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Evidence for the influence of conspecific chemical cues on *Aphthona nigriscutis* (Coleoptera: Chrysomelidae) behaviour and distribution

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Abstract. Although the distribution of biological control agents may have a significant effect upon their impacts, the mechanisms regulating these distributions are often unknown. Such is the case with *Aphthona nigriscutis*, a classical biological control agent of leafy spurge in North America. These beetles assume aggregated distributions at some sites but disperse rapidly at others. The potential influence of plant and insect-factors upon aggregation and dispersal was investigated to try to explain these observations. Male beetles produce a putative aggregation pheromone. Responses of conspecifics to male-associated cues are greater when beetles are feeding on host plants. Densities of beetle groups greatly impact their attractiveness. Males are more sensitive to dispersal cues and females are more sensitive to congregation cues.

Key words: aggregation, *Aphthona nigriscutis*, biological control, congregation, dispersal, flea beetle, leafy spurge, pheromone, weeds

Introduction

The flea beetle *Aphthona nigriscutis* Foudras (Coleoptera: Chrysomelidae: Alticinae) was introduced to North America from Hungary in 1983 as a classical biological control agent of the introduced rangeland weed, leafy spurge (*Euphorbia* spp. near *esula*) (Bourchier et al., 2002). Clumped distributions of these beetles have been documented shortly after release (Tansey, 2001) and are maintained during the early stages of population buildup at release sites. At some release sites, we have observed adult *A. nigriscutis* completely defoliating host plants near the point of release, then spreading outward in a circular pattern reminiscent of the 'solitary population wave' described by Kovalev (1990) for *Zygogramma suturalis* F. (Coleoptera: Chrysomelidae). On other established sites they disperse to form multiple new colonies (Bob Carlson, pers. comm.).

Aggregated distributions caused by conspecific cues are more appropriately referred to as congregations (Turchin, 1999). Aggregated distributions may allow herbivorous insect populations to avoid Allee effects, which result in low population growth due to an inability to find mates, overcome host defenses, or avoid predators at low densities (Allee, 1931). These are of particular concern when relatively small numbers of insects are introduced as classical biological control agents (Grevstad, 1999).

Evidence for the influence of attractive conspecific cues in aggregation has been shown for several other chrysomelids. Zhang and McEvoy (1996) suggested a female-produced sex pheromone that attracted male *Longitarsus jacobaeae* Waterhouse. Peng et al. (1999) demonstrated that male *Phyllotreta cruciferae* Goeze were attractive to both sexes in field bioassays, suggesting a male-produced aggregation pheromone. Smyth and Hoffmann (2003) also found behavioural evidence for a male-produced aggregation pheromone in *Acalymma vittatum* (F.).

In other phytophagous insect groups, production of and response to conspecific cues is influenced by sex and population density. Male *Dendroctonus frontalis* Zimmermann (Col.: Scolytidae) select host plants and, through a synergy of pheromones and host plant volatile chemicals, attract females (Wood, 1982). Males also produce a compound to repel other males from colonised host plants (Rudinski, 1973). Females also produce this compound, though in lesser concentrations. It is repulsive to males at relatively low concentrations and repellent to both sexes at higher concentrations (Renwick and Vité, 1969). Pheromone responses in *D. frontalis* may also be enhanced by host plant volatiles (Wood, 1982).

Aggregation and dispersal behaviours in *A. nigriscutis* are poorly understood but may be subject to the influence of conspecific chemical cues. Bartelt et al. (2001) isolated six male-specific sesquiterpenes from *P. cruciferae*. They also isolated these and two more from adult male *Aphthona flava*, *A. czwalinae*, and *A. cyparissiae*. Five of these compounds stimulated the antennae of male and female *A. flava* in electroantennograph tests, indicating a potential role as pheromone components. Blends of these compounds occurred in species-specific proportions in the *Aphthona* spp. tested. Compounds associated with leafy spurge plants also stimulate *A. flava* antennae (Bartelt et al., 2001).

In light of these findings, it is likely that host plant volatiles and/or an interaction between putative pheromones and host plant volatiles may also influence *A. nigriscutis* congregation and dispersal behaviour. In our study, laboratory and field trapping bioassays were used to find evidence for the influence of conspecific chemical cues on adult *A. nigriscutis*. These tests were performed with a range of beetle densities to investigate production of potential aggregation cues and assess the effect of beetle density on the production or reception of these cues. Inter and intra-sex responses were also

evaluated at different densities to determine roles of each sex and effects of conspecific density in production of cues associated with congregation. Here, we present evidence for a male-produced aggregation pheromone. Results of this study also indicate the importance of beetle density on production of attractive cues, and so on congregation and dispersal behaviour, and that plant factors increase the attractiveness of beetle cues.

Materials and methods

Beetles and plants

Aphthona nigriscutis were collected from an established population in Edmonton, Alberta and were maintained on potted leafy spurge plants in mesh cages in a laboratory at 21 ± 2 °C. Beetles were sexed based on the presence or absence of a distinctly lobate terminal abdominal sternite with a median dark line (LeSage and Paquin, 1996).

Leafy spurge plants were originally propagated from seed collected from a site near Cardston, AB in 1991. Differences in feeding preferences of *A. nigriscutis* adults for biotypes of leafy spurge have been demonstrated and can occur among plants collected from the same site (Tansey, 2001), so a single plant provided all of the root material for the propagation of plants for these tests. Cut root pieces approximately 2 cm long by 3 mm were planted in moistened Sunshine Professional™ potting mix in 15 cm diameter pots in 1998. After 1 year of growth roots from these plants were cut and planted in separate pots. Plants intended for beetle maintenance and field assays were grown in 6 in round pots and those for olfactometer tests in 5 in round pots. Plants were maintained in a greenhouse for the duration of the study.

Laboratory bioassays: olfactometer

A no-choice olfactometer (Figure 1) was designed for this study. The olfactometer consisted of a cylindrical Plexiglas™ chamber and a 1 m by 1 cm internal diameter glass tube connected by flexible Tygon™ tubing. An electric pump forced air through the chamber and carried volatiles emitted from test materials through a glass tube to its open end. Air passed through an activated charcoal filter to remove volatiles from laboratory air, distilled water to ensure consistent humidity and an airflow regulator before being pumped through the olfactometer. Air flowed through the system at 0.2 l/min corresponding to a velocity of 4.2 cm/s through the glass tube. Beetles usually responded to this airflow by moving up the glass tube. The glass tube was marked at 10 cm intervals and suspended on a metal support frame at a 15° angle. This slight

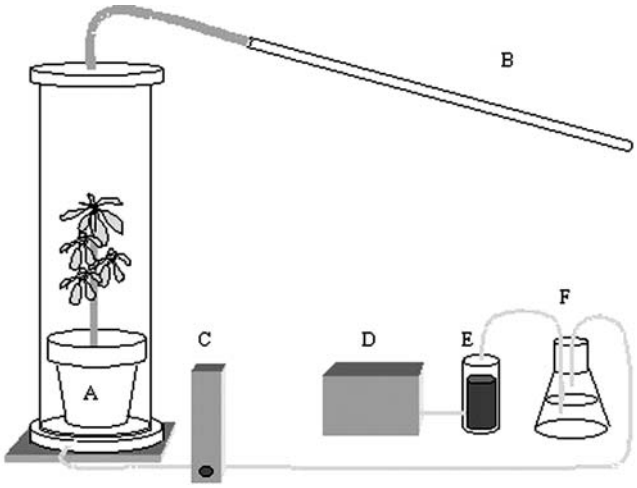

Figure 1. Olfactometer: (A) Test material and source of volatiles. (B) Glass tube. Beetles are introduced to the open end and their movements up the tube are recorded. (C) Air-flow regulator. (D) Electric air pump. (E) Activated charcoal filter. (F) Distilled water.

upward slope encouraged the movement of *A. nigriscutis* into the tube rather than the laboratory. The apparatus received continuous illumination from two 40 W fluorescent lamps placed 10 cm above its highest point.

All tests were performed in July and August 1999 in a laboratory at 21 ± 2 °C between 0900 and 2000 h (MST). Individual *A. nigriscutis* were introduced to the open end of the glass tube. The end of the tube was covered for 5 s to prevent immediate escape. The distance traveled by each beetle up the glass tube in 2 min was recorded. Beetles that moved out of the apparatus and those that remained stationary were assigned distances of zero. Distinctions between reduced attraction and active repellence were not made. All beetles were randomly chosen from a laboratory colony and starved for 24 h before each test. Beetles were not sexed for these tests unless stated. When plants and beetles were transferred to the test chamber, visual counts were performed, and in rare cases where beetles were lost or died, they were replaced. The chamber, flexible and glass tubing were washed between tests with 70% ethanol, rinsed with distilled water and dried for a minimum of 30 min. Responses of 40 beetles to each test material were assessed.

Attractiveness of volatiles from undamaged and mechanically damaged plants was assessed. In this test, 2, 4, 8 and 16 leaves per plant were damaged by removing approximately 50% of leaf tissue with scissors. These plants were transferred to the olfactometer immediately following damage. In a second test, randomly selected groups of 20, 40, 60, 80, 100 and 120 *A. nigriscutis* fed

on leafy spurge plants for 24 h. After this period, plants and beetles were transferred to the olfactometer and the responses of randomly selected conspecifics were compared to those to undamaged plants.

Intra- and inter-sex attraction was tested at both low and high densities. In this study groups of 20 or 120 *A. nigriscutis* of each sex fed separately on plants for 24 h prior to the test. Responses of each sex to these groups and plants were assessed and compared to undamaged plants. Since Peng and Weiss (1992) suggested that *P. cruciferae* aggregation pheromone was secreted in its frass we evaluated the attractiveness of *A. nigriscutis* frass to conspecifics. Filter paper was fitted around the base of a leafy spurge plant to collect frass dropped from plants by groups of 20 or 120 males or females for 24 h. Beetles were removed and the filter paper was placed into an empty chamber. The attractiveness of this filter paper was compared to that of an untreated filter paper. Although this test was intended to evaluate the potential attractiveness of beetle frass, filter paper may also have adsorbed volatiles from chamber air, or from direct contact with beetles.

Overall, responses of *A. nigriscutis* to damaged and intact host plants, conspecifics at different densities with and without host plants, small and large groups of males and females and filter paper that had been exposed to males and females at different densities were used to assess influences and potential interaction between plant and beetle cues.

Laboratory bioassays: vial test

A. nigriscutis were sexed (LeSage and Paquin, 1996) and sealed inside a 1.5 cm by 6 cm glass vial containing a 2 cm by 6 cm piece of filter paper. The paper conformed to the shape of the vial so that approximately half of the vial's inner surface was covered. After 24 h the beetle was removed. Another sexed *A. nigriscutis* was introduced to the apparatus and the vial's open end was connected to the open end of another vial containing an untreated piece of filter paper. This beetle was allowed 30 min to acclimate. Its position, in one vial or the other was then recorded as binomial data at 15 min intervals for 3 h. A beetle located on the treated side was scored as '1'. There were a minimum of 30 replicates for each combination of males and females. Beetles fed on caged plants in the laboratory for at least 24 h before each test.

Field-trapping experiment

The attractiveness of some materials examined in laboratory olfactometer tests was assessed in the field with a trapping study. Tests were conducted along the bottom of a hill parallel to previous release sites in Edmonton, Alberta. Each trap consisted of a 60 cm diameter plastic saucer filed with soapy water

(approximately 20 ml commercial liquid dish soap). Test materials, consisting of potted leafy spurge plants grown to heights of 40–50 cm in a greenhouse, or pots of soil mix, were placed in an inverted 12 cm diameter plastic saucer in the centre of each trap. Pots were covered with a fine cloth mesh bag to allow volatiles out, retain test insects and exclude field insects (Figure 2).

Two tests were conducted; each compared the numbers of males and females and total numbers of *A. nigriscutis* captured among treatments. In the first test, a potted plant, a pot of soil mix, a mechanically damaged plant and two plants with *A. nigriscutis* feeding damage (a group of 20 randomly selected beetles fed for 24 h) were used as bait. *A. nigriscutis* but not their frass were removed from one plant just before this test. Plants were mechanically damaged by cutting small pieces from 16 leaves just prior to each test.

In the second test captures with 20 male *A. nigriscutis* on plants, 20 female *A. nigriscutis* on plants, moistened soil mix and undamaged plants were compared. In both tests baits were replicated four times and arranged in a modified randomized block design. Traps were arranged at the bottom of the hill along a mowed strip, 5 m apart in a line and approximately 2 m below a dense strip of leafy spurge. Traps were examined after 48 h.

Figure 2. Soapy-water trap. (A) Test material and source of volatiles. (B) Tray filled with soapy water. (C) Cloth mesh bag. (D) Pin flag to hold up the cloth mesh bag. (E) Inverted plastic tray to keep test material above the water level.

Table 1. Results of the vial preference test

Responding sex	Sex used to treat filter paper	N_T mean ± s.e. (n = 30)	*t*-test for H_0:N_T = 6
Male	Male	5.40 ± 0.9B	p = 0.516l
Male	Female	5.20 ± 0.77B	p = 0.3095
Female	Male	8.53 ± 0.77A	p = 0.0026
Female	Female	7. 20 ± 0.70AB	p = 0.0966

N_T is the total number of occasions out of the 12 sampling times on which the responding beetle was found in the treated side. Values followed by different letters are significantly different ($p < 0.05$, Kruskal–Wallis pairwise comparisons).

Data analysis

Results of the olfactometer bioassays were transformed by $\sqrt{(x + 0.5)}$ to stabilize the variance. Beetle responses to test materials were compared by ANOVA. Means were separated using Student-Newman–Keul's test at the 5% level of significance. Comparisons of the responses of beetles to groups of conspecifics with and without plants were made by ANOVA of the full factorial design. Untransformed responses of beetles to test materials in field trapping tests were compared by ANOVA of the randomized complete block design and means separated by SNK.

Results from the vial preference test (Table 1) were analyzed with each combination of beetle sexes considered as a separate treatment. The response variable N_T was the total number of occasions out of the 12 sampling times on which the responding beetle was found in the treated side. The expected value of N_T in the absence of a response to the treated filter paper is 6. As this variable was not normally distributed, Kruskal–Wallis one-way non-parametric anova was used to detect treatment differences.

Results

Laboratory bioassays: olfactometer

A. nigriscutis demonstrated similar responses for undamaged and mechanically damaged plants ($p = 0.11$, data not shown).

Beetles were more attracted to 20 randomly chosen conspecifics on plants than to other groups ($p < 0.01$). Plants with increasing numbers of conspecifics were successively less attractive; plants with 120 beetles were significantly less attractive than uninfested plants. Without plants, groups of 20 beetles were found to be most attractive and responses decreased as group size increased.

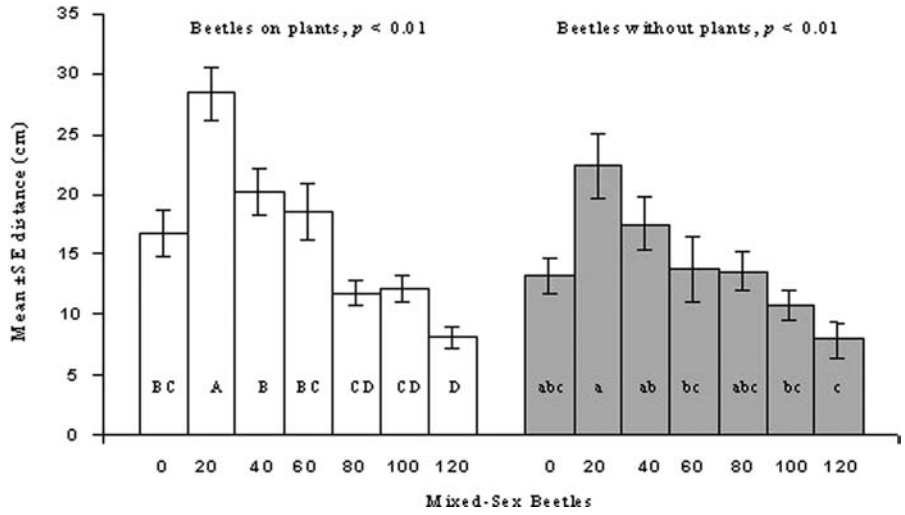

Figure 3. Responses of *A. nigriscutis* to conspecifics upon plants and conspecifics without plants. Analyses were performed on $\sqrt{(x + 0.5)}$ transformed data. Letters indicate SNK grouping of transformed data. There are no significant differences among like-lettered groups.

Response to 20 beetles without plants was significantly greater than to 120 ($p < 0.01$) (Figure 3).

Groups of beetles are significantly more attractive to conspecifics when on plants (plant presence or absence: $F = 10.34$, $p < 0.01$. Beetle numbers: $F = 17.43$, $p < 0.01$). A lack of significant interaction between beetle numbers and plant presence or absence ($F = 1.21$, $p = 0.30$), suggests that patterns of beetle response to increasing densities of conspecifics are similar with or without plants (Figure 3).

Male and female *A. nigriscutis* responded similarly to undamaged plants ($p = 0.21$) (Figure 4). Female beetles responded similarly to groups of 20 or 120 conspecific females feeding upon plants and undamaged plants ($p = 0.75$). There were differences ($p < 0.01$) in the responses of male *A. nigriscutis* to females. Males were less attracted to 120 than to 20 female *A. nigriscutis* or to undamaged plants. There were also differences in the responses of both female and male *A. nigriscutis* to males on plants ($p < 0.01$ for each). Female beetles were most attracted to 20 males. Male *A. nigriscutis* were more attracted to 20 and less attracted to 120 males than to an undamaged plant (Figure 4). Responses of female and male beetles to male conspecifics on plants were also compared. There were differences ($p < 0.01$) in the overall response of females and males to males. Females moved greater distances up the olfactometer tube toward male *A. nigriscutis* on plants than did males. There was also a significant interaction of beetle sex and beetle numbers ($p < 0.01$). Males were

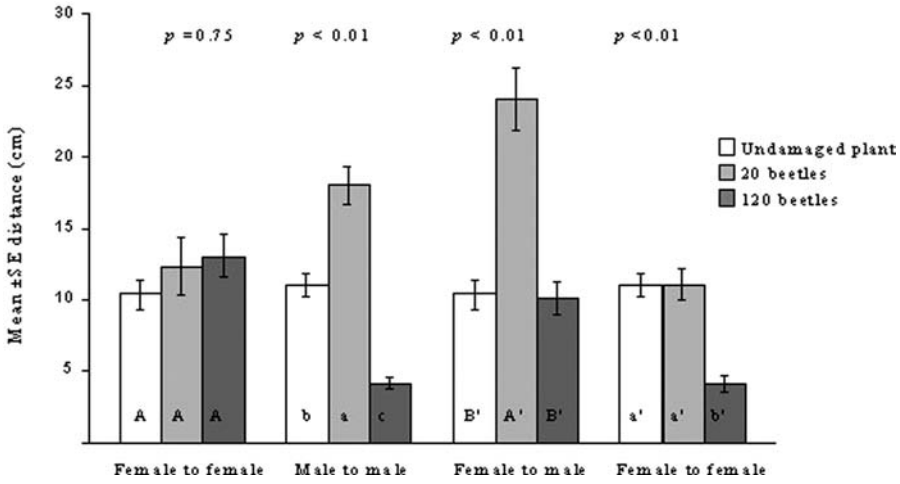

Figure 4. Responses of beetles of each sex to each sex of conspecifics on plants. Analyses were performed on $\sqrt{(x + 0.5)}$ transformed data. Letters indicate SNK grouping of transformed data. There are no significant differences among like-lettered groups.

apparently more sensitive to repellence or cessation of attractive cues and females to attractive cues.

There were differences in responses of female *A. nigriscutis* to filter paper exposed to males ($p < 0.01$) (Figure 5). This filter paper was more attractive to female beetles at densities of both 20 and 120 beetles per plant than controls. Filter paper exposed to male beetles was also more attractive to male beetles at densities of 120 beetles per plant ($p < 0.01$) than at 20 beetles per plant or to untreated filter paper. Filter paper exposed to female *A. nigriscutis*, despite the negative influence of high densities of females on plants to conspecific males, did not influence male behaviour ($p = 0.13$). Since there were no differences in the responses of females to females on plants, responses of females to filter paper exposed to females were not tested.

Laboratory bioassays: vial test

There was a significant difference among treatments (Kruskal–Wallis statistic = 12.29, $p < 0.01$). This appeared to be due to an attraction of females to male cues (Table 1). A one-sample *t*-test was also used to detect whether the values of N_T were significantly different from 6; only the value for females responding to males was significant. Females also showed slight but non-significant attraction to female cues. There was no evidence in this test for a response of males to cues from either sex.

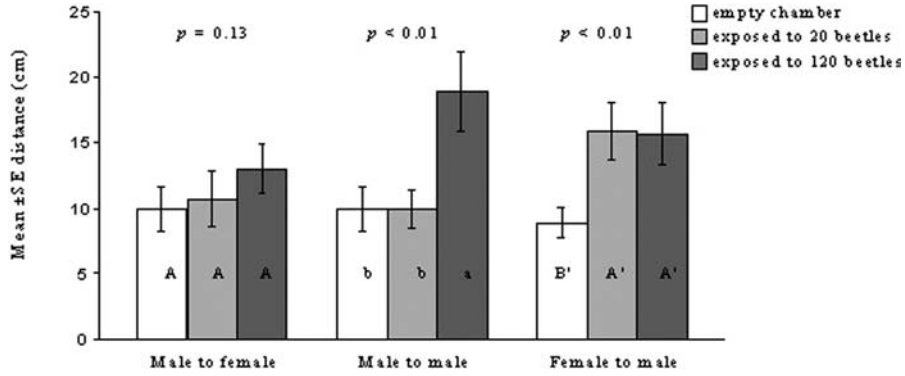

Figure 5. Responses of beetles of each sex to filter paper exposed to each sex. Analyses were performed on $\sqrt{(x + 0.5)}$ transformed data. Letters indicate SNK grouping of transformed data. There are no significant differences among like-lettered groups.

Field-trapping experiment

In the first experiment, significant differences in the total numbers of *A. nigriscutis* captured were observed among baits ($p < 0.01$). The greatest numbers of beetles were captured in traps baited with 20 conspecifics feeding upon a plant. The fewest beetles were captured in traps baited with undamaged plants or potted soil. Traps with beetle frass on plants or mechanically damaged plants captured an intermediate number of beetles. Traps baited with 20 conspecifics captured the most males ($p < 0.01$) and females ($p < 0.01$) (Figure 6).

In the second experiment, more *A. nigriscutis* were captured in traps baited with male conspecifics feeding upon a plant than those baited with females on a plant, undamaged plants or soil ($p < 0.01$). There were significant differences in the numbers of females ($p = 0.04$), but not of males ($p = 0.11$), captured with each bait. More female *A. nigriscutis* were captured with males as bait than with potted soil (Figure 7). There were also significant block effects for numbers of males, females and total numbers of *A. nigriscutis* captured (males $p < 0.01$, females $p = 0.04$, total $p < 0.01$), suggesting a clumped distribution of beetles at the site when this test was run. In addition, many fewer beetles were captured when the second test was run. We believe this difference can be attributed to the much cooler temperatures at the time of this test. *P. cruciferae* requires a minimum temperature of 14 °C for flight (Lamb, 1983). Temperature may also affect *A. nigriscutis* flight and mobility.

Discussion

Evidence from this study supports three conclusions. First, that male *A. nigriscutis* produce attractive chemical cues, to which females are more

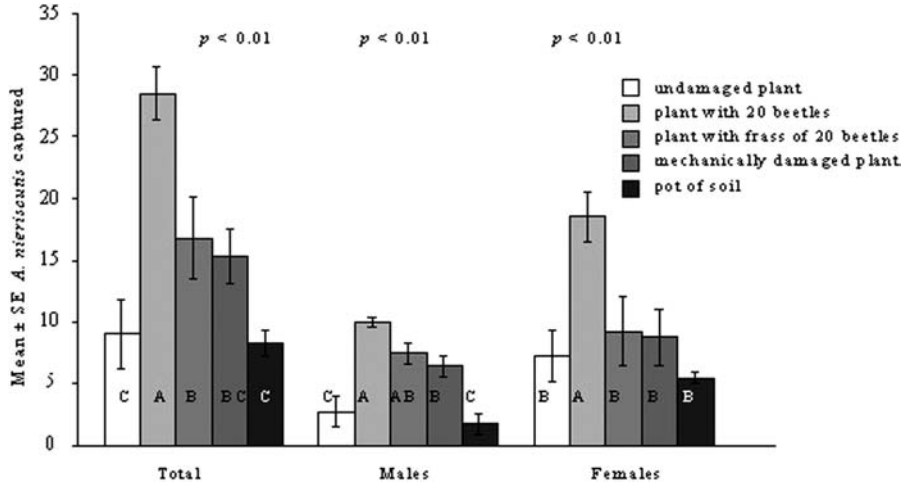

Figure 6. Field-trapping test 1. Mean total, male and female beetles captured in traps baited with different test materials. Letters indicate SNK grouping. There are no significant differences among like-lettered groups.

sensitive to than males. Secondly, that density influences the attractiveness of beetle congregations, such that a repellent effect is apparent at high densities; males are more sensitive to this repellent effect. Thirdly, that plant factors increase the attractiveness of congregation cues.

Mixed-sex groups of *A. nigriscutis* on plants were highly attractive to conspecifics at relatively low densities (20 per plant) in olfactometer and

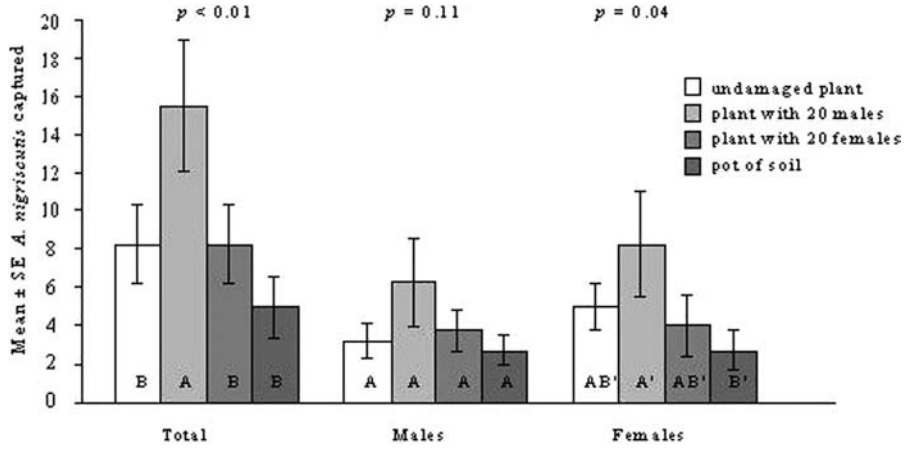

Figure 7. Field-trapping test 2. Mean total, male and female beetles captured in traps baited with different test materials. Letters indicate SNK grouping. There are no significant differences among like-lettered groups.

field-trapping tests, while in olfactometer tests, responses of beetles diminished as conspecific density increased.

Significant differences in responses of *A. nigriscutis* to same-size groups of conspecifics with or without plants were detected. Peng and Weiss (1992) suggested that *P. cruciferae* requires contact with and feeding on a suitable host plant for production of its aggregation pheromone. However, *A. nigriscutis* that fed for 24 h produce attractive cues even in the absence of plants. In vial tests, filter paper that had been exposed to male *A. nigriscutis* attracted significantly more females than untreated filter paper. Groups of 20 *A. nigriscutis* without host plants were significantly more attractive than groups of 120 in olfactometer tests. Although mixed-sex groups of *A. nigriscutis* on plants were more attractive to conspecifics than groups without plants, production of attractive cues cannot be attributed entirely to the host plant.

These results suggest an interaction of plant and beetle factors. Male *D. frontalis* select host plants and, through a synergy of pheromones and host plant volatile chemicals, attract females (Wood, 1982). Significantly more male *A. nigriscutis* were captured with field traps baited with damaged plants than with undamaged plants. Bartelt et al. (2001) isolated a leafy spurge compound that stimulated the antennae of *A. flava* in electroannenograph tests. Although distinguishing additive from synergist effects of beetle and plant volatiles without isolating these compounds is difficult, synergistic plant compounds are generally neutral or slightly repellent alone but very attractive when in combination with other compounds (Lance, 1983 cited in Ahmad, 1983). Because volatile emissions from mechanically damaged test plants were attractive to *A. nigriscutis* males in the field, the mode of interaction of attractive beetle cues and plant volatiles is likely additive.

Undamaged or heavily mechanically damaged plants did not influence behaviour of randomly selected *A. nigriscutis* in olfactometer tests. However, mechanically damaged plants were significantly more attractive to *A. nigriscutis* in field trapping tests. The skewed sex ratio (approximately 75% female) of the tested population of *A. nigriscutis* (Tansey, 2001) could have obscured male responses to volatiles from damaged plants in olfactometer tests. The best evidence for this are the greater though not significantly greater numbers of beetles captured in traps baited with mechanically damaged plants relative to pots of soil of undamaged plants.

Responses of both sexes diminished as densities of males on plants increased. 120 male and 120 female *A. nigriscutis* per plant were significantly less attractive to males than undamaged plant controls. Responses of female *A. nigriscutis* to groups of 120 males were significantly less than to plants with 20 males though not less than to undamaged plants. In this study, males are more sensitive to cues associated with dispersal or cessation of aggregation while females are more sensitive to congregation cues. Females showed greater

responses to males in olfactometer tests and significantly more female beetles were captured with field traps baited with males and plants than with those baited with females and plants or undamaged plants. The numbers of males captured by male-baited field traps were not significantly higher than other traps.

In olfactometer tests of treated filter paper, responses of males were significantly greater than untreated filter paper at 120 male conspecifics per plant. The attraction of females was greater than control plants at 20 and 120 males per plant. Males release attractive compound(s). Attractive cues may be released in frass alone, though once released they may have been adsorbed by filter paper. In addition, beetles may have contacted and potentially contaminated filter paper discs. No reduction in attractiveness with increasing conspecific density was evident in the responses of either sex to treated filter paper. A gradual decrease in attractiveness of groups of beetles as group size increases could be explained as a reduction in attractiveness and/or onset of repellence associated with an increased concentration of an aggregation pheromone. Attractive conspecific compounds are repellent to *D. frontalis* at higher concentrations (Renwick and Vité, 1969). Frass or filter paper that adsorbed male-produced congregation cues may not release them in sufficient quantities to reduce attraction.

For *A. nigriscutis*, the production or stimulation of distinct cues influences congregation and its cessation or the onset of repellence. Although groups of male *A. nigriscutis* become less attractive to females as their densities increase, no such effect was detected in responses of females to females. Females did not produce attractive cues in these tests so an effect of reduced response with an increase in attractive cues exuded by them is unlikely.

Reduced attraction may be influenced by changes in plant volatiles associated with an increase in beetle density and feeding pressure. Tobacco (*Nicotiana attenuata*) plants up-regulate production of nicotine in response to wounding. However, when fed on by *Manduca sexta* (Lepidoptera: Sphingidae) larvae, these plants down-regulate this direct defense in favour of production of volatiles that attract predaceous bugs and reduce oviposition by adult moths. Tobacco plants distinguish attacks by these caterpillars from other herbivores through the introduction of fatty acid amino acid conjugates (FAC's) in oral secretions (Baldwin, 2001 and references therein).

Groups of 120 male *A. nigriscutis* per plant repel both males and females. Groups of 120 females also reduce the attractiveness of test material to males though not to levels significantly below those of undamaged plants. The responses of males to 120 males per plant and 120 females per plant are approximately equivalent. So too are responses of females to 120 males and 120 females. Equivalent numbers of males and females should cause comparable damage and elicit the same responses from host plants. An example of

sex-specific induction of plant secondary chemistry by a foliage feeder is unknown to us. Differences in responses of males and females to equivalent numbers of conspecifics can likely be attributed to each sex's sensitivity to the same cue. This cue may be attributed to changes in plant volatiles or be of insect origin. Male and female *D. frontalis* produce a compound that repels other males from colonised host plants (Rudinsky, 1973). It is repellent to both sexes at higher concentrations (Renwick and Vité, 1969).

The influence of a male-produced aggregation pheromone has been demonstrated for the flea beetle *P. cruciferae* (Peng et al., 1999). Results of Bartelt et al. (2001) also suggest the influence of male-produced semiochemicals on other *Aphthona* spp. though their tests could not determine the functions of isolated bioactive compounds. An example of a dispersal pheromone has yet to be demonstrated in the Alticinae though the effects of crowding and resource depletion on dispersal have been demonstrated for the goldenrod beetle, *Trirhabda virgata* (Coleoptera: Chrysomelidae). Female *T. virgata* were more likely to disperse both when male beetles were absent and when host plants were heavily defoliated (Herzig, 1995). Herzig and Root (1996) suggest that this dispersal of females from heavily defoliated plants is a strategy to disperse their offspring to areas that have not been subject to over-exploitation.

A. nigriscutis collected from the Edmonton site were approximately 75% female (Tansey, 2001). Disproportionate attraction of female beetles to congregations would further skew local sex ratios thus collection sex ratios may not necessarily reflect those of an entire population. The biased sex ratio of *A. nigriscutis* does not impede local population growth. At the Beverly Bridge release site in Edmonton, Alberta, initial releases of hundreds of beetles grew to populations of hundreds of thousands within a few years (Stromme et al., 2000) apparently confounding potential Allee effects. This rapid population growth in spite of a relative scarcity of males suggests that *A. nigriscutis* employs a polygamous mating strategy.

Results of this study, particularly density dependent responses indicates that conspecific cues have the potential to contribute to the congregation behaviour demonstrated shortly after release (Tansey, 2001), solitary population wave movement and dispersal behaviour of *A. nigriscutis*. Congregation and repellence may be complementary strategies used to maintain appropriate local population densities high enough to benefit from aggregated distributions but not so high as to completely deplete resources. Following resource depletion, members of dense *Aphthona* populations tend to disperse and colonize new weed stands (R.B. Carlson, pers. comm.). These new colonies might become sources of immigrants for established colonies as their populations grow and they in turn disperse. In addition, local extinctions are common among insect species with aggregated distributions (Hanski, 1994). A congregation of *A. nigriscutis* may suffer some catastrophe, leaving a patch of resources open to

re-colonisation. Dispersal from densely populated local patches would encourage re-colonisation of patches where beetle have become extinct (Herzig and Root, 1996).

Roles of leafy spurge volatiles and semiochemicals isolated from *Aphthona* spp. by Bartelt et al. (2001) should be studied to determine if their behavioural effects and the circumstances of their production correspond to those of the conspecific and plant cues suggested by the results of this study.

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