Preliminary Study of Pear Ester Toxicity When Consumed by Polistes dominula (Hymenoptera: Vespidae)

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Research

Preliminary study of pear ester toxicity when consumed by *Polistes dominula* (Hymenoptera: Vespidae)

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Abstract

The European paper wasp, *Polistes dominula* (Christ) (Hymenoptera: Vespidae), directly competes with native *Polistes* and is a safety concern for workers and homeowners. A previous investigation of *Polistes* behavior found that a portion of wasps died after consuming a solution of pear ester, a compound commonly found in fruits that is considered non-toxic to humans. The goal of our study was to corroborate those preliminary observations and investigate the toxicity of pear ester to *P. dominula*. Two experiments were performed. First, 3 wasp castes were fed 17% sugar water, or sugar water mixed with 0.5%, 1.0%, 5.0%, or 10.0% (w/v) solutions of pear ester. Mortality after 24 h ranged from 0% among wasps fed sugar water only, to >10% among wasps that were fed pear ester. Mortality increased logarithmically with pear ester concentration. Pear ester toxicity differed among wasp castes, with <5% mortality recorded among fall gynes to >10% mortality among workers. The second experiment attempted to define the relationship between morbidity and volume of pear ester consumed by *P. dominula* workers. Although results confirmed that ingestion of pear ester causes death or paralysis of *P. dominula* workers, there was no clear relationship between volume of ingested pear ester and wasp morbidity. About 80% of *P. dominula* were unaffected by pear ester despite consuming large quantities of the solution. This is the first study to demonstrate lethal effects of pear ester compound and may indicate its potential as an insecticide for paper wasps.

Key Words: paper wasp; ethyl-(2E,4Z)-deca-2,4-dienoate; ethyl decadienoate; morbidity

Resumen

La avispa cartonera europea, *Polistes dominula* (Christ) (Hymenoptera: Vespidae), compite directamente con las *Polistes* nativas y, además, es una preocupación de seguridad para trabajadores y familias en sus casas. Una investigación previa sobre el comportamiento de *Polistes,* encontró que algunas de las avispas morían después de consumir una solución de éster de pera, un compuesto comúnmente encontrado en frutas y que no es considerado tóxica para humanos. El objetivo de nuestro estudio fue corroborar esas observaciones preliminares e investigar la toxicidad del éster de pera para *P. dominula.* Se realizaron 2 experimentos. Primero, 3 castas de avispas fueron alimentadas con soluciones de 17% agua azucarada, o agua mezclada con 0.5%, 1.0%, 5.0% y 10.0% (p/v) con éster de pera. La mortalidad después de 24 horas varió desde 0% entre avispas alimentadas solamente con agua azucarada, a >10% entre avispas a las cuáles se les dio éster de pera. La mortalidad aumentó logarítmicamente con la concentración de éster de pera. La toxicidad del éster de pera varió entre las castas de avispas, con una mortalidad registrada <5% entre hembras reproductivas de otoño a >10% entre obreras. El segundo experimento intentó definir la relación entre morbilidad y volumen de éster de pera consumido por obreras de *P. dominula*. Aunque los resultados confirmaron que la ingesta de éster de pera causa la muerte o parálisis de obreras de *P. dominula*, no hubo una relación clara entre el volumen ingerido de éster de pera y morbilidad. Cerca del 80% de *P. dominula* no fue afectado por el éster de pera, a pesar, de haber consumido grandes cantidades de la solución. Este es el primer estudio que demuestra que la ingestión de éster de, pero por un insecto causa parálisis o muerte. Este es el primer estudio que demuestra efectos letales del compuesto de éster de pera y puede indicar su potencial como insecticidas para avispas papeleras.

Palabras Clave: avispa cartonera; etilo-(2E,4Z)-deca-2,4-dienoato; decadienoato de etilo; morbilidad

Polistes dominula (Christ; Hymenoptera: Vespidae), the European paper wasp, is a primitively eusocial species native to Mediterranean Europe (Beverley n.d.; Liebert et al. 2006; Weiner et al. 2012). Matthias et al. (2008) list its distribution as central Europe to Mongolia and China, south to northern Africa, Israel, Iran, Afghanistan, Pakistan, and northern India with introduction into western Australia. In the United States, it was first introduced to the Boston area in the 1970s (Beverley n.d.; Liebert et al. 2006; Buck et al. 2008; Beggs et al. 2011; Cranshaw et al. 2011; Baker & Potter 2020) and has since spread throughout the United States and the Canadian provinces to become one of the most

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abundant paper wasps in North America (Borkent & Cannings 2004; Beggs et al. 2011; Cranshaw et al. 2011). It was first reported in Washington state by Landolt and Antonelli (1999). The geographic range of *P. dominula* has expanded since the 1980s to include South Africa (Veldtman et al. 2012), Chile (Willink & Chiappa 1993), Argentina (Sackmann et al. 2003), and western Australia (Liebert et al. 2006).

Since its introduction to the United States, *P. dominula* has negatively impacted native species of *Polistes* (Liebert et al. 2006; Beggs et al. 2011). The presence of *P*. *dominula* has elevated predation pressure on preferred prey, leading to direct competition with native species of *Polistes*. For example, studies in Michigan showed that *P. dominula* has displaced the indigenous North American paper wasp *Polistes fuscatus* (Fabricius) through competition for local resources (Gamboa et al. 2002, 2004, 2005; Liebert et al. 2006). Furthermore, *P. dominula* builds nests around structures where they are a health risk to workers and homeowners. These wasps exhibit defensive behavior and will protect themselves and their nests with painful stings when threatened (Landolt & Antonelli 2003).

Polistes dominula occur as several morphologically similar castes that vary in behavior and seasonal occurrence: alpha queens, subordinate queens, and non-reproductive female workers. This species overwinters as mated fall gynes in the Pacific Northwest United States. Spring queens emerge in Apr and May to begin constructing nests and laying eggs. Worker populations and nest sizes increase in Jun and Jul. Fall gynes and males are produced in Aug to Oct, and mated gynes locate hibernacula for overwintering.

While many products have been developed to effectively control yellowjacket populations, managing *Polistes* paper wasps has proven more difficult (Day & Jeanne 2001; Dvořák & Landolt 2006; Dvořák 2007; Landolt et al. 2007; Davis et al. 2012; Demichelis et al. 2014; Babcock et al. 2017). Heptyl butyrate, the leading commercial lure in pestiferous wasp traps, is not an attractant for *Polistes* (Davis et al. 1973; MacDonald et al. 1973; Gambino & Loope 1992; Landolt 1998; Landolt et al. 2003; Buteler et al. 2018). In addition, as *Polistes* feed on live insect prey, many of the baits that utilize or mimic animal protein do not work because they rely on yellowjacket scavenging behavior to be effective (Grant et al. 1968; Spurr 1995; Sackmann et al. 2001; D'Adamo et al. 2003; Wood et al. 2006; Buteler et al. 2018; Choe et al. 2018). Consequently, developing successful population control methods for *P. dominula* will require new strategies.

In a recent study investigating the learning behavior of *Polistes*, it was found that the odorous plant-based chemical, pear ester, was potentially toxic to paper wasps when consumed at low concentrations (Asche 2021). Pear ester (ethyl-(2E,4Z)-deca-2,4-dienoate) has low mammalian toxicity (Sigma Aldrich 2020) and is widely used by both the food and perfume industries (Shakhmaev et al. 2017). The commercial product is derived from Bartlett pears (Jennings et al. 1964; Shakhmaev et al. 2017; Sigma Aldrich 2020), though it also occurs naturally in apple, grape, and quince (Sigma Aldrich n.d.). In studies of *Polistes* odor recognition (Asche 2021), pear ester was selected to test associative learning because it was believed to be an odor paper wasps would likely encounter while foraging for nectar; therefore, wasps would be capable of detecting it at low concentrations. During that study, wasps were fed a sugar water solution infused with 1% pear ester to evaluate whether paper wasps could learn to associate a unique odor with a food reward. Some wasps fed the solution experienced paralysis and death, while wasps that were provided sugar water only remained healthy. Those observations suggested that ingestion of pear ester compound leads to morbidity of *P. dominula*, but these behavioral studies were not designed to specifically test toxicity of pear ester to wasps.

The goal of our study was to experimentally test whether ingestion of pear ester by *Polistes* wasps leads to wasp mortality. Two experiments were performed to investigate pear ester toxicity on *P. dominula*. The first experiment investigated the impact of pear ester on mortality of different wasp castes (fall gynes, spring queens, and workers). The second experiment attempted to quantify the volume of a pear ester solution a wasp would need to consume to experience mortality or sub-lethal effects.

Methods and Materials

WASP COLLECTION

Wasps were collected either individually with an aerial net or by extracting entire nests from the surface of structures into disposable plastic containers. Wasps were transported from collection sites in a Styrofoam container filled with ice packs to keep specimens from overheating (Gillaspy 1971). Once at the laboratory, wasps were stored in a refrigerator (4 °C) until they were sorted by species and sex. Wasps were then placed in metal cages with mesh sides (Collapsible Cage, BioQuip, Rancho Dominguez, California, USA) and were provided deionized (DI) water and a 17% w/v sugar water solution made with granulated sugar. Humid conditions within the cages were provided by placing an inverted baby food jar of DI water on the top of each cage. These jars had a paper towel rubber-banded over the mouth, allowing water to keep the paper saturated until the jar dried out. The cages that the wasps were held in had an average temperature of 20.77 °C and average relative humidity of 43.80%. The room was illuminated by natural light from the windows in the laboratory and overhead florescence during daytime hours. Cages were checked daily, and dead wasps were removed.

EXPERIMENT #1, DISH FEEDING EXPERIMENTS

Prior to beginning each experimental replicate, 5 live wasps were placed inside six 20 × 20 × 20 cm metal cages (Collapsible Cage, BioQuip, Rancho Domeinguez, California, USA). Five of these cages represented the treatment groups and the 6th cage served as the control. Treatment and control wasps were starved for 24 h. After the starvation period, treatment wasps were fed DI water (4 cotton balls saturated and placed on a Fisherbrand™ sterilized polystyrene Petri dish) and a 17% w/v sugar water solution with pear ester (4 cotton balls saturated and placed on a Fisherbrand™ sterilized polystyrene Petri dish). Control wasps were fed DI water (4 cotton balls saturated and placed on a Fisherbrand™ sterilized polystyrene Petri dish) and a 17% w/v sugar water solution without pear ester added (4 cotton balls saturated and placed on a Fisherbrand™ sterilized polystyrene Petri dish). These cotton balls were left in the cages for 24 h. During this time, wasps could freely roam within the enclosure and interact with the food and water. After 24 h, the food and water were removed, and the number of dead wasps was counted.

Experiments were performed from Jul 2019 to Oct 2020, and 260 cages (each having 5 wasps) were used in this experiment. Three wasp castes (fall gynes, workers, and spring queens) were each subject to 20 replicates at each of 4 concentrations of the pear ester solution (0.5%, 1.0%, 5.0%, and 10.0%) and at the control (0% pear ester, only 17% w/v sugar water). Once exposed to pear ester, wasps were not re-used for any additional testing.

Adult mortality was compared among castes and pear ester concentrations by logistic regression using the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc. 2013). Events/trials syntax (number of dead wasps/number of wasps in a cage) was included as the dependent variable, and caste, pear ester concentration, and the main effect interaction was included as the fixed effects. Cage was included as the random variable. The water control was not included in the final statistical analysis because none of the wasps in these cages died. Log regression function was used to describe the relationship between mean mortality and pear ester concentration.

EXPERIMENT #2, J-TUBE FEEDING EXPERIMENTS

Experimental arenas were constructed from plastic snap caps (length 5 cm, diameter 3 cm). Each snap cap held a single wasp, which had exclusive access to a pear ester feeding solution. The solution was administered via a 77.86 microliter glass capillary tube (length 75 mm, inner distance 1.1–1.2 mm) heated over a flame and bent into a j-shape. The j-shaped capillary tube was inserted into the snap cap through a small slit cut into the lid and held in place by a firm cotton plug stopper with a slit cut into one side (Fig. 1). After filling the tube with pear ester solution, the meniscus was marked with a pen for later measurement of how much solution each wasp consumed. Design of the j-shaped capillary tube feeding strategy was based on the methods of Landolt et al. (1991).

Prior to beginning the experiment, each wasp was starved for 48 h. The duration of the starvation period was longer for this test compared to the dish feeding experiment because the testing period was shorter, and the researchers wanted to increase the chances that the wasps would feed. Wasps were then placed into the snap cap with the j-shape capillary tube inserted to allow them to feed. Each experiment lasted 3 h, during which time, 6 wasps in 6 snaps were tested. Five *P. dominula* workers were provided a 10% pear ester feeding solution and a single control wasp was given only sugar water. After the 3 h exposure time, the j-tubes containing the food were removed from the vials containing the wasps, and the amount of consumed solution was calculated using the change in position of the solution's meniscus within the tube. Once the experiment was completed, any dead or paralyzed wasps were documented. To determine sub-lethal effects, wasps were observed for an additional 24 h after the experiment was completed to see if any paralyzed wasps recovered. Experimental units were individual wasps. Fifteen replicates were performed, utilizing a total of 75 treatment wasps and 15 control wasps for this experiment.

The amount of ingested pear ester was categorized into 3 groups: <5 µL, 5–10 µL, or >10 µL. Proportions of wasps that were alive, paralyzed, or dead after ingestion of each categorized amount of pear ester were analyzed by contingency table analysis (PROC FREQ) using SAS 9.4 (SAS Institute Inc. 2013). Statistical differences between treatments were assessed based on the Cochran-Mantel-Haenszel row mean score statistic (Stokes et al. 2000).

Results

EXPERIMENT #1, DISH FEEDING EXPERIMENTS

There was no significant main effect interaction (x^2 = 1.7; df = 6; *P* = 0.110), indicating that patterns between pear ester concentration and wasp mortality were similar among wasp castes. There were significant differences in mortality among wasp castes after ingestion of pear ester (x^2 = 12.1; df = 2; *P* = 0.002; Fig. 2A). The highest mortality was observed among workers (total dead wasps, n = 52/100), and the lowest mortality was observed among fall gynes (total dead wasps, n = 13/100). Mortality of spring queens (total dead wasps, n = 24/100) were intermediate to those of fall gynes and workers. Pear ester concentration also influenced mortality of wasps, regardless of wasp caste (x^2 = 9.4; df =3; *P* = 0.024; Fig. 2B). Mortality varied from 0% in water controls to over 10% among wasps exposed to the highest concentration of pear ester (Fig. 2B). Regression analysis showed a logarithmic relationship between wasp mortality and pear ester concentration with increased mortality with increasing concentration (Fig. 2B).

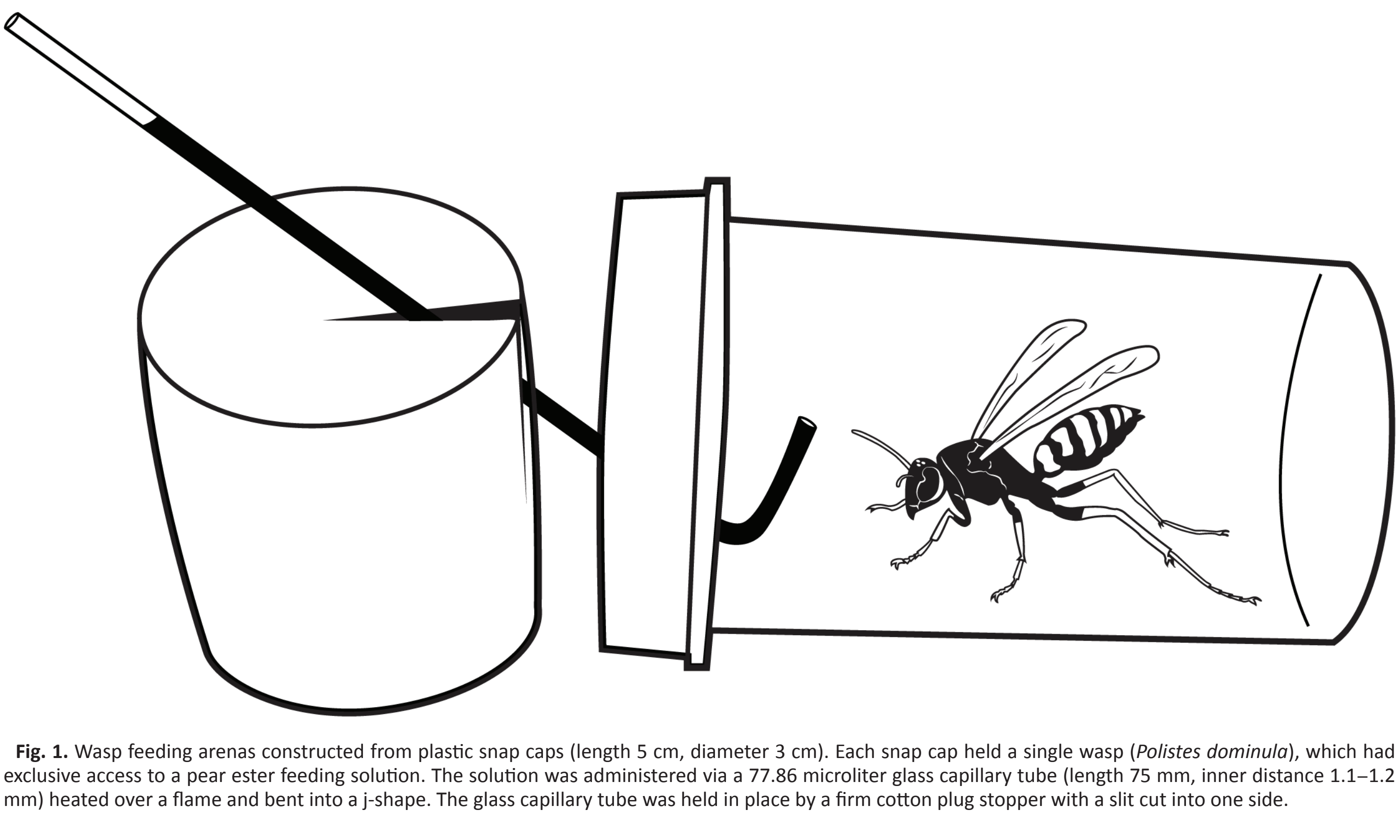

Fig. 1. Wasp feeding arenas constructed from plastic snap caps (length 5 cm, diameter 3 cm). Each snap cap held a single wasp (*Polistes dominula*), which had exclusive access to a pear ester feeding solution. The solution was administered via a 77.86 microliter glass capillary tube (length 75 mm, inner distance 1.1–1.2 mm) heated over a flame and bent into a j-shape. The glass capillary tube was held in place by a firm cotton plug stopper with a slit cut into one side.

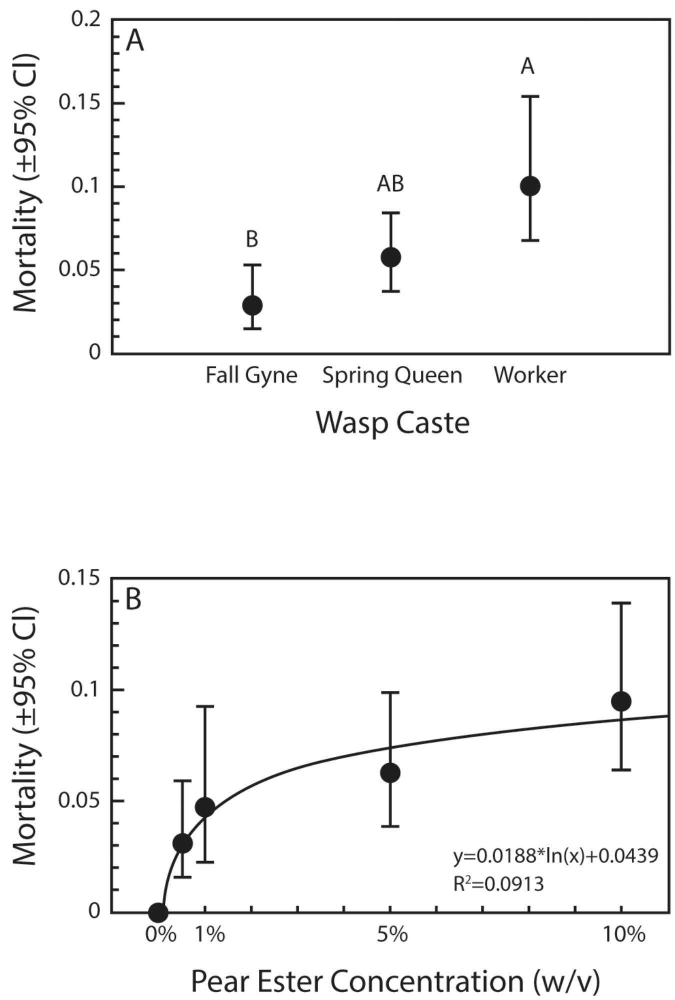

Fig. 2. Mortality of different *Polistes dominula* castes after ingestion of pear ester regardless of concentration (A), and mortality of wasps following ingestion of different concentrations of pear ester (B). Different letters denote significant differences among treatments at the α=0.05 confidence level.

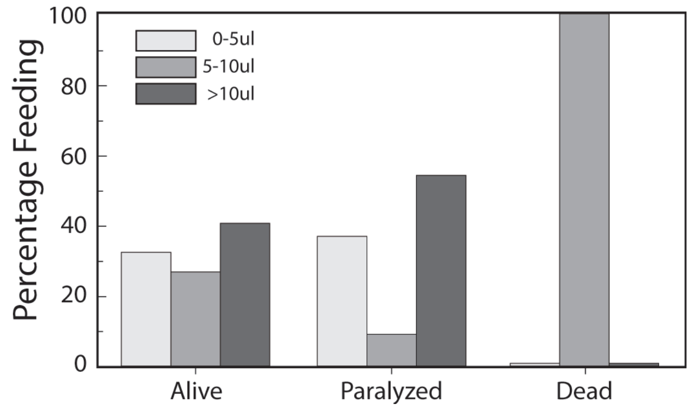

Fig. 3. Percentage of *Polistes dominula* that were alive, paralyzed, or dead after ingesting <5 µL, 5–10 µL, or >10 µL of pear ester solution.

EXPERIMENT #2, J-TUBE FEEDING EXPERIMENTS

None of the wasps provided 17% sugar water without pear ester showed signs of morbidity after the 3 h experiment was completed. Overall, wasps ingested a mean of 10.6 µL of 10% pear ester, with volumes that ranged from 2.1 µL to 37.4 µL. Fifty-nine of these wasps (78.7%) appeared completely unharmed after the experiment was completed. Five wasps died after the 3 h experiment was completed, and 11 wasps were paralyzed. Paralyzed wasps exhibited a number of symptoms, including inability to walk, body tremors, or complete lack of movement. Of those 11 paralyzed wasps, 9 regained mobility after 24 h but 2 wasps remained paralyzed and eventually died. There were no differences among living (unaffected) wasps that ingested <5 µL, 5–10 µL, or >10 µL. Likewise, there was not a significant difference among paralyzed wasps that ingested various amounts of pear ester, although >50% of the paralyzed wasps consumed more than 10 µL of pear ester solution. All dead wasps had consumed between 5–10 µL of pear ester (Fig. 3).

Discussion

Results of feeding assays (Experiment #1) corroborated earlier observations made during a *Polistes* wasp behavior study (Asche 2021) that ingestion of pear ester by *P. dominula* increases wasp mortality. Results also showed a concentration-dependent relationship between pear ester concentration and mortality of *P. dominula*. This is the first study to demonstrate lethal effects of pear ester ingestion on an insect. Pear ester occurs in certain fruits, including apples, pears, and grapes, albeit at lower concentrations than tested in our study. This compound is recognized as safe for human consumption, having a pear-like taste and odor.

There were differences among wasp castes in mortality following ingestion of pear ester, which varied from <5% for fall gynes to greater than 10% for workers, which may reflect foraging behavior differences between the groups tested. Recently mated fall gynes overwinter and do not engage in nest construction or foraging to feed immatures before spring so maybe less likely to consume the treated sugar water. In contrast, workers and spring queens both engage in nest construction and foraging (Evans & West-Eberhard 1973).

Experiment #2 attempted to quantify the volume of pear ester solution a wasp would need to consume to die or become paralyzed. We chose to use *P. dominula* workers because they displayed the highest mortality in Experiment #1. In addition, the concentrations were set at 10% pear ester with the belief that this would increase the number of moribund wasps after each 3 h experiment was completed. Overall, results corroborated findings from the feeding dish assays by demonstrating that ingestion of pear ester caused mortality among *P. dominula* workers. However, many wasps that ingested large volumes of pear ester remained unaffected by the end of the assay, with no clear relationship between morbidity and volume of ingested pear ester. Most wasps exhibiting signs of morbidity (paralysis or death) had consumed greater than 5 µL of pear ester. None of the wasps that died consumed more than 10 µL of pear ester solution, suggesting that low quantities caused relatively rapid death among the most susceptible wasps.

It is possible that many wasps are either immune to the effects of pear ester ingestion, or that pear ester combined with other unaccounted for variables may be causing paralysis or death. Unfortunately, research investigating the impact of insecticides on Vespidae wasps are limited and this is the first study testing pear ester as a potential insecticide. However, volatile pear ester has been used successfully for mating disruption of Lepidoptera (Knight et al. 2019). Without previous studies to compare these results to, it is hard to speculate why some wasps experienced morbidity while others did not. However, honey bees (*Apis mellifera* L.), another social Hymenoptera, have been the subject of many insecticide experiments and these studies may be worth examining. Researchers have found that interactions between pesticides and other physical stressors, such as disease, can impact honey bee morbidity (Poquet et al. 2016; O'Neal et al. 2018). Furthermore, it has been shown that honey bee workers that performed different tasks (foraging, nursing) are impacted differently by pesticide exposure (Barascou et al. 2022). These findings may explain some of the results of this study and should be considered when designing future methods for testing insecticide efficacy consumed by *Polistes*. As the wasps used in these experiments were field collected, we cannot account for their age as well as potential pathogens or pesticide exposure prior to collection.

In conclusion, we provide evidence that ingestion of pear ester by *P. dominula* causes paralysis or death to 10–20% of *P. dominula* workers under laboratory conditions. Ingestion of pear ester affected a smaller proportion of fall gynes and queens compared with workers, perhaps due to differences in foraging behavior. Additional work is required to quantify the amount of consumed pear ester that is lethal to one-half (50%) of wasps exposed to it (LD_{50}) and the mode of action. This is the first study to demonstrate lethal effects of pear ester compound and may indicate its potential as an insecticide for paper wasps.

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