

Attraction of Lovebugs (Diptera: Bibionidae) to Visual and Olfactory Stimuli¹

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Abstract We evaluated volatile organic compounds and colors for attractancy to adult lovebugs, *Plecia nearctica* Hardy, under field conditions in central and southern Florida. In olfactory tests, sticky traps placed at 10 m intervals (1 m height) and baited with the floral compound phenylacetaldehyde (PAA), essential oil anethole and anisaldehyde were highly attractive to both sexes of lovebugs during spring and fall flights. However, PAA was superior, capturing at least 3 times as many lovebugs in direct comparisons. Methyl salicylate, eugenol and benzaldehyde were weak attractants, whereas geraniol and citrus oil were not attractive. Heptaldehyde, 1-phenylethanol and acetophenone also were not attractive in tests that included PAA. In visual studies with unbaited sticky traps, lovebugs were most attracted to different hues of yellow and white at both high population densities (spring flight) and low population densities (fall flight). There was little statistical difference among the remaining colors (green, blue, red and black), although black traps were consistently the least attractive. We hypothesize that attraction shown in our studies is related to feeding behavior in this insect.

Key words *Plecia nearctica*, floral lures, monitoring traps, color, phenylacetaldehyde

Native to Central America, the lovebug, *Plecia nearctica* Hardy (Diptera: Bibionidae), has become established in Costa Rica, Guatemala, Honduras, Mexico and the southeastern U.S.A., with a current distribution extending to all states bordering the Gulf of Mexico as well as Georgia and North and South Carolina (Denmark et al. 2010). Lovebug larvae are detritovores, feeding on decomposing plant materials in pastures and various other moist vegetated areas (Buschman 1976). However, this insect is best known for its biannual flight periods, where large numbers of adults can be observed mating, hence the common name.

In Florida, each adult generation of lovebugs lasts about 4 wks in April-May and August-September, with a smaller third flight in December in southern Florida (Cherry and Raid 2000). During these times, large populations of day-flying adults swarming alongside roadways can become a nuisance for motorists, due to the splattering of flies on vehicles. Adults also are attracted to freshly-painted surfaces and may become an annoyance in rural or suburban areas adjacent to breeding areas (Denmark et al. 2010). The extensive breeding areas in low-value crops make area-wide control uneconomical in most cases, although cultural control methods such as removal of crop residue or improved drainage might reduce breeding populations. However, the

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role of adults in pollination and larvae in recycling organic wastes also suggest that lovebugs are beneficial species in some cases (D'Arcy-Burt and Blackshaw 1991, Hetrick 1970).

Due to their pest status, the development of effective monitoring and trapping tools for adult lovebugs is of interest. Because a sex pheromone has not been discovered and this day-flying insect does not respond to UV or tungsten light traps (Callahan et al. 1985), the identification of volatile attractants has received attention. Previously described attractants include the aromatic oil anethole (Cherry 1998), the floral compound phenylacetaldehyde (PAA) (Arthurs et al. 2012), aromatic (aldehyde) components of automobile exhausts irradiated with UV-light (Callahan and Denmark 1973, Callahan et al. 1985), as well as localized heat sources (Whitesell 1974). However, the relative attractiveness among these different volatile compounds to lovebugs is unknown, and compounds attractive to other Diptera have not been tested for lovebug attraction.

Color also has been noted in previous adult lovebug studies. Callahan and Denmark (1973) reported that freshly-painted buildings, especially light-colored ones, were attractive to adults. Thornhill (1976) used sticky traps painted white to measure dispersal of lovebug adults. Cherry (1998) used yellow sticky traps to measure adult lovebug attraction to anethole and to determine their seasonal flight (Cherry and Raid 2000). Most recently, Arthurs et al. (2012) used green sticky traps to determine lovebug attraction to floral lures. In spite of these earlier reports, color attraction of lovebugs has not been determined.

Here we compare known and prospective volatile lures, as well as color, for attractiveness to adult lovebugs under field conditions. Such information will help understand the biology and help improve monitoring strategies for this insect.

Materials and Methods

Olfactory studies. Experiments were conducted during 2012, in open grassy areas, mostly Bahiagrass (*Paspalum notatum* Flügge), in central Florida at the Mid-Florida Research and Education Center near Apopka, Orange Co., FL. Yellow sticky traps (12 × 8 cm Pestrapp™, Hummert International, Earth City, MO) were baited with various compounds in a randomized block design to test their attractiveness to adult lovebugs. Traps were suspended vertically from a horizontal boom mounted 1-m above the ground on a PCV pole driven into the ground. All compounds were dispensed separately (750 µL) in hollow polyethylene stoppers (Kimble, Vineland, NJ) attached directly adjacent to the sticky trap. An empty stopper served as a control treatment.

In the first test, we compared previously reported attractants for *P. nearctica* (PAA, anethole and heptaldehyde) along with 1-phenylethanol and acetophenone, compounds with reported attraction to other Diptera (Kamm et al. 1987). There were 6 replicates with traps placed at 10 m spacing and treatment blocks separated by at least 200 m. Tests were conducted in May when adult lovebugs were observed flying. Traps were collected after 72 h; insects were counted and sex ratio was determined as described previously (Cherry 1998). The experiment was conducted twice. Further tests were conducted in the fall flight (September) to test additional aromatic compounds known to be attractive to a wide range of insects, including 'essential' oils eugenol, geraniol, the wintergreen oil methyl salicylate (MESA), anisaldehyde, the floral chemical benzaldehyde as well as a processed

citrus oil (Orocit, Oro-Agri Inc., Trophy Club, TX). A final test directly compared the most attractive compounds from the previous 3 tests, i.e., PAA, anethole and anisaldehyde. Methods for placing and counting traps were as for the spring tests, except there were only 5 replicates in fall tests. All chemicals (except Orocit) were reagent grade purchased from Aldrich Chemical Co. (Milwaukee, WI) or Fisher Scientific Co. (Fair Lawn, NJ).

Visual studies. Tests to determine color preferences of lovebugs were conducted during 2012 on St. Augustinegrass, *Stenotaphrum secundatum* Kuntze, at the Everglades Research and Education Center at Belle Glade, Palm Beach Co., FL. Each replicate consisted of traps in a 3×2 pattern with 10 m spacing and blocks separated by at least 30 m. Traps were 20×20 cm clear plastic plates (Salad Ware, Amscan Inc., Elmsford, NY). Each plate was spray painted 1 of 6 colors (yellow, white, green, blue, red and black) and later coated with Tangle-trap Sticky Coating™ (Contech Enterprises Inc., Victoria, B.C., Canada). Traps were hung 1 m high.

All 6 colors were tested in each test with 5 replicates per test. Different hues of a color have been shown to affect attractancy of insects (Crook et al. 2012). Hence, each test used one different color hue and/or reflectivity finish (i.e., flat, satin, or glossy) for each color. Reflectivity could not be directly compared between colors because only black and white existed in all 3 finishes (flat, satin, and glossy). The object of using the 18 color variations ($= 6 \text{ colors} \times 3 \text{ variations/color}$) was to determine if any colors were consistently attractive in spite of different hues and reflective finishes. Test One was conducted from April 20 to May 9 and tests Two and Three from September 6 to October 10. These time periods correspond to the 2 major flight periods of the lovebugs in southern Florida (Cherry and Raid 2000).

Statistical analysis. Differences in numbers of lovebugs captured among treatments was determined by one-way ANOVA and Fisher's separation at $P < 0.05$ with log (n+1) transformation used if needed (SAS 2012). Chi-square analysis with Yates correction was used to determine if the sex ratio of captured individuals differed among the various treatments.

Results

Olfactory studies. Traps containing PAA lures collected significantly more lovebugs compared with other traps in both the first and second spring test, although populations had declined somewhat by the second test (Table 1). There were no differences among the remaining treatments. Traps containing anethole and anisaldehyde were most attractive in fall tests, capturing 5 - 6 times as many lovebugs as unbaited traps (Table 2). MESA, eugenol and benzaldehyde were weak attractants for lovebugs, with a small (50 - 80%) but significant increase in traps baited with these compounds, whereas geraniol and citrus oil were not attractive. The final olfactory test conducted during the peak flight period showed that, among the most attractive lures, PAA was clearly the most attractive compound capturing at least 3 times as many lovebugs as anethole or anisaldehyde (Table 3). Overall, among 8,559 lovebugs collected in olfactory tests, 4,612 (53.9%) were female. There was no statistical association between the sex of lovebug and treatment in which they were caught in spring tests ($\chi^2 \geq 4.8$, d.f. = 5, $P > 0.05$) nor the first fall test ($\chi^2 = 2.4$, d.f. = 7, $P > 0.05$). However in the final test, more females were caught in PAA (58.4% female) and anethole (58.2%) baited traps compared with anisaldehyde (52.4%) or unbaited (53.8%), ($\chi^2 = 8.5$, d.f. = 3, $P < 0.05$).

Table 1. Adult lovebugs (mean \pm SEM) collected on sticky traps baited with different lures during the spring flight (Apopka).

Lure	Test 1*	Test 2
Unbaited	12.2 \pm 5.9B	1.3 \pm 0.7B
PAA	185.3 \pm 23.0A	30.8 \pm 8.8A
Anethole	22.8 \pm 8.3B	1.0 \pm 0.4B
Heptaldehyde	12.8 \pm 5.8B	1.5 \pm 1.0B
1-phenylethanol	8.3 \pm 2.0B	1.3 \pm 0.7B
Acetophenone	10.5 \pm 2.8B	0.5 \pm 0.3B

* Means in a column followed by the same letter are not significantly different ($P < 0.05$, Fishers LSD test). ANOVA test 1 ($F_{5,30}=13.5$, $P < 0.0001$), test 2 ($F_{5,30}=14.8$, $P < 0.0001$).

Visual studies. Significantly more lovebugs were captured on yellow and white traps (Table 4) than any other color in the spring flight period (Test 1). Different hues or reflective finishes for the 6 colors were used during the fall flight period when lovebug populations were lower (Tests 2, 3). However, again yellow and white caught more lovebugs than other colors with significant differences between various colors in both tests (Table 4). There were no significant differences in lovebug catches on white versus yellow traps in any of the 3 tests. Overall, these data show that yellow and white were broadly attractive to lovebugs in both different hues and reflectivity finishes at both high population densities (spring flight) and low population densities (fall flight). There was little difference among the remaining colors in adults caught, although black was consistently the least attractive. The sex ratio was determined using methods described by Cherry (1998), and random samples of 100 adults taken from all yellow and white traps indicated had an even sex ratio ($\chi^2 = 0.26$, d.f. = 1, $P = > 0.05$).

Table 2. Adult lovebugs collected on sticky traps baited with different lures during the fall flight (Apopka).

Lure	Mean \pm SEM*
Unbaited	20.0 \pm 5.7D
MESA	31.2 \pm 4.6BC
Anethole	93.4 \pm 17.8A
Eugenol	32.0 \pm 4.7BC
Geraniol	20.8 \pm 3.3CD
Citrus oil	21.4 \pm 1.7CD
Anisaldehyde	121.8 \pm 16.7A
Benzaldehyde	36.0 \pm 3.2B

* Means in a column followed by the same letter are not significantly different ($P < 0.05$, Fishers LSD test). ANOVA ($F_{7,32}=17.2$, $P < 0.0001$).

Table 3. Adult lovebugs collected in 72 h on sticky traps baited with different lures during the fall flight (Apopka).

Lure	Mean \pm SEM*
Unbaited	63.4 \pm 19.8C
PAA	642.0 \pm 36.6A
Anethole	194.4 \pm 35.8B
Anisaldehyde	141.6 \pm 26.8B

* Means in a column followed by the same letter are not significantly different ($P < 0.05$, Fishers LSD test). ANOVA ($F_{3,16}=23.3$, $P < 0.0001$).

Discussion

Our olfactory studies confirm earlier reports (Arthurs et al. 2012, Cherry 1998) that PAA and anethole are attractants for male and female lovebugs, and provide a first report of anisaldehyde being attractive to this species. Anisaldehyde, which is also attractive to the mosquito *Aedes albopictus* Skuse (Hao and Sun 2011), is prepared commercially by oxidation of methoxytoluene (p-cresyl methyl ether) but is produced by oxidation of anethole, so it may act on the same receptors in lovebugs. However, PAA was by far the most attractive compound, capturing at least 3 times, and up to 30 times, more lovebugs compared with the other materials. It also appears that within a localized area (i.e., the 10 m trap spacing), the more potent PAA may also effectively 'mask' the attractiveness of other materials (i.e., anethole in Table 1). The same trend was not observed in the last test (Table 3), although we also noted that PAA-baited

Table 4. Adult lovebugs (mean \pm SEM) caught on sticky traps of different colors during the spring and fall (Belle Glade).

Color*	Test 1**	Test 2	Test 3
Yellow	191.2 \pm 44.2 A	3.4 \pm 0.8 AB	4.2 \pm 1.8 A
White	146.0 \pm 29.0 A	6.4 \pm 1.9 A	3.8 \pm 0.4 AB
Green	53.2 \pm 9.5 B	1.8 \pm 0.9 BC	1.0 \pm 0.6 BC
Blue	33.6 \pm 9.1 B	0.6 \pm 0.4 BC	3.3 \pm 1.4 AB
Red	27.2 \pm 3.7 B	1.8 \pm 1.1 BC	0 \pm 0 C
Black	13.0 \pm 2.9 B	0 \pm 0 C	0 \pm 0 C

* In test 1, yellow = satin Hubbell house golden maize, white = flat white, green = gloss luscious green, blue = gloss indigo cloth, red = glass cut ruby and black = gloss black. In Test 2, yellow = gloss gold abundance, white = gloss white, green = satin leafy rise, blue = satin indigo streamer, red = gloss classic red, and black = flat black. In Test 3, yellow = satin whipped apricot, white = satin white, green = gloss Montpelier palmetto green, blue = gloss exotic sea, red = satin bright red, and black = satin black. All paints are products of Valspar Corporation, Wheeling Illinois.

** Means in a column followed by the same letter are not significantly different ($P < 0.05$, Fishers LSD test). ANOVA test 1 ($F_{5,24}=10.8$, $P < 0.0001$), test 2 ($F_{5,24}=5.0$, $P < 0.005$), test 3 ($F_{5,24}=4.2$, $P < 0.01$).

traps became completely inundated with lovebugs in this test, physically preventing additional specimens being captured.

The reason PAA elicits such a strong response from lovebugs is uncertain. One hypothesis is that PAA activates feeding-based receptors on antennal sensillae, as this compound occurs naturally in many flowers, such as oakleaf hydrangea, on which lovebugs feed (Arthurs et al. 2012). Callahan and Denmark (1973) noted that lovebugs (found away from highways) appeared to fly in a searching pattern for food or water. PAA has been isolated from many flowering plants and shrubs (Haynes et al. 1991, Heath and Manukian 1992, Shaver et al. 1997) and is known to be attractive to other insects, such as noctuid moths (Meagher 2001). However, we also note that many different insects use 2-phenylacetaldehyde in their chemical communications systems (<http://www.pherobase.com>), suggesting the possibility that other behavioral responses may also be involved.

The lack of attractancy to heptaldehyde in our field tests was unexpected, as this compound (irradiated with a UV light source) was reported among the most attractive aldehydes to lovebugs in laboratory studies (Callahan et al. 1985). Those authors proposed that specific aldehydes released from vehicle exhausts mimic natural lovebug oviposition attractants released from decaying vegetation. However, because heptaldehyde was included in tests with PAA, the possibility that its attractancy was 'masked' by the more potent compound (PAA) cannot be discounted. For the same reason, the lack of any positive response of lovebugs to 1-phenylethanol, a potent attractant for mushroom flies, *Megaselia* spp. (Kamm et al. 1987), and the closely-related acetophenone cannot be considered definitive, as these compounds also were tested in the vicinity of traps containing PAA. It would be necessary to test all materials with unbaited traps only before confirming the lack of any attraction to lovebugs.

To our knowledge, the response of bionid flies to different colors has not been previously reported. We observed that lovebugs were most attracted to yellow and white. Similar observations have been reported for other day-active Diptera. Captures of tephritid fruit flies were higher on yellow and white spheres compared with various darker colors (Vargas et al. 1991, Cornelius et al. 1999). Onion fly, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae), preferentially oviposited on yellow-colored surrogate onion stems over other colors (Harris and Miller 1983). Yellow rectangles and green spheres (mimicking nuts) were considered the best combinations for monitoring walnut husk fly, *Rhagoletis completa* Cresson, suggesting that shape, as well as color, are important for monitoring purposes (Riedl and Hislop 1985). The reason that lovebugs are attracted to certain colors is unknown. However, one hypothesis is that color response might relate to flower feeding behavior. Many dipterans have UV receptors that are sensitive in the 340–360 nm range (Allan et al. 1987), and yellow flowers reflect a high proportion of UV light (Guldborg and Atsatt 1975). Male lovebugs, which have larger and structurally-distinct divided compound eyes, probably have better long-range vision compared with females (Zeil 1983). Although we did not observe a higher proportion of male lovebugs captured in any tests, we noted that the majority of males were captured as a part of a mating pair, where the female typically leads the male in coitus.

It should be noted that substantially fewer lovebugs were caught on color traps (Belle Glade) in the fall flight than the spring flight. This is consistent with R.H.C.'s observation that few lovebug adults were seen throughout Palm Beach Co. in general in the fall. In an earlier study, Cherry and Raid (2000) showed lovebug flight numbers approximately equal between spring and fall flights in Palm Beach Co. The reasons for

this decline are unknown, but followed unusually high rainfall in the region during Hurricane Isaac. The drowning of immature lovebug stages or disease epizootics favored by the wet conditions might explain this population decline. Kish et al. (1974) reported a decrease in larval populations associated with 5 species of fungi resulting in reduced lovebug flight in spring 1973. Unfortunately, this report is somewhat speculative since flight data, percentage infections, pathogenicity of the fungi, or other parameters possibly causing an epizootic were not recorded. However, Kish et al. (1974) and our study highlight our general lack of understanding concerning factors affecting lovebug abundance.

Our findings may help improve monitoring approaches for lovebugs and facilitate the development of traps to help control localized nuisance populations, such as those occurring in gardens, parks or other areas. Additional studies are needed to optimize the design of traps to maximize catch of lovebugs over an extended time period (several weeks), whereas minimizing by-catch of unwanted insects. Because PAA tends to evaporate, the deployment of this lure in some type of wax or rubber matrix might extend its release period. Using white or yellow materials would enhance attraction to the traps.

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