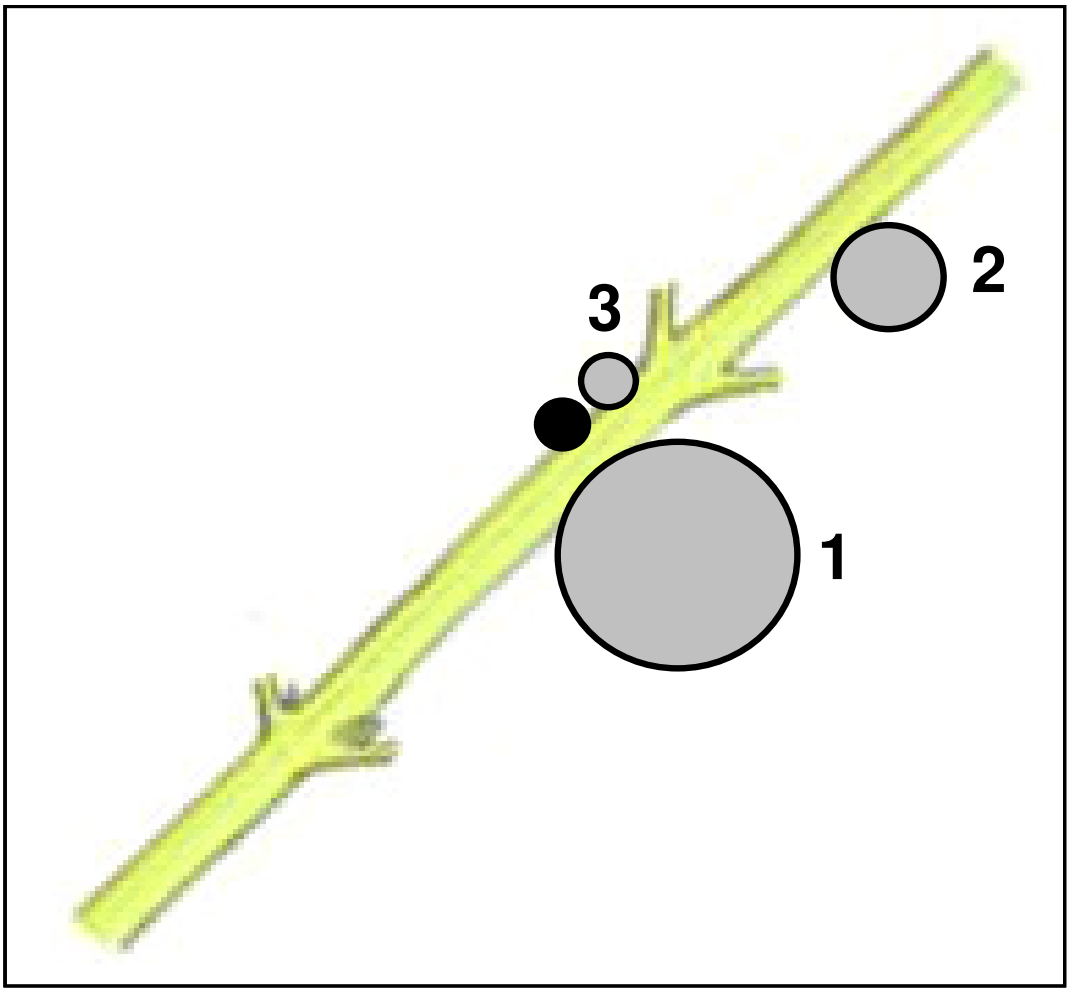

Figure 1.2. Positioning of the egg masses laid after 48 hours relative to the initial egg mass(es) (colored in black). Areas of the gray circles areas correspond to the percentages of egg masses laid 1) on the underside of the twig, within the portion of the twig initially infested; 2) on the underside of the twig outside of portion of the twig initially infested; and 3) on the upper side of the twig, adjacent to the portion of the twig initially infested.

evidence that clusters of *P. viburni* egg masses observed in the field along terminal twigs of susceptible shrubs can be the work on several females successively infesting a same twig, spatially coordinating their egg masses to form aligned clusters. However, we have to keep in mind that these results were obtained under no-choice conditions of non-choice: in order to determine if *P. viburni* oviposition behavior is truly aggregative, it would be necessary to investigate how *P. viburni* females position their egg masses when given the choice between infested and non infested twigs.

Authors who have studied *P . viburni* oviposition have suggested that the egg cap of *P. viburni* egg masses is made of "frass mixed with chewed bark" (Lühmann 1934, Hilker 1992). In our trials, during the production of the egg cap, females used their terminal abdominal segments to apply and smear the protective anal secretion over the cavity, but no chewing was done to incorporate chewed bark in the secretion. However, it is possible that bark fragments coming from the cavity itself were caught in the secretion during its deposition. It is also possible that the *P. viburni* population we worked with differed in some aspects of its oviposition behavior from the populations Lühmann (1934) and Hilker (1992) studied. For example, Lühmann observed that *P. viburni* females sometimes chewed the cavity for their egg mass with their heads facing away from gravity, which was never observed during our trials.

Slight differences in oviposition behavior might occur among geographically distinct *P. viburni* populations in its native range. A comparison with *P. viburni* oviposition behavior in its introduced range could be of importance in helping to identify the area of origin of the *P. viburni* population that initially established in North America, which remains unknown at this point.

When confronted with an unnatural situation where egg masses are oriented on the upper side of the twig and not the underside, *P. viburni* females laid the vast majority of their egg masses on the underside, in close proximity to the portion of the

twig initially infested. This is strong evidence that both gravity and presence of other egg masses act as positional stimuli for *P. viburni* females. Females were very reluctant to lay eggs against gravity (only 4.2% of the egg masses were laid on the upper side of twigs). This suggests that, while clues associated with the presence of egg masses laid by conspecifics lead *P. viburni* females to select a portion of the twig for oviposition, gravity is the most important stimulus used to position the new egg mass. I hypothesize that positioning egg masses on the underside (shaded side) of the twig might help the egg cap to retain moisture, which would provide humidity to *P. viburni* eggs.

Possible directions for future research on *P. viburni* oviposition behavior include comparing modalities of oviposition behavior in *P. viburni* populations from different areas of its native and introduced range, and investigating oviposition preferences at the twig, shrub, and host species levels.

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CHAPTER 2

STAYING TOGETHER FOR THE KIDS: AGGREGATIVE OVIPOSITION BEHAVIOR OF A PHYTOPHAGOUS BEETLE AND THE POTENTIAL BENEFIT OF OVERCOMING PLANT DEFENSES

Abstract

Communal oviposition has been reported for species belonging to a variety of insect orders, but the adaptive value of this behavior is rarely well understood. Our study focused on *Pyrrhalta viburni*, a phytophagous beetle laying eggs in the terminal twigs of *Viburnum* shrubs. To test whether *P. viburni* oviposition is communal or not, we conducted laboratory oviposition choice-tests. Females preferred to lay eggs in twigs already infested by conspecifics (3.0 ± 0.5 egg masses/twig) than on non-infested twigs (0.7 ± 0.3), and consistently positioned their egg masses adjacent to existing ones, showing clear evidence of aggregative oviposition. To determine the relationship between aggregative oviposition and plant wound response, an observational study was conducted in the field on naturally infested *V. dentatum* shrubs. The vast majority (94.4%) of the infested twigs had more than 1 egg mass, and 21% had 20 egg masses or more. There was a negative correlation between plant wound response and number of egg masses and a positive correlation between twig mortality and number of egg masses, suggesting that *P. viburni* benefits from aggregative oviposition by killing twigs to overcome plant defenses.

Introduction

Unrelated conspecifics depositing eggs in a common oviposition site, a behavior referred to as “communal”, “social”, or “gregarious” oviposition in the

literature (Costa 2006), has been reported to occur in a variety of species in the animal world [e.g. works of Radder and Shine (2007) on reptiles and Waldman (1982) on wood frogs]. Among insects, evidence of social oviposition has been reported for relatively few species, and occurs in several orders (Costa 2006) including, but not limited to, Psocoptera (New 1985), Hemiptera (Melksham 1984), and Lepidoptera (Reed 2005).

Whether communal oviposition is driven by environmental constraints (e.g. scarcity of oviposition sites), arises as a stochastic consequence of reproductive behavior (e.g. aggregation for mating), or bears an adaptive value is not always clear. Potential benefits of increased clutch size, a direct consequence of communal oviposition, have been suggested and summarized by Stamp (1980). They include prevention of egg dessication, enhancement of egg aposematism, prey dilution effect, minimization of female search time, and facilitation of larval aggregation. Larval aggregations and larval group-feeding in general have received considerable attention of researchers studying phytophagous insects. Several hypotheses suggested to explain their adaptive value (Fordyce and Nice 2004) include increasing thermoregulatory ability, maximization of resource use, circumvention of physical plant defenses (e.g. trichomes), accelerated larval growth due to enhanced plant quality, and increase of larval defenses against natural enemies.

Viburnum leaf beetle, *Pyrrhalta viburni* (Paykull) (Insecta: Coleoptera: Chrysomelidae), is an interesting species for studying ovipositional ecology. A univoltine species overwintering as eggs, *P. viburni* lays eggs in round cavities (approx. 1 mm in diameter and 1 mm deep) excavated in the tissue of terminal twigs of susceptible *Viburnum* shrubs. Each cavity is filled with an average of 8 eggs (Weston et al. 2008), and covered with a frass-like secretion (2-4 mm in length). The entire oviposition process takes on average 135 minutes per egg mass, and females

typically produce 1 egg mass per day (unpublished data). Females can lay eggs their entire lifetime, producing on average 75 egg masses per female under laboratory conditions (Weston et al. 2008). Twig mortality due to *P. viburni* oviposition is commonly observed, but plants are not defenseless against oviposition; production of wound tissue that either overgrows the egg mass or squeezes the eggs out of the egg cavity has been frequently observed in response to oviposition under both laboratory and field conditions.

P. viburni oviposition is suspected to be communal; under field conditions, clusters of large numbers of egg masses are often found aligned on the underside of twigs, suggesting that several females contributed to the formation of such clusters (see Chapter 1).

The present study had two objectives: a) determine if *P. viburni* females prefer to oviposit on twigs previously infested by conspecifics via laboratory choice-tests, and b) establish the relationship between number of egg masses per twig and plant defenses under field conditions.

Material and methods

Laboratory oviposition assays. All *P. viburni* adults and plant material used for these experiments were collected in the Ithaca area (NY, USA) on naturally infested *V. dentatum* shrubs during July-August 2007. Adults were kept on fresh *V. dentatum* shoots in plastic containers (30 cm x 22 cm x 10 cm) with a screen lid kept at 22°C under a 15:9 (L:D) light regime until needed for experimental purposes.

Our choice-test setting was as follows: two mated *P. viburni* females were placed in a plastic cylindrical container (9.5 cm x 21 cm) with a screen lid containing two *V. dentatum* (L.) twigs uniform in length (12 cm) and diameter (2.5 mm), and bearing a pair of leaves as a food source. One twig was non-infested, and the other

was infested with 1-4 egg masses laid by conspecifics in the laboratory during the 24 h preceding the trials. The number of egg masses on all twigs and position of the new egg masses relative to the existing ones on infested twigs were recorded after 24 and 48 h. When recording the position of new egg masses, we considered that an egg mass was adjacent to an existing one if it had been laid within 5 mm. Ten replicates were used for this experiment.

Differences in numbers of egg masses laid on non-infested and infested twigs after 48 h were analyzed by performing a paired *t*-test (Statistix 9.0, Analytical software 2008). To determine if the distribution of egg masses laid on infested twigs after 24 and 48 h was random or aggregated, we performed multinomial tests (Statistix 9.0, Analytical software 2008) testing the hypothesis that the observed frequency of egg masses laid adjacent to existing ones (=adjacent egg-laying) was not different from the expected frequency of adjacent egg-laying if egg masses were distributed at random. To calculate the expected frequencies of adjacent egg-laying, we assumed that each twig initially had 24 potential oviposition sites (12 cm twig length divided by 5 mm per egg mass). We then subtracted from this total number of oviposition "slots" those slots initially occupied by egg masses, and counted the number of available slots that were adjacent to existing egg masses. Dividing the number of adjacent slots available by the total number of available slots yielded the expected frequency of adjacent egg-laying per twig. This calculation had to be made twice, at the beginning of the experiment and again after 24 h since egg masses laid during the first 24 h decreased the number of available slots and increased the expected frequency of adjacent egg-laying.

Field observations. During winter 2006, twigs of 37 *V. dentatum* shrubs naturally infested with *P. viburni* were sampled in a field setting in the Ithaca area (NY, USA). Shrubs were 8-10 years old, had never been treated with systemic

insecticides, and had not been treated with foliar insecticides against *P. viburni* adults the previous year. Twenty terminal twigs were randomly cut from each shrub (=740 twigs total). For each twig, the following parameters were recorded: twig vitality (living or dead), number of intact egg masses, and number of egg masses surrounded by wound tissue. Percentage wound response was then calculated as the number of egg masses encased in wound tissue divided by the total number of egg masses laid on the twig.

The vitality of twigs was gauged by observing the presence of green tissue under the bark or buds of the twig; if a twig possessed green tissue at both ends, it was considered alive. If a twig infested with egg masses possessed green tissue on the portion basal to the egg masses but no green tissue on the distal portion, it was considered killed by *P. viburni* oviposition. The relationships between the number of egg masses and percentage wound response, and between the number of egg masses and percentage twig mortality were established using non-linear regression (Statistix 9.0, Analytical software 2008). To perform these analyses, twigs sampled from all shrubs were divided into categories of infestation depending on the number of old egg masses present (i.e. 1-2, 3-4… 23-24, 25+). The number of twigs that died due to *P. viburni* oviposition was then enumerated, and the percentage of twig mortality calculated for each category.

Results

Laboratory oviposition assays. *P. viburni* females laid significantly more egg masses on twigs already infested (3.0 $\pm$ 0.5) (mean $\pm$ SE) than on non-infested twigs (0.7 $\pm$ 0.3) ($t = 3.74$, $P = 0.0023$) (Fig. 2.1). The expected frequencies of adjacent egg-laying were 0.20 and 0.22 after 24 and 48 h, respectively. The observed frequencies of adjacent egg-laying were 0.75 after 24 h (9 egg masses out of 12 laid

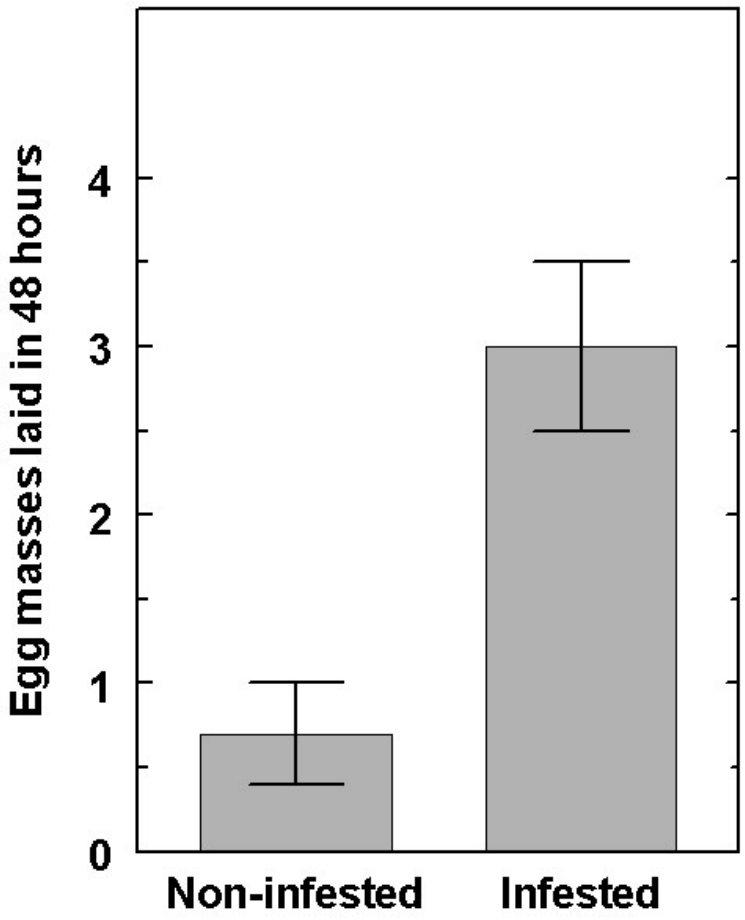

Figure 2.1. Egg masses (mean $\pm$ SE) laid by *P. viburni* on previously infested and non-infested twigs in a laboratory choice test. Oviposition on infested twigs was significantly higher than that on non-infested twigs as determined by a paired *t*-test ($P < 0.05$).

adjacent to existing ones) and 0.88 after 48 h (15 egg masses out of 17) (Fig. 2.2). Observed frequencies were statistically different from expected frequencies after 24 h ($\chi^2 = 29.4$, $P < 0.0001$) and 48 h ($\chi^2 = 39.6$, $P < 0.0001$), indicating that egg masses deposited on infested twigs were not placed at random.

Field observations. Of the 740 twigs sampled for this study, 9 were damaged during transportation and excluded from the analysis. Of the 731 twigs remaining, 500 were infested with *P. viburni* egg masses (68.4%) and 231 were non-infested (31.6%). The number of egg masses on infested twigs averaged 13.0 $\pm$ 0.4, ranging from 1 to 64 egg masses (Fig. 2.3). Only 5.6% of the infested twigs had only 1 egg mass, and 21% had 20 egg masses or more.

There was a significant negative correlation between the number of egg masses per twig and percentage wound response ($F = 28.2$, $P < 0.001$), and a significant positive correlation between number of egg masses and twig mortality ($F = 55.35$, $P < 0.0001$). A logarithmic regression explained the most variation for both regressions ($r^2 = 0.96$ and 0.99, respectively) (Fig. 2.4).

Discussion

Our results show that *P. viburni* females clearly exhibit aggregative oviposition behavior, preferring to lay eggs on twigs already infested by conspecifics when given a choice, and positioning their egg masses adjacent to existing egg masses in 81.5% of cases. This precise spatial coordination of egg-laying to form aligned clusters of egg masses leads us to employ the term "aggregative" oviposition rather than "communal" or "gregarious" oviposition, the two latter terms somewhat implying the idea of simultaneous oviposition, which can indeed occur in nymphalid butterflies (Reed 2005) but is unlikely to be the case in our system. *P. viburni* oviposition has an important time cost and females are constrained in the number of egg masses they can

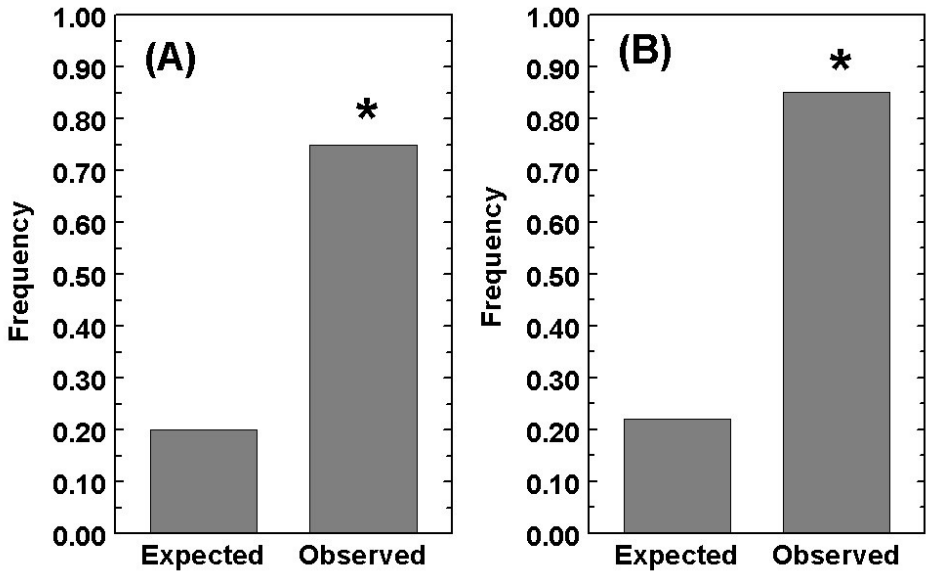

Figure 2.2. Frequencies of expected and observed adjacent egg-laying by *P. viburni* on previously infested twigs after (A) 24 h and (B) 48 h in laboratory assays. Bars with an asterisk indicate significantly greater oviposition than expected if oviposition were random as determined by a multinomial test ($P < 0.05$).

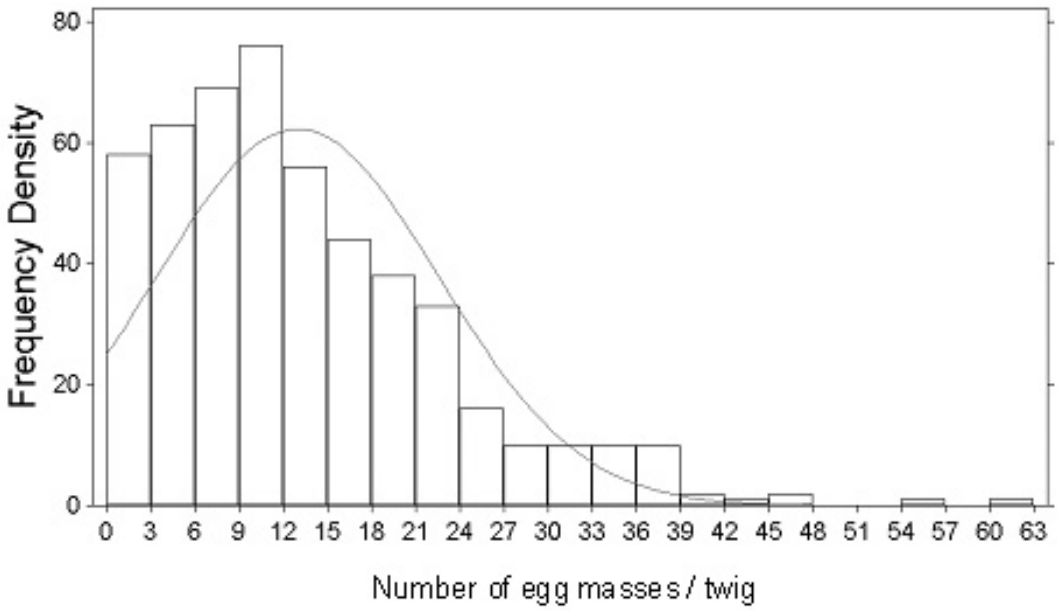

Figure 2.3. Histogram of frequencies of numbers of *P. viburni* egg masses found on naturally infested *V. dentatum* twigs in the field. The smooth line indicates a normal distribution.

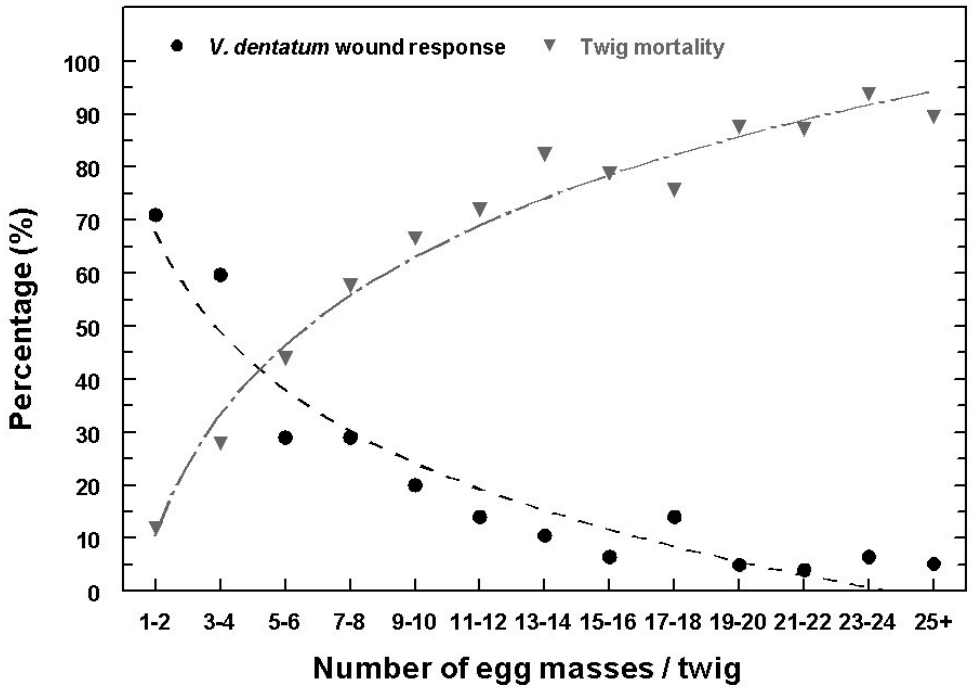

Figure 2.4. Percentage wound response of *V. dentatum* twigs and twig mortality depending on number of egg masses of *P. viburni* per twig.

produce per day; the formation of aggregates of egg masses along twigs is thus more likely to result from successive ovipositional bouts spaced in time rather than simultaneous ones, especially in the case of large clusters.

Our field observations support the conclusions drawn from the laboratory assays. *P. viburni* egg masses were rarely found isolated on twigs, and 21% of the twigs had more than 20 egg masses, suggesting the contribution of several females ovipositing on the same twigs. It is unlikely that this high density of egg masses per twig was due to scarcity of oviposition sites because 31.6% of the total twigs sampled were non-infested.

Why do *P. viburni* aggregate their egg masses? While several potential benefits among those summarized by Stamp (1980), such as prey dilution effect and minimization of female search time cannot be ruled out, our study suggests that the main benefit of this behavior might be overcoming plant defenses. Figure 4 shows that wound response significantly decreases as the number of egg masses per twig increases. In other words, the more egg masses laid on a twig, the less effective the plant response. The inverse relationship between twig mortality and number of egg masses per twig (i.e. rapid increase in twig mortality with increasing number of egg masses) suggests the following mechanism: each *P. viburni* egg mass is excavated through the bark and therefore damages the vascular system of the twig. When multiple egg masses are laid, vascular tissues are eventually disrupted and the twig dies, disabling its capacity to produce wound tissue.

Of course, this proposed mechanism might be an oversimplification of reality. Many factors such as chemical compounds produced by the beetle during oviposition or by the plant in response to oviposition could play a role in plant wound response and twig mortality and would be worth investigating in the future. Similarly, additional studies including a direct measure of *P. viburni* fitness in relation to

aggregative oviposition are needed to conclude that this behavior is associated with increased fitness.

Due to some highly distinctive aspects of its ovipositional ecology, *P. viburni* stands out even among insect species showing communal oviposition. The tight association of *P. viburni* oviposition with plant defenses, the act of oviposition itself being a way to circumvent wound response, has not, to our knowledge, been documented previously for any other communal species. However, cooperation of adults to overcome plant defenses is not uncommon in the insect world. For example, numerous species of bark beetles (Coleoptera: Scolytinae) exhibit adult aggregation and colonial breeding, size of the starting colony being a determinant in the success of overcoming tree defenses (i.e. resin production) and egg survivorship (Byers 1989a). High densities of periodical cicadas ovipositing in young twigs often result in high twig mortality, tree wound response appearing to be density dependent (Williams and Simon 1995).

In such systems, a basis for more adequate comparisons with *P. viburni* behavior certainly exists with future research prospects. For example, the costs of aggregative behavior, including intraspecific competition, have received considerable attention from bark beetle researchers (Byers 1989b) and would be a fascinating topic to investigate regarding *P. viburni* oviposition.

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CHAPTER 3

CHAPTER 3 STIMULI ASSOCIATED WITH VIBURNUM LEAF BEETLE [*PYRRHALTA VIBURNI* (PAYKULL)] AGGREGATIVE OVIPOSITION BEHAVIOR

Abstract

Viburnum leaf beetle, *Pyrrhalta viburni* (Paykull) (Coleoptera: Chrysomelidae), oviposits in terminal twigs of *Viburnum* shrubs. Females excavate round cavities they fill with eggs and cover with a cap made of a frass-like secretion. Twigs react to oviposition by producing wound tissue that overgrows the egg mass. *P. viburni* oviposition behavior is aggregative: females prefer to lay egg masses on twigs previously infested by other females and to position their egg masses adjacent to existing ones. Female oviposition preferences were studied in a series of choice tests under laboratory conditions using non-infested, naturally-infested, and artificially manipulated *Viburnum opulus* twigs.

The three components of the egg mass (cavity, eggs, and egg cap) were not sufficient to elicit complete aggregation oviposition response (twig preference + position preference) when presented separately. Cavity and egg cap elicited positional preference, but not the artificial cavity, artificial egg cap, or frass, suggesting that cues might be associated with insect saliva and egg cap secretion. Females preferred to lay eggs on twigs that had produced wound tissue in response to previous oviposition, but did not show positional preference for wound tissue. We conclude that twig choice and positional preference for oviposition are likely to be dissociated, and that twig wound response might be used by *P. viburni* females to locate twigs already infested and possibly less defended against subsequent oviposition.

P. viburni females preferred to lay egg masses on heavily infested twigs rather than on lightly-infested twigs. When given twigs with similar numbers of egg masses, females did not show preference for twigs infested with their own egg masses, and laid more egg masses on twig infested by multiple females than on twigs infested by single females. We conclude that *P. viburni* females are able to distinguish egg masses laid by conspecifics, and we discuss the possible ecological implications of this behavior.

Introduction

Signals and cues from conspecifics can influence insect oviposition behavior in different ways. Previously-infested sites can be deterrent to ovipositing females; this has been documented for phytophagous insects trying to avoid intraspecific competition among larvae, especially if a food source is limited (Prokopy 1981). Avoidance of hosts already infested by conspecifics can also of crucial importance for many species of parasitoids in order to avoid superparasitism (Ueno 1994, McKay and Broce 2004). On the other hand, insect species that benefit from aggregation might be attracted to sites already infested by conspecifics. Several studies have shown that phytophagous insects can be attracted by herbivory-induced plant volatiles (Loughrin et al. 1996, Prokopy and Roitberg 2001, Shiojitri and Takabayashi 2003, Hilker and Meiners 2006). The hypothesized benefits of preferring damaged plants range from finding suitable host plants and mates (Prokopy and Roitberg 2001, Loughrin et al. 1996) to diluting the effect of predators and parasitoids (Shiojitri and Takabayashi 2003). In addition to herbivory-induced plant responses, egg deposition itself can attract females to infested sites. In the mosquito *Culex tarsalis* (Diptera: Culicidae), gravid females are attracted to ponds already infested by other females, a pheromone released during oviposition being responsible for attraction (Shorey 1973). Olfactory pheromones leading females to gregarious oviposition sites have also been

documented for the desert locust *Schistocerca gregaria* (Homoptera: Acrididae) and the sheep blowfly *Lucilia cuprina* (Diptera: Calliphoridae) (Shorey 1973).

The viburnum leaf beetle, *Pyrrhalta viburni* (Coleoptera: Chrysomelidae), is a pest of shrubs belonging to the genus *Viburnum.* It is native to Eurasia and invasive in the northeastern U.S. and southeastern Canada. Both larvae and adults are leaf feeders and can kill susceptible shrubs after 2 to 4 years of repeated defoliations. *P. viburni* females lay their eggs in round cavities (1-2 mm diameter, 1 mm deep) they excavate in the terminal twigs of viburnum shrubs. Each cavity contains an average of 8 eggs (Weston et al. 2008) and is closed with a cap made of a brown frass-like secretion (2-4 mm diameter) that we will refer to as "egg cap" in the rest of the study.

P. viburni oviposition behavior is aggregative; females prefer to lay eggs on twigs already infested by other females, and position their egg masses adjacent to existing ones. The main benefit of this behavior is thought to be overcoming plant defenses; infested *Viburnum* twigs can respond to *P. viburni* oviposition by producing wound tissue that either overgrows the egg mass or squeezes the eggs out of the egg cavity, but the intensity of wound response is negatively correlated with egg mass density on an individual twig (Chapter 2).

Through a series of choice tests under laboratory conditions using naturally infested and artificially manipulated twigs, our study aimed to determine a) what components of the egg mass influence twig preference and egg mass positioning, b) whether the quantity of egg masses influences twig preference, and c) if *P. viburni* females can distinguish their own egg masses from egg masses of conspecifics.

Material and methods

Insects and plant material

All *P. viburni* adults used for the experiments in this study were collected in

the Ithaca area (NY, USA) on naturally infested plants during summer, 2007. Insects were transferred to *Viburnum dentatum* (L.) and *V. opulus* (L.) shoots in rectangular plastic containers (30 cm x 22 cm x 10 cm) with a vented lid and kept at 22°C under a 15:9 (L:D) light regime until needed for experiments. Each adult was used only once for experimental purposes. All the plant material used for the choice tests came from unmanaged *V. opulus* shrubs located in the Ithaca area. All plants serving as a source of twigs shared similar characteristics in terms of age (8-10 years), had never been treated with systemic insecticides, and had not been treated with foliar insecticides during the two previous growing seasons.

Choice test setting

In the laboratory, choice tests were performed in August-September, 2007 with mated *P. viburni* females. Two females were placed in a cylindrical container (9.5 cm diam. x 21 cm height) with a screen lid (=oviposition container) containing two *V. opulus* twigs homogeneous in length (12 cm) and diameter (3 mm). Numbers of egg masses on both twigs were recorded after 48 hours.

Manipulation of egg mass components. In this series of choice tests, female were given the choice between a non-infested twig and a treatment twig (8-12 replicates per treatment). Treatments were: a) Egg mass: either 1 or 2 egg masses laid on each twig by 2 *P. viburni* females in an oviposition container during the 24 h preceding the trials; b) Cavity: twigs bearing a single natural egg mass cavity, but no eggs or egg cap. In order to prepare these twigs, 2 *P. viburni* females were placed in an oviposition container during the 24 h preceding the trial. If a female was observed engaging in the oviposition process, she was allowed to chew the egg mass cavity but removed before the subsequent steps of the process (egg-laying and secretion of the egg cap); c) Egg cap: twigs bearing natural egg caps, but no cavities or eggs. Egg caps were removed from natural egg masses with a metal probe and glued to treatment

twigs using a tiny droplet of Elmer's glue. Each treatment twig bore two egg caps adjacent to one another; d) Eggs: twigs bearing natural eggs, but no cavities or egg cap. Eggs were removed from natural egg masses with a metal probe and glued to treatment twigs using a tiny droplet of Elmer's glue. Each treatment twig bore a minimum of 8 eggs gathered from one single egg mass; e) Artificial egg mass: twigs bearing faux egg caps matching the dimensions of natural egg caps. Faux egg caps were shaped directly on non-infested twigs using a mix of brown water-color paint and Elmer's glue prepared beforehand. Each twig bore two faux egg caps adjacent to one another; f) Artificial cavity: twigs bearing a single artificial cavity matching the approximate dimensions of a natural cavity. Artificial cavities were excavated in non-infested twigs using a sterilized scalpel blade in the 24 h preceding the trials; g) Wound response: twigs bearing wound tissue produced in reaction to egg masses but no intact egg masses. In order to prepare this treatment, twigs infested with 1-5 egg masses were kept in an incubator at 22° C in water-filled plastic tubes until wound tissue was produced that covered all of the egg masses (14 d). Control twigs were cut the same day as treatment twigs and kept under the same conditions until trials; h) Frass: twigs bearing frass of female beetles but no egg masses. Frass was collected by scraping the inside of a container containing only females with a small paintbrush and applied on uninfested twigs in one circular spot, mimicking the dimensions of a natural egg mass. The frass was applied in the 24 h preceding the trials; i) Egg mass vs. Wound response: twigs were prepared as for these two treatments separately, with the exception of twig age. It took 20 d to observe production of wound tissue for the wound response twigs used for this test. Twigs for the egg mass treatment were also 20-d old but had only been infested in the 24 h preceding the trials.

For each individual treatment, positions of new egg masses relative to treatments were recorded after 24 and 48 h exposure to treatment twigs. When recording positions of

new egg masses, we considered an egg mass to be adjacent to a treatment if it had been laid within 5 mm of it.

Egg mass as a quantitative stimulus. Females were given the choice between a twig with a low density of egg masses (1-5) and a twig with a high density of egg masses (>10). Twigs with low densities of egg masses were prepared by placing 2 *P. viburni* females on an uninfested twig in an oviposition container for 24 h prior to the trial. Twigs with high densities of egg masses were prepared by placing 5 *P. viburni* females on an uninfested twig in an oviposition container 72 h prior to the trial (11 replicates).

Influence of oviposition by conspecifics. Two different choice tests were conducted for this experiment. In the first test, one *P. viburni* female was given the choice between a twig with her own egg masses and a twig with egg masses of a single conspecific female. Twigs were infested 48 h prior to the trial. Tested females were removed from the twig where they initially oviposited 24 h before the trial and placed in a container with *V. dentatum* leaves as food until the trial. The number of egg masses was similar on both twigs (1-2) (10 replicates).

In the second choice test, females were given the choice between a twig where a single female had oviposited and a twig where multiple females had oviposited. To prepare the multiply infested twigs, successive females were placed in an oviposition container for 24 h with a twig that was initially uninfested (or 48 h if no egg masses had been laid after 24 h) the week preceding the trials. A multiply infested twig was considered ready for a trial when 5 different females had oviposited on it. Numbers of egg masses on twigs used for this choice test were similar within each pair of twigs (i.e. there was never a difference of more than 2 egg masses between the single infested twig and the multiply infested twig) (9 replicates).

Statistical analysis

Differences in the quantities of egg masses laid on both twigs after 48 h were analyzed using a complete block design (ANOVA: Statistix 9.0, Analytical software 2008), with each replicate counting as a block. Data were transformed using the square root transformation to meet the assumption of the model. To determine if the distribution of egg masses laid on treatment twigs after 24 and 48 h was random or aggregated around treatments, multinomial tests were performed (Statistix 9.0, Analytical software 2008), testing the hypothesis that observed frequency of egg masses laid adjacent to treatments (=adjacent egg-laying) was not different from the expected frequency of adjacent egg-laying if the distribution was random. To calculate the expected frequencies of adjacent egg-laying, we assumed that each twig initially had 24 potential oviposition sites (12 cm twig length divided by 0.5 cm egg mass length). We then subtracted, from this total of oviposition "slots", the number of slots initially occupied by treatments, and counted the number of slots available adjacent to the treatments. Dividing the number of adjacent slots available by the total number of unoccupied slots gave the expected frequency of adjacent egg-laying per treatment twig. Since the number of slots occupied by treatments was not the same for all treatments, expected frequencies of adjacent egg-laying varied depending on treatments.

Results

Manipulation of egg mass components. *P. viburni* females laid significantly more egg masses on twigs previously infested by conspecifics ($F = 8.86$, $P = 0.02$) and laid their egg masses adjacent to existing egg masses in 63% of the cases ($\chi^2 = 55.26$, $P < 0.0001$) (Table 3.1). None of the three egg mass components (cavity, eggs, or egg cap) influenced twig preference separately. However, cavity and egg cap induced a positional preference: when *P. viburni* females laid egg masses on twigs bearing

cavities or egg caps, they laid their egg masses adjacent to the cavity or the egg cap in 50% ($\chi^2 = 38.77$, $P < 0.0001$) and 65% ($\chi^2 = 45.90$, $P < 0.0001$) of the cases, respectively. None of the artificial manipulations treatments (artificial cavity, artificial egg mass, or frass) induced twig or positional preference. Females laid significantly more egg masses on twigs that exhibited wound responses ($F = 47.74$, $P < 0.001$), but did not position their egg masses adjacent to wound tissue ($\chi^2 = 0.25$, $P = 0.62$). When given a choice between twigs previously infested by conspecifics and twigs that exhibited wound responses, *P. viburni* females laid comparable quantities of egg masses on both types of twigs ($F = 1.58$, $P = 0.24$), but preferentially positioned their egg masses next to existing ones on previously infested twigs ($\chi^2 = 21.27$, $P < 0.0001$).

Egg mass as a quantitative stimulus. When given a choice between twigs with low egg mass densities and twigs with high egg mass densities, *P. viburni* females laid more egg masses on twigs with high egg mass densities ($F = 5.46$, $P = 0.04$) (Table 3.2).

Influence of conspecifics. When given a choice between their own egg masses and egg masses from a single conspecific, *P. viburni* females did not show preference ($F = 0.89$, $P = 0.37$) (Table 3.2). However, females laid more egg masses on twigs where multiple conspecifics had laid egg masses than on twigs where single conspecifics had laid egg masses ($F = 5.79$, $P = 0.04$).

Discussion

P. viburni females showed aggregative oviposition behavior on *V. opulus* under laboratory conditions, preferring to lay egg masses on twigs previously infested

Table 3.1. Mean number of egg masses laid by *P. viburni* females in choice tests between control (non-infested) and treatment twigs in 48 hours, and frequencies of adjacent egg-laying on treatment twigs.

		Mean egg masses laid per twig ± SE				Proportion of adjacent egg-laying		
Treatments	N	Control	Treatment	*P*		Expected	Observed	*P*
Egg mass	10	0.60 ± 0.27	2.10 ± 0.43	0.02		0.11	0.63	<0.0001
Egg cap	10	2.40 ± 0.67	1.70 ± 0.34	0.59		0.10	0.65	<0.0001
Cavity	12	1.00 ± 0.42	1.40 ± 0.34	0.51		0.10	0.50	<0.0001
Eggs	8	1.25 ± 0.25	1.00 ± 0.38	0.49		0.10	0.11	1.00
Artificial egg cap	10	1.60 ± 0.30	0.80 ± 0.36	0.09		0.10	0	0.29
Artificial cavity	10	1.70 ± 0.63	1.30 ± 0.40	0.75		0.10	0	0.29
Frass	10	1.80 ± 0.44	1.30 ± 0.47	0.57		0.10	0.17	0.29
Wound response	8	0.25 ± 0.44	3.00 ± 0.60	<0.001		0.20	0.17	0.62
		Egg mass	Wound response	*P*		Expected	Observed	*P*
Egg mass vs. Wound response	10	1.40 ± 0.420	0.70 ± 0.26	0.24	Egg mass	0.17	0.62	<0.0001
					Wound response	0.14	0.25	0.28

Table 3.2. Mean number of egg masses laid after 48 hours by *P. viburni* females in choice tests with twigs bearing low and high numbers of egg masses, twigs infested by themselves or other females, and twigs infested by one or multiple females.

		Mean egg masses laid per twig ± SE		
Choice test	N	Low	High	*P*
Low vs. high	11	0.64 ± 0.28	1.55 ± 0.28	0.04
Choice test	N	Self	Other	*P*
Self vs. other	10	2.00 ± 0.40	1.40 ± 0.45	0.37
Choice test	N	Single	Multiple	*P*
Single vs. multiple	9	1.00 ± 0.47	2.67 ± 0.50	0.04

by conspecifics and to position their egg masses adjacent to existing ones, confirming previous results obtained with *V. dentatum* under laboratory conditions (unpublished data). The three components of the egg mass (cavity, eggs, or egg cap) were not sufficient separately to elicit a "complete" aggregation response (i.e. twig preference and position preference); however, the cavity and egg cap were sufficient to elicit a positional preference.

It is somewhat surprising that eggs themselves were not stimulatory at all to *P. viburni* females. Eggs containing marking pheromones that are used by females to locate host-plants already infested by conspecifics have been documented in several other systems (Hilker and Meiners 2002, Li and Ishikawa 2005). However, we must keep in

mind that *P. viburni* females are unlikely to encounter exposed eggs under field conditions when searching for oviposition sites, eggs being always protected by the egg cap under natural conditions unless an accident occurs or wound tissue expels the eggs outside the cavity. In addition, in other systems natural egg deposition on host-plants has been documented to be sufficient to elicit plant physical or chemical response (Hilker and Meiners 2006), but the way we applied eggs on treatment twigs was unnatural and therefore might not have triggered a normal plant response.

Interestingly, our artificially manipulated treatments (artificial cavity, artificial egg cap, and frass) did not elicit twig preference or positional preference. Artificial cavities, despite being similar in size to natural ones, did not elicit the positional preference observed with natural cavities. We hypothesize that females might utilize cues from insect saliva, or plant chemicals induced by insect saliva (Haukioja 1991), when looking for a spot to position their egg masses. Another possibility is that we were not able to reproduce precisely enough the physical damage caused by *P. viburni* mandibles because we used a scalpel blade so that females failed to recognize our

artificial cavities as egg masses cavities. Additional studies manipulating directly saliva of *P. viburni* females could help answering these questions.

Our faux egg caps were not stimulatory to *P. viburni* females, suggesting that that the stimuli eliciting partial or complete aggregation response are not only visual: a brown bump of the dimensions of an egg mass is not sufficient to stimulate females to oviposit.

Frass did not elicit the positional preference observed with egg caps, suggesting that egg caps contain cues, possibly chemical, that are not contained in frass. Egg caps also differ from frass in the way that pieces of bark (created in the process of chewing the egg cavity) are often mixed with the frass-like secretion produced by females when making the egg cap (Zorin 1931). Mixed cues coming from both twig bark and the secretion might be responsible for the positional preference observed with natural egg caps.

Twigs that were initially infested and that produced wound tissue overgrowing the initial egg masses were more attractive to females, who laid more egg masses on these twigs than on non-infested twigs. However, females did not position their egg masses adjacent to the portions of the twigs showing wound tissue (i.e. initial oviposition slots). It is important to note that wound tissue produced by twigs under laboratory conditions and under field conditions is different in some respects. Wound tissue produced in the laboratory is white-grey, almost translucent, and has no precise shape, while wound tissue observed on twigs in the field is usually light brown, hardened, and elongated around the initial egg mass. It is therefore possible that *P. viburni* females did not recognize the wound tissue produced under laboratory conditions.

Why do *P. viburni* females prefer twigs that produced wound tissue? It may seem an illogical choice for females to favor twigs that exhibited a strong defensive

reaction against previous oviposition. However, plant defensive reaction has been shown to be negatively correlated with egg mass density (Chapter 2), and twigs that already exhibited wounding responses might be less defended against subsequent oviposition. Cues used by females responding to wound tissue are less likely to be visual in the case of our study (due to the nature of the wound tissue produced under laboratory conditions). It seems likely that chemical cues coming from i) plant wound tissue itself, ii) traces of initial oviposition, iii) secondary metabolites produced in response to initial oviposition, or a combination of i), ii), and iii) might have played a role in twig attractiveness.

Overall, results from the "manipulation of egg mass components" experiment suggest that the two components of the aggregation response (twig choice + position preference) are dissociated, and that different cues are used by females to choose twigs for oviposition and to position their egg masses on these twigs. The exact nature of these cues is still speculative at this point, and further experiments looking at the chemistry of egg masses and both non-infested and infested twigs would be needed to test the suggested hypotheses.

Our results also show that egg masses act as a quantitative stimulus: the more egg masses on a twig, the more attractive the twig was to the females. Mechanistically, increased stimulation might be due to higher levels of cues present on heavily infested twigs. From an ecological perspective, preferring twigs with higher densities of egg masses makes sense if we keep in mind the negative correlation between egg mass density and plant wounding response. Eggs laid on such twigs might have increased survivorship due to decreased plant response. However, other factors such as intraspecific competition among hatching larvae might decrease the benefit of laying eggs on heavily infested twigs. A close relative to *P. viburni*, the elm leaf beetle, *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae), has been shown to be attracted

to volatiles produced by lightly infested elm leaves (feeding damage and egg deposition), but to avoid heavily infested leaves (Meiners et al. 2005). In the case of *P. viburni*, it would be interesting to investigate if there is a threshold in egg masses density above which females stop discriminating among twigs, or even start discriminating against twigs that are more heavily infested.

Females did not show a preference for twigs containing their own egg masses, but did lay more egg masses on twigs infested by multiple females than on twigs infested by single females. Since the same numbers of egg masses were present on twigs infested by a single female and twigs infested by multiple females, a quantitative difference in the cues females received from both twigs would not explain this preference. However, there might have been a qualitative change: cues associated with *P. viburni* egg masses might vary from individual female to individual female, and egg masses laid by multiple females might elicit different plant responses than when only a single female oviposits.

From an ecological perspective, favoring twigs where multiple females have laid egg masses is not obvious to interpret. Plant wounding response might be negatively correlated, not only with egg mass density, but also with the number of females laying egg masses. In the field, egg mass density and number of females ovipositing on individual twigs are likely to be positively correlated. Therefore, presence of cues from different females might be an additional indicator that females use to locate twigs less defended against oviposition. It is also possible that preferring twigs infested by multiple females does not bear an adaptive value and is only a consequence of higher physiological stimulation.

In conclusion, our study sheds lights on several previously undocumented aspects of *P. viburni* oviposition preferences. Different components of the egg mass stimulus clearly elicited different responses from females, with the egg cap and cavity

causing positional preference. Interestingly, the importance of the egg cap for host-marking had already been documented for an insect showing an oviposition comparable to *P. viburni*, the pepper weevil *Anthonomus eugenii* (Coleoptera: Curculionidae): *A. eugenii* females lay their eggs in small cavities they dig in flower buds or small fruits of their host-plants, the cavities being sealed by an "oviposition plug" made of a hardened clear anal secretion. Addesso et al. (2007) showed that *A. eugenii* females are deterred by previously infested fruits, and that oviposition plugs and female frass are sufficient to deter oviposition. Oviposition plugs were stronger deterrents than eggs or frass. In both *P. viburni* and *A. eugenii* systems, further analyses looking for example at the chemistry of egg cap/oviposition plug components is still needed to identify the elicitors of females response.

Viburnum wound response to oviposition and increased attractiveness of responding twigs to *P. viburni* females adds to the growing body of literature documenting plant responses to egg deposition and herbivore responses to oviposition-induced plant compounds (Hilker and Meiners 2006). In our system, the plant wound response directly affects early ovipositing females by displacing or destroying the initial egg masses, but might have a cost by attracting more ovipositing females to the weakened twig, a situation similar to response by *Pinus* trees to bark beetles infestations. "Primary" bark beetles attacking healthy trees are most often killed by tree defensive responses (resin flow, toxins), but a rapid communal attack of subsequent beetles attracted by pheromones from early beetles and plant volatiles may overcome tree defenses (Borden 1974, Byers, 1989). However, there is an important difference in scale between both systems. On one hand, bark beetle adults settle for one single tree that will provide support for the entire colony. On the other hand, field-collected *P. viburni* females lay in average 75 egg masses over their lifetime (Weston et al. 2008) and are likely to lay their egg masses on multiple twigs on the same or

different *Viburnum* shrubs. One missing piece of information in our system is the plant response to *P. viburni* oviposition at the shrub level: do *Viburnum* shrubs react systemically to oviposition? Do twigs surrounding a twig exhibiting plant response also become attractive to *P. viburni* females? Our study focused on the twig level. Field studies looking at ovipositional preferences and plant responses at the shrub level would certainly broaden our understanding of the ovipositional ecology of this important pest.

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CHAPTER 4

OVERCOMING PLANT WOUND RESPONSE: A REALIZED BENEFIT OF AGGREGATIVE OVIPOSITION FOR VIBURNUM LEAF BEETLE

Abstract

The viburnum leaf beetle, *Pyrrhalta viburni* (Paykull) (Coleoptera: Chrysomelidae), an emerging landscape pest in northeastern North America, exhibits an aggregative oviposition behavior, conspecifics laying egg masses on terminal *Viburnum* twigs in aligned clusters. In this study, naturally infested twigs of *V. dentatum*, *V. opulus*, and *V. x bodnantense* with varying diameters and densities of egg masses were selected in the field and monitored for twig mortality, twig wound response, and larval emergence to determine whether there was a fitness benefit of aggregative oviposition. Mean egg survivorship and twig mortality increased with increasing level of infestation, while twig wound response decreased with increasing level of infestation for the three species tested. Egg survivorship was negatively correlated with wound response, and was consistently higher on twigs that died than on twigs that remained alive. Overall, these results suggest that, by aggregating their egg masses, *P. viburni* females overcome twig wound response, often killing the twig. Among the host plants tested, the native *V. dentatum* was the least defended against *P. viburni* oviposition (decreased wound response for twigs 2 mm and 3 mm thick) and the least tolerant (increased twig mortality). Lack of defenses and low tolerance in *V. dentatum* could have facilitated the spread and accentuated the damage due to *P. viburni* in areas of northeastern North America where *V. dentatum* is abundant.

Introduction

Aggregations in non-social arthropods are a general phenomenon that has been documented for a wide range of taxa (i.e. reviews by Wertheim et al. 2005, Costa 2006). Circumstantial aggregations driven by scarcity of food or high population densities must be separated from active aggregations driven by behavioral response of individuals to the presence of conspecifics or aggregation pheromones emitted by conspecifics. Active aggregations are of greater importance from an evolutionary perspective because they imply an adaptive value of aggregation for gregarious species.

The chemistry of aggregation pheromones and the mechanisms leading to aggregation have been abundantly documented for a number of species (e.g. Byers 1991, Tillman et al. 1999). However, studies showing realized benefits of aggregation, especially under natural conditions, are less common. Four major types of benefits arising from aggregation have been described (Wertheim et al. 2005): mate-finding, protection from natural enemies, protection from environmental conditions, and increased resource use efficiency. Increase in resource use efficiency can occur via physiological feeding benefits such as optimal intake of food in groups compared to solitary individuals (Prado and Tjallingii 1997), or via the necessity of multiple simultaneous attacks to overcome host resistance (Byers 1989).

Benefits of aggregation can be dependent on environmental conditions and population dynamics. At low population densities, aggregative behavior can help overcoming Allee effects (Stephens and Sutherland 1999). By facilitating mate-finding and/or host exploitation, aggregative behavior can help populations to establish in newly colonized environments. However, at high population densities, costs of gregariousness such as intraspecific competition may arise and counteract aggregation benefits (Byers 1989, Grimaldi and Jeanike 1984).

The viburnum leaf beetle, *Pyrrhalta viburni* (Paykull) (Coleoptera: Chrysomelidae), is a chrysomelid native to Eurasia and invasive in the northeastern U.S. and southern Canada (see Weston et al. 2007 for a summary of biology and invasion history). Both larvae and adults are leaf feeders and can kill susceptible plants after 2 to 4 years of repeated extensive defoliation. *P. viburni* feed only on shrubs belonging to the genus *Viburnum*, but not all *Viburnum* species are equally susceptible to the pest. Susceptible species such as the arrowwood viburnum (*V. dentatum*) and the American cranberrybush (*V. opulus* var. *americanum*, formerly known as *V. trilobum*), both native to North America, often become totally defoliated due to feeding damage in the *P. viburni* invasive range. However, many *Viburnum* species show reduced or no defoliation under field conditions, and are considered moderately susceptible or resistant (Weston et al. 2007).

P. viburni is a univoltine species overwintering as eggs, and lays eggs in round cavities (1-2 mm diameter, 1 mm deep) excavated in the terminal twigs of viburnum shrubs. Each cavity contains an average of 8 eggs (Weston et al. 2008) and is closed with a cap made of a brown secretion produced by the females (2-4 mm length). *P. viburni* oviposition behavior is aggregative; females prefer to lay eggs on twigs already infested by other females and position their egg masses adjacent to existing ones (see Chapters 1 and 2). As a result, *P. viburni* egg masses are usually found in the field in clusters of 5-20 egg masses. Larger clusters are not rare if *P. viburni* populations are high, and clusters of as many as 50 egg masses have been observed (personal observation). Infested *Viburnum* twigs can respond to *P. viburni* oviposition by producing wound tissue that either overgrows the egg mass or squeezes the eggs out of the egg cavity (Chapter 3). This wound response, commonly observed in the field, takes several weeks under laboratory conditions on cut *Viburnum* twigs (personal observation).

The aim of our study was to determine if *P. viburni* benefit from aggregative oviposition by overcoming wound response of *Viburnum* spp. We measured egg survivorship and plant wound response on twigs naturally infested with varying numbers of *P. viburni* egg masses.

We focused our study on three *Viburnum* species: arrowwood viburnum, *V. dentatum* (native to North America and susceptible to *P. viburni*), European cranberrybush viburnum, *V. opulus* (native to Europe and susceptible) and dawn viburnum, *V. x bodnantense* (native to Asia and resistant).

Material and methods

Viburnum shrubs used for this study were from experimental plantings located in the Ithaca, New York area. Shrubs were 8-10 years old, had never been treated with systemic insecticides, and had not been treated with foliar insecticides against *P. viburni* the previous year. Breeding populations of *P. viburni* had been established at the site since 2001, the year of appearance of *P. viburni* in the Ithaca area. In August 2007, terminal twigs of viburnum shrubs belonging to the three host species included in the study were monitored for *P. viburni* natural egg infestation. Twigs with the following infestation levels were selected: low (1-5 egg masses), moderate (6-10), high (11-15), and very high (15+). Number of egg masses present and twig diameter to the nearest millimeter (i.e. 2, 3, or 4 mm in our sample) were recorded. In total, 348 twigs were selected: 91 for *V. dentatum*, 185 for *V. opulus*, and 72 for *V. x bodnantense* (Table 4.1). Selected twigs were caged with sleeve nets (70 by 30 cm) to prevent further oviposition by *P. viburni* females. All twigs were alive at the time of twig selection. At the end of the growing season, once *P. viburni* adults could no longer be found in the field (October 2007), the sleeve nets were removed and the twigs were each marked with a plastic band.

Table 4.1. Table of replicates used for the study. Numbers represent the number of twigs selected for each combination of host species / infestation level / diameter.

Species	Diameter	Infestation level (number of egg masses / twig)				Total
		Low (1-5)	Mod (6-10)	High (11-15)	Very high (>15)	
V. x bodnantense	2 mm	12	17	3	3	35
	3 mm	6	5	5	4	20
	4 mm	9	1	4	3	17
	Subtotal	**27**	**23**	**12**	**10**	**72**
V. dentatum	2 mm	10	7	3	5	25
	3 mm	12	13	10	9	44
	4 mm	9	7	3	3	22
	Subtotal	**31**	**27**	**16**	**17**	**91**
V. opulus	2 mm	16	23	13	18	70
	3 mm	34	21	16	21	92
	4 mm	8	7	5	3	23
	Subtotal	**58**	**51**	**34**	**42**	**185**
	Total	**116**	**101**	**62**	**69**	**348**

In April 2008, just before larval emergence, twigs selected the previous summer were removed and brought to the laboratory. For each twig, the following parameters were recorded: twig vitality (living or dead), number of egg masses intact, and number of egg masses impacted by wound tissue. The vitality of the twig was gauged by observing the presence of green tissue under the bark or buds of the twig: if a twig presented green tissue at both ends, it was considered alive. If a twig infested with egg masses presented green tissue on the portion basal to the egg masses but no green tissue on the distal portion, it was considered to have been killed by *P. viburni* oviposition. Finally, if a twig presented no traces of green tissue at either end, regardless of the position of *P. viburni* egg masses, it was considered dead due to a cause other than *P. viburni* oviposition. We will refer to this last category as "natural twig mortality" in the rest of the study. Twig wound response was then calculated as the number of egg masses impacted by wound tissue divided by the total number of egg masses on the twig.

Twigs were kept in individual Petri dishes (8.5 cm diameter x 2.5 cm height) with a moistened filter paper and screen lid at 22°C under a 15:9 (L:D) light regime. Twigs were monitored daily for larval emergence until the end of the egg hatch period (28 April-8 May). Percentage egg survivorship per egg mass was calculated for each twig as 100 × the number of larvae emerged / (8 × total number of egg masses laid), 8 being the average number of eggs per egg mass (Weston et al. 2008).

Statistical analysis

The effects of the independent variables infestation level, host species, and twig diameter on the dependant variables twig wound response and egg survivorship were analyzed using two separate 3-way analyses of variance (ANOVAs). All the 2nd and 3rd degree interactions were included in the model. Twig wound response data

were transformed using the arcsine transformation to meet the assumptions of the model. Means were compared using the LSD all-pairwise comparisons procedure at $\alpha = 0.05$ (Analytical Software, 2008). The correlations between twig wound response and egg survivorship, and between infestation level and twig mortality (excluding natural twig mortality) for all species were investigated using linear and non-linear regression models (Analytical Software 2008). Differences among twig mortality percentages depending on twig diameter and host species were analyzed using a chi-square tests; For each level of infestation, twig mortality percentages were compared using the multiple comparison for proportions procedure at $\alpha = 0.01$ (Analytical Software 2008). The effects of twig mortality on twig wound response and egg survivorship were analyzed separately for each host species using Kruskal-Wallis nonparametric ANOVA (Analytical Software 2008).

Results

Influence of infestation level, twig diameter, and host species on twig wound response

There was a significant effect of infestation level on twig wound response ($F_{3, 280} = 43.4$, $P < 0.0001$). Twig wound response was the highest on twigs with low levels of infestation (93.7 $\pm$ 2.3%) (mean + SE), and the lowest on twigs with very high levels of infestation (54.0 $\pm$ 5.0%) ($P < 0.05$) (Fig. 4.1A). There was no significant effect of any of the interactions between infestation level and the other terms of the model ($P > 0.05$)

Host species ($F_{2, 280} = 15.1$, $P < 0.0001$) and the interaction between host species and twig diameter ($F_{4, 280} = 7.8$, $P < 0.0001$) had significant effects on twig wound response. For twigs 2 mm thick, the effect of host species was significant ($F_{2, 96} = 11.68$, $P < 0.0001$); wound response was higher on *V. x bodnantense* (89.4 $\pm$ 3.8%)

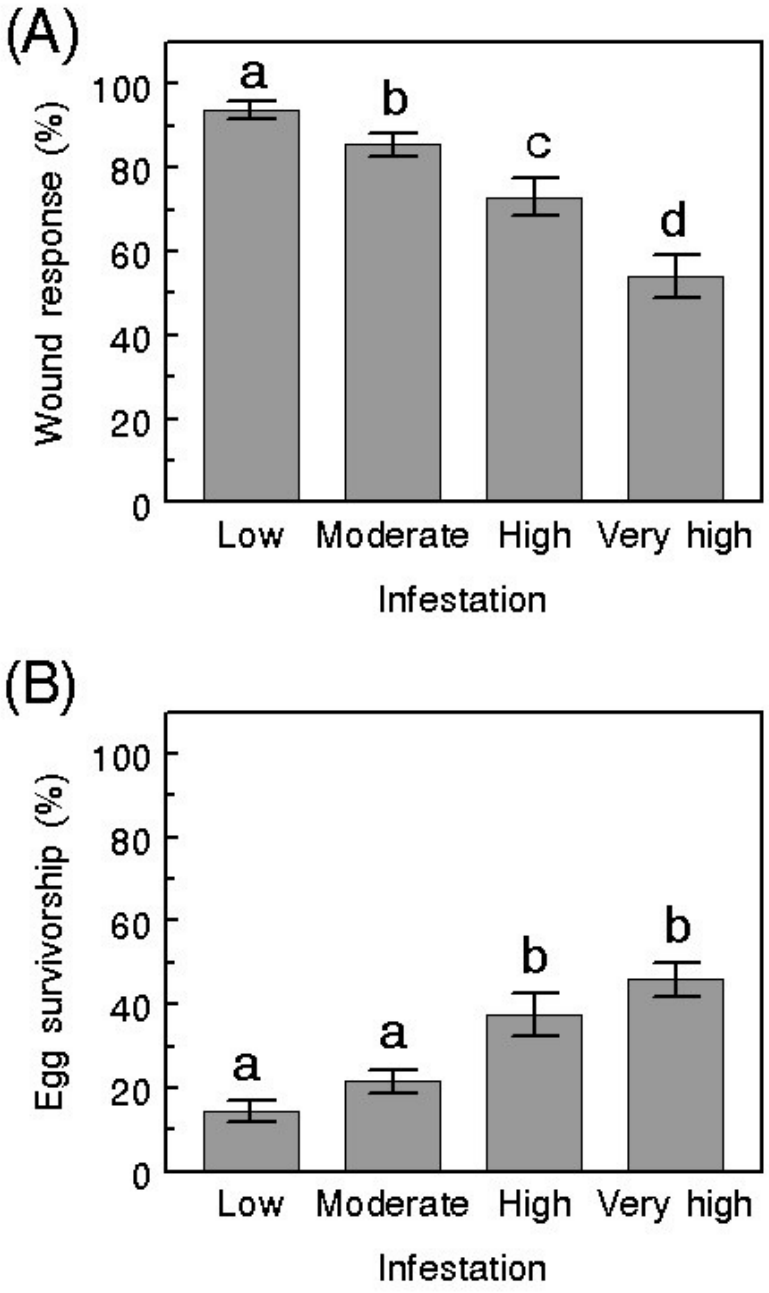

Figure 4.1. (A) Twig wound response (mean ± SE), and (B) *P. viburni* egg survivorship (mean ± SE) depending on infestation level. Means followed by the same letter are not significantly different ($P>0.05$, general ANOVA, LSD all-pairwise comparison procedure). Low infestation = 1-5 egg masses / twig, Moderate = 6-10, High = 11-15, Very high = >15.

and *V. opulus* (88.1 ± 3.4%) than on *V. dentatum* (51.4 ± 9.1%) ($P < 0.05$) (Fig. 4.2A). For twigs 3 mm thick, the effect of host species was again significant ($F_{2,128} = 14.82$, $P < 0.0001$); wound response was higher on *V. x bodnantense* (93.0 ± 4.2%) and *V. opulus* (87.2 ± 3.0%) than on *V. dentatum* (60.6 ± 5.8%) ($P < 0.05$). However, for twigs 4 mm thick, the effect of host species was not significant ($F_{2, 59} = 1.84$, $P = 0.17$). None of the other terms in the model (twig diameter or 2nd or 3rd degree interactions) had a significant effect on twig wound response ($P > 0.05$).

Influence of infestation level, twig diameter, and host species on egg survivorship

Infestation level had a significant effect on egg survivorship ($F_{3, 280} = 19.4$, $P < 0.0001$). Egg survivorship was higher on twigs with high (37.5 ± 5.1%) and very high (45.9 ± 4.2%) levels of infestation than on twigs moderately (21.5 ± 2.9%) or lightly (14.4 ± 2.7%) infested ($P < 0.05$) (Fig. 4.1B).

Host species ($F_{2, 280} = 38.95$, $P < 0.0001$), twig diameter ($F_{2, 280} = 7.73$, $P < 0.0001$), and the interaction between these two terms ($F_{4, 280} = 3.18$, $P = 0.014$) had a significant effect on egg survivorship. For twigs 2 mm thick, the effect of host species was significant ($F_{2, 96} = 29.66$, $P < 0.0001$); egg survivorship was highest on *V. dentatum* (59.0 ± 8.1%), lower on *V. opulus* (27.3 ± 3.89%), and lowest on *V. x bodnantense* (6.3 ± 1.9%) ($P < 0.05$) (Fig. 4.2B). For twigs 3 mm thick, the effect of host species was significant ($F_{2, 128} = 15.15$, $P < 0.0001$); egg survivorship was highest on *V. dentatum* (47.2 ± 4.3%), lower on *V. opulus* (23.5 ± 4.2%), and lowest on *V. x bodnantense* (6.1 ± 2.8%) ($P < 0.05$). However, for twigs 4 mm thick, the effect of host species was not significant ($F_{2, 59} = 2.08$, $P = 0.13$). None of the other 2nd degree or 3rd degree interaction terms had a significant effect on egg survivorship ($P > 0.05$).

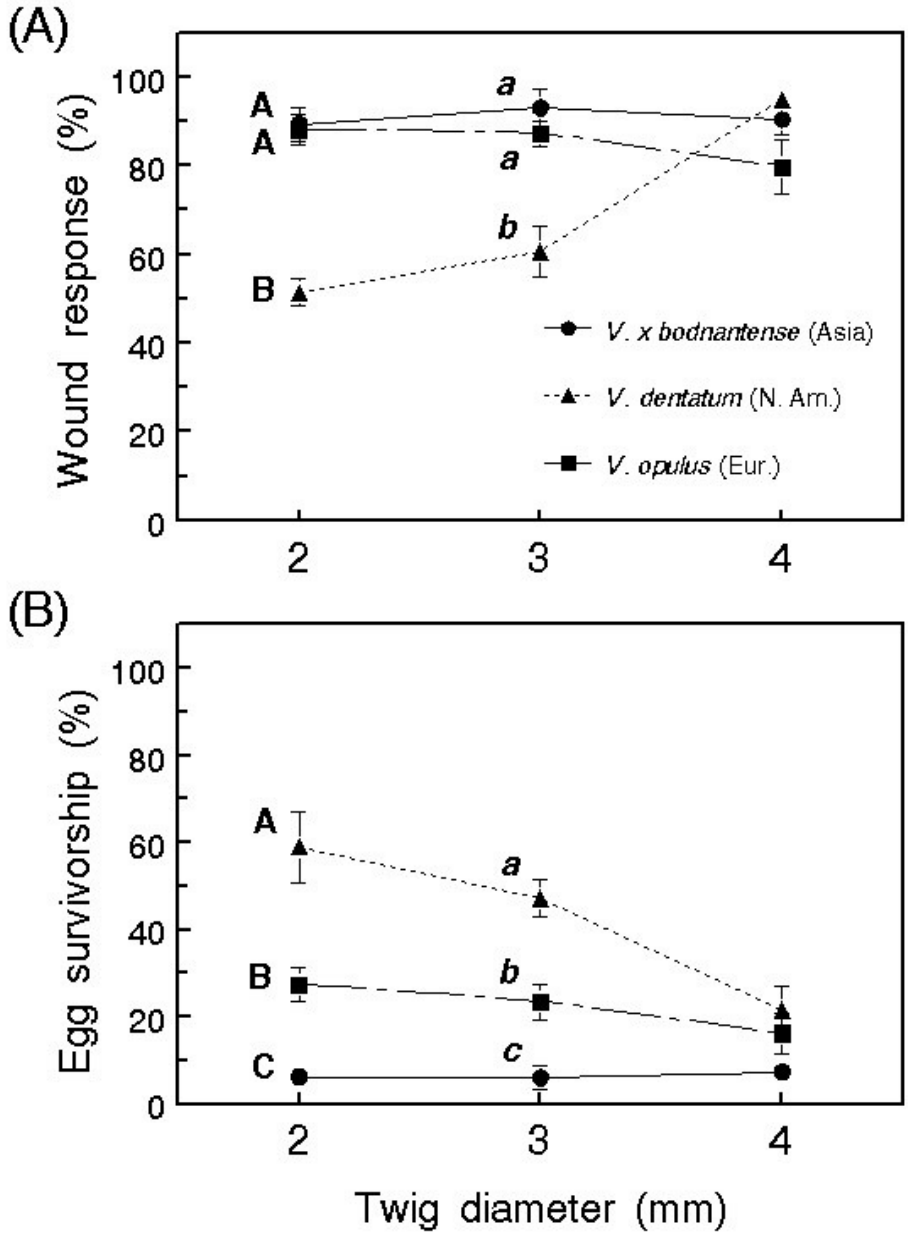

Figure 4.2. Effect of twig diameter (mm) and host species on (A) twig wound response (mean ± SE) and (B) *P. viburni* egg survivorship (mean ± SE). For the diameters 2 mm and 3 mm, means followed by a different letter are significantly different ($P<0.05$, general ANOVA, LSD all-pairwise comparison procedure). There was no significant difference between the means for diameter 4 mm.

Correlation between egg survivorship and twig wound response

Overall, egg survivorship was negatively correlated with wound response (F = 324.62, $P < 0.0001$, $r^2 = 0.52$). The highest egg survivorship percentage was achieved on twigs exhibiting 0-10% wound response (87.5 $\pm$ 7.3%) (mean $\pm$ SE), and the lowest on twigs exhibiting 90-100% wound response (11.6 $\pm$ 1.3%) (Fig. 4.3).

Twig mortality and egg survivorship.

There was an overall positive association between egg infestation and percentage twig mortality (excluding natural twig mortality) across the three Viburnum species tested ($y = -3.3 + 1.6x + 0.4x^2$, $R^2 = 0.95$) (Fig. 4.4). Chi-squared tests showed an effect of host species on twig mortality depending on infestation: at low, moderate, and high infestation levels, the effect on host species on twig mortality was not significant ($P > 0.01$), but at the very high infestation level, twig mortality was significantly higher ($\chi^2 = 10.6$, $P = 0.005$) on *V. dentatum* (82.3%) than on *V. x bodnantense* (20.0%) (Fig. 4.5A). There was also a significant interaction between twig diameter and level of infestation; at low, moderate, and high levels of infestation, the effect of twig diameter on twig mortality was not significant ($P > 0.01$), but at very high levels of infestation, twig mortality was higher ($\chi^2 = 12.1$, $P = 0.002$) for twigs 2 mm thick (70.6%) and 3mm thick (75.0%) than on twigs 4 mm thick (11.1%) (Fig. 4.5B).

There was a significant effect of twig mortality on twig wound response and egg survivorship for the three host species tested. On *V. x bodnantense*, twig wound response was higher ($F_{1,70} = 7.6$, $P = 0.008$) on living twigs (92.6 + 2.1%) than on twigs killed by *P. viburni* oviposition (57.5 + 16.6%). Egg survivorship was higher ($F_{1,70} = 7.8$, $P = 0.007$) on killed twigs (26.2 + 10.3%) than on living twigs (5.32 + 1.1%). On *V. dentatum*, results were similar: twig wound response was higher ($F_{1,89} = 75.8$, $P < 0.0001$) on living twigs (83.7 + 3.2%) than on twigs killed by *P. viburni*

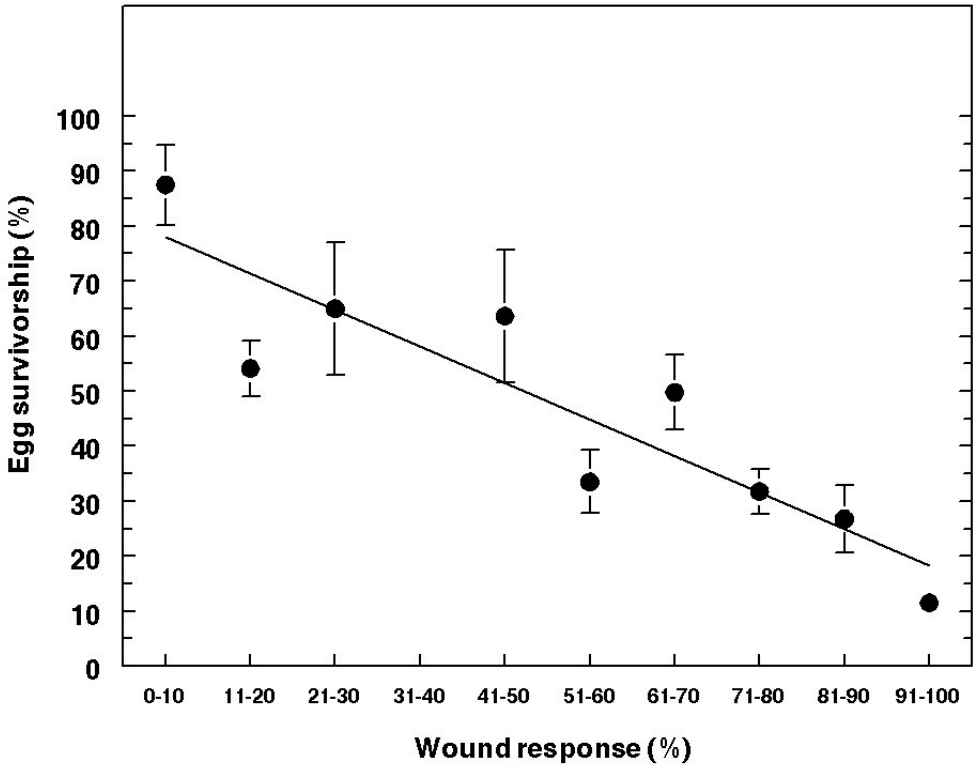

Figure 4.3. *P. viburni* egg survivorship (%) depending on twig wound response (%) (means ± SE).

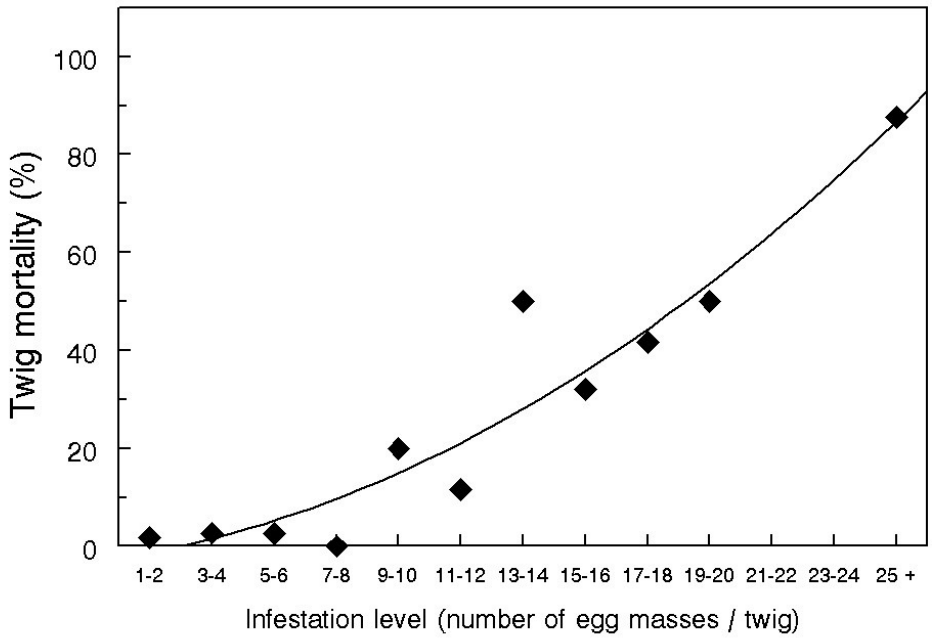

Figure 4.4. Twig mortality (%) depending on infestation level (i.e. number of egg masses per twig).

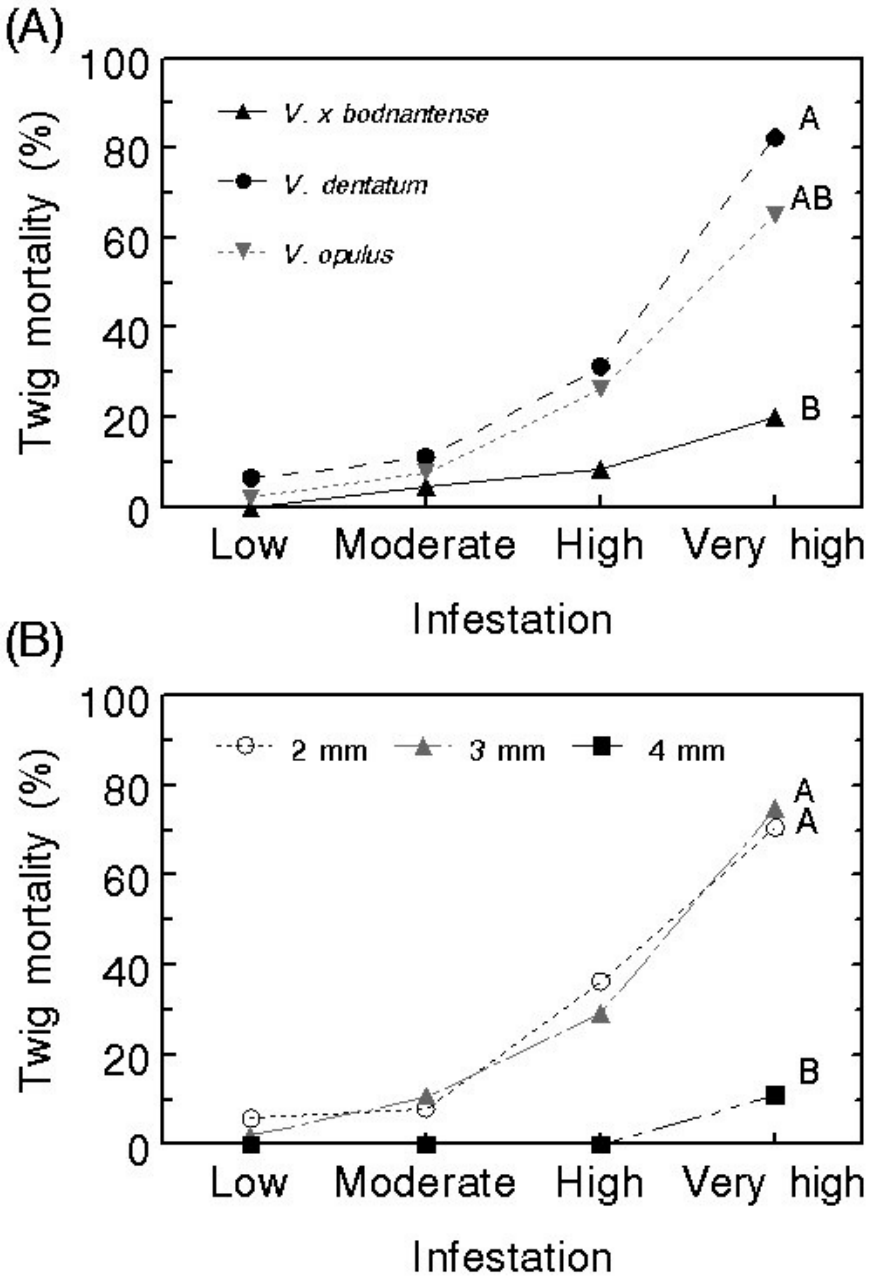

Figure 4.5. Twig mortality (%) at different infestation levels depending on (A) host species and (B) twig diameter. For very high infestation, percentages followed by the same letter are not significantly different (Chi-square test, multiple comparisons for proportions procedure, $\alpha = 0.01$). For low, moderate, and high levels of infestation, there were no significant differences between the percentages ($P > 0.01$)

oviposition (18.1 + 5.6%). Egg survivorship was higher ($F_{1,89} = 40.0$ $P < 0.0001$) on killed twigs (74.8+5.3%) than on living twigs (33.3 + 3.7%).

Natural twig mortality was only found in our *V. opulus* sample. Out of 186 *V. opulus* twigs collected, 56 (30.1%) died of other causes than *P. viburni* oviposition. Twenty-nine of these twigs (51.8%) had produced an inflorescence during the growing season and had desiccated by the end of the summer. The cause of death of the remaining twigs could not be determined. There was a significant difference in twig wound response ($F_{2,183} = 118.7$, $P < 0.0001$) and egg survivorship ($F_{2,183} = 104.6$, $P < 0.0001$) depending on twig mortality and cause of twig death. Wound response was highest on living twigs (90.3 $\pm$ 2.1%), lower on killed twigs (66.9 $\pm$ 5.8%) and lowest on twigs that died of natural mortality (21.1 $\pm$ 4.2%) ($P < 0.05$) (Fig. 4.6A). Egg survivorship was lower on living twigs (15.7 $\pm$ 2.1%) than on twigs killed by *P. viburni* oviposition (57.2 $\pm$ 7.4%) and those dying from natural causes (87.2 $\pm$ 15.7%) ($P < 0.05$) (Fig. 4.6B).

Discussion

The significant effect of infestation level on egg survivorship is evidence of a realized fitness benefit of aggregative oviposition behavior for *P. viburni*; the greater the density of egg masses on a twig, the higher the overwintering survivorship (Fig. 4.1). The hypothesis that aggregative oviposition helps *P. viburni* to overcome plant defenses is supported by the result that twig wound response significantly decreases as infestation level increases (Fig. 4.1A). The strong negative correlation between twig wound response and egg survivorships suggests that wound response alone can have a major impact on *P. viburni* fitness: egg survivorship was more than seven times higher on twigs showing low levels of wound response (1-10%) than on twigs showing very

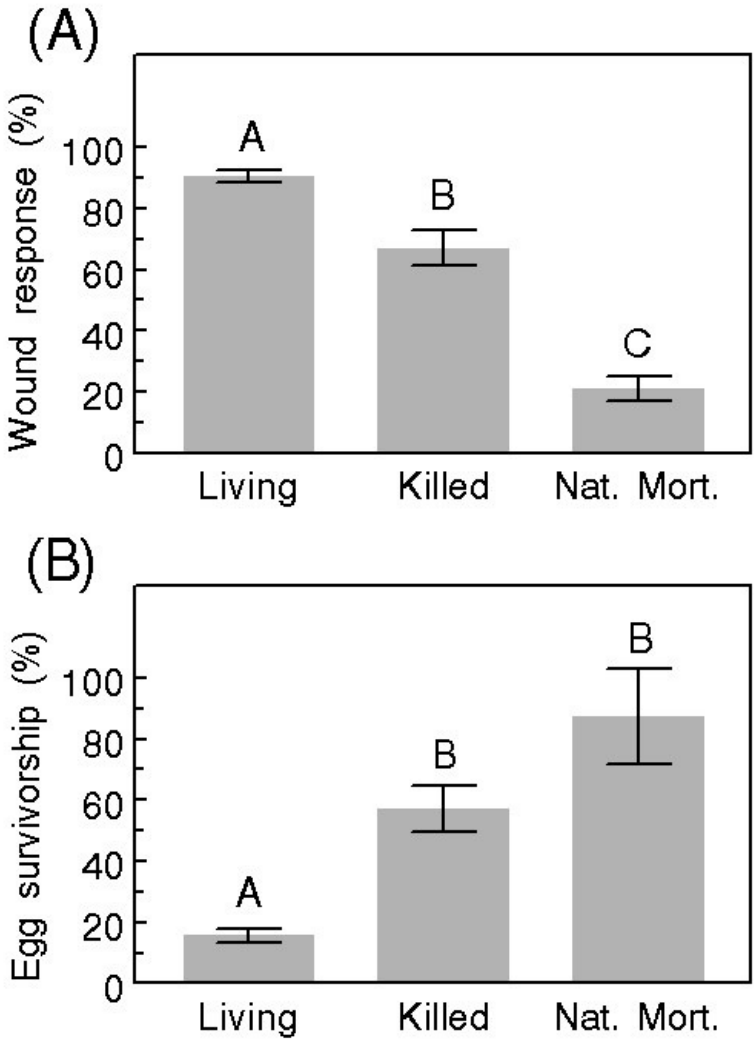

Figure 4.6. (A) Twig wound response (mean ± SE) and (B) *P. viburni* egg survivorship (mean ± SE) on *V. opulus* for: living twigs, twigs killed by *P. viburni* oviposition, and twigs that died naturally. Means followed by the same letter are not significantly different ($P > 0.05$, Kruskal-Wallis one-way ANOVA).

high or total wound response (90-100%) (87.5 and 11.6%, respectively) (Fig. 4.3). Wound tissue usually overgrew the egg mass(es), squishing the eggs or making them fall to the ground in the process. Exposed eggs expelled by wound tissue sometimes remained attached to the twig and survived overwintering, explaining why egg survivorship was not always zero when wound response was complete (100%).

How does aggregation help overcoming plant defense? In bark beetles that mass- attack conifers (Coleoptera: Curculionidae: Scolytinae), single adults have very little chance to successfully colonize a host tree due to tree defense mechanisms (resin flow and toxins). However, multiple simultaneous attacks eventually kill the tree by disrupting sap flow and clogging water-conducting tissues (often in association with symbiotic fungi), thus rendering the tree suitable for feeding by both larvae and adults (Byers 1989). For *P. viburni*, effects of aggregation seem to work in a similar fashion, although at a smaller scale. We found a positive correlation between infestation level and twig mortality, twig mortality reaching 87.5% for twigs bearing 25 egg masses or more (Fig. 4.4). The mechanism behind twig mortality might be comparable to debilitation of tree defenses caused by mass-attacking bark beetles: each egg mass excavated through the bark damages the vascular system of the twig, and it seems likely that multiple oviposition eventually disrupts the vascular tissues enough to cause the twig to die, at the same time disabling the capacity of the twig to produce wound tissue in response to oviposition. In addition, we cannot rule out a possible role for plant pathogens in twig mortality, which seems quite likely given that each egg mass represents a potential site of pathogen introduction. The importance of twig mortality for *P. viburni* oviposition success is supported by the fact that egg survivorship was consistently higher for twigs that died due to *P. viburni* oviposition compared to twigs that survived oviposition for the three host species tested.

Overall, our data suggest that overcoming plant defenses may be, for *P.*

viburni, mainly a matter of killing the infested part of the attacked host. However, while bark beetles need to kill entire trees in order to establish their colonies, *P. viburni* only affects egg-infested twigs. Killing entire plants would be disastrous for *P. viburni*, since both larvae and adults need living leaf material to feed and develop. Therefore, *P. viburni* adults are caught in a trade-off between the benefit of killing twigs via oviposition for egg survivorship, and the cost of reducing the amount of food for emerging larvae. It is worth mentioning that only the portion of infested twig distal to oviposition sites is killed, the basal portion remaining connected to the vascular system of the plant. Thus, killing a twig via oviposition does not mean eliminating the totality of the plant material that could develop from that twig the following season.

The presence of natural twig mortality in our sample was particularly interesting. Twigs that died naturally were only present in the *V. opulus* sample, and the majority (51.8%) were twigs that bore an inflorescence and naturally desiccated by the end of the growing season. The cause of death for the remaining twigs that died naturally could not be determined with certainty, but it is common for plants extensively defoliated by *P. viburni* larvae and adults typically to not re-grow entirely, and the plants we used for the study suffered high levels of damage due to *P. viburni* feeding during the summer of 2007. On twigs that died naturally, egg survivorship was as high as on twigs killed by oviposition, and higher than on living twigs (Fig. 4.6B). Wound response was even lower on these twigs than on twigs killed by oviposition (21.1% vs 66.9%). These results show that there is a realized fitness benefit of ovipositing on twigs that die naturally and that, in the case of twigs bearing inflorescences, there is a possible cost for *V. opulus* plants of producing inflorescences by increasing the number of favorable oviposition sites for *P. viburni*. The occurrence of natural twig mortality on *V. dentatum*, *V. x bodnantense*, or other *Viburnum* species, has yet to be determined; inflorescences were rare on *V. dentatum* and *V. x*

bodnantense during our study and were not represented in our sample.

This study shows the importance of two additional factors on egg survivorship, twig wound response, and twig mortality: host species and twig diameter. Overall, the thickest twigs (i.e. 4 mm) showed higher levels of defense and reduced mortality (Figs. 2A and 5B). Considering that the surface area of vascular tissue increases with twig diameter, it seems logical to conclude that thicker twigs are capable of tolerating more egg masses and producing increased amounts of wound tissue. However, while the effect of twig diameter on wound response was pronounced on *V. dentatum*, it was far less obvious on *V. opulus* and *V. x bodnantense* (Figs. 2A). For these two species, twigs of all diameters showed comparably high levels of wound response, and twig mortality was consistently low for *V. x bodnantense*, independent of the level of infestation (Fig. 4.5A).

Overall, the North American species *V. dentatum* was the least resistant among the host species tested, and the most favorable for egg survivorship. Levels of wound response were comparable on *V. x bodnantense* and *V. opulus*, but egg survivorship was lower on *V. x bodnantense* for twigs 2 mm and 3mm thick than on *V. opulus* (Fig. 4.2B). There was therefore a detrimental effect of *V. x bodnantense* on egg survivorship independent of wound response. Further studies investigating structural and/or chemical particularities of *V. x bodnantense* twigs would be necessary to understand why this *Viburnum* species is such a poor host for *P. viburni* eggs.

The fact that the only North American *Viburnum* species tested was also the least resistant to oviposition seems to support the hypothesis formulated by Herms and Mattson (1992) that plants that have not shared an evolutionary history with a pest have lower defenses against herbivory. However, we have to be cautious: the most resistant species to *P. viburni* oviposition in our study, *V. x bodnantense*, is a hybrid between *V. farreri* and *V. grandiflorum*, two Asian species. The co-evolutionary

history of these species with *P. viburni* is hard to assess given the lack of information on the extent of the range of *P. viburni* in Asia and the presence of two closely related *Pyrrhalta* species, *P. humeralis* (Chen) and *P. annulicornis* (Baly), also feeders on viburnums in Southeast Asia. In addition, the genus *Viburnum* contains more than 160 species worldwide so we cannot draw general conclusions regarding the entire genus from the three species tested in our study.

The low level of resistance to oviposition in *V. dentatum,* one of the most common viburnums in natural areas of northeastern North America certainly would facilitate the initial establishment and ongoing spread of *P. viburni* populations in its introduced range. Relatively low levels of egg aggregation are needed to at least partially overcome *V. dentatum* twig wound response, reducing the number of females needed to successfully colonize new sites and overcome possible Allee effects. Overall, our study shows that the impact of twig wound response on *P. viburni* fitness can be dramatic. Extending our research to more *Viburnum* species of different origins and levels of susceptibility would certainly improve our understanding of the ecology and populations dynamics of this emerging pest in both its native and introduced range.

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CHAPTER 5

OVIPOSITION BY *PYRRHALTA VIBURNI* (PAYKULL) ON DEAD PLANT MATERIAL: SUCCESSFUL REPRODUCTIVE STRATEGY OR MALADAPTIVE BEHAVIOR?

Abstract

Viburnum leaf beetle, *Pyrrhalta viburni* (Paykull, 1799), is a Eurasian chrysomelid recently introduced to North America, where it has become a major landscape pest. *P. viburni* deposits eggs in the terminal twigs of infested viburnum shrubs, in small cavities that are then covered by the female with a frass-like secretion. In the field, fresh *P. viburni* egg masses are sometimes laid on dead plant material, prompting the current study to investigate the frequency and proximate causes of this behavior. In the field, *P. viburni* females were found to lay significantly more eggs on live twigs than on dead twigs, and to lay more eggs on dead twigs that had been infested the previous growing season and contained remains of old egg masses than on non-infested dead twigs. In laboratory choice-tests, females laid significantly more eggs on dead twigs if they contained remains of old egg masses, but did not show preferences between young and old infested twigs. We conclude that the presence of remains of egg masses deposited during the previous growing season is stimulatory to *P. viburni* females, and triggers oviposition on dead plant material.

Introduction

Viburnum leaf beetle, *Pyrrhalta viburni* (Paykull, 1799), is a chrysomelid belonging to the subfamily Galerucinae and the tribe Galerucini. Native to Eurasia, *P. viburni* was accidentally introduced to North America where it has become a major pest of native and exotic viburnum shrubs in nurseries, managed landscapes, and

natural habitats. Although early records indicate that the beetle was present in Canada as early as 1924 (Majka and LeSage 2007), it was not observed causing serious damage until 1978 when it was found in Ottawa, Ontario and Hull, Quebec (Canada), defoliating *Viburnum opulus* (L.) shrubs (Becker 1979). Observed in the USA for the first time in Maine in 1994, *P. viburni* has spread in all New England states, New York, Pennsylvania, and Ohio (Weston and Hoebeke 2003). A separate introduction has occurred on the west coast of North America, where the beetle has been reported in British Columbia (Canada), and the state of Washington (USA) (Weston et al. 2008).

Despite a recent rise in interest due to its pest status in North America, very little has been published on the biology of *P. viburni*. The most extensive studies existing on the subject are the works of Zorin (1931), Lühmann (1934), Balachowsky (1963), and Weston et al (2007). One of the most remarkable features of the biology of *P. viburni* is its distinctive oviposition behavior. Females lay their eggs in cavities that they excavate in the terminal twigs of susceptible shrubs. The female chews away the bark and hollows out the egg cavity in the pith (Hilker 1992) before laying an average of 8 eggs per egg cavity (Weston et al. 2008). The egg cavity is then closed by a "cap" made of a frass-like secretion produced by the female. It can take up to a few hours for a female to make a complete egg mass (egg cavity + eggs + cap), chewing the cavity being the most time-consuming step of the process (personal observation). *P. viburni* females can produce 1-3 egg masses per day (unpublished data), and 75 on average over their lifetime (Weston et al. 2008).

By concealing their eggs inside twigs, *P. viburni* females protect them from most potential natural enemies. The cap also sponges and stores moisture, thereby providing humidity for the eggs. This is especially important since *P. viburni* overwinters as eggs, and the eggs have to go through an obligate period of diapause of

several months in the field before hatching in the spring (Weston and Diaz 2005).

It was recently discovered that *P. viburni* females prefer to lay eggs on twigs already infested with egg masses of conspecifics, and position their egg masses adjacent to existing masses (Chapter 2). This aggregation behavior leads to the formation of clusters of egg masses linearly arranged along terminal twigs of infested shrubs. The main benefit of this behavior is thought to be overcoming plant defenses. Indeed, it has been observed that plants can respond to the wounding caused by *P. viburni* oviposition by producing undifferentiated tissue at the wounding site, forming a small mass of wound tissue that either overgrows the egg mass or squeezes the eggs out of the egg cavity. When multiple egg masses are laid along a twig, vascular tissues are eventually disrupted and the twig dies, disabling its capacity to produce wound tissue. Twigs killed by repeated oviposition are commonly observed in the field on many viburnum species attacked by *P. viburni*.

Infested shrubs do not suffer only twig mortality due to oviposition. Both larvae and adults of *P. viburni* are leaf feeders, and high levels of infestation of both stages can lead to complete defoliation. Repeated defoliation quickly depletes the reserves of nutrients stored in the roots, and defoliated plants typically do not re-grow entirely after defoliation (in fact, plants may die after 2-3 consecutive years of defoliation). In this paper, we will refer to this phenomenon of partial shrub mortality due to depletion of plant reserves as *dieback*. The terms “dead twig” and “dead plant material” will be used exclusively to describe shrub parts that suffered dieback (i.e. excluding twigs that were directly killed by *P. viburni* oviposition).

Although dead twigs do not produce leaves, we have observed fresh egg masses of *P. viburni* laid in such twigs in the field on *V. dentatum* (L.) and *V. trilobum* (L.) [= *V. opulus* (L.) var. *americanum* (Aiton)] shrubs. Oviposition on dead plant material is highly unusual for phytophagous insects, and was unknown for *P. viburni*.

The objectives of the study reported here were to document the frequency of occurrence of *P. viburni* oviposition on dead viburnum twigs in the field and to understand the stimulatory process leading to this behavior via laboratory choice-tests using dead and alive, infested and non-infested plant material.

Material and methods

Insects and plant material.

All *P. viburni* adults used for the laboratory experiments in this study were collected in the Ithaca area (NY, USA) on naturally infested plants during summer 2008. Insects were transferred to *V. dentatum* and *V. trilobum* shoots in rectangular plastic containers (30 cm x 22 cm x 10 cm) with a vented lid and kept at 22°C under a 15:9 (L:D) light regime until needed for experiments. Each adult was used only once for experimental purposes. All the plant material used for the oviposition choice-tests came from unmanaged *V. dentatum* shrubs located in the Ithaca area. All plants serving as a source of twigs shared similar characteristics in terms of age (8-10 years) and history of *P. viburni* infestation, and had not been treated with pesticides for the previous several years.

Observational study.

To study the frequency of occurrence of oviposition on dead plant material under field conditions, we conducted an observational study using naturally infested *V. trilobum* shrubs that had been exposed to severe levels of defoliation due to *P. viburni* in preceding years. Our study site was located in the Allegheny National Forest (PA, USA). In March 2008, 25 shrubs from the study site were selected for twig sampling. For each shrub, 20 terminal twigs were randomly selected and cut at the base. Occasional supernumerary twigs were kept in the analysis: in total, we used 510 twigs for this study. For each twig sampled, the following parameters were measured: twig

length and diameter, vitality, and number of new (from the past growing season) and old (from previous growing seasons) egg masses. The vitality of the twig was gauged by observing the presence of green tissue under the bark or buds of the twig: if a twig presented green tissue at both ends, it was considered alive. If a twig infested with egg masses presented green tissue on the basal portion but no green tissue on the distal portion, it was considered killed by *P. viburni* oviposition the current growing season. Finally, if a twig presented no traces of green tissue at either end, independent of the position of *P. viburni* egg masses, it was considered dead due to dieback. The distinction between new and old egg masses on the twigs was made by observing the eggs contained in the egg cavity: new egg masses laid during the past growing season contained turgid eggs, round in shape and orange colored. Moreover, the cap was almost always present. Old egg masses, which only occurred on twigs that suffered dieback, contained empty chorions of hatched eggs or unhatched shriveled eggs of a white or pale yellow color, and the egg mass cap was sometimes absent.

For each shrub, the level of dieback was calculated by dividing the number of dead twigs by the total number of twigs sampled. The relationship between the average number of new egg masses/twig and level of shrub dieback was analyzed via linear regression. The difference between mean number of new egg masses present on live plant material and dead plant material was analyzed with Kruskal-Wallis nonparametric ANOVA (Statistix 9.0). The differences in percentages of new infestation between live and dead plant material and between dead plant material that presented old egg masses and non-infested dead plant material were analyzed using Chi-square tests. The relationship between the number of old egg masses present on dead plant material and probability of being infested with new egg masses was established by performing a regression. In order to perform this regression, the dead plant material sampled from all shrubs was divided into 6 categories depending on the

number of old egg masses present in the twigs: 0, 1-2, 3-4, 5-6, 7-8, or 9-10. The number of twigs in each category that contained new egg masses was then counted. A 2nd order polynomial term (quadratic regression) was added to the model because the data appeared non-linear.

Laboratory choice-tests.

In the laboratory, a series of choice-tests was performed in August 2008 to determine the oviposition preferences of *P. viburni* females. Two mated females were placed in a cylindrical container (9.5 cm x 21 cm) with a vented lid containing two treatment twigs for 48 hours. After 48 hours, the females were removed and the total number of egg masses laid on both twigs was counted. All the twigs used for the choice-tests were *V. dentatum* twigs, homogenous in length (12 cm) and diameter (2.5 mm). Each twig belonged to one of the following categories of age and infestation, which corresponded to our four treatments: 1) y*oung infested*: Live twigs containing egg masses laid during the current growing season, 2) y*oung non-infested*: Live twigs not containing any egg masses, 3) *old infested*: dead twigs containing remains of old egg masses, and 4) *old non-infested*: dead twigs not containing egg masses.

We tested the following combinations of treatments: young infested vs young non-infested, old infested vs old non-infested, young infested vs old infested, and young non-infested vs old non-infested. We used a total of 10 replicates for each choice-test, except for the old infested vs old non-infested series (14 replicates). We removed the leaves from the young twigs tested in order to avoid any influence of the presence of food on the stimulatory process leading the choice of a twig for oviposition, except for the young infested vs young non-infested series, which was the only choice-test where two live twigs were used and for which two leaves were left on each of the twigs tested. For all the other combinations, food was provided to females during the trials in the form of leaves left at the bottom of the container; the cut ends

of leaves were held in a plastic floral pick to keep them hydrated. For each treatment combination, the difference between the numbers of egg masses laid on each treatment was analyzed using a paired *t*-test.

Results

Observational study.

The mean level of dieback per shrub was 57.0%, with values ranging from 9.5 to 100.0%. The average number of new egg masses per twig was 1.6 $\pm$ 3.6 (mean $\pm$ S.D.) for the total sample, 2.6 $\pm$ 4.3 for live plant material only, and 0.8 $\pm$ 2.8 for dead plant material only. The difference in the number of new egg masses laid on live and dead plant material was statistically significant (Kruskal-Wallis nonparametric ANOVA, $P < 0.0001$). There was a negative correlation between twig dieback and number of new egg masses laid / twig for both the totality of twigs sampled ($P = 0.045$, $r^2 = 0.12$) and dead twigs only ($P = 0.044$, $r^2 = 0.13$) (Fig. 5.1).

Among the 510 twigs sampled in the study, 221 presented green tissue (alive or killed by *P. viburni* oviposition the current growing season) and were considered live plant material (43.3%); 289 were dead (56.7%). The percentage of live twigs infested with new egg masses (62.9%) was significantly higher than the percentage of dead twigs with new infestation (16.6%) ($\chi^2 = 115.14$, $P < 0.0001$). Among dead twigs, the percentage of new infestation for twigs that were infested the previous year (and thus contained old egg masses) (30.94%) was higher than for non-infested dead twigs (6.54%) ($\chi^2 = 29.44$, $P < 0.0001$). There was a significant positive quadratic regression between the number of old egg masses present on a twig and the probability of new infestation ($P = 0.014$, $r^2 = 0.90$) (Fig. 5.2).

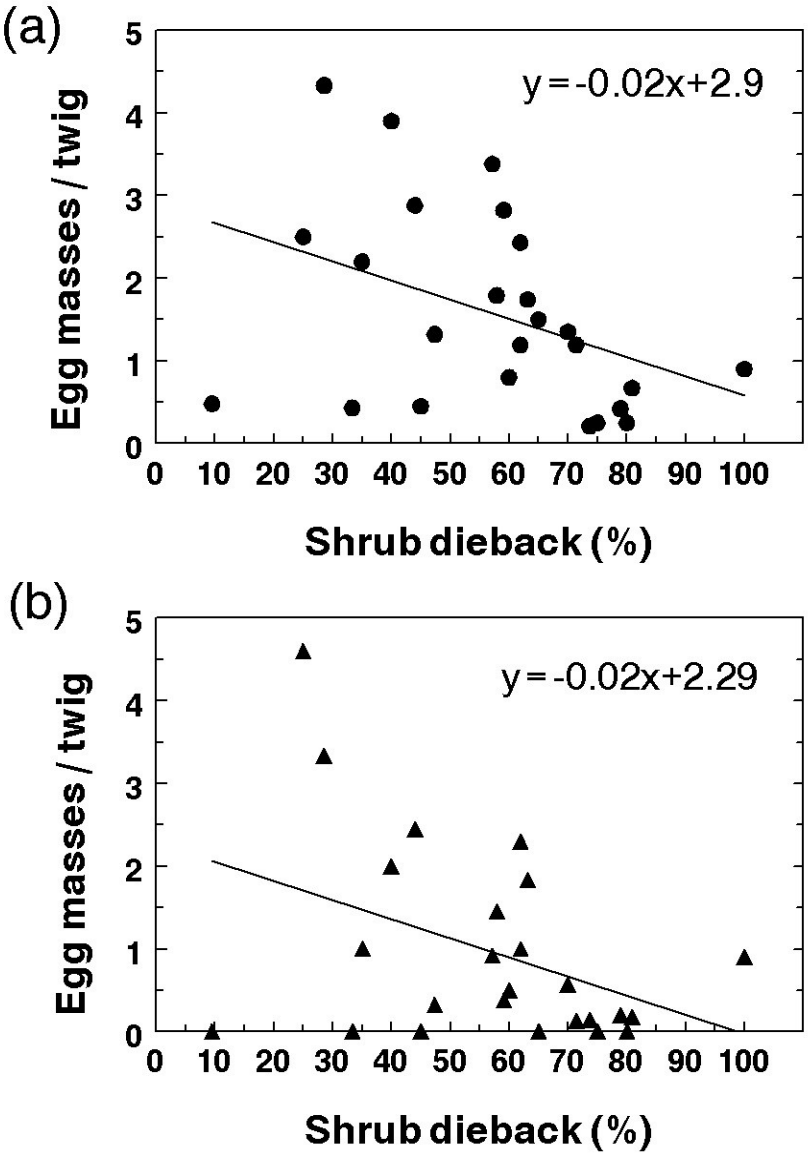

Figure 5.1. Mean number of new egg masses / twig depending on the level of shrub dieback for (a) the totality of twig sampled, and (b) dead twigs only.

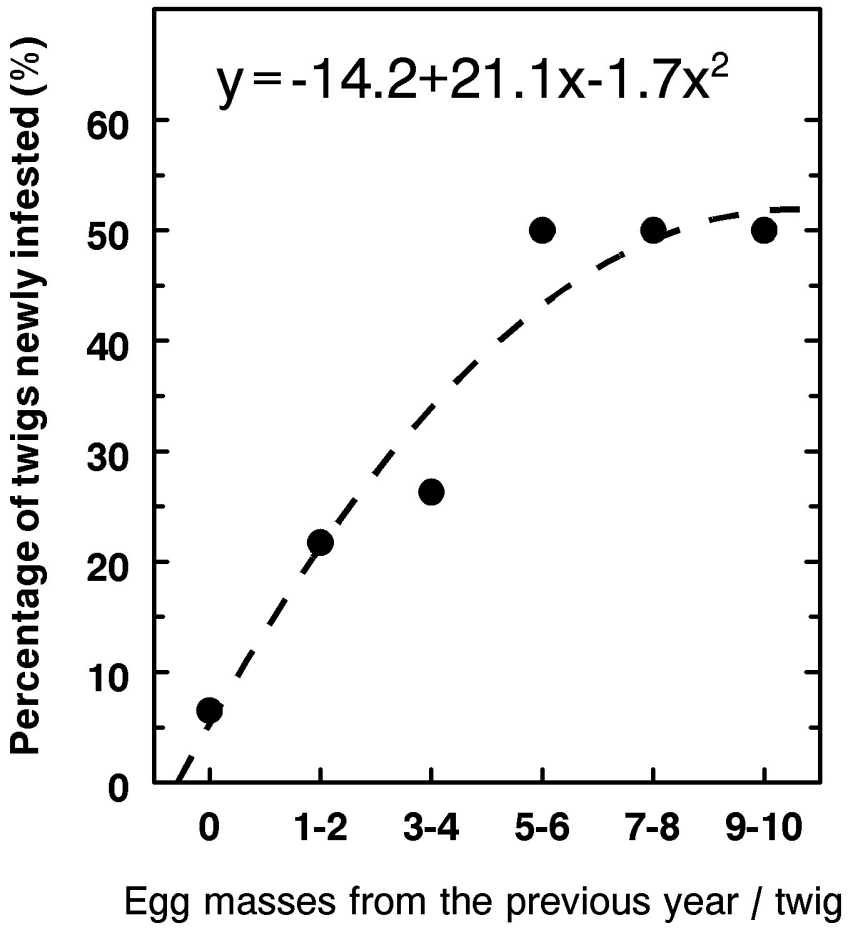

Figure 5.2. Percentage of dead twigs newly infested depending on number of egg masses deposited the previous year(s).

Laboratory choice-tests.

When given a choice, females laid significantly more egg masses on previously infested twigs than on non-infested twigs. This was true for both young twigs (young infested vs young non-infested: $t = 3.71$, $P = 0.0049$) and old twigs (old infested vs old non-infested: $t = 2.69$, $P = 0.019$). There was no significant difference between the numbers of egg masses laid on young infested twigs vs. old infested twigs ($t = 1.00$, $P = 0.34$), and between numbers of egg masses laid on young non-infested twigs vs. old non-infested twigs ($t = -0.68$, $P = 0.51$) (Fig. 5.3).

Discussion

Chrysomelid beetles and other species in the order Coleoptera in general are extraordinarily variable in how they lay their eggs, and insertion of eggs in the plant tissue is not unique to *P. viburni*. Among chrysomelids, species of the neotropical genus *Coelomera* (Galerucinae) lay their eggs into the hollow stem of palm trees. *Argopistes sexvittatus* (Alticinae) lays eggs into small holes bored into the leaf and covered with a brown, hard secretion. *Phaedon cochlearidae* (Chrysomelinae) uses a similar strategy, but the cavity in this case is not covered by a secretion (see Hilker 1994 for a review of egg protection in Chrysomelidae).

For some of these species, the plant reaction in response to oviposition has also been described. For example, plant tissue wounded by the oviposition of *Zeugophora subspinosa* into a leaf reacts with production of necrotic formations (Hilker 1994). Oviposition on dead plant material, however, is much more unusual for phytophagous insects. Intuitively, it seems like a poor choice for females to lay eggs on a site where no living tissues will grow, and therefore no food will be provided for the future larvae. It is not uncommon for chrysomelid adults to lay eggs away from their host-

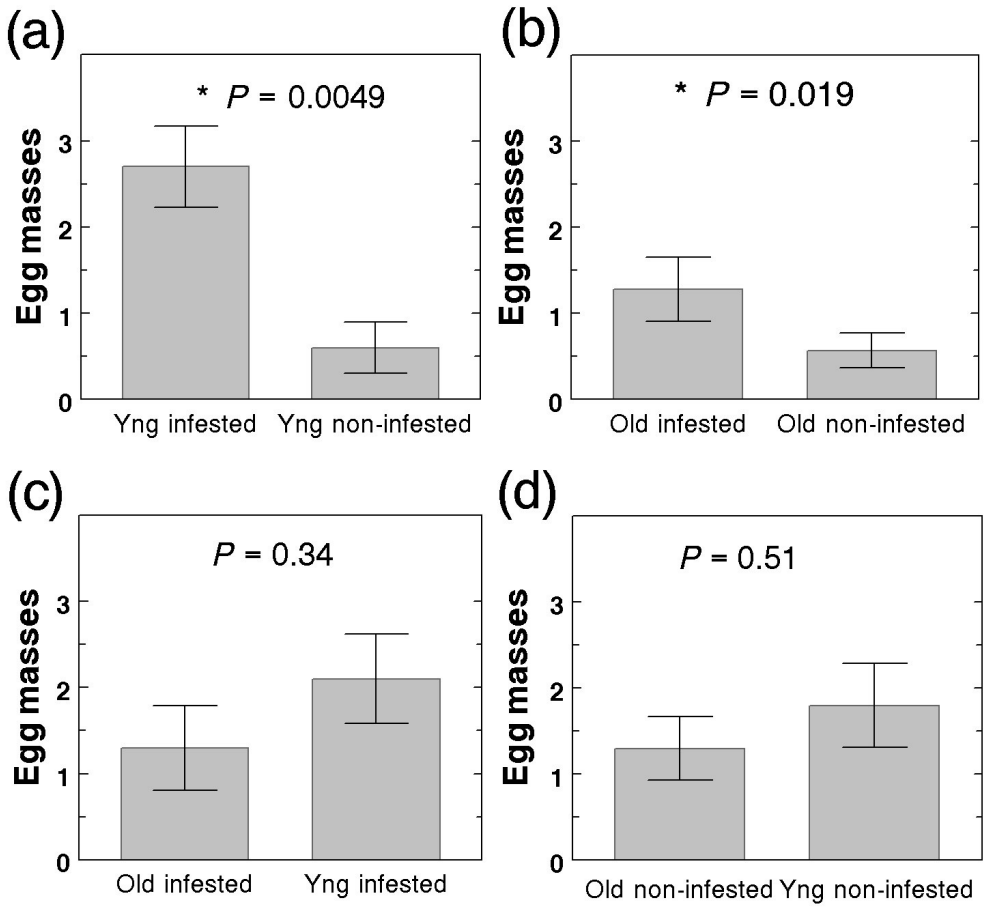

Figure 5.3. Average egg masses laid on treatment twigs (± SE) after 48 hours for the following combinations of treatments: (a) Young infested vs. Young non-infested, (b) Old infested vs Old non-infested, (c) Old infested vs Young infested, and (d) Old non-infested vs Young non-infested.

plant (Selman 1994), often in the soil, but the larvae of these species are usually mobile and able to forage for their own food from the 1st instar. In the case of *P. viburni*, a relevant question to ask is: does oviposition on dead plant material reflect a reproductive strategy, or is it accidental, and possibly detrimental for the fitness of the insect?

Our observational study revealed that oviposition on dead plant material is relatively minor and that females show a significant ovipositional preference for live twig material under field conditions (16.6% and 62.9% of the total of dead and live twigs, respectively, were newly infested). The negative correlations between mean oviposition and percentage shrub dieback were parallel for the entire twig samples and dead plant material only (Fig. 5.1), suggesting that *P. viburni* females do not change their preferences in twig choice depending on shrub dieback. The negative correlations observed are likely to be an effect of lower densities of adults, and consequently decreased levels of oviposition, on shrubs that exhibit high levels of dieback and therefore constitute less favorable feeding sites for adults.

An interesting finding of our observational study is the influence of infestation from the previous year (or years) on probability of new infestation. Dead twigs showing signs of past infestation (presence of old egg masses) were more often infested with new egg masses than dead twigs not bearing old egg masses. Moreover, there was a positive correlation between the number of old eggs masses present on dead twigs and the probability of new infestation (Fig.2); this suggests that the presence of old egg masses stimulates *P. viburni* females to lay eggs on dead plant material, and that the response to the level of stimulus released by old egg masses is quantitative. It is interesting to note that the correlation between the number of old egg masses and the probability of new infestation seems to reach a plateau, the maximum percentage of new infestation being apparently close to 50%. This can be interpreted

in a couple of ways. First, old egg masses might not all remain stimulatory to the females; exposure to environmental conditions might have resulted in egg masses becoming less stimulatory (e.g. by losing chemical constituents or the egg cap itself). Secondly, it is possible that not all females respond to the stimuli presented by old egg masses; some females might only be stimulated by fresh egg masses. These two hypotheses are not mutually exclusive.

The choice-tests conducted under laboratory conditions confirmed the results of the observational study. *Pyrrhalta viburni* females laid significantly more eggs on infested old twigs than on non-infested old twigs, confirming that old egg masses are still stimulatory to females for at least one year after their production. There was no difference in quantity of egg masses laid on old infested twigs and young infested twigs, suggesting that the attractiveness of newly laid egg masses and old egg masses to *P. viburni* females is similar. Finally, the lack of difference between the quantity of egg masses laid on young and old non-infested twigs seemed to indicate that the age and vitality of the twigs was not a factor driving twig choice for oviposition. However, we must be careful with this last conclusion: young non-infested twigs offered to the females in our choice-test setting were not exactly equivalent to young twigs they are likely to encounter in the field, since we removed leaves from these twigs before our tests. Presence of optimal food in their microenvironment has been shown to influence oviposition behavior of *P. viburni* females (unpublished data), and it is likely that, in the field, presence of leaves on young twigs might make them more stimulatory for oviposition than dead twigs, as found in the results of our observational study.

Overall, our results suggest that oviposition of *P. viburni* females on dead plant material is an indirect consequence of aggregative oviposition behavior: females prefer to lay egg masses on twigs already infested by conspecifics, and the long-lasting stimulatory effect of egg masses leads them to lay eggs on twigs infested the

previous growing season, even if, during the interim, these twigs have died.

Finally, it is important to keep in mind that *P. viburni* is less likely to encounter dead viburnum shrubs under natural conditions in its native range, where the insect occurs in lower numbers and is less harmful to its host-plants, than in North America, where infested susceptible shrubs are often killed after a few years. Therefore, *P. viburni* females might not have adapted (yet?) to the presence of dead plant material and remains of egg masses laid during the previous growing season in their ovipositional environment.

Even if the choice of dead plant material for oviposition appears accidental, it is not necessarily maladaptive. Although dead twigs seem to be poor quality sites for egg-laying due to the absence of food in the microenvironment for the offspring, newly hatched larvae might be able to migrate to live twigs and leaves and survive. One might expect an increase in the severity of dieback to make it more difficult for larvae to find suitable twigs, and thus could increase competition for the remaining food among young larvae. Little is known about the foraging capacity of 1st instar *P. viburni* larvae, and additional experimentation on this particular aspect of their larval biology is required to test these hypotheses.

Oviposition on dead twigs might also be beneficial for the insect by being an alternative way to avoid plant defenses. Indeed, production of wound tissues by attacked plants might be a major source of mortality for *P. viburni* eggs (unpublished data). Dead twigs are unable to react to oviposition, and egg survivorship and larval hatching rate might be higher on dead twigs than on live twigs, assuming that dead twigs provide the same degree of protection for overwintering as live twigs.

In conclusion, our study suggests that oviposition on dead plant material by *P. viburni* females occurs as a result of the long-lasting attractiveness of old egg masses deposited during previous growing seasons, but additional experiments measuring the

fitness of females ovipositing on live and dead plant material are required before making conclusions on the adaptive value of such behavior.

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CHAPTER 6

EVALUATION OF THE EGG PARASITOID *APROSTOCETUS* SP. (HYMENOPTERA: EULOPHIDAE) AS A BIOLOGICAL CONTROL AGENT AGAINST VIBURNUM LEAF BEETLE [*PYRRHALTA VIBURNI* (PAYKULL)]

Abstract

Pyrrhalta viburni, an invasive chrysomelid native to Eurasia, poses a major threat to viburnums in North America, killing shrubs in natural habitats and managed landscapes as it spreads through the northeastern U.S. and southern Canada. In a study conducted in 2000 in Switzerland, the most abundant natural enemy of *P. viburni* was an unidentified egg parasitoid belonging to the genus *Aprostocetus*. In 2008, a study was conducted under quarantine conditions to determine the longevity and host specificity of *Aprostocetus* sp. *Aprostocetus* sp. females lived 53.8 days on average, the maximum longevity being 133 days, and successfully parasitized the following non-target species: *Galerucella nymphaeae*, *Neogalerucella.pusilla/N. calmariensis*, and *Plagiometriona clavata*. Parasitism rates on these species did not exceed 3.1%. Due to its lack of host specificity, I think that the egg parasitoid *Aprostocetus* sp. should not be considered for introduction as a biological control agent against *P. viburni* in North America. Additional surveys for natural enemies in the native range of *P. viburni* are needed to identify potential biological control agents of this invasive pest.

Introduction

Viburnum leaf beetle [*Pyrrhalta viburni* (Paykull)] is a chrysomelid that feeds exclusively on plants belonging to the genus *Viburnum*. Native to Eurasia, it was

accidentally introduced to North America where it was recorded for the first time in 1924 in Nova Scotia, Canada (Majka and LeSage 2007). However, it was not reported damaging native viburnums until the late 1970s. Since then, *P. viburni* has steadily expanded its range through southeastern Canada and northeastern U.S., killing susceptible viburnum shrubs in natural habitats and ornamental settings as it spreads. Both larvae and adults are leaf-feeders, and repeated extensive defoliation kills infested shrubs by depletion of their reserves (Weston et al. 2007).

P. viburni is a univoltine species and overwinters as eggs. Eggs are deposited in round cavities chewed in the terminal twigs of infested shrubs and covered with a protective cap secreted by the females. The cap is thought to store moisture and provide humidity to the eggs in addition to protecting them from harsh weather conditions and most natural enemies. In North America, larvae emerge in early May, soon after *Viburnum* bud break, and require 3 to 4 wk to develop. They then crawl to the soil to pupate. Adults emerge late June-early July, and are relatively long-lived, persisting in the field until frost or depletion of their food sources. Adults mate and lay eggs repeatedly over their entire lifetime.

In its native range, *P. viburni* is considered an occasional pest of European viburnums, mainly *Viburnum opulus* and *V. lantana*, but is not nearly as destructive as in North America. Several predators and parasitoids of *P. viburni* that are present in Eurasia but absent in North America are mentioned in the literature (Zorin 1931) and may play a role in regulating *P. viburni* populations, but little is known of their biology and impact on *P. viburni*.

In 2000, a collaborative project between Cornell University and CABI Bioscience Switzerland Centre was initiated to evaluate the presence and importance of European natural enemies of *P. viburni*. In a preliminary survey conducted in 2000 in Switzerland, three parasitoid species were found. The most abundant was an

unidentified egg parasitoid belonging to the genus *Aprostocetus* (Eulophidae), with a parasitism rate of 8%. The two remaining species were adult parasitoids, the tachinid *Medina collaris* Fallen and an unidentified braconid, and were found in very low numbers (M. Kenis, personal communication). From the results of this study, it was decided to investigate the potential of *Aprostocetus* sp. as a biological control agent. Egg parasitoids have been successfully used for biological control purposes in the past (e.g. Smith 1996).

In 2008, with the help of the European Biological Control Laboratory (EBCL) (Montpellier, France), viburnum twigs infested with *P. viburni* egg masses were collected in Switzerland, France, and The Netherlands and sent to the Sarkaria Arthropod Research Laboratory (SARL) (a quarantine facility at Cornell University in Ithaca, NY) for rearing of *Aprostocetus* sp. The present study was conducted with *Aprostocetus* sp. that emerged at the SARL in 2008 with two objectives: a) to determine the longevity of *Aprostocetus* sp. females under laboratory conditions, and b) to determine the specificity of *Aprostocetus* sp. against non-target chrysomelid species present in North America.

Material and methods

Collection and rearing. Infested *Viburnum* twigs were collected in unmanaged areas (e.g. old fields, woods, forest understories) in northern France (Willems), southern France (Montferrier-sur-Lez), Switzerland (Delemont) and The Netherlands (Nieuwegein) in March-April 2008 (Table 6.1). Infested twigs were stored at 4°C at the EBCL after collection and then sent to the SARL on 13 May 2008. After they had arrived at the SARL, egg masses were kept at 22°C and monitored daily for larval and parasitoid emergence.

Adult parasitoids were sexed and placed in individual glass vials (4.5 x 1.5

cm). To ensure that the females used for the experiments were mated, a male was placed with each female for 48 hours before the beginning of the experiments. A small droplet of honey was added to each rearing vial as source of food, and was replaced if needed.

Longevity trials. Fifty mated female *Aprostocetus* sp. were monitored every day for survivorship. In addition to an undiluted droplet of honey, *P. viburni* egg masses were added to each rearing vial and replaced every few days. *P. viburni* egg masses served as a source of hosts for females and as a source of food; host feeding, the use of a host insect as a food source by an adult parasitoid (Jervis and Kidd 1986), has been observed for *Aprostocetus* sp. with eggs of *P. viburni* (F. Herard, personal communication).

Host specificity trials. Eggs of seven chrysomelid hosts were used for these trials:

- *P. viburni*. Viburnum leaf beetle eggs were used as a positive control. Egg masses used during the trials had been laid during summer 2007 in the Ithaca area (NY, USA), collected during winter 2008, and stored at cold temperature until needed for the trials. *P. viburni* egg masses are laid inside the terminal twigs of viburnum shrubs and each egg mass contains an average of 8 eggs (Weston et al. 2008). Infested portions of twigs containing 2-15 egg masses (i.e. 16-120 eggs) were presented to the parasitoids during the trials.
- *Galerucella nymphaeae* L. Native to Europe and North America, *G. nymphaeae* feeds on water lilies (*Nymphaea* spp.). Its host range also includes the water chestnut *Trapa natans*, invasive species in North America. Adults and eggs were collected in May 2008 on white water lilies (*Nymphaea odorata)* in the Ithaca area and reared in a laboratory colony. *G. nymphaeae* egg masses are laid on the surface of the host leaves. Portions of leaves

containing one egg mass (4-26 eggs) were presented to the parasitoids during the trials.

- *Neogalerucella calmariensis* L. / *N. pusilla* Duftschmid. Native to Europe, these two species were introduced in North America in the early 1990s as biological control agents against purple loosestrife *(Lythrum salicaria)*. The two species are very similar and their eggs are virtually undistinguishable (B. Blossey, personal communication). Adults were collected in May 2008 in the Ithaca area on *L. salicaria* plants and reared in a laboratory colony. In our colonies, the two species were not separated, and will be considered as a single host for the rest of the paper. *N. calmariensis* / *N. pusilla* eggs are laid in small groups on the surface of the host leaves and twigs. Portions of leaves or twigs containing one egg mass (1-15 eggs) were presented to the parasitoids during the trials.
- *Plagiodera versicolora* Laicharting. Native to Eurasia, *P. versicolora* is an invasive pest of willows (*Salix* spp.) and poplars (*Populus* spp.) in North America. Adults were collected in May 2008 in the Ithaca area on willows and reared in a laboratory colony. *P. versicolora* eggs are laid on the surface of host leaves. Portions of leaves containing one egg mass (1-32 eggs) were presented to the parasitoids during the trials.
- *Leptinotarsa decemlineata* Say. Native to North America, the Colorado potato beetle (*L. decemlineata)* is a worldwide pest of potatoes and other plants in the family Solanaceae. Adults were collected in May 2008 in the Freeville area (NY, USA) and reared in a laboratory colony. *L. decemlineata* eggs are laid on the surface of host leaves. Portions of leaves containing one egg mass (1-32 eggs) were presented to the parasitoids during the trials.

Table 6.1. Collections of *P. viburni* egg masses in Western Europe in 2008

Collection site	Collection dates	Number of egg masses collected
Montferrier-sur-lez (France)	2 March, 20-22 March	approx. 200
Willems (France)	17-23 March	approx. 1,000
Nieuwegein (The Netherlands)	22 March	139
Delemont (Switzerland)	25 April	379

- *Plagiometriona clavata* Fabricius. Native to North America, the clavate tortoise beetle (*P. clavata)* feeds on various plants in the family Solanaceae. Adults were obtained from a local laboratory colony in May 2008 and reared in a laboratory colony. *P. clavata* eggs are laid individually in sacs deposited on the surfaces of leaves. Portions of leaves containing one to three egg sacs (1-3 eggs) were presented to the parasitoids during the trials.
- *Lema daturaphila* Kogan and Goeden. Native to North America, the three lined potato beetle (*L. daturaphila)* is an occasional pest of potatoes and other plants in the family Solanaceae. Adults were borrowed from a local laboratory colony in May 2008 and reared in a laboratory colony. *L. daturaphila* eggs are laid on the surface of host leaves. Portions of leaves containing one egg mass (1-18 eggs) were presented to the parasitoids during the trials.

Each host-specificity trial consisted in exposing an individual mated *Aprostocetus* sp. female to one egg mass (or several in the case of *P. viburni*) of one of the chrysomelid species tested for a period 24 h. After 24 h, exposed eggs were removed and monitored for parasitoid and host larval emergence. A trial was considered successful if at least 1 adult parasitoid emerged from the exposed egg mass, and parasitism rate was calculated as the number of parasitoids emerged divided by the total number of eggs exposed. Percentage host larval emergence was calculated as the number of host larvae emerging divided by the total number of eggs exposed to the parasitoid. A total of 50 individual mated females were used repeatedly during 14 d. Because of parasitoid mortality and variability in availability of host eggs, not all 50 females could be used every day. On average, each *Aprostocetus* sp. female was used for 9.3 $\pm$ 0.5 trials. The sequence of host species that females were exposed to day after day was randomly chosen among the host eggs available in laboratory colonies.

Out of the 50 females tested, 48 had at least one trial with *P. viburni* eggs, and 46 had at least one trial with *P. viburni* and a non-target host species.

All the chrysomelid species tested, native or introduced, occur naturally in the northeastern U.S. For the needs of the host specificity trials, each chrysomelid colony was checked daily for presence of eggs. Eggs were used for the host specificity trials only if they had been produced in the 24 h preceding a trial. The only exceptions were *G. nymphaeae*, for which the egg masses used were field collected for most of the trials (13 out of 21), and *P. viburni*, for which the egg masses used had been field collected the previous winter.

Results

A total of 352 *Aprostocetus* sp. adults emerged from the 379 egg masses imported from Switzerland, and 54 emerged from the 139 egg masses imported from The Netherlands. No *P. viburni* larvae or parasitoids emerged from the egg masses imported from France. Assuming an average of 8 eggs per *P. viburni* egg mass (Weston et al. 2008), this corresponds to parasitism rates of 11.6% (Switzerland) and 4.9% (The Netherlands), respectively. Parasitoids emerged 2 to 3 wk after *P. viburni* larval emergence. Females used for the longevity trials survived 54.1 $\pm$ 24.7 d (mean $\pm$ SD), the minimum longevity being 13 d and the maximum longevity being 133 d (Fig. 6.1).

Results of the host specificity trials showed that *Aprostocetus* sp. females successfully parasitized eggs of four of the seven hosts they were exposed to: *P. viburni*, *G. nymphaeae*, *N. calmariensis* / *N. pusilla*, and *P. clavata* (Table 6.2). Only males emerged from parasitized eggs of *G. nymphaeae* and *N. calmariensis* / *N. pusilla*. Parasitism levels were very low: from 0.6% for *P. viburni* to 3.1% for *P. clavata*. Larval emergence was consistently lower for species successfully parasitized

(from 9.8% for *P. viburni* to 21.3% for *G. nymphaeae*) than for species *Aprostocetus* sp. failed to parasitize (from 48.1% for *L. daturaphila* to 83.1% for *P. versicolora*) (Fig. 6.2). *P. clavata* eggs did not hatch under laboratory conditions, whether eggs were exposed to *Aprostocetus* sp. or not.

Out of the 46 females tested at least once with *P. viburni* and a non-target species, 20 (43.5%) did not lay viable eggs at all, 17 (37.0%) laid viable eggs only on *P. viburni* eggs, 6 (13.0%) laid viable eggs on both *P. viburni* and a non-target species, and three (6.5%) laid viable eggs only on a non-target species. Out of the three females that laid viable eggs only on a non-target species, two laid viable eggs on *G. nymphaeae*, and one laid viable eggs on *N. pusilla / N. calmariensis*.
Host-feeding was directly observed on three of the hosts: *P. viburni*, *G. nymphaeae*, and *N. calmariensis / N. pusilla.* Modalities of host-feeding were similar for the three hosts; after puncturing the host egg chorion with the ovipositor, *Aprostocetus* sp. females fed on the droplet of vitellus coming through the puncture. Host-feeding was sometimes followed by parasitization of the same egg (i.e., competitive host-feeding).

Discussion

Classical biological control relies on the idea of re-establishing the natural balance that regulates a pest in its native range, assuming that one or multiple natural enemies are responsible for controlling the pest populations (Hajek 2004). In the case of *P. viburni*, the relatively innocuous nature of the pest in its native range, contrasting with its destructive power in North America, and the presence of several natural enemies only occurring in Eurasia seemed to support this assumption.

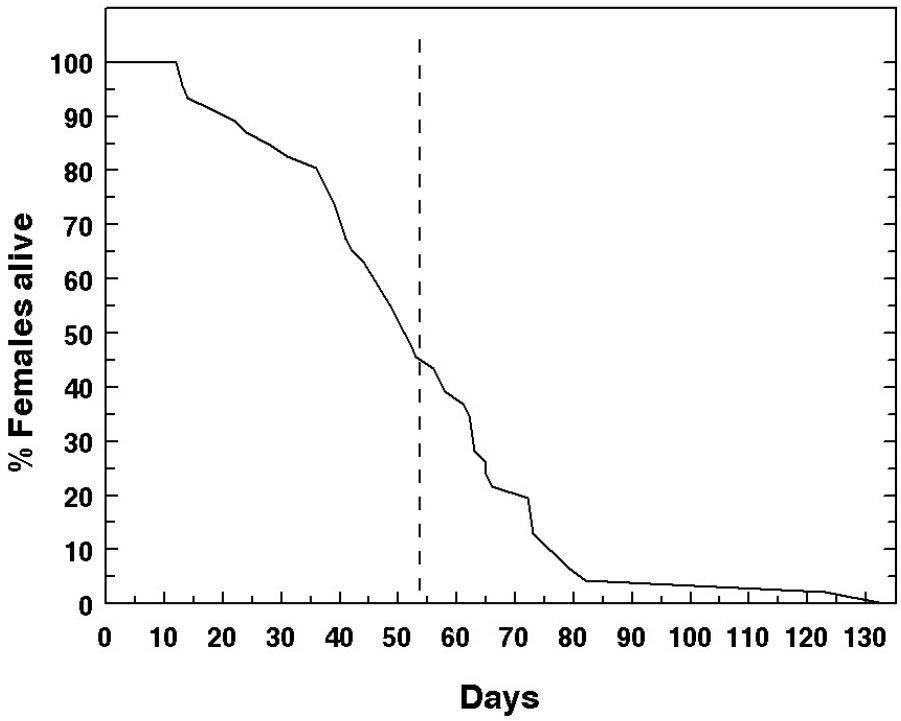

Figure 6.1. Longevity of *Aprostocetus* sp. females under laboratory conditions. Vertical bar indicates mean longevity.

Table 6.2 Parasitism of *Aprostocetus* sp. on various chrysomelid hosts under laboratory conditions. (Note: 2nd generation parastioids whose sex could not be determined were not included in the sex ratio analysis). Each trial consisted in exposing 1 mated *Aprostocetus* sp. female to 1 egg mass of a host species for 24 h.

Host species	Females tested	Successful females (%)	Total trials	Successful trials (%)	Parasitoids emerged	% Parasitism	Sex ratio (m : f)
P. viburni	48	22 (45.8)	292	50 (17.1%)	83	0.6	72 : 3
G. nymphaeae	21	4 (19.1)	21	4 (19.1%)	9	2.0	9 : 0
N. calmariensis / *N. pusilla*	41	5 (12.2)	58	5 (8.3%)	7	2.5	7 : 0
P. versicolora	36	0 (0.0)	40	0 (0%)	-	-	-
L. decemlineata	17	0 (0.0)	17	0 (0%)	-	-	-
P. clavata	24	1 (4.2)	27	1 (3.7%)	1	3.1	0 : 1
L. daturaphila	18	0 (0.0)	18	0 (0%)	-	-	-
Total			478	60	100		84 : 4

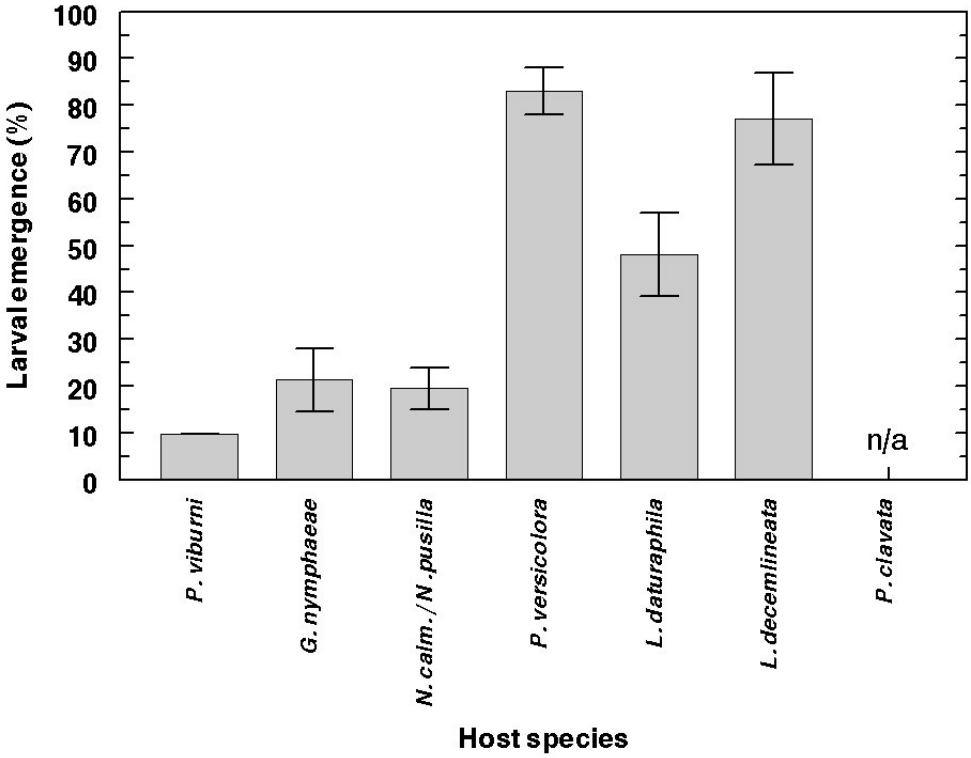

Figure 6.2. Larval emergence from eggs exposed to *Aprostocetus* sp. under laboratory conditons (mean $\pm$ SE).

The egg parasitoid *Aprostocetus* sp., was determined in 2000 as the most promising biological control agent candidate against *P. viburni* (M. Kenis, personal communication), but two major aspects of its biology needed to be clarified before establishing its value and suitability for introduction in North America: its life cycle and host specificity.

Little is known about the life cycle of *Aprostocetus* sp. Adults emerge from parasitized eggs 2 to 3 weeks after *P. viburni* larval emergence (i.e., mid-May), as recorded by Kenis in 2000 in Switzerland. *P. viburni* egg masses are parasitized sometime during the period of *P. viburni* egg production from mid-July to September. Between parasitoid emergence and first availability of its host (*P. viburni* eggs), there is a gap of approximately two months. Our longevity trials showed that average longevity of *Aprostocetus* sp. females is close to two months (54.1 d) and that some individuals survived much longer (up to 133 d). Therefore, it is theoretically possible that the life cycle of *Aprostocetus* sp. consists of one generation of adults solely parasitizing *P. viburni* eggs. However, the fact that survivorship of *Aprostocetus* sp. steadily declined after 13 d does not fit this hypothesis well. Longevity might also be lower in the field due to the negative impact of weather, natural enemies, scarcity of food, etc. on *Aprostocetus* sp. survivorship. An alternative hypothesis of the *Aprostocetus* sp. life cycle is that the parasitoid has a first generation on one (or several) secondary host(s) during the period between adult emergence from *P. viburni* eggs and availability of fresh *P. viburni* egg masses for a second generation of these parasitoids.

Host specificity trials confirmed that, although parasitism was low, *Aprostocetus* sp. can develop in more than one host species. In total, females successfully parasititzed three of the six non-target hosts they were exposed to: *G. nymphaeae, N. calmariensis* / *N. pusilla,* and *P. clavata*. In addition to the results of

our trials, successful parasitization of *Pyrrhalta luteola* (Mueller) eggs by *Aprostocetus* sp. females collected in France was observed at the EBCL in 2007 (F. Herard, personal communication), giving further credence to the idea of a broad host-range for *Aprostocetus* sp. Among the non-target hosts parasitized, *P. clavata* is the most distantly related to *P. viburni* within the family Chrysomelidae: *P. viburni*, *P. luteola*, *G. nymphaeae*, and *N. calmariensis* / *N. pusilla* all belong to the Galerucinae subfamily, whereas *P. clavata* belongs to the subfamily Cassidinae.

Parasitism rates on the non-target species were consistently low and only *Aprostocetus* sp. males emerged from *G. nymphaeae* and *N. calmariensis* / *N. pusilla* parasitized eggs. This might be an artifact of the small number of successful trials obtained with these two hosts (9 and 7, respectively), or due to the quality of the hosts themselves. Hymenopteran parasitoids can control the sex of each individual offspring and often make decisions regarding sex of offspring based on perceived host quality (Quicke 1997). A general trend followed by idiobiont parasitoids, whose hosts do not develop after parasitization, is that male eggs are laid preferentially in smaller hosts and females in larger hosts (Tanaka et al. 1992). *N. calmariensis* / *N. pusilla* eggs are slightly smaller than *P. viburni* eggs, while *G. nymphaeae* eggs are approximately the same size. The exclusive production of *Aprostocetus* sp. male offspring on these two non-target hosts might therefore be due to female choice. Alternatively, the exclusive production of male offspring might be due to failed development of *Aprostocetus* sp. female offspring within the hosts or unsuccessful female mating prior to trial (i.e. non-fertilized eggs only produce male offspring), or the fact that the eggs used were very young. The fact that most of the eggs of *N. calmariensis* / *N. pusilla* and *G. nymphaeae* did not hatch after exposure to *Aprostocetus* sp. (Fig. 6.2) could be an indicator of failed parasitism. Host-feeding by the female parasitoids, observed on these two hosts, could also have played a role in egg survivorship. However, since we

did not record the larval emergence of host eggs not exposed to parasitoids, we cannot draw conclusions about the effect of parasitoids on reduced larval emergence of *N. calmariensis* / *N. pusilla* and *G. nymphaeae*.

The parasitism rate observed on *P. viburni* eggs during the host specificity trials was very low, with a male-biased sex ratio, and only 45.8% of the females exposed to *P. viburni* eggs produced viable eggs (Table 6.2). These results may suggest that *P. viburni* is not really a preferred host for *Aprostocetus* sp. females. Alternatively, parasitism of *P. viburni* might have been low because eggs presented to the parasitoids during the trials had been laid the previous year and had had an artificially extended chilling period (approx. 3 months longer than under natural conditions), which might have reduced their quality as hosts. Fresh *P. viburni* egg masses could not be used because they were not available in the field at the time of the trials. In addition, the egg caps of *P. viburni* egg masses used for the trials were not removed and might have kept some eggs out of the reach of the *Aprostocetus* sp. females tested. Finally, the rearing and/or trial conditions might not have been optimal for successful parasitism.

Our host-specificity trials were limited in resources and time. Ideally, every female included in the trials should have been exposed to every host, and host order should have been determined to have comparable numbers of females exposed at first to *P. viburni* or to a non-target species. Using the same females repeatedly might also have impacted the results. 43.5% of the females exposed to *P. viburni* and at least one non-target did not lay viable eggs at all, and it is impossible to draw conclusions about whether or not these females were physically able to lay viable eggs. Therefore, the question of whether *Aprostocetus* sp. was not able to successfully parasitize *P. versicolora*, *L. decemlineata*, and *L. daturaphila* in our trials because of unsuitability of the host or quality of the females tested is important to consider. However, the fact

that a proportion of the females tested for *P. versicolora* (55.5%), *L. decemlineata* (47.1%), and *L. daturaphila* (44.4%) laid viable eggs on *P. viburni* or another non-target gives us confidence that the absence of parasitism observed on this three species is likely to be due to host unsuitability. Finally, exposing *Aprostocetus* sp. females to eggs laid in the 24 h preceding the trials for most of the hosts might have impacted the results of the trials: host age is known to influence egg parasitoid preferences and parasitism success (Miura and Kobayashi 1998). It would have been preferable to expose *Aprostocetus* sp. females to eggs varying in age for each host tested.

Overall, the results of our study showed that *Aprostocetus* sp. is not specialized on *P. viburni,* and can successfully attack several chrysomelids present in North America. Direct mortality due to parasitism was consistently low on the non-target species attacked in our trials. Indirect mortality, possibly due to failed parasitism and host-feeding, might occur although further studies are necessary to evaluate the potential impact of these effects. There is therefore a risk that, if *Aprostocetus* sp. was released in North America as a biological control agent against *P. viburni*, it may negatively impact populations of non-target species, and this should be prevented (Louda et al. 2003). Negatively impacting *N. pusilla/N. calmariensis* populations could potentially jeopardize the success of the biological control program against purple loosestrife in North America, which could be ecologically disastrous. There might be ecological barriers preventing *Aprostocetus* sp. from parasitizing other chrysomelid species under field conditions, but given the temporal overlap between the presence of adult *Aprostocetus* sp. and eggs of the non-target species in the field, and potential spatial synchrony (*G. nymphaeae*, *N. calmariensis* / *N. pusilla,* and *P. viburni* can all inhabit wetlands, for example), the risk of ecological overlap does not seems trivial. Therefore, I think that *Aprostocetus* sp. should not be considered for introduction in North America as a biological control agent against *P. viburni*.

The unsuitability of *Aprostocetus* sp. as a biological control agent should not put an end to the efforts made toward classical biological control of *P. viburni* in North America. Future research should focus on enlarging the area of exploration for natural enemies of *P. viburni* in its native range, especially areas that are climatically similar to the northeastern U.S. and southeastern Canada, to identify new potential biological control agents of this invasive pest.

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CHAPTER 7

IMPLICATIONS OF THIS RESEARCH FOR MANAGEMENT OF VIBURNUM LEAF BEETLE IN NORTH AMERICA

Summary

In this concluding chapter, I give an overview of the implications and potential applications of the research described in the previous chapters toward management of viburnum leaf beetle, *Pyrrhalta viburni* (Paykull), in North America. I begin by describing the current situation of *P. viburni* in its native and introduced range in light of the most recent observations made in North America and the findings of a few observational studies conducted in Europe in the last few years. I then list the current management tools used by home owners, nurserymen, and landscape managers for control of *P. viburni*. Efforts made to develop a classical biological control program against *P. viburni* are then summarized with the first conclusions drawn from recent research and future priorities. The last two sections of the chapter are devoted to a description of the possible applications of oviposition studies for *P. viburni* management, focusing mostly on enhancing viburnum resistance to oviposition, and the future research needs for pest management.

Viburnum leaf beetle situation in North America and Europe

It is commonly accepted that *P. viburni* was introduced to North America along with infested viburnum plants imported from Eurasia to Canada for ornamental purposes. However, the precise date and circumstances of its arrival are still unclear.

For many years, it was thought that the first occurrence of *P. viburni* in Canada dated from 1947 (Becker 1979), but a recent study by Majka and Lesage (2007) show that *P. viburni* individuals were collected in 1924 in Nova Scotia. Breeding populations of the insect were not observed until 1978 when it was found in Ottawa, Ontario and Hull, Quebec (Canada), defoliating *V. opulus* shrubs (Becker 1979). This marks the beginning of the *P. viburni* invasion wave: since then, the insect has steadily increased its distribution in the south of Canada and was first reported from the U.S. in Maine in 1994. It now occurs in all New England states, New York, Pennsylvania, Michigan, and Ohio. A separate introduction occurred on the west coast of North America, with reports of *P. viburni* in British Columbia in 2001 and the state of Washington in 2002 (Weston et al. 2007). The introduction was likely from transportation of infested nursery stock from eastern Canada.

Three main hypotheses can be formulated to explain the gap of over 50 years between the first records of *P. viburni* in North America and the beginning of the invasion wave: (a) The individuals from early records did not establish long-term breeding populations and died out, and the individuals that became invasive in the 1970s originated from a separate, later introduction. (b) The individuals from early records did establish breeding populations that remained at low densities, and the individuals that became invasive in the 1970s originated from a separate, later introduction. (c) The individuals from early records established breeding populations and remained at low densities until the late 1970s, when they started to outbreak and became invasive. The third hypothesis is not uncommon for biological invasions. There is often a lag between initial establishment and increase and spread of an invader, a period referred to as “lag phase” (Crooks 2005). A lag phase can occur for a number of reasons, including Allee effect, founder effect, environmental and demographic stochasticity, and inbreeding. These effects are often linked with the

small size of the original established population and can impact the ability of the established population to produce enough propagules to invade nearby environments, thus restricting invasiveness.

Now that the invasion wave of *P. viburni* has started, one of the main questions that remain is: Where will it stop? So far, the insect has spread as rapidly as 25 mi (40 km)/yr along large bodies of water (Weston and Hoebecke 2003), and it can be expected to expand its range until scarcity of suitable hosts or climatic conditions stop it. *P. viburni* populations present in North America have a required chilling period of several months that is necessary to overwinter (Weston and Diaz 2005), which will certainly limit the southward spread of the insect. It has been hypothesized that spread of *P. viburni* is likely to stop somewhere in the southern Appalachians (Weston et al. 2007). Until then, the invasion wave of *P. viburni* will probably continue in the dramatic fashion we are witnessing now: killing the vast majority of susceptible viburnum (e.g. *V. dentatum*, *V. trilobum*, and *V. rafinesquianum*) in natural settings and unmanaged environments after a few years of repeated defoliation.

In Europe, viburnum leaf beetle is not considered a major pest, and is more commonly referred as an "occasional problem" for *V. opulus* and *V. lantana* in managed landscapes. Relatively few biologists have studied *P. viburni* biology, ecology, and behavior (mainly Zorin 1931, Lühmann 1934, and Balachowsky 1963), presumably because of the relative harmlessness of the insect for native viburnums and lack of economic impact in its native range. Several natural enemies of *P. viburni* have been reported in Europe, mostly by Zorin in Russia (1931), and M. Kenis in Switzerland (2000, unpublished). In total, one egg parasitoid [unidentified *Aprostocetus* sp. (Hymenoptera: Eulophidae)] (Zorin 1931), one larval parasitoid [unidentified *Closterocerus* sp. (Hymenoptera: Braconidae)] (Zorin 1931), and three adult parasitoid [unidentified *Leiophron* sp. and *Centistes* sp. (Hymenoptera:

Braconidae), *Medina collaris* (Diptera: Tachinidae)] (Zorin 1931, M. Kenis, personal communication), plus a few generalist predators (M. Kenis, personal communication) have been reported. It is generally assumed that this assemblage of natural enemies exclusive to Eurasia is responsible for regulating *P. viburni* in its native range, preventing it from reaching outbreaking population densities.

The facts summarized above seem to depict *P. viburni* as a relatively classic invader: an herbivorous insect innocuous in its native range removed from the impact of its natural enemies and becoming invasive in its introduced range after a lag phase, endangering its new host(s). However, recent observations made in Europe and North America seem to indicate that the *P. viburni* story might not be so simple.

First of all, it appears that *P. viburni* is not as harmless as presumed in Europe. Field observations recently collected by M. Kenis in Switzerland in 2000 and 2008; R. Beenen in The Netherlands in 1996 and 2008; F. Herard in the southern part of France in 2006, 2007 and 2008; and myself in France, Switzerland, and Belgium in 2006, 2007, and 2008 have shown that *P. viburni* is abundant in the vast majority of the sites sampled, in managed landscapes as well as natural areas, and that defoliation due to larvae and adults feeding is often noticeable (5-10% defoliation) and sometimes spectacular (>75% defoliation) on local viburnums. I personally witnessed two outbreaks of *P. viburni* adults in the Northern part of France in the summer of 2006: in both cases, thousands of beetles totally defoliated dozens of large *V. opulus* shrubs, feeding even on the bark of the shrubs once the leaves were consumed, illustrating the severity of the infestation. Several landscape managers and nurserymen in the area told me they regularly use chemical control to keep *P. viburni* populations down and reduce defoliation levels, illustrating the recurrence of this “minor” problem. However, it must be added that both outbreaks I witnessed were very localized and occurred in urban environments, which might have been more stressful for the plants

and/or *P. viburni* natural enemies and therefore more favorable to a flush of *P. viburni* populations.

Overall, *P. viburni* populations in Western Europe can be described as "stable at relatively high densities", common and often abundant almost everywhere viburnums are present, and damaging but rarely lethal to the plants infested. The only geographic exception we noted to this rule was the Southern part of France, where *P. viburni* is rarer and causes minor damage. This may be due to the combination of a less adequate climate (Mediterranean area) and the fact that *V. tinus*, a suboptimal host, is the dominant viburnum species in this area.

Second, although several natural enemies have been reported attacking *P. viburni* in Europe, their impact on *P. viburni* populations seems relatively minor. As mentioned in the previous chapter, the egg parasitoid *Aprostocetus* sp. appears to be the most abundant natural enemy in Western Europe, and its natural parasitism rates average 8 to 10% in Switzerland and the Netherlands (2000 and 2008 data). The other parasitoids observed by Zorin (1931) and Kenis (2000) seem either opportunistic (the adult parasitoid *Medina collaris*), or have a minor impact (adult parasitoids of *Centistes* sp.). Kenis noted the absence of larval parasitism in his 2000 study as "surprising" and the overall parasitism as "disappointing". In 2006, I was unable to retrieve a single adult parasitoid from samples of over 3,000 *P. viburni* adults collected in several locations of the Northern and Eastern parts of France. Zorin, despite describing three parasitoids of *P. viburni* and recording higher egg parasitism than Kenis (up to 35%), concluded that "the weather conditions are the main regulator of the guelder rose beetle (i.e. *P. viburni*) reproduction". In addition to parasitoids, a few generalist predators such as the pentatomids *Arma custos* (Fabricius) and *Pentatoma rufipes* L. (Hemiptera: Pentatomidae) have been observed by Kenis (2000) and me (2006), but predation on *P. viburni* was very minor. Pathogens of *P. viburni*

are a vastly unexplored topic. In North America, an unidentified fungus has been observed killing pupating *P. viburni* larvae in the soil during wet springs (personal observation). In Europe, *P. viburni* laboratory colonies are sometimes infested by an unidentified fungus (M. Kenis, personal communication), but the occurrence of such infestations under natural conditions has not yet been documented.

From these observations (high levels of abundance and damage, poor assemblage of natural enemies, and uncertain impact), Herard formulated the hypothesis that *P. viburni* might have arrived in Western Europe only recently (evolutionarily speaking) and that its "true" native range might be further east in Asia (personal communication). A few additional pieces of evidence seem to support this hypothesis.

First, at least two species closely related to *P. viburni*, *P. annulicornis* (Baly) and *P. humeralis* (Chen), both feeding on viburnums, occur in Southeast Asia (Korea, Japan, NE China, E. Siberia, and Sakhalin for *P. annulicornis*, Korea, Japan, China, and Taiwan for *P. humeralis*) (Park and Lee 2004). *P .humeralis* is considered a major pest of *V. awabuki* in Japan. Satoh (2002) mentions a third *Pyrrhalta* sp. feeding on viburnums in Japan, *P. esakii*, but information on that species is scarce. The idea that the four *Pyrrhalta* species feeding on viburnums might have had a common ancestor in Asia does not seem unreasonable.

Second, Asian viburnums are in general more resistant to *P. viburni* defoliation and oviposition than European and North American species (with the exception of the European *V. tinus*), suggesting that Asian species might have shared a coevolutionary history with viburnum leaf beetle (or its hypothetical ancestor). Interestingly, *P. viburni* and the three Asian "sister species"--*P. annulicornis*, *P. humeralis*, and *P. esakii*--have a lot in common in terms of general biology and life cycle (e.g. univoltine species, both larvae and adults are leaf feeders), but differ in their oviposition

strategies. *P. viburni* lays its eggs in cavities dug inside the terminal twigs while *P. humeralis* lays eggs as a mass under the bark at the base of branches (Satoh 2002, Shinkaji et al. 1987), and *P. esakii* lays "small single egg masses on branch tips" (Satoh 2002). I was unable to find information about *P. annulicornis* oviposition behavior. Did divergent oviposition strategies shape the speciation of these species, and did plant defenses play a role in this process? These questions remain to this point a fascinating but speculative topic of investigation.

Finally, the situation of viburnums in North America might not be as desperate as the desolating spectacle of seas of defoliated viburnums in natural areas hit by the pest. *P. viburni* has been present in the U.S. for 14 years and we are now starting to accumulate information on the short-term future of *Viburnum* populations hit by the invasion wave. It appears that *P. viburni* populations, being dependent of the presence of viburnums to survive, quickly decline after depleting their food sources in the areas they invade, which is not surprising. More interestingly, a growing body of field observations provides evidence of viburnum recovery in the years following *P. viburni* departure from an invaded area (P. Weston, W. Nemeth, V. Nuzzo, personal communication). Viburnum recovery comes from new shrubs emerging from seeds, and more importantly from a regrowth, sometimes spectacular, of shrubs, including the highly susceptible arrowwood viburnum (*V. dentatum)* and American cranberrybush (*V. trilobum),* supposedly killed by *P. viburni*. Quantitative data showing the degree of recovery of viburnum shrubs post-invasion wave have yet to be collected, but would be very valuable for conservation biologists.

It is also important to keep in mind that not all North American viburnums are highly susceptible to *P. viburni*. Several native species such as *V. lentago*, *V. prunifolium*, and *V. lantanoides* are only moderately susceptible to the beetle (i.e. show varying levels of damage but usually not killed) and do not seem at risk of being

eliminated from the northeastern U.S. landscape.

These somewhat optimistic considerations deserve to be addressed, but do not minimize the need for efficient tools for controlling *P. viburni* in managed landscapes and keeping its populations down in natural environments. The fact that some viburnums are able to recover from years of severe *P. viburni* defoliation does not mean that the beetle will not eventually increase again in these areas to restart the boom/bust cycle. In addition to the cost of pest management and replacement of killed shrubs in managed settings, the loss or reduction of native viburnums in natural areas is likely to have ecological consequences whose magnitude has yet to be determined. For example, many arthropod species are associated with viburnums and suffer from the loss of their host plants. Reduction in viburnum fruit availability, a primary source of food for many migratory songbirds, may also seriously affect small mammals and songbird abundance in both stopover and overwintering habitats.

Existing management options against viburnum leaf beetle

Several management options are available to control *P. viburni* populations, depending on the time of the year, life stage targeted, and size of the infestation. From fall to early spring, pruning out egg-infested twigs significantly reduces larval populations; application of horticultural oil (4%) on infested twigs a few weeks before egg hatch reduces egg survivorship by suffocating the egg masses (Weston et al. 2007). In May, control of larval populations can be achieved by application of insecticidal soap; such applications need to be made to the undersides of the leaves, where the small larvae feed, because this product requires direct contact with the insect to be effective. Insecticidal soap decreases in efficacy as the larvae grow, and is completely ineffective against adults. Several broad-spectrum insecticides (e.g. imidacloprid, malathion, carbaryl, and pyrethrins) are very effective against larvae and

adults when applied to the foliage. Soil drenches of imidacloprid provide 100% control for two years or more after a single application (Weston et al. 2007). One biological control agent, the generalist predator *Podisus maculiventris* Say (Hemiptera: Pentatomidae), is also available commercially and can significantly reduce larval damage if a predator/prey ratio of roughly 1:100 is established (Desurmont and Weston 2008). However, this control method is rather expensive due to the large number of predators needed to tackle heavy infestations, and is unlikely to provide complete control.

In nurseries and managed landscapes, impact of *P. viburni* can also be reduced by plant management: not all viburnum species are susceptible to the pest, and planting resistant varieties decreases the need for pest management. A list of viburnum species and their susceptibility to *P. viburni* can be found at the Cornell Viburnum Leaf Beetle Citizen Science website: http://www.hort.cornell.edu/vlb/suscept.html.

All of these management options are not applicable in natural areas, for practical and/or environmental reasons. Replacing destroyed plants with more resistant native viburnums as part of revegetation programs in invaded areas would be valuable but probably is limited in scope unless it is strongly supported financially. To provide long-term control of *P. viburni* populations in natural areas without regular human intervention, classical biological control remains to this day the only viable option.

Classical biological control of viburnum leaf beetle: conclusions and prospects

Investigating the potential of natural enemies of *P. viburni* for classical biological control purposes has been by far the most difficult aspect of my Ph.D research and would not have been possible without the collaboration of two European biological control experts: Franck Herard from the European Biological Control Laboratory (EBCL) Montpellier (France) and Marc Kenis from the CABI Biocentre

Delemont (Switzerland). In addition to providing time, material support, and expertise for a project with very limited funding, their knowledge has taught me a great deal about biological control in practice and their unflagging enthusiasm has been a great source of motivation to carry on the project.

The time I have had at my disposal to study the natural enemy we chose to focus on, the egg parasitoid *Aprostocetus* sp. under quarantine conditions at the Sarkaria Arthropod Research Laboratory (SARL, Cornell University, Ithaca) was brief (May-August 2008), but allowed me to come to a definitive conclusion concerning this particular natural enemy: it should not be considered for introduction to North America for biological control of *P. viburni*. The host-range of *Aprostocetus* sp. is too broad and this parasitoid could potentially impact several native or introduced chrysomelids in the Northeastern U.S and Canada. Successful parasitism of two of the non-targets, *Neogalerucella calmariensis* and *N. pusilla,* raised the biggest concern because these species have been introduced from Europe as biological control agents and are part of a successful program of control of purple loosestrife, *Lythrum salicaria,* in North America (see chapter 6 for details).

As mentioned earlier, *Aprostocetus* sp. is the most abundant natural enemy of *P. viburni* reported in the European literature, and its unsuitability as a biological control agent is certainly unfortunate. However, this does not mean that efforts toward classical biological control of *P. viburni* in North America should be abandoned.

First, the species identification of *Aprostocetus* sp. used in my studies has yet to be conducted. It has been suggested that the egg parasitoids of *P. viburni* might actually belong to two different species, *A. suevius* and *A. celtidis* (Graham 1987, Beenen 1997), differentiated mostly by the length of the ovipositor sheath (far exserted in *A. suevius*, slightly exserted in *A. celtidis*). If this is the case, one could imagine that the two species might differ in terms of life cycle and host-specificity.

However, Zorin, in his 1931 study, proposed a different interpretation of the biology of *P. viburni* egg parasitoid. He describes two forms of *Aprostocetus* sp., each corresponding to one generation, the insect being presented as bivoltine:

"Summer females, which parasitize eggs of *P. viburni* have a long ovipositor, with which they easily pierce the caps that cover the egg sac holes in the branches. Females of the generation that overwinter (i.e. spring generation) have a short ovipositor and parasitize the eggs of other species of *Galerucella*, e.g. *G. tenella*, and *G. nymphaeae*, as they are deposited directly on the leaves."

A couple of observations made at SARL in 2008 seem to corroborate Zorin's interpretation. First, the *Aprostocetus* sp. females we worked with emerged from overwintering *P. viburni* egg masses, which correspond to Zorin's spring generation, had a relatively short ovipositor and were able to successfully parasitize the other *Galerucella* sp. they were exposed to, *G. nymphaeae* and *G. pusilla*/*G.calmariensis*. Second, when exposed to *P. viburni* eggs, our females showed consistently low parasitism rate (0.6% average total) and seemed to only be able to attack the more exposed eggs of the egg masses (personal observation), while Kenis observed that even the more concealed eggs are found parasitized in the field under natural conditions (personal communication).

Second, a major part of the *P. viburni* native range (or supposed native range) has not been explored for presence of natural enemies. According to Balachowsky (1963), *P. viburni* is widespread over northern and temperate Europe, as well as southernmost Siberia, but is absent in eastern Siberia and northern China. From my interactions with entomologists and botanists based in Eurasia, the most eastern recent direct observation of *P. viburni* occurred in Novosibirsk, southern Siberia, close to the borders of Kazakhstan and Mongolia in 2007 (N. Kirichenko, personal communication).

The studies of Zorin (1931) and Kenis (2000, unpublished) on natural enemies of *P. viburni* took place in the area of St Petersburg (northwest Russia) and Switzerland, respectively, leaving a large part of the *P. viburni* range unexplored. Future surveys should focus on Eastern Europe and Asia, especially areas that are climatically similar to northeast U.S. and southern Canada. Investigations should not be restricted to parasitoids and should include other types of natural enemies such as predators and pathogens.

Finally, the presence of other chrysomelids closely related to *P. viburni* feeding on viburnums in Eurasia, i.e. *P. annulicornis*, *P. humeralis*, and *P. esakii*, opens new perspectives for classical biological control of *P. viburni*. The possibility of creating a new association, in this case introducing an exotic natural enemy to control an exotic pest it has not coevolved with, deserves to be examined. Efforts should be made to identify natural enemies of these three *Pyrrhalta* species, especially if no other natural enemy of *P. viburni* is suitable for introduction as a biological control agent in North America.

In conclusion, the disappointing results obtained from evaluating the potential of *Aprostocetus* sp. as a biological control agent against *P. viburni* should not dissuade us from pursuing efforts to develop a classical biological control program against *P. viburni* in North America. This will certainly be a long-term project, especially since so little is known about *P. viburni* in the eastern part of its native range. Therefore, classical biological control is not suited for the immediate needs for management tools from landscape managers and gardens owners. However, as mentioned earlier, it might be the only possible way to save from destruction thousands of native susceptible viburnums that are widespread in the natural areas of North America.

What we have learned from oviposition studies

The oviposition behavior of *P. viburni* has been abundantly described and discussed in the previous chapters. Here, I would like to summarize the main conclusions we draw from studying *P. viburni* oviposition behavior as well as some of the potential applications of this research.

Viburnum resistance to oviposition, i.e. the ability to produce wound tissue that kills or displaces the eggs within the egg mass cavity, plays a major role in *P. viburni* egg survivorship and overall fitness. Results from a field experiment including *V. dentatum*, *V. opulus*, and *V. x bodnantense* showed that the overall egg survivorship decreases from almost 90% on twigs showing no reaction to less than 10% on twigs showing 100% reaction (see chapter 4). These results demonstrate that, in addition to top-down forces such as natural enemies, bottom-up forces such as plant resistance may participate in regulating *P. viburni* populations.

Viburnum resistance to ovipostion is species specific. In addition to purely quantitative differences in the ability to produce wound tissue in response to oviposition (i.e. the number of egg masses a twig can react to before being killed), I have documented that the host species itself can play a role in host survivorship independent of production of wound tissue (e.g. overall reduced egg survivorship on *V. x bodnantense* compared to *V. dentatum* and *V. opulus*, chapter 4). Overall, trials have shown that North American species such as *V. dentatum* and *V. rafinesquianum* are less resistant to oviposition than European viburnums (*V. opulus*, *V. lantana*, and *V. tinus*), and Asian viburnums (*V. x bodnantense*, and *V. setigerum*). North American viburnums also suffer greater twig mortality due to oviposition, and therefore have lower tolerance to oviposition damage than viburnum species from other origins. These findings may partially explain the success of *P. viburni* as an invader in North America (greater fitness on poorly defended hosts) and the intriguing equilibrium at

relatively high population densities observed in western Europe (plant resistance to oviposition might prevent *P. viburni* populations from reaching outbreak densities).

The European *V. tinus* stands out among viburnums as the only species capable of an induced increased wound response in response to oviposition (Fig 7.1). For all the other species tested, percentage wound tissue production goes down as the number of egg masses per twig goes up, showing a benefit of aggregating egg masses for *P. viburni* females. On *V. tinus*, aggregative oviposition has a cost, which may lead females to select for non-infested twigs to lay egg masses, limiting the ability of *P. viburni* populations to build high densities on this particular species. It would be tempting to suggest that induced increased wound tissue production may have evolved in *V. tinus* as a result of coevolution with *P. viburni*. However, we have to be careful, as *V. tinus* has other unique traits: it is, for example, the only evergreen species included in our studies. Therefore, *V. tinus* is the only species that we tested that has active sap flow during winter, which might influence the wounding response process. Further experiments investigating wound response of other evergreen viburnums not having coevolved with *P. viburni* as well as the specificity of *V. tinus* wound response (directed or not toward *P. viburni* egg masses) would help address this question.

Viburnum resistance/susceptibility to oviposition is dependent on particular plant and twig traits that vary between and within *Viburnum* species. For example, we have shown that twig diameter plays a role in oviposition resistance for *V. dentatum*, thicker twigs being more resistant to oviposition, while diameter is not significantly associated with oviposition resistance for *V. opulus* and *V. x bodnantense*. Some other plant traits such as the structure of inflorescences also play a role by providing optimal oviposition sites for *P. viburni* females or not (see chapter 4). From an applied standpoint, these results open the perspective of selecting traits linked with oviposition

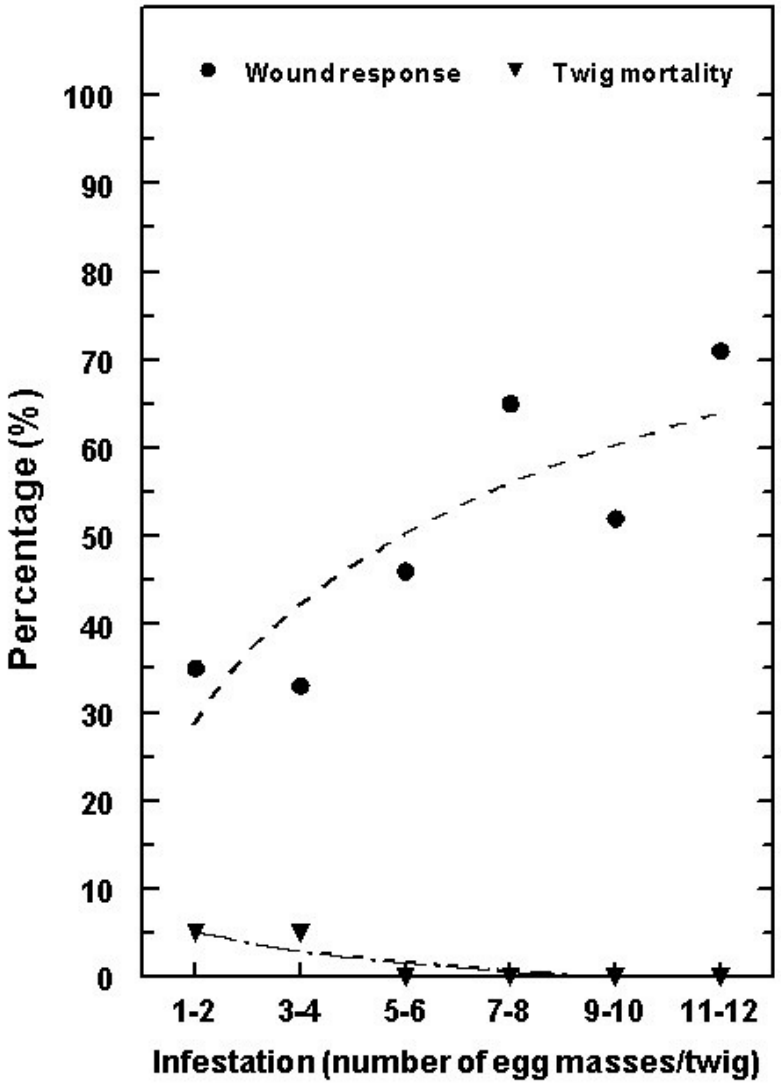

Figure 7.1. Twig mortality and twig wound response (%) depending on infestation on *V. tinus*.

resistance to develop or promote *Viburnum* varieties less susceptible to *P. viburni*. This can be done through screening existent varieties of ornamental and native species found in North America or developing new varieties via plant breeding. Twig diameter may be a suitable candidate for selection of *V. dentatum* strains, but the high plasticity of this trait, dependent on plant age and vigor and other environmental factors (e.g. sun exposure, soil fertility, etc.) may complicate horticultural selection for resistance to *P. viburni*.

Ovipositional resistance does not seem a priori based on the same traits that determine larval and adult performance: For example, *V. lantana* is a suitable host for larval and adult feeding but is very resistant to oviposition, while *V. rafinesquianum* is not optimal for larval performance but is poorly defended against oviposition (unpublished).

Finally, oviposition studies have shed light on some of *P. viburni* oviposition preferences and part of the process of oviposition site selection. It appears that *P. viburni* females discriminate among viburnum species when given a choice, and that these differences are scale-dependent. In laboratory multiple choice-tests including six *Viburnum* species (*V. dentatum*, *V. trilobum*, *V. lentago*, *V. setigerum*, *V. x bodnantense*, and *V. carlesii*), we found that *V. x bodnantense* was consistently preferred for oviposition, although *V. dentatum* and *V. trilobum* were preferred for feeding (unpublished). However, in the field, oviposition on *V. x bodnantense* is scarce while most of the egg masses are deposited on *Viburnum* species preferred for feeding (e.g. *V. dentatum*, *V .trilobum*, *V. opulus*, and *V. sargentii*) (personal observation). This suggests that the oviposition site selection process might be divided in two successive steps for *P. viburni*: (a) selection of a suitable microenvironment based on availability of food and (b) selection of a suitable twig for oviposition. In laboratory choice-tests, *P. viburni* females were in thepresence of optimal food (*V.*

trilobum and *V. dentatum*) and preferentially laid egg masses on the most stimulatory twigs (*V. x bodnantense*, for unknown reasons). In the field, isolated *V. x bodnantense* shrubs do not represent a microenvironment with optimal food and therefore likely do not stimulate *P. viburni* females to engage in the process of twig selection, resulting in low levels of oviposition on that species.

This attempt to explain *P. viburni* oviposition site selection is speculative and would necessitate further research and experiments to be validated. In terms of applications to pest management, *P. viburni* site selection behavior could potentially be exploited through a push-pull strategy. Push-pull strategies involve the behavioral manipulation of insect pests via the integration of stimuli to make the protected resource unattractive or unsuitable (push) while attracting pests to an attractive source (pull) (see Cook et al. 2007 for a review of push-pull strategies). Applied to *P. viburni* in the context of revegetation programs or landscape management, one could imagine plantings of susceptible native viburnums (the resource to protect) mixed with varieties that either repel *P. viburni* from feeding and ovipositing in the area or attract individuals present in the area, preventing them from damaging the susceptible shrubs. In the latter scenario, an ideal variety to mix with the susceptible one should have the following characteristics: preferred for adult feeding, preferred for adult oviposition, and unsuitable for larval development. *V. x bodnantense* corresponds to two of these criteria: our trials have shown that its twigs are preferred for oviposition when in close proximity to *V. dentatum*, while larvae perform very poorly, i.e. show reduced larval survivorship and pupal weight, when feeding on its foliage (unpublished).

More generally, investigating the bases of *P. viburni* oviposition preferences, especially the chemical cues associated with oviposition stimulation and deterrence, might have some applied value by providing a basis to develop chemical oviposition deterrents. Compared to other chemicals such as broad-spectrum insecticides,

oviposition deterrents usually have the advantage of being non-toxic and target-specific.

The future of *P. viburni* research

In concluding this chapter, we can classify the needs for future research for management of *P. viburni* in North America using three categories, with adoption depending on how quickly results are needed and how long support is provided:

- Short term projects should focus on improving existing management tools against *P. viburni* and developing integrated pest management programs. For example, application of oil against egg masses in the spring is efficient but labor-intensive compared to standard insecticide spray applications due to the necessity to reach the undersides of the twigs, which are bare at the time of the application and do not retain oil as leafed twigs would. As another example, the generalist predator *P. maculiventris* may not be able to provide complete control of *P. viburni* and may be too costly for inundative biological control, but could potentially be used in the context of conservation biological control. The use of *P. maculiventris* aggregation pheromones to attract endemic populations of the predator to infested viburnums has certain potential and deserves to be closely examined.
- Projects based on enhancing viburnum resistance to *P. viburni*, in particular resistance to oviposition, will take more time to develop than improving existing control techniques. The three main potential applications of research on oviposition resistance mentioned earlier, i.e. screening/selecting for resistant varieties, developing push/pull strategies, and searching for oviposition deterrents, are all conceptual at this point. Research is needed to validate the preliminary data gathered and results must be applied to the field.

In the long term, efforts invested toward the enhancement of viburnum oviposition resistance might provide less-toxic, environmentally friendly tools for managing *P. viburni* in natural areas and managed landscapes.

- Long term efforts to control *P. viburni* should focus on classical biological control. Exploration of the eastern part of the native range of *P. viburni* is critical to identify new potential biological control agents and should constitute a top priority.

In addition to this applied research, the ecology and behavior of *P. viburni* and the associated plant/insect interactions constitute a fascinating system from a theoretical point of view. From the complexities of the aggregative oviposition behavior of *P. viburni* females to the evolutionary history of the *Viburnum* genus and the role *P. viburni* played in shaping viburnum traits, numerous research challenges wait to be examined.

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