

## Research Article

# Capture of Nontarget Flies (Diptera: Lauxaniidae, Chloropidae, and Anthomyiidae) on Traps Baited with Volatile Chemicals in Field-Crop Habitats

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Received 30 April 2016; Accepted 18 July 2016

Academic Editor: G. Wilson Fernandes

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Volatile chemicals increased trap catch of flies from the families Lauxaniidae [*Homoneura bispina* (Loew) and *Camptoprosopella borealis* Shewell], Chloropidae (*Ocella* sp.), and Anthomyiidae (*Delia* spp.) in field crops. With lauxaniids, baiting with 2-phenylethanol on cotton-roll dispensers increased catch of *H. bispina* in two corn plot tests, and methyl salicylate increased trap catch in one test. Traps baited with methyl salicylate increased the catch of *C. borealis*. When using plastic-sachet dispensers, traps baited with methyl salicylate caught more *H. bispina* than ones baited with 2-phenylethanol, whereas traps baited with 2-phenylethanol caught more *C. borealis* than those with methyl salicylate. For chloropids, traps baited with 2-isopropyl-3-methoxypyrazine greatly increased catch of *Ocella* flies in corn and soybean. With anthomyiids, catch of male *Delia* flies in wheat increased with 2-phenylethanol on cotton rolls and with either 2-phenylethanol or methyl salicylate using plastic dispensers. In soybean, 2-phenylethanol formulated on cotton rolls or in plastic dispensers increased catch of male *Delia* flies, but methyl salicylate did not affect trap catch. Trap catch of female *Delia* flies did not vary among chemicals. In another test in soybean, trap catch of both male and female *Delia* flies was greater with 2-phenylethanol than with other volatile chemicals.

## 1. Introduction

Chemical cues are important to the basic life history of insects in activities such as locating food, finding mates, and recognizing suitable habitat [1]. However, the chemical ecology of many insects is still poorly understood. This is particularly true for noneconomic species, as methodical studies on their chemical ecology are often lacking.

Although systematic, hypothesis-driven research can produce novel information about insect chemical ecology, new knowledge may sometimes arise from unexpected sources [2]. This is frequently the case for many flight-capable insects that are captured unwittingly in traps provisioned with volatile chemicals intended to attract one or a few target species of insects [3, 4]. Substantial bycatch of a particular nontarget species may reveal a novel association between that species and the chemical [5–7]. Thus, this bycatch may be used to expand knowledge regarding the chemical ecology of various nontarget insects [4, 6].

Large bycatches of nontarget flies were sometimes unwittingly encountered in a study [8] that I conducted to evaluate responsiveness of arthropod natural enemies to various volatile chemicals among various field-crop habitats. Significant numbers of flies from three different families (Lauxaniidae, Chloropidae, and Anthomyiidae) were captured, and some captures represented potentially novel associations with volatile chemicals. The objective of this paper is to report on the associations of volatile chemicals with trap catch of the nontarget flies in my study.

## 2. Materials and Methods

Nontarget flies were captured unexpectedly on traps baited with individual volatile chemicals (Table 1; Figure 1) in seven separate field tests (Table 2) in 2004 and 2005 near Brookings, SD. As the catch of these flies was unexpected, their response to the volatile chemicals was evaluated on an ad hoc basis

TABLE 1: Volatile chemicals tested for attraction to beneficial insects in agricultural plots near Brookings, SD.

Volatile chemical	Alternate chemical name		Dosage, dispenser	
<i>Year 2004</i>				
Camphor	CAM	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one	100 mg, cotton roll	Sigma-Aldrich, St. Louis, MO
Ethanol (nonattractant control)	ETH		100 mg, cotton roll	Sigma-Aldrich, St. Louis, MO
2-Isopropyl-3-methoxypyrazine	IMP	2-Isopropyl-3-methoxypyrazine	100 mg, cotton roll	Sigma-Aldrich, St. Louis, MO
2-Phenylethanol	PE	2-Phenylethanol	100 mg, cotton roll	Sigma-Aldrich, Milwaukee, WI
Terpineol (mixed isomers)	TERP	2-(4-Methyl-1-cyclohex-3-enyl)propan-2-ol	100 mg, cotton roll	Sigma-Aldrich, St. Louis, MO
<i>trans</i> -Caryophyllene	TC	<i>trans</i> -(1R,9S)-8-Methylene-4,11,11-trimethylbicyclo-undec-4-ene	100 mg, cotton roll	Spectrum Chemical, Gardena, CA
<i>Year 2005</i>				
4-Allylanisole	4AA	1-Methoxy-4-(2-propenyl)benzene	100 mg, cotton roll	Sigma-Aldrich, Milwaukee, WI
Ethanol (nonattractant control)	ETH		100 mg, cotton roll	Sigma-Aldrich, St. Louis, MO
Ethanol (nonattractant control)	ETCL		2 mg, commercial dispenser	ChemTica USA, Durant, OK
Eugenol	EUG	3-(3-Methoxy-4-hydroxyphenyl)prop-1-ene	100 mg, cotton roll	Sigma-Aldrich, Milwaukee, WI
Isoeugenol	ISO	3-(3-Methoxy-4-hydroxyphenyl)prop-2-ene	100 mg, cotton roll	Spectrum Chemical, Gardena, CA
Methyl salicylate	MS	Methyl 2-hydroxybenzoate	100 mg, cotton roll	Sigma-Aldrich, Milwaukee, WI
	MSCL		2 mg, commercial dispenser	ChemTica USA, Durant, OK
2-Phenylethanol	PE	2-Phenylethanol	100 mg, cotton roll	Sigma-Aldrich, Milwaukee, WI
	PECL		2 mg, commercial dispenser	ChemTica USA, Durant, OK

when meaningful numbers of the flies were caught in particular tests. The tests were conducted in 0.5 to 1.5-ha plots of spring wheat, corn, and soybean at the Eastern South Dakota Soil and Water Research Farm (44°19'N, 96°46'W, 500-m elevation). Crops in the plots were grown using common agronomic practices, with no insecticide applied to plots from the planting through the sampling periods each year.

Yellow sticky traps (Pherocon AM, Trecé, Adair, OK) were used for the tests. Each trap was folded along its midline so that two faces of adhesive surface were exposed for capturing insects. The traps were deployed individually on 1-m tall stakes and set just above the canopy ( $\approx 0.7$ -m ht.) in spring wheat and soybean plots and at the top of the stake in corn plots.

Traps were baited either with a cotton roll (3.8 cm long; Patterson, St. Paul, MN) impregnated with 100 mg of a volatile chemical or with a controlled-release, plastic sachet containing 2 mg of chemical (2-phenylethanol or methyl salicylate). A chemical was applied to a cotton roll by pipette as either stock solution or with the camphor treatment after dissolution in ethanol. Ethanol was used as the control. Wicks

were prepared in the morning and clipped on a nonadhesive face of traps in the field a few hours later. The traps were deployed 10- to 30-m apart in the plots for various 2-day periods. Individual baited traps served as replicates, and treatments of volatile chemicals were replicated four to 10 times depending on the number tested and plot size.

The traps were retrieved from plots at the end of each test and taken to the laboratory and stored in refrigerators, and nontarget flies on them were identified and counted within a few days. The flies were identified to genus or species, depending on the condition of specimens on the traps; sex of the specimens was determined for Anthomyiidae by their dimorphic compound eyes (females, dichoptic; males, holoptic) [9]. Counts of nontarget flies for each 2-day trap period were subjected to separate analyses of variance by species and, for Anthomyiidae, by sex, using a generalized linear mixed model (PROC GLIMMIX [10, 11]). Treatment means were separated by the LSMEANS feature with Tukey-Kramer adjustment. Zero counts for the camphor treatment were omitted in order to support the robustness of parametric analyses in tests 1 and 2 [11].

TABLE 2: Tests of the attractancy of volatile chemicals to beneficial insects in agricultural plots near Brookings, SD.

Test	Crop	Date	Volatiles tested <sup>1</sup>	Flies evaluated
1	Corn	Aug 2–4, 2004	CAM, IMP, PE, TC, TERP, ETH	<i>Homoneura bispina</i> (Lauxaniidae); <i>Olcella</i> sp. (Chloropidae)
2	Soybean	Aug 17–19, 2004	CAM, IMP, PE, TC, TERP, ETH	<i>Olcella</i> sp.
3	Wheat	Jun 15–17, 2005	PE, PECL, MS, MSCL, ETH	Male <i>Delia</i> spp. (Anthomyiidae)
4	Corn	Jul 13–15, 2005	IMP, MS, PE, ETH	<i>H. bispina</i> , <i>Camptoprosopella borealis</i> (Lauxaniidae)
5	Corn	Jul 13–15, 2005	PECL, MSCL, ETCL	<i>H. bispina</i> , <i>C. borealis</i>
6	Soybean	Jul 14–16, 2005	PE, PECL, MS, MSCL, ETH	Male <i>Delia</i> spp.
7	Soybean	Jul 20–22, 2005	4AA, EUG, ISO, PE, ETH	Male and female <i>Delia</i> spp.

<sup>1</sup>See Table 1 for abbreviations.

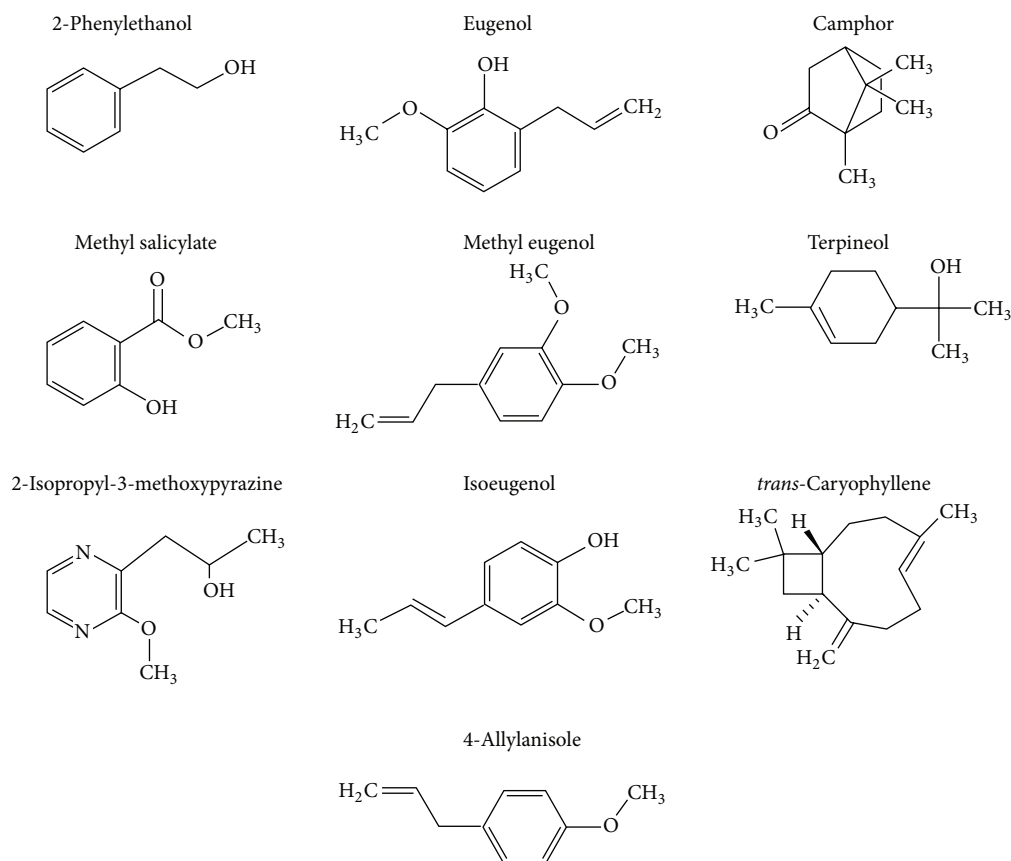


FIGURE 1: Chemical structures of test compounds.

### 3. Results

**3.1. Overview.** Significant numbers of flies from the families Lauxaniidae, Chloropidae, and Anthomyiidae were captured among the various tests. Trap counts of these flies often varied significantly among the volatile chemicals used to bait the traps. The results for each of these families are reported separately in the following.

**3.2. Lauxaniidae.** Trap catch of two species of lauxaniid flies, *Homoneura bispina* (Loew) and *Camptoprosopella borealis* Shewell, varied with volatile attractants in three tests in corn plots. In 2004 (test #1), trap catch of *H. bispina* varied with

volatile attractant ( $F = 94.63$ ; d.f. = 5, 53; and  $P < 0.0001$ ). More *H. bispina* were caught on traps baited with 2-phenylethanol compared with those baited with any other compound (Figure 2). Trap catch did not differ among the other volatile compounds, except that terpineol-baited traps had fewer *H. bispina* than ones baited with ethanol.

A follow-up test (#4) in 2005 also showed that trap catch of *H. bispina* varied with volatile chemicals ( $F = 13.31$ ; d.f. = 3, 36; and  $P < 0.0001$ ). Results confirmed that 2-phenylethanol increased trap catch of *H. bispina* over other volatiles tested and showed that trap catches were higher on traps baited with methyl salicylate compared with those baited with ethanol or 2-isopropyl-3-methoxypyrazine (IMP) (Figure 3(a)).

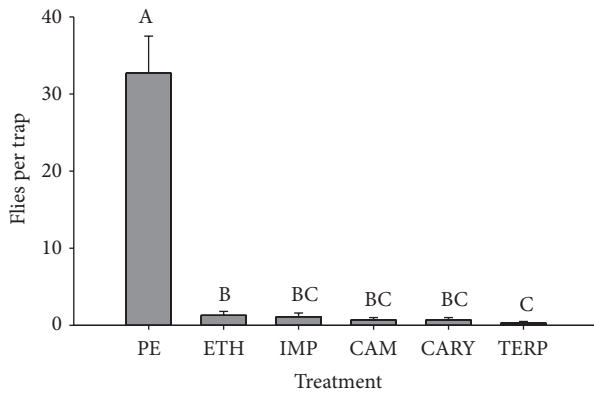


FIGURE 2: Mean number of adult *Homoneura bispina* per sticky trap ( $\pm$ SE) baited with volatile chemical from Aug 2 to Aug 4, 2004, in a corn plot near Brookings, SD (test #1). Bars without the same letter above have means that differ significantly. PE = 2-phenylethanol, ETH = ethanol (control), IMP = 2-isopropyl-3-methoxyppyrzazine, CAM = camphor, CARY = *trans*-caryophyllene, and TERP = terpineol, each at 100 mg on a cotton roll. *Homoneura bispina*, corn, 2004.

Catch of *H. bispina* did not differ between ethanol and IMP.

In addition, trap catch of *C. borealis* differed among volatiles in test #4 ( $F = 5.20$ ; d.f. = 3, 36; and  $P = 0.004$ ). Traps baited with methyl salicylate caught more *C. borealis* than those baited with ethanol (Figure 3(b)). Catch of *C. borealis* on traps baited with 2-phenylethanol or IMP did not differ from that on traps baited with either methyl salicylate or ethanol, and trap catch did not differ between 2-phenylethanol and IMP.

Trap catch of *H. bispina* and *C. borealis* each varied among volatiles in a final test using controlled-release lures (test #5). However, trap catch of lauxaniids was largely converse to that using cotton rolls as lures in test #4. That is, traps baited with methyl salicylate caught more *H. bispina* than those baited with 2-phenylethanol or ethanol (Figure 4(a);  $F = 4.41$ ; d.f. = 2, 12; and  $P = 0.037$ ), whereas traps baited with 2-phenylethanol caught more *C. borealis* than those baited with methyl salicylate or ethanol (Figure 4(b);  $F = 5.16$ ; d.f. = 2, 12; and  $P = 0.024$ ).

**3.3. Chloropidae.** Counts of an *Ocella* sp. varied among attractants in corn and soybean plots in 2004 (corn, test #1:  $F = 188.66$ ; d.f. = 4, 44; and  $P < 0.0001$ ; soybean, test #2:  $F = 73.42$ ; d.f. = 4, 15; and  $P < 0.0001$ ), as the counts were significantly more abundant on traps baited with IMP compared with traps baited with other volatile compounds. In corn, IMP-baited traps caught a mean ( $\pm$ SE) of 103.4 ( $\pm$ 12.6) *Ocella* flies per trap, whereas counts averaged  $\leq 3.3$  *Ocella* flies per trap for each of the other treatments (Figure 5(a)). In soybean, IMP-baited traps caught a mean ( $\pm$ SE) of 83.8 ( $\pm$ 9.1) *Ocella* flies per trap, whereas counts averaged  $\leq 2.0$  *Ocella* flies per trap for other treatments (Figure 5(b)). Traps baited with IMP and other attractants in 2005 (test #3) did not capture sufficient numbers of *Ocella* flies for analysis.

**3.4. Anthomyiidae.** Trap catch of adult *Delia* spp. varied by volatile chemicals in three tests. In spring wheat (test #3), catch of male *Delia* flies was greater on traps baited with 2-phenylethanol on a cotton wick than with all other treatments (Figure 6(a);  $F = 27.47$ ; d.f. = 4, 25; and  $P < 0.001$ ). Traps baited with 2-phenylethanol or methyl salicylate dispersed from a controlled-release dispenser captured more male *Delia* flies than traps baited with ethanol or methyl salicylate dispersed from cotton rolls. Numbers of female *Delia* flies did not vary among attractants ( $P = 0.059$ ).

In soybean (test #6), the catch of male *Delia* flies was greater on traps baited with 2-phenylethanol on a cotton wick or in a controlled-release dispenser than on traps with other treatments (Figure 6(b);  $F = 6.84$ ; d.f. = 4, 20; and  $P = 0.001$ ). The numbers of male *Delia* flies did not differ among traps baited with a controlled-release lure of methyl salicylate or ones baited with cotton rolls imbued with ethanol or methyl salicylate. Trap catch of female *Delia* flies did not vary among volatile chemicals ( $P = 0.16$ ).

In a second test in soybean (test #7), the trap catch of both male ( $F = 17.25$ ; d.f. = 4, 20; and  $P < 0.001$ ) and female *Delia* flies ( $F = 4.20$ ; d.f. = 4, 20; and  $P = 0.013$ ) varied among volatile chemicals. More male and female *Delia* flies were captured on traps baited with 2-phenylethanol than on those baited with other volatile chemicals (Figure 7); trap catch of *Delia* spp. did not vary among eugenol, isoeugenol, 4-allylanisole, or ethanol.

## 4. Discussion

Some of the findings in this study represent novel reports of attractancy for particular volatile compounds to the two lauxaniid species, *Homoneura bispina* and *Camptoprosopella borealis*, and to the chloropid flies in the genus *Ocella*. However, previous reports have documented responses of *Delia* flies to traps baited with volatile attractants such as 2-phenylethanol [12]. Results of this study are compared below with findings of other studies for each group of flies.

**4.1. Lauxaniidae.** The attractancy of 2-phenylethanol and methyl salicylate to lauxaniids is novel, but the basis for their attraction is unclear. Consideration of lauxaniid biology and the natural occurrence of 2-phenylethanol and methyl salicylate may allow for inference about the basis of attraction for these two volatiles. Specific knowledge about the biology of *H. bispina* and *C. borealis* is lacking. Adult lauxaniids generally feed on fungi [13] and visit flowers [14], and larvae are typically saprophagous among fallen leaves, straw, rotting wood, and bird nests [15]. With regard to the chemicals, 2-phenylethanol is a compound associated with decaying vegetation [16], and methyl salicylate is induced in plants by herbivory to act as a volatile attractant of herbivore natural enemies [17]. However, 2-phenylethanol and methyl salicylate are also floral volatiles that attract pollinators [17, 18]. Thus, given the biology of lauxaniids, the increased trap catch of *H. bispina* and *C. borealis* to 2-phenylethanol and methyl salicylate may reflect an attraction to these compounds as floral attractants or as decaying vegetation that

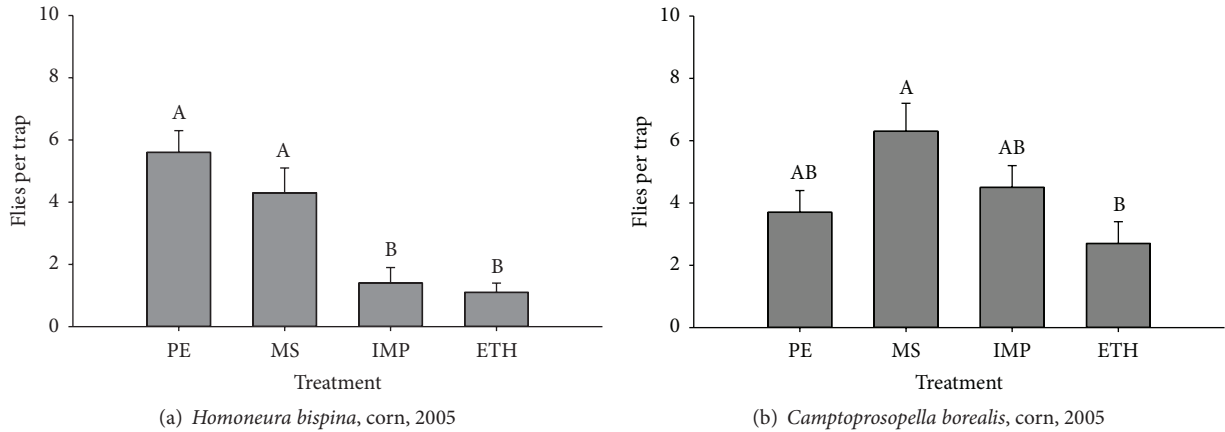


FIGURE 3: Mean number of lauxaniid flies per sticky trap ( $\pm$ SE) baited with volatile chemicals from Jul 13 to Jul 15, 2005, in a corn plot near Brookings, SD (test #4). For each species, bars without the same letters above them indicate that the means differ significantly. PE = 2-phenylethanol, MS = methyl salicylate, IMP = 2-isopropyl-3-methoxypyrazine, and ETH = ethanol (control), each at 100 mg on a cotton roll. (a) Adult *Homoneura bispina*; (b) adult *Camptoprosopella borealis*.

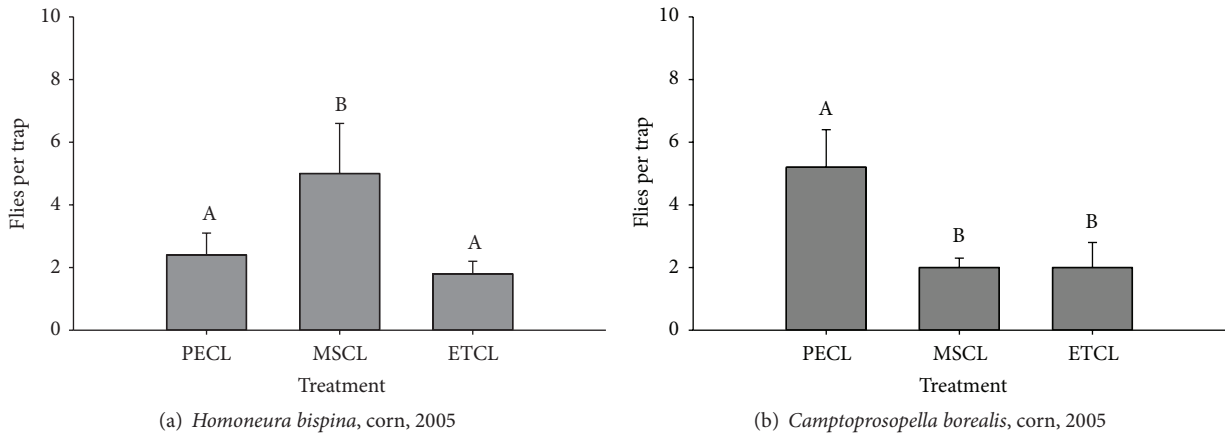


FIGURE 4: Number of lauxaniid flies per sticky trap ( $\pm$ SE) baited with 2 mg in controlled-release dispensers of ethanol (control) = ETCL, methyl salicylate = MSCL, or 2-phenylethanol = PECL in corn plots near Brookings, SD, Jul 13–15, 2005 (test #5). (a) Adult *Homoneura bispina*; (b) adult *Camptoprosopella borealis*. For each species, bars without the same letter above them indicate that the means differ significantly.

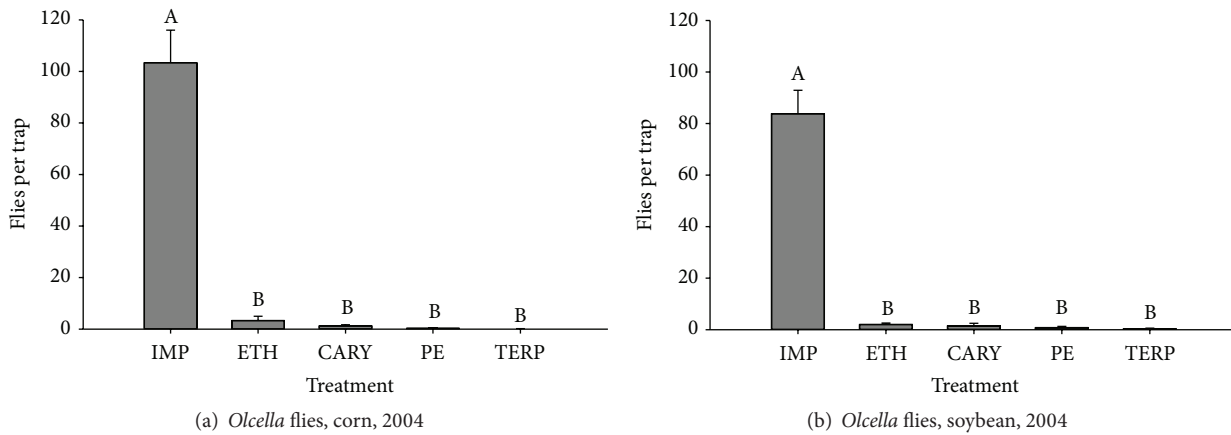


FIGURE 5: Mean number of adult *Olcella* sp. flies per sticky trap ( $\pm$ SE) baited with 100 mg of volatile chemicals in plots near Brookings, SD. Chemicals dispensed on cotton rolls. Bars without the same letters above them indicate that the means differ significantly. (a) Aug 2–4, 2004, corn plot (test #1). (b) Aug 17–19, 2004, soybean (test #2). IMP = 2-isopropyl-3-methoxypyrazine, ETH = ethanol (control), CARY = *trans*-caryophyllene, PE = 2-phenylethanol, and TERP = terpineol. Zero counts for camphor (CAM) traps not included.

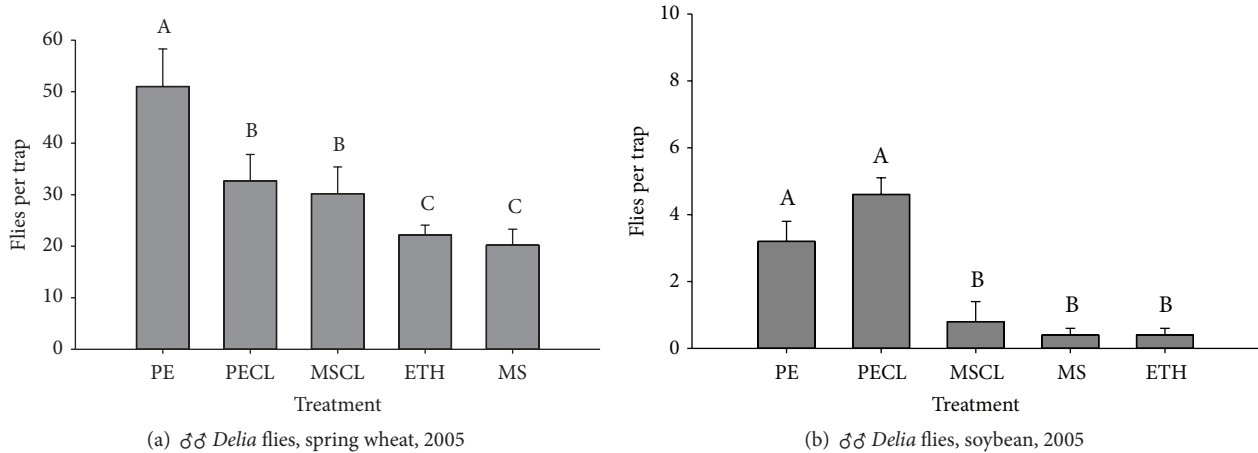


FIGURE 6: Mean number ( $\pm$ SE) of male *Delia* flies captured near Brookings, SD, on sticky traps baited with a volatile attractant. (a) Spring wheat, Jun 15–17, 2005 (test #3). (b) soybean, Jul 14–16, 2005 (test #6). PE = 2-phenylethanol, MS = methyl salicylate, and ETH = ethanol (control), each at 100 mg on a cotton roll; PECL = 2-phenylethanol and MSCL = methyl salicylate, each at 2 mg on a controlled-release lure. For each graph, bars without the same letter above them indicate significant differences between means.

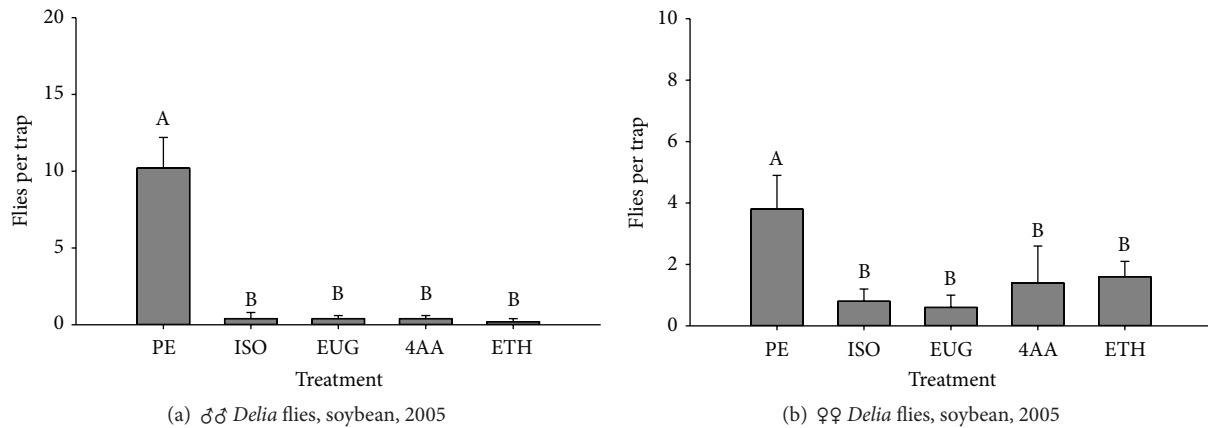


FIGURE 7: Mean number ( $\pm$ SE) of male (a) and female (b) *Delia* flies captured on sticky traps baited with volatile chemical, Jul 20–22, 2005, in a soybean plot near Brookings, SD (test #7). For each sex, bars without the same letters above them indicate that the means differ significantly. PE = 2-phenylethanol, EUG = eugenol; ISO = isoeugenol; 4AA = 4-allylanisole, and ETH = ethanol (control), each at 100 mg on a cotton roll.

may indicate suitable larval habitat to ovipositing females. The differential responses of the two species to these compounds emanating from either cotton rolls or controlled-release septa indicate that concentration and release rate influence lauxaniid response. Both cotton and plastic have been used effectively in studies with volatile attractants and flies; however, dispenser type may be a factor affecting trap catch, as different dispensers release volatile compounds at different rates [19, 20]. Further study is needed to clarify these relationships.

**4.2. *Ocella* Flies.** The results of my study constitute the first report of attractancy of 2-isopropyl-3-methoxy-pyrazine (IMP) to *Ocella* sp. (and other insects). It significantly attracted large numbers of *Ocella* sp. in two tests in August 2004 but failed to catch meaningful numbers of *Ocella* sp. in a July 2005 test. The phenology of *Ocella* flies is not known for field crops in South Dakota. However, as sampling of insects

directly relates to their abundance and activity, and trap catch can reflect the temporal dynamics of insects [21, 22], I hypothesize that the disparity in trap catch between years may have been due to differences in abundance or activity of *Ocella* flies at the respective times of year (July versus August) when the tests were run. I suggest future tests across a broader time frame to determine the temporal dynamics involved in attraction of *Ocella* flies to IMP.

The basis of attractancy of IMP to *Ocella* sp. is unclear. IMP is a pungent volatile and structural analog of methoxyalkylpyrazines (MAP), which are defensive compounds of aposematic insects [23, 24]. As such, IMP was originally intended as a repellent control in screening volatile compounds for natural enemy responsiveness, when its unexpected attractancy to *Ocella* was discovered. Other studies have shown that *Ocella* spp. are attracted to various volatile compounds [6, 25–27]. For instance, *Ocella trigramma* (Loew) is attracted to hexyl butyrate and (*E*)-2-hexenyl



butyrate, defensive compounds in the metathoracic scent glands of stink bugs (Hemiptera: Pentatomidae) [27] that seep out upon death [28]. Furthermore, chloropid flies are attracted to stink bugs and other true bugs (Hemiptera) that emit defensive secretions when being trapped in spider webs [29], and Zhang and Aldrich [27] have suggested that these defensive compounds function as kairomones to scavenging flies. Accordingly, I hypothesize that the increased trap catch of *Olcella* flies in my study was due to analogous attraction of these flies to IMP as a defensive secretion and suggest further testing of IMP and other MAPs for their attractancy to chloropids.

Alternatively, however, if *Olcella* flies are attracted to defensive compounds of trapped insects (e.g., in spider webs [27]), their attraction to IMP-baited traps could have ultimately been influenced by the capture of other insects on those traps. However, capture of other insects was not appreciable on IMP-baited traps in the present study. Thus, the capture of high numbers of *Olcella* flies was likely due to their direct attraction to IMP.

**4.3. *Delia* Flies.** Previous reports have shown attractancy of 2-phenylethanol to *Delia* spp., and male *Delia* are particularly responsive to this compound [12, 16, 30]. 2-Phenylethanol was shown to be a key component of decomposing onion pulp that attracted onion flies [*D. (=Hylemya) antiqua* (Meigen)] and seedcorn maggot flies [*D. (=Hylemya) platura* (Meigen)]. Correspondingly, attraction of *Delia* spp. to 2-phenylethanol has been documented in onion [12, 16, 30] but also in snap bean fields [12]. However, my study is the first known to document a trapping response of *Delia* spp. to 2-phenylethanol in wheat and soybean fields. Some species, such as *D. platura* and *D. florilega* (Zetterstedt) (bean seed maggot), are pests of seedling soybean [31], especially in fields where ovipositing females are attracted to soil that has had manure applied or green plant material incorporated [32]. Traps baited with attractants such as 2-phenylethanol might be useful as a monitoring tool to assess *Delia* populations in soybean fields [12]. However, tests may be needed to determine how the presence of competing volatiles associated with manure and decaying green plant material might affect the catch of traps baited with synthetic volatile attractants.

## 5. Conclusions

In summary, three types of flies were caught serendipitously in this study on yellow sticky traps that had been baited with a relatively limited range of attractants and placed in a limited number of field-crop habitats at a single location in eastern South Dakota. As such, there is a rich set of follow-up questions that may be pursued systematically in future studies at various geographic locations. These studies may include tests of chemical analogs and varied dosages of the volatiles used here in order to determine structure-activity relationships and sensitivity involved in the flies' attraction. Future studies may be conducted to determine if response varies by sex and reproductive status, particularly for lauxaniids and *Olcella* flies. In addition, future studies may

also test attractancy among dispensers with different release rates [19] and across different trap designs, including traps ones that leave flies intact upon removal and thereby facilitate researchers' ability to readily identify flies to species. Finally, additional studies on the basic biology of *H. bispina*, *C. borealis*, and *Olcella* flies are needed to make inferences about the bases for responses to volatile chemicals. The suggested studies are likely to greatly expand knowledge about the chemical ecology of these groups of flies.

## Disclosure

The paper reports research results only. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by USDA and does not imply its approval to the exclusion of other products that may also be suitable.

## Competing Interests

The author declares that there are no competing interests regarding the publication of this paper.

## Authors' Contributions

Eric Beckendorf, David Mills, Kendra Jensen, Hanna Fetzer, Joshua Pedro, and Ryan Rubbelke provided technical assistance. Stephen Gaimari and Jon Kieckhefer identified flies. Mark West advised on statistical analysis. Sharon Papiernik and Fathi Halaweish provided advice on drawing chemical structures. Jan Menely, Michael Catangui, and Leslie Hammack advised on attractants. Eric Beckendorf, Lauren Hesler, Deirdre Prischmann-Voldseth, Leslie Hammack, and Sharon Papiernik graciously reviewed drafts of this paper.

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