

POLISTES SPP. (HYMENOPTERA: VESPIDAE) ORIENTATION TO WINE AND VINEGAR

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ABSTRACT

Attractants are sought for trapping of *Polistes* spp. paper wasps when they are pestiferous. The serendipitous capture of *Polistes metricus* Say and *Polistes bellicosus* Cresson in traps baited with a wine/vinegar mixture for spotted wing drosophila, *Drosophila suzukii* (Matsamura) prompted experiments to determine the nature of the wasp response. Both wasp species were captured in subsequent field tests in traps baited with the same mixture of wine plus vinegar, and not in unbaited traps. *Polistes bellicosus* responses to wine, vinegar, ethanol (as a major volatile of wine), and acetic acid (as a major volatile of vinegar) were evaluated using a Y-tube olfactometer. In the olfactometer, *P. bellicosus* wasps were attracted to wine and not to vinegar. They also preferred wine alone to wine with vinegar, and were attracted to ethanol. Female wasps were deterred by acetic acid. In field tests comparing traps baited with wine, vinegar and a combination of the 2 materials, *P. bellicosus* and *Polistes fuscatus* (Fab.) were captured in traps baited with wine, but were not trapped with vinegar. The inclusion of vinegar with wine did not improve bait attractiveness in the field. We conclude that the paper wasp response to the *D. suzukii* bait of wine plus vinegar was largely the result of the wasp response to wine. This work constitutes the first demonstration of an attractive bait that can be used to trap *P. bellicosus* and *P. metricus* in situations where they are pestiferous, and suggests a potential source of a chemical attractant based on wine volatiles.

Key Words: *Polistes*, paper wasp, attractant, bait, trap

RESUMEN

Las avispas de papel, *Polistes metricus* Say y *Polistes bellicosus* Cresson, fueron capturadas en Mississippi en trampas cebadas con una mezcla de un vino Merlot y un vinagre de arroz que se pone en el campo para la mosca drosophila de alas manchadas, *Drosophila suzukii* (Matsumura). En una prueba de campo subsiguiente, las avispas *P. bellicosus* y *P. metricus* quedaron atrapadas de nuevo con la mezcla de vino y vinagre, pero no en las trampas sin cebo. La respuesta de *Polistes bellicosus* al vino, vinagre, etanol (como un importante volátil de vino) y ácido acético (como un importante volátil de vinagre) fue evaluada utilizando un olfatómetro de clase tubo-Y. En el olfatómetro, las avispas *P. bellicosus* fueron atraídas por el vino pero no por el vinagre. También prefirieron vino solo al vino con vinagre, y fueron atraídas por el etanol, pero disuadidas por el ácido acético. En una prueba de campo comparando trampas cebadas con el vino, vinagre y una combinación de los 2 materiales, *P. bellicosus*, *P. metricus* y *Polistes fuscatus* fueron capturadas en trampas cebadas con el vino, pero no fueron capturadas con vinagre. La inclusión de vinagre con el vino no mejoró el atractivo del cebo. Llegamos a la conclusión de que la respuesta de las avispas de papel hacia el cebo de vino y vinagre para la mosca drosophila de alas manchadas fue el resultado de la respuesta de la avispa al vino solo. Este trabajo constituye la primera demostración de un cebo atractivo que se puede utilizar para atrapar *P. bellicosus* y *P. metricus* en situaciones cuando son una molestia y sugiere una fuente potencial de un atrayente químico basado en compuestos volátiles del vino.

Palabras Clave: *Polistes*, avispa de papel, atrayente, cebo, trampa

Social wasps in the genus *Polistes* are sometimes referred to as paper wasps, a term which also refers to a much broader group of Vespidae (Greene & Caron 1980). For our purposes, we use the term paper wasp exclusively to refer to species of *Polistes*. Paper wasps are predators of other insects and thus are often considered beneficial. They have been evaluated for potential to control lepidopteran larvae on vegetable crops (Lawson et al. 1961; Gillaspay 1979), and their numbers can be artificially increased for this purpose with the use of appropriate nest boxes (Gould & Jeanne 1984). However, under certain circumstances, some species of paper wasps achieve a significant pest status.

At times, paper wasps damage fruit crops, are a stinging hazard, and can be invasive. For example, *Polistes dominula* (Christ) in North America damages ripening cherries, grapes, and other thin skinned fruits (Galvan et al. 2008; Cranshaw et al. 2011). *Polistes olivaceus* (DeGeer) damages grapes in Iraq (Al-Mahdawi & Al-Kihani 2011) and India (Saxena 1970). *Polistes dominula* is a stinging hazard in part because of a propensity to build nests within and on man-made objects and structures (Silagi et al. 2003). *Polistes fuscatus* (Fab.), *Polistes metricus* Say, *Polistes bellicosus* Cresson and *Polistes dorsalis* (Fab.) can be a nuisance and a hazard in autumn when they swarm at tall structures (Reed & Landolt 1991). These swarm sites appear to be female overwintering locations, and males are active at the same sites, presumably to intercept arriving females. Some species of *Polistes* have greatly expanded distributions and abundances, facilitated by human traffic and trade. *Polistes dominula* is spread throughout much of North America (Hathaway 1981), and has partially displaced native paper wasps such as *P. fuscatus* (Gamboia et al. 2002). *Polistes versicolor* is introduced to the ecologically sensitive and historic Galapagos Islands, where it competes with native vertebrate predators for insect prey (Causton et al 2006). Other examples of invasive paper wasps are *Polistes aurifer* Saussure throughout the Hawaiian Islands (Carpenter 2008) and *Polistes chinensis antennalis* Pérez in New Zealand (Clapperton et al. 1996).

Mitigation of social wasp numbers and pest status can sometimes be achieved with poison baits (Chang 1988; Hanna et al. 2012) or lures for traps that are based on food materials (Dvorak & Landolt 2006; Ross et al. 1984; Silveira et al. 2005; Spurr 1995, 1996) or chemical attractants (Davis et al. 1969, 1973; Landolt 1998). However, there are no demonstrated food baits or chemical attractants that might be used to trap most paper wasps. Exceptions are *P. aurifer* and *P. fuscatus* which are attracted to acetic acid with isobutanol (Landolt 1999; Landolt et al. 1999; Reed & Landolt 2003).

Polistes paper wasps forage at times for non-prey types of foods; principally carbohydrate-rich or sweet materials (Barrows 1979; Beckmann and Stucky 1981; Cranshaw et al. 2011; Hardy 1988; Martinson et al. 2013; Young 1984; MacKenzie 2004; MacKenzie et al. 2006). Paper wasps have been captured in traps baited with fermented sweet materials (Wegner & Jordan 2005, Dvorak & Landolt 2006, Dvorak 2007, MacKenzie 2004), but the wasp response has not been demonstrated experimentally. Carbohydrate-foraging behavior of paper wasps might provide a basis for discovery of chemical attractants that could be used to detect or manage pest species.

We observed the capture of numbers of paper wasps in traps baited with wine and vinegar as part of a series of field tests to develop a chemical attractant for spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Cha et al. 2013). The identification of *D. suzukii* antennal-active compounds from the wine/vinegar bait, using an electroantennal detector (EAD), ultimately generated a good chemical attractant for the fly when tested in the field. The combination of wine and vinegar had been shown to be a superior bait for *D. suzukii* (Landolt et al. 2012) and was then used as a positive control for the field testing of chemical blends. The incidental captures of paper wasps in those *D. suzukii* trapping tests provided the starting hypothesis for this work, that *P. bellicosus* and *P. metricus* are attracted to volatile compounds emitted from the wine and vinegar bait. These two species of *Polistes* were targeted in our studies because they dominated the wasp trap catch in the *D. suzukii* field tests in Mississippi (Cha et al. 2013) and they are among the species that are pestiferous at towers in the southeast U.S (Reed & Landolt 1991). The objectives of our subsequent field and laboratory experiments were to determine the respective roles of wine and vinegar in paper wasp responses to the mixture of the two materials, and any roles of ethanol and acetic acid, the most abundant organic volatiles of wine and vinegar respectively.

MATERIALS AND METHODS

General Procedures for Field Trapping Studies

Three field tests were conducted using Trap-pitt® or dome traps (Agrisense Ltd., Pontypridd, UK), that are shaped like the McPhail trap design (Newell 1936). The dome traps have an opaque yellow plastic bottom receptacle that holds a liquid bait or drowning solution, a 6 cm diam hole and funnel in the center of the trap bottom for insect entry to the trap, and a clear plastic top cover. All traps contained either 300

mL of a liquid food type bait, 300 mL of water, or 300 mL of aqueous solutions of ethanol or acetic acid. Also, all traps contained soap (0.0125% Palmolive Clear and Clean Spring Fresh Dishwashing Soap, Colgate-Palmolive Co., New York, New York) to reduce surface tension and boric acid (1%, Fisher Scientific, Santa Clara, California) to suppress decomposition of captured insects in the trap liquid. Polypropylene vials (4 mL, Nalge Nunc, Rochester, New York) that were used as chemical dispensers were suspended from the top inside of the trap by a wire. The wine used for all experiments was Carlo Rossi Reserve Merlot (Modesto, California), and the vinegar used was Safeway Select Rice Vinegar (Pleasanton, California).

This paper reports results for captures of *Polistes* paper wasps in traps, and results for other types of wasps are not reported here.

Field Experiment 1. Observation of Wasps Trapped with a Mixture of Wine and Vinegar

This experiment was part of the study to develop chemical lures for *D. suzukii* (Cha et al. 2013). The objective of this test was to determine *D. suzukii* responses to 3 blends of EAD-active chemicals that are volatiles from Merlot wine and rice vinegar. Trap treatments were the wine/vinegar mixture (300 mL per trap of 60% wine and 40% vinegar), chemical blend 1 (acetic acid, ethanol, acetoin, grape butyrate, methionol, isoamyl lactate, 3-phenylethanol, and diethyl succinate), chemical blend 2 (acetic acid, ethanol, acetoin, grape butyrate, and 2-phenylethanol), and chemical blend 3 (acetic acid, ethanol, acetoin, ethyl lactate, and methionol). Acetic acid (1.6%) and ethanol (7.2%) were mixed with the drowning solution of the trap for the chemical blend treatments. For chemical blend 1, acetoin, (23%), grape butyrate (1%), methionol (2%), isoamyl lactate (1%), 2-phenylethanol (47%), and diethyl succinate (26%) were mixed at the proportions indicated and dispensed as one mL from a single 4 mL vial with a 3 mm diam hole. For chemical blend 2, acetoin (54%), grape butyrate (7%) and 2-phenylethanol (39%) were mixed at the proportions indicated, and dispensed as one mL from a single 4 mL vial with a 3 mm hole. For chemical blend 3, acetoin, ethyl lactate, and methionol were dispensed from separate 4 mL vials, each with a 3 mm hole. Acetoin was mixed 1:1 with water and loaded as 2 mL per vial. Ethyl lactate and methionol were each loaded at one mL per vial.

A randomized complete block design was used, with 10 blocks of the 4 treatments maintained from 24 Jul to 7 Aug 2012. Traps were placed at a height of 1 m, and traps were 20 m apart within a block. Traps were checked

and drowning solutions and liquid baits were replaced weekly. Trap sites were in Stone Co., Mississippi, in experimental and commercial blueberry fields. There were no unbaited traps as negative controls because the objective of this test was to compare chemical lures to the wine/vinegar mixture that is a bait for *D. suzukii* (Landolt et al. 2012; Cha et al. 2013).

Field Experiment 2. Wasps Trapped with a Mixture of Wine and Vinegar

Traps baited with the same mixture of wine and vinegar (with soap and boric acid) were compared to traps with a drowning solution containing soap and boric acid and no bait or lure. Five replicates of this comparison were maintained at 3 locations: Moody Air Force Base in Lanier Co., Georgia, Shaw Air Force Base in Sumter Co., South Carolina, and the University of Florida, Gainesville, Alachua Co., Florida. Traps were maintained during Feb and Mar. Numbers of wasps per trap were summed over the 4 weeks of the test, to provide 5 replicates per location and 20 replicates for the test.

Field Experiment 3. Wasps Trapped with Wine, Vinegar, and the Combination of Wine and Vinegar

This experiment tested the hypotheses that wine and vinegar are attractive to paper wasps and the 2 materials are positively interactive or co-attractive. Traps were baited with 1) the drowning solution as a blank or control, 2) 60% wine in water, 3) 40% vinegar in water, and 4) a 60:40 mixture of wine and vinegar. Five replicates of this 4-treatment comparison were set up on the campus of the University of Florida, using a randomized complete block design. Traps were on stakes at a height of 1 m, with 10 m between traps within a block. The Florida experiment was maintained from 22 Jul to 9 Sep 2013. Five replicates of the same experiment were maintained in Tulsa, Oklahoma from 24 Sep to 22 Oct 2013. At both locations, trap baits were replaced weekly.

General Procedures for Laboratory Olfactometer Studies

Four laboratory experiments were conducted using a Y-tube type olfactometer, similar to that used by MacKenzie et al. (2006, 2008). *Polistes bellicosus* wasps used in olfactometer tests were acquired in late September into November 2012 from nests in Gainesville, Florida and near Valdosta, Georgia., and from inside of a shed at the Mississippi State University McNeill Research Farm, Pearl River Co., Mississippi, and again from the McNeill Farm in November 2013. Wasps

were held in groups of 20-25 wasps per steel screen cage (20 × 20 × 20 cm) in a greenhouse. Greenhouse illumination was supplemented by overhead halogen lamps on a 16:8 L:D cycle, and was kept at 25 ± 1 °C. Each cage was provisioned with 5.3 cm diam plastic petri dishes with water on cotton balls and sugar water on cotton balls, and a 50 mL water jar that wetted paper toweling on the cage top. Wasps were moved to a clean cage without sugar water (starved) 24 h prior to their use in assays, were placed in a controlled environment room 3 to 5 h before assays, and then transferred to 20 mL plastic polystyrene vials (1 wasp per vial) 1 h before assays. Bioassays were conducted at 24 °C, and 64% RH, with overhead fluorescent lighting, between 10 am and 2 pm. Treatment solutions were placed in Petri plates in each holding chamber of the olfactometer. Wasps were tested individually, as a series of 10 wasps, with each wasp observed for up to 2 min. A choice by a wasp was indicated upon its first entry into an arm, at which time the assay was ended and the wasp removed. After the testing of 10 wasps, the treatment chambers, arms of the olfactometer Y, and connecting tubing were switched from right to left, and a second series of 10 wasps were assayed. After assays, wasps were returned to cages with water and sugar water, in the greenhouse. Wasps were not re-used in bioassays within a 72 h period. Olfactometer glassware and tubing was cleaned between assay days with detergent water (Micro-90), a water rinse, and then an acetone rinse. Cleaned glassware was baked in an oven at 350 °C for 4 -12 h before use.

As in field tests, all olfactometer experiments used Carlo Rossi Reserve Merlot (Modesta, California), and Safeway Select Rice Vinegar (Pleasanton, California) as the wine and vinegar treatments.

Laboratory Experiment 1. Male *P. bellicosus* Olfactometer Response to a Mixture of Wine and Vinegar

We tested the hypothesis that *P. bellicosus* wasps are attracted to a mixture of wine and vinegar in comparison to water, as a single 2-choice test in the olfactometer. One mL of a 60% wine and 40% vinegar solution was placed in a petri dish in one holding chamber, and one mL of water was placed in a Petri dish in the other holding chamber. The assay procedure described above for the testing of 10 wasps was conducted 14 times for a total of 140 wasps assayed.

Laboratory Experiment 2. Male and Female *P. bellicosus* Olfactometer Responses to Wine, Vinegar, and Wine plus Vinegar Mixed

We tested the hypotheses that male wasps are preferentially attracted to the combination

of wine and vinegar, compared to wine alone, or vinegar alone, as two 2-choice tests in the olfactometer. The first test compared wasp responses to one mL of a mixture of 60% wine and 40% vinegar placed in a petri dish in one holding chamber, and one mL of 60% wine in water was placed in a Petri dish in the other holding chamber. The second test compared one mL of a mixture of 60% wine and 40% vinegar to one mL of 40% vinegar in water. For each test, the assay procedure for 10 male wasps was conducted 6 times, providing 60 male wasps tested.

These tests were then completed in their entirety with female *P. bellicosus* wasps, again assaying 10 wasps 6 times, providing 60 female wasps tested.

Laboratory Experiment 3. Male and Female *P. bellicosus* Olfactometer Responses to Wine, Vinegar, Ethanol, and Acetic Acid

We tested the hypotheses that male wasp attraction to the mixture of wine and vinegar is due to ethanol in the wine and acetic in the vinegar, as two 2-choice tests in the olfactometer. First, we compared a mixture of vinegar plus wine to a mixture of vinegar plus ethanol (ethanol substituted for wine), and then compared wine plus vinegar to wine plus acetic acid (acetic acid substituted for vinegar). For the first test, one mL of a mixture of 40% vinegar and 60% wine was placed in one chamber and one mL of a mixture 40% vinegar with 7.4% ethanol in water was placed in the other chamber. For the second test, one mL of a mixture of wine and vinegar was compared to one mL of a mixture of 60% wine with 1.6% acetic acid in water. For each of the 2 experiments, the assay procedure for testing 10 wasps was conducted 6 times for males, providing 60 male wasps assayed.

This experiment was then conducted with female *P. bellicosus*. For the comparison of wine plus vinegar versus wine plus acetic acid, 12 sets of 10 females were assayed, providing 120 wasps tested. For the comparison of wine plus vinegar versus ethanol plus vinegar, 6 sets of 10 females were assayed, providing 60 wasps tested.

Laboratory Experiment 4. Male and Female *P. bellicosus* Olfactometer Responses to Ethanol and Acetic Acid

We tested the hypotheses that ethanol is attractive to the wasps, and that acetic acid is deterrent to the wasps. These hypotheses were suggested by the results of the preceding olfactometer experiments. Four 2-choice tests were conducted. The first test evaluated wasp response to a one mL dose of a solution of 7.4% ethanol

and 1.6% acetic acid versus one mL of a solution of 7.4% ethanol. The second test compared a one mL dose of a solution of 7.4% ethanol and 1.6% acetic acid versus a one mL dose of 1.6% acetic acid. The third test compared a one mL dose of 7.4% ethanol to water, and the fourth test compared a one mL dose of 1.6% acetic acid to water. For each 2-choice treatment comparison, 6 sets of 10 male wasps were tested, providing 60 male wasps assayed.

Subsequently, all four 2-choice tests were conducted again using female *P. bellicosus*. For each treatment comparison, 6 sets of 10 female wasps were assayed, as described above for males.

Data for trapping comparison were summed for the duration of the experiment for each replicate. Data for multi treatment comparisons of experiments 1 and 3 were subjected to an ANOVA, and treatment means were separated by Tukey's test. Data for the 2 treatments of experiment 2 were compared by a Student's *t* test. Data for treatments of all olfactometer tests were compared using the Chi-Square test. Statistical analyses were conducted using StatMost Statistical Software (DataMost 1995).

RESULTS

Field Experiment 1. Observation of Wasps Trapped with a Mixture of Wine and Vinegar

The numbers of *P. metricus* and *P. bellicosus* in traps baited with wine plus vinegar were significantly greater than traps baited with any of the chemical blends ($F = 19.10$, $P < 0.001$, $df = 3,36$; $F = 6.97$, $P < 0.0001$, $df = 3,36$; respectively) (Table 1). In this test, 73 female *P. metricus*, 45 female *P. bellicosus*, and 2 female *Polistes carolina* (L.) were captured.

Field Experiment 2. Wasps Trapped with a Mixture of Wine and Vinegar

Numbers of *P. bellicosus*, *P. metricus*, and *P. fuscatus* captured in traps baited with wine plus vinegar were significantly greater than numbers

in traps that were unbaited (Table 2). Twenty-five female *P. bellicosus*, 63 female *P. metricus*, 15 female *P. fuscatus*, 14 female *P. dorsalis*, 5 female *Polistes major* (Beauvois) 2 female *P. carolina*, and 1 female *Polistes exclamans* (Vierick) were captured in traps baited with wine and vinegar, while 1 *P. bellicosus* only was captured in the unbaited traps.

Field Experiment 3. Wasps Trapped with Wine, Vinegar, and the Combination of Wine and Vinegar

The numbers of paper wasps captured in this test were small. However, in Florida, numbers of *P. bellicosus* trapped with wine were greater than in unbaited traps ($F = 3.45$, $P = 0.03$, $df = 3,16$), and in Oklahoma the numbers of *P. fuscatus* trapped with wine and with wine plus vinegar were greater than in unbaited traps ($F = 4.93$, $P = 0.01$, $df = 3,16$) (Table 3).

Laboratory Experiment 1. Male *P. bellicosus* Olfactometer Response to a Mixture of Wine and Vinegar

Significantly more male wasps selected the olfactometer arm with air flow from over one mL of wine plus vinegar (Mean \pm SEM = $50 \pm 0.7\%$), compared to the arm with airflow over one mL of water ($1.9 \pm 0.5\%$) ($t = 6.64$, $df = 13$, $P < 0.001$). Eighty-nine of 140 wasps tested entered into one of the arms of the olfactometer, while 51 wasps did not enter an arm (did not make a choice).

Laboratory Experiment 2. Male and Female *P. bellicosus* Olfactometer Response to Wine, Vinegar, and Wine plus Vinegar Mixed

Significantly more males chose the olfactometer arm with airflow over wine alone compared to the airflow over the combination of wine and vinegar ($\chi^2 = 22.2$, $df = 5$, $P < 0.001$) (Fig. 1A). Forty-eight of 60 wasps tested moved into an arm of the olfactometer. In the second comparison, significantly more males chose the combination of wine and vinegar over vinegar alone ($\chi^2 = 16.9$,

TABLE 1. MEAN (\pm SE) NUMBERS OF PAPER WASPS CAPTURED PER TRAP BAITED WITH A MIXTURE OF WINE AND VINEGAR (WV) AND BLENDS OF VOLATILE COMPOUNDS FROM WINE AND VINEGAR HEADSPACE THAT WERE ANTENNAL ACTIVE FOR THE SPOTTED WING DROSOPHILA.

Wasp species	Wine Vinegar mix	Chemical blend 1 ^a	Chemical blend 2 ^b	Chemical blend 3 ^c
<i>P. bellicosus</i>	2.6 \pm 0.6 a	0.5 \pm 0.2 c	1.3 \pm 0.4 b	0.4 \pm 0.2 c
<i>P. metricus</i>	5.2 \pm 0.9 a	1.2 \pm 0.4 b	0.9 \pm 0.4 bc	0 c

Means within a row followed by the same letter are not significantly different by Tukey's Test, at $P < 0.05$.

^aChemical blend 1 consisted of acetic acid, ethanol, acetoin, grape butyrate, methionol, isoamyl lactate, 3-phenylethanol, and diethyl succinate.

^bChemical blend 2 consisted of acetic acid, ethanol, acetoin, grape butyrate, and 2-phenylethanol.

^cChemical blend 3 consisted of acetic acid, ethanol, acetoin, ethyl lactate, and methionol.

TABLE 2. MEAN (\pm SE) NUMBERS OF PAPER WASPS CAPTURED PER TRAP BAITED WITH A MIXTURE OF WINE AND VINEGAR. BLANK TRAPS CONTAINED THE DROWNING SOLUTION AND NO BAIT.

Wasp species	Blank	Wine/Vinegar mix	<i>t</i> , <i>df</i> , <i>P</i>
<i>P. annularis</i>	0	0.65 \pm 0.28	1.76, 19, 0.09
<i>P. bellicosus</i>	0.1 \pm 0.1	1.25 \pm 0.43	2.23, 19, 0.04
<i>P. dorsalis</i>	0	0.70 \pm 0.26	1.56, 19, 0.14
<i>P. fuscatus</i>	0	0.75 \pm 0.22	3.47, 19, 0.003
<i>P. major</i>	0	0.15 \pm 0.15	1.00, 19, 0.33
<i>P. metricus</i>	0	3.15 \pm 0.75	3.98, 19, 0.0008

df = 5, *P* = 0.004) (Fig. 1A). Fifty-four of 60 wasps tested moved into one of the olfactometer arms.

More females chose airflow over wine compared to wine plus vinegar ($\chi^2 = 26.8$, df = 5, *P* < 0.001) (Fig. 1B), and all 60 wasps tested moved into an arm of the olfactometer. More females chose wine plus vinegar versus vinegar ($\chi^2 = 20.1$, df = 5, *P* = 0.001) (Fig. 1B). Fifty-nine of 60 wasps tested moved into an arm of the olfactometer.

Experiment 6. Male and Female *P. bellicosus* Olfactometer Responses to Wine, Vinegar, Ethanol, and Acetic Acid

There was no difference between the numbers of male wasps choosing the combination of wine and vinegar compared to the combination of wine and acetic acid ($\chi^2 = 4.6$, df = 5, *P* = 0.47) (Fig. 1C). Forty-three of the 60 wasps tested moved forward and into one of the olfactometer arms (Fig. 1B). There was a preference for wine plus vinegar compared to ethanol plus vinegar ($\chi^2 = 15.5$, df = 5, *P* < 0.001) (Fig. 1C). In that test, 57 of 60 wasps tested moved up the olfactometer stem and into one of the arms.

Fewer females chose airflow over wine plus vinegar compared to wine plus acetic acid ($\chi^2 = 26.4$, df = 11, *P* = 0.006) (Fig. 1D), and 115 of 120 wasps tested moved into an arm of the olfactometer. More females chose airflow over wine plus vinegar compared to vinegar plus ethanol ($\chi^2 = 25.1$ df = 5, *P* < 0.001) (Fig. 1D). Fifty-nine

of 60 wasps tested moved into an arm of the olfactometer.

Laboratory Experiment 4. Male and Female *P. bellicosus* Olfactometer Responses to Ethanol and Acetic Acid

Male *P. bellicosus* generally oriented to ethanol and did not show a significant orientation to acetic acid. When given the choice of ethanol versus water, significantly more male wasps chose ethanol ($\chi^2 = 18.7$, df = 5, *P* < 0.001) (Fig. 2A). When given the choice of ethanol plus acetic acid versus ethanol, significantly more male wasps chose ethanol ($\chi^2 = 32.8$, df = 5, *P* < 0.001) (Fig. 2D). When given the choice of acetic acid versus water, male wasps did not show a preference ($\chi^2 = 5.5$, df = 5, *P* = 0.36) (Fig. 2B). When given the choice of ethanol plus acetic acid versus acetic acid alone, wasps did not show a preference ($\chi^2 = 0.6$, df = 5, *P* = 0.99) (Fig. 2D).

Female *P. bellicosus* were attracted to ethanol and were deterred by acetic acid. When given the choice of ethanol plus acetic acid versus ethanol, significantly more female wasps chose ethanol ($\chi^2 = 29.4$, df = 5, *P* < 0.001) (Fig. 2B) and when given the choice of ethanol versus water, significantly more female wasps chose ethanol ($\chi^2 = 13.4$, df = 5, *P* = 0.02) (Fig. 2D). When given the choice of acetic acid versus water, significantly more female wasps chose water ($\chi^2 = 20.0$, df = 5, *P* = 0.001) (Fig. 2B). There was no significant difference between numbers of female wasps choosing

TABLE 3. MEAN (\pm SE) NUMBERS OF PAPER WASPS CAPTURED PER TRAP BAITED WITH WINE ALONE, VINEGAR ALONE, OR THE COMBINATION OF WINE AND VINEGAR. BLANK TRAPS CONTAINED THE DROWNING SOLUTION AND NO BAIT.

Wasp species	Blank	Wine	Vinegar	Combination
Gainesville, Florida				
<i>P. bellicosus</i>	0 a	1.8 \pm 0.9 b	0.2 \pm 0.2 a	0 a
<i>P. metricus</i>	0 a	1.0 \pm 0.3 b	0.4 \pm 0.2 ab	0.6 \pm 0.4 ab
Tulsa, Oklahoma				
<i>P. fuscatus</i>	0 a	2.6 \pm 0.8 bc	1.0 \pm 0.3 ab	3.8 \pm 1.2 c

Means within a row followed by the same letter are not significantly different by Tukey's Test, at *P* < 0.05.

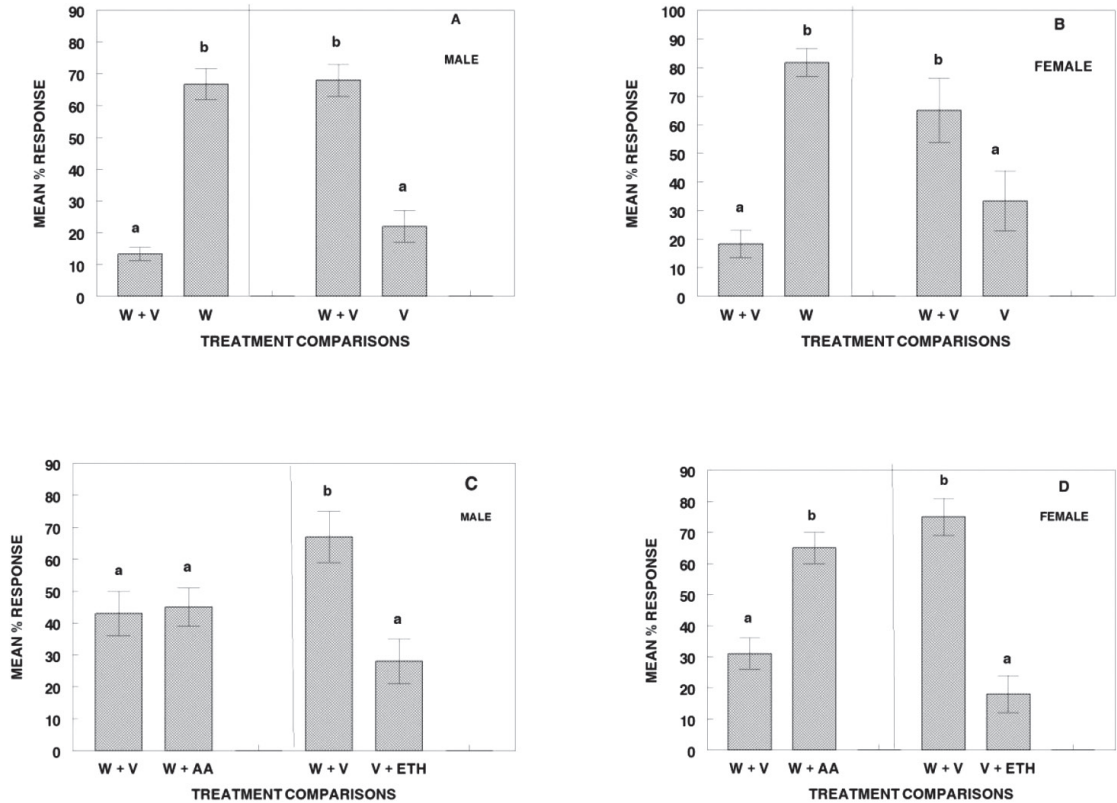


Fig. 1. Mean percentages of *Polistes bellicosus* wasps responding to comparisons of wine (W) + vinegar (V) versus wine, wine + vinegar versus vinegar, for males (A) and for females (B). Mean percentages of *P. bellicosus* wasps responding to comparisons of wine + vinegar versus wine + acetic acid (AA), and wine + vinegar versus ethanol (ETH) + vinegar for males (C) and for females (D). All assays were conducted in a Y-tube olfactometer.

ethanol plus acetic acid versus acetic acid alone ($\chi^2 = 4.3$, $df = 5$, $P = 0.50$) (Fig. 2D).

Voucher specimens of *Polistes annularis* (L.), *P. bellicosus*, *P. dorsalis*, *P. fuscatus*, and *P. metricus* are deposited in the M. T. James Entomological Collection, Washington State University, Pullman, Washington.

DISCUSSION

In the first field experiment numbers of *P. bellicosus* and *P. metricus* were trapped, suggesting that these wasps are attracted to wine and vinegar. This observation was intriguing because there are no prior reports of attractants for these wasps, and the same two species are among those that swarm at towers (Reed & Landolt 1991). Results of this field test prompted our hypothesis of paper wasp attraction to volatile chemicals emitted from those materials.

The response of these two wasps to this same bait in the second trapping experiment

confirmed their attraction to wine plus vinegar. Baits, lures, and traps might be useful for reducing numbers of these wasps in circumstances where they are pestiferous (Reed & Landolt 1991). The combination of acetic acid and isobutanol is attractive to *P. aurifer* and *P. fuscatus* (Landolt 1999; Reed & Landolt 2003) and small numbers of paper wasps have been trapped with beer, fruit juices, and other similar baits (i.e., De Souza et al. 2011; Dvorak 2007; Dvorak & Landolt 2006; Wegner & Jordan 2005), however low numbers of paper wasps were captured in these studies. Our field results provide a first indication of a useful bait for trapping *P. metricus* and *P. bellicosus*, and a potential source for the isolation and identification of a chemical attractant for these species.

As discussed above, the testing of the combination of wine and vinegar was initiated as a study of *D. sukuzii* attractants, and wasps were trapped with that combination of materials. Results of our laboratory tests indicate that *P. bellicosus* are attracted to wine and are not

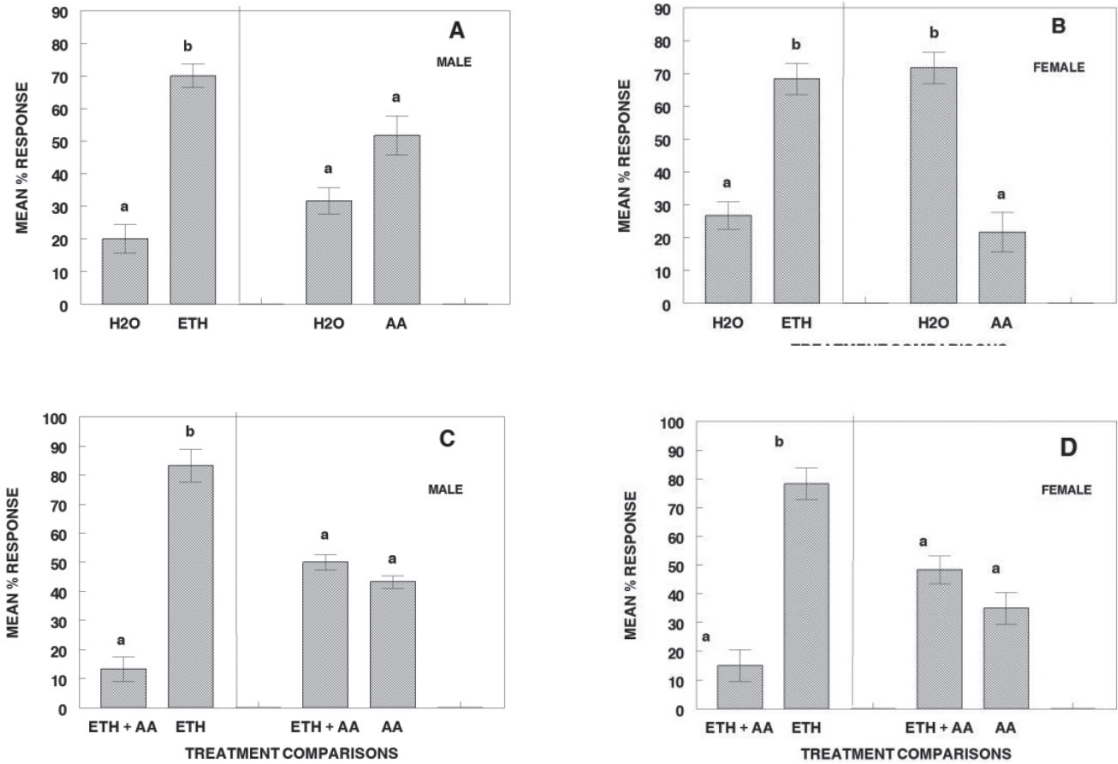


Fig. 2. Mean percentages of *Polistes bellicosus* wasps responding to comparisons of water (H₂O) versus ethanol (ETH), and water versus acetic acid (AA), for males (A) and females (B). Mean percentages of *P. bellicosus* wasps responding to comparisons of ethanol + acetic acid versus ethanol, and ethanol + acetic acid versus acetic acid, for males (C) and females (D). All assays were conducted in a Y-tube olfactometer.

attracted to vinegar. These findings were verified in subsequent field tests that demonstrated attraction to wine and not vinegar by the same wasp species, with a similar response seen for *P. metricus*. Given the olfactometer results, it is somewhat surprising that so many of these wasps were captured in the field with the combination of wine and vinegar. It is possible to have results in the field that differ somewhat from those of a laboratory assay, because the behavior leading to capture in a trap in the field differs from the behavior observed in a Y-tube olfactometer. Also, this discrepancy may be a result of choices available to the wasps under the different experimental conditions of the laboratory and the field. In the olfactometer, the wasps were presented with 2 odors simultaneously, and in the field they may be exposed to only one trap odor at a time. The combined results of our laboratory and field tests indicate that pursuit of the isolation and identification of volatile chemicals eliciting paper wasp attraction to the wine and vinegar mixture need only consider the volatiles of the wine, and not

the vinegar. Similarly, for the trapping of *P. bellicosus* using food grade materials, wine would best be used without the addition of vinegar. We might expect similar patterns of response by *P. metricus* to wine and to vinegar, but additional work is needed to determine if this is the case.

Ethanol is the most abundant volatile organic chemical in wine (12% in this case) and acetic acid is the most abundant volatile organic chemical in vinegar (5% in this case). We sought then to determine what if any role these 2 chemicals play in paper wasp orientation to the wine/vinegar mixture, using laboratory olfactometer assays. The results paralleled the results with the wine and vinegar, with a wasp preference for ethanol, and not for acetic acid. The wasps also showed a clear preference for wine plus vinegar versus ethanol plus vinegar, indicating a role of additional wine volatiles in wasp orientation to wine. Female wasps showed a preference for wine plus vinegar compared to wine plus acetic acid, suggesting attractive volatiles in vinegar (not acetic acid). Although we did not see a similar response by males to wine

plus vinegar versus wine plus acetic acid, Cha et al. (2012) found overlap in the wine and vinegar volatiles that are EAD-active for *D. suzukii*. It may be hypothesized that these paper wasps are also attracted to a mixture of volatiles that are shared in part by this wine and this vinegar. Alcoholic beverages, such as beer (Dvorak 2007), have been used as baits to trap social wasps, but there are no experimental demonstrations of social wasp attraction to ethanol, and there are no reports of wasp attraction to other volatile chemicals of either alcoholic beverages or vinegars. Further analysis of the neurophysiological and behavioral responses of paper wasps to wine volatiles is needed to determine an attractive blend of chemicals.

Out of practical necessity, our experiments involved different wasp sexes, castes, and species. All olfactometer assays were conducted with male *P. bellicosus*, and 3 of the 4 olfactometer experiments were repeated using female *P. bellicosus* gynes. *Polistes metricus* however was the most abundant paper wasp in traps in field tests, but with *P. bellicosus* consistently trapped also. The first trapping test was conducted in summer with males, worker females, and potentially new gynes present. The second field test was conducted in spring when no males were present, and only spring gynes were present to respond. The third field test was conducted in late summer and early autumn, when more new gynes and males are active. We used *P. bellicosus* males in olfactometer tests because we fortuitously acquired large numbers of males from late-season nests and aggregations in Georgia and Florida. However, we must keep in mind the potential for variance in foraging behavior and olfactory responses among males, gynes, and workers of a paper wasp species. Similarly, the different species of *Polistes* captured in traps may also vary in their foraging behavior and behavioral responses to these baits. Care must be taken to not extrapolate too far from these experimental results, without additional work to validate those findings.

This work is based on observations of paper wasp responses to man-made food materials, but the orientation to these types of fermented baits is likely to be food-finding behavior that wasps use in nature to locate fermented sweet materials. Paper wasps do feed at carbohydrate-rich foods in the field, such as fruits, saps, honeydews of sucking insects, and both floral and extrafloral plant nectaries (Al-Mahdawi & Al-Kihani et al. 2011; Barrows 1979; Beckmann & Stucky 1981; Cranshaw et al. 2011; Galvan et al. 2008; Hardy 1988; MacKenzie 2004; Martinson et al. 2013; Saxena 1970; Young 1984). These wasps, and other insects as well, probably use volatile chemical cues from microbes such as yeasts as indicators of a suitable car-

bohydrate-rich food source. Davis et al. (2012) showed yellowjacket (*Vespula* spp.) attraction to a widespread yeast and to volatile chemicals from that yeast. We expect that *Polistes* paper wasps locate carbohydrate foods by orienting to volatile byproducts of microbial fermentation, which also leads them to man-made fermentation products such as wine. The pursuit then of the isolation and identification of these microbial odorants should yield chemical attractants that match the attractiveness of either the natural food sources or the man-made food baits.

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