# Semiochemical Communication Between Yellowjacket Wasps and their Yeast Symbionts

**by**

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## Abstract

My research investigated whether (i) symbiotic yeasts isolated from the digestive tract of social wasps, (ii) commercial yeasts, or (iii) the volatiles these yeasts produce can be used as trap baits for capturing yellowjackets. I found that adding brewer's yeast to dried fruit and fruit powder enhanced attraction of yellowjackets in Argentina. I also found that the two yeast species *Hanseniaspora uvarum* and *Lachancea thermotolerans*, isolated from North American yellowjackets and grown on grape juice-infused agar, attract yellowjackets. *Lachancea thermotolerans* in admixture with fruit powder was also attractive and expressed an additive effect when combined with a commercial wasp lure. Synthetic analog blends of the volatiles produced by *H. uvarum* growing on grape juice-infused media and *L. thermotolerans* growing on fruit powder were both attractive to western yellowjackets, but not to other yellowjackets. In summary, symbiotic yeasts and their semiochemicals, respectively, show potential as yellowjacket trap baits or lures.



**Keywords**: *Vespula*; yellowjacket trapping; symbiotic yeast; *Hanseniaspora uvarum*; *Lachancea thermotolerans*; brewer's yeast; semiochemical communication; invasive species

*Dedicated to the often overlooked but incredibly fascinating world of insects.*

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## List of Acronyms

| | |
|---|---|
| DF | Dried fruit mixture |
| DNA | Deoxyribonucleic acid |
| FP | Fruit powder mixture |
| GC-MS | Gas chromatography–mass spectrometry |
| HB | Heptyl butyrate |
| PCR | Polymerase chain reaction |
| SB | Synthetic blend |
| SLS | Sodium lauryl sulfate |
| YPD | Culture media made from yeast extract, peptone, and dextrose |

# Glossary

| | |
|---|---|
| Agar | A nutrient-rich growth medium used for culturing microorganisms |
| Attractant | A stimulus which causes an organism to approach it |
| Bait | A food or other desired substance used to attract an organism to a specific location |
| Eusocial | A form of social behaviour in which (*i*) generations overlap, (*ii*) members participate in cooperative brood care, and (*iii*) individuals are separated into distinct reproductive and non-reproductive castes, or roles |
| Lure | A semiochemical composition used to attract an organism to a specific location |
| Mutualism | A type of symbiosis in which both organisms involved in the relationship accrue a benefit from the association |
| Olfactory | Relating to odour |
| Queen | An actively reproductive female member of a social insect species |
| Semiochemical | A chemical which conveys a message |
| Symbiosis | A relationship in which two organisms live in close association |
| Trap | A container used to capture and retain organisms |
| Trophallaxis | The exchange of regurgitated liquid food between social insect colony members |
| Volatile | A compound which easily evaporates from a substance and becomes airborne |
| Worker | A non-reproductive member of a social insect species |
| Yellowjacket | A type of social wasp belonging to the genera *Vespula* or *Dolichovespula* |

# Chapter 1.

# Introduction

## 1.1. Yellowjacket wasps

### 1.1.1. Life history

Yellowjackets are truly eusocial insects within the Vespinae subfamily (Hymenoptera: Vespidae). Two genera, *Dolichovespula* and *Vespula,* comprise the yellowjacket group. Most species are patterned with black and yellow stripes, although some are black and white. Members of the *Dolichovespula* genus typically build aerial nests, whereas members of the *Vespula* genus are mainly ground-nesting (Akre *et al.*, 1980). Colonies generally last a single season, and the largest nests can be tended by more than ten thousand adult wasps (Edwards, 1980).

Yellowjacket nestmates comprise a reproductive queen, sterile female workers, and brood. In the Northern hemisphere, mated queens emerge from diapause in the spring and initiate a nest. Each queen locates a suitable site and begins to construct a nest out of bark and wood fibers. Nest architecture consists of multiple cell combs enclosed by a nest envelope. The queen lays a small batch of eggs into cells of the initial comb and forages for nectar and protein to feed her larvae once they hatch. When the first group of offspring emerge as adult wasps, the queen gradually stops foraging and focusses on laying more eggs. The newly emerged worker wasps assume foraging, nest construction, and brood care duties. Nest activity peaks in July and August. In late summer, new reproductive wasps (gynes and males) are produced. These reproductive castes leave the nest and mate; following this, each mated gyne seeks a sheltered crevice where she overwinters and enters diapause. Males die soon after mating. By early October,

yellowjacket activity subsides as the nests start to die off (Akre *et al*., 1980; Edwards, 1980; Matsuura & Yamane, 1990).

Yellowjackets aggressively defend their nests from predators and other dangers. They do so by inflicting painful stings on any potential attackers. Stinging involves a female yellowjacket injecting venom from her poison gland into a target using her modified ovipositor (Akre *et al.*, 1980). Yellowjacket venom is chemically complex, including histamine, 5-hydroxytriptamine, dopamine, and noradrenaline (Geller *et al.*, 1976; Edwards, 1980). When nests are disturbed, yellowjackets also release a nest defense pheromone that recruits nest mates and facilitates coordinated attack of the prospective nest predator (Landolt *et al.*, 1995, 1999; Ibarra Jimenez *et al.*, 2016).

Yellowjackets forage for both protein and carbohydrate resources; they commonly consume other arthropods, fruit, nectar, and human foodstuffs. Members of the *Vespula rufa* species group tend to be predaceous on insect protein, whereas members of the *Vespula vulgaris* species group are generalist scavengers on various resources, and thus tend to be more pestiferous (Akre *et al.*, 1980).

### 1.1.2. Ecological function

Yellowjackets commonly prey on other arthropods such as larval Lepidoptera and Coleoptera, adult Diptera, spiders, and other Hymenoptera (Harris & Oliver, 1993; Sackmann *et al.*, 2000). For some arthropod species, yellowjackets are a primary predator and can have major impacts on population sizes (Beggs, 2001). For this reason, they may be considered beneficial under certain circumstances, as they reduce populations of some forest defoliators and agricultural pests. Some studies have even contemplated the use of yellowjackets as a biological control organism for the control of pests in agricultural fields (Donovan, 2003).

Yellowjackets also act as a source of food for mammals such as bears, skunks, racoons, badgers, moles, and coyotes (Akre *et al.*, 1980; Matsuura & Yamane, 1990). These animals dig up underground nests or attack aerial nests to feed on the protein-rich yellowjacket larvae. Racoons in particular seem to be voracious predators of yellowjacket nests in North America (Matsuura & Yamane, 1990; Ibarra Jimenez *et al.*, 2016). Some

birds also feed on yellowjacket foragers as they enter or exit the nest entrance (Akre *et al.*, 1980).

Nectar is a common source of energy for yellowjackets, and as a result they are effective pollinators for certain plant species (Edwards, 1980). For example, the late-summer inflorescences of common ivy, *Hedera helix*, are thought to rely heavily on *Vespula* species for pollination (Jacobs *et al.*, 2009), and some species of orchids are exclusively pollinated by yellowjackets (Cheung *et al.*, 2009).

## 1.2. Pest status of yellowjackets

### 1.2.1. Invasive species

Several species of yellowjackets have become invasive throughout the world. Overwintering queens are commonly spread through the transport of goods being shipped by air (Edwards, 1980). They are also thought to be spread by the movement of Christmas trees and other plants, as they often shelter underneath tree bark during diapause (Hollingsworth *et al.*, 2009). The German yellowjacket, *Vespula germanica*, and common yellowjacket, *Vespula vulgaris*, are two of the most common and widespread invasive species. Some species of yellowjackets have become invasive in countries with no native yellowjackets, such as Argentina and New Zealand, where they have become prevalent nuisance pests and have adversely impacted communities and ecosystems (Beggs, 2001; Beggs *et al.*, 2011). In Hawaii, the invasive western yellowjacket, *Vespula pensylvanica*, has reached high population levels due to the lack of any natural predators (Hollingsworth *et al*., 2009).

### 1.2.2. Impact on ecosystems

Both *V. germanica* and *V. vulgaris* are invasive to New Zealand (Thomas, 1960; Beggs, 2001) and Argentina (Willink, 1980; Masciocchi *et al*., 2010). In New Zealand, *V. vulgaris* has been shown to outcompete endemic bird species for insect honeydew, an important carbohydrate resource in beech forests. This has impacted the behaviour of the birds and may have also reduced their population sizes. *Vespula vulgaris* has also greatly

affected population sizes of several native arthropods that it preys on within these same beech forests (Beggs, 2001). In Argentina, both species of invasive yellowjackets have reached very high population sizes, rapidly spread across the country (Masciocchi & Corley, 2013) and become a significant nuisance to humans. *Vespula pensylvanica* has adversely impacted ecosystems in Hawaii by displacing several native species of solitary Hymenoptera, either by directly preying on these species or through competition for food resources (Wilson & Holway, 2010).

### 1.2.3. Impact on humans

Female yellowjackets possess a very painful sting that they use to deter any perceived threat to themselves or their nest (Akre *et al.*, 1980). They frequently sting humans, causing thousands of hospital visits every year (Langley *et al*., 2014). They can also cause life-threatening anaphylactic shock in people with an allergy to hymenopteran venom (Bonay *et al*., 1997; Faux *et al*., 1997; Vetter *et al*., 1999). Between 1999 and 2007, bees and wasps caused 509 deaths and accounted for 28.2% of all animal-related deaths in the United States (Forrester *et al.*, 2012). Yellowjackets in the genus *Vespula* build underground nests which are very cryptic and easy to step on accidentally. This can trigger an alarm response from the thousands of yellowjackets within the nest, posing a serious hazard to anyone nearby. In late summer, yellowjackets can build up very high population levels. Their presence in such high densities, especially in invaded areas, has delayed logging and agricultural projects, and has also resulted in school and recreational park closures (Akre *et al.*, 1980).

Yellowjackets are also pests of the beekeeping industry, as they will attack honey bee hives to feed on honey and bee larvae (Clapperton *et al*., 1989; de Jong, 1990). This causes them to become significant apicultural pests, thus impacting the pollination and subsequent yield of agricultural crops and orchard trees (Edwards, 1980).

## 1.3. Pest management of yellowjackets

### 1.3.1. Control tactics

Several different methods are used to control pestiferous yellowjacket populations. One of the most common methods is the use of attract-and-kill traps. In this tactic, a bait serves to draw yellowjackets into a trap containing several inches of a surfactant-laced liquid. Once a yelllowjacket enters the trap, it is unable to escape and eventually falls into the liquid, where it drowns (Landolt & Zhang, 2016). Alternatively, toxic baiting strategies can be used. In this tactic, an attractive bait laced with a pesticide is placed inside a baiting station which allows wasps to freely forage on the bait and bring portions of bait back to the nest, where it is fed to nestmates. In this way, the pesticide is spread through the nest and has the potential to kill more than just the foraging wasp (MacDonald *et al*., 1976; Akre *et al*., 1980).

Both attract-and-kill traps and toxic baiting stations require a bait which is highly attractive to the target yellowjacket species. In attract-and-kill tactics, this bait can be replaced with a synthetic chemical lure. These lures are often made to mimic the odorants emanating from attractive food sources (Day & Jeanne, 2001).

### 1.3.2. Known attractants

Although baits such as fermented fruit (Day & Jeanne, 2001; Dvořák & Landolt, 2006) or meats (Ross *et al.*, 1984; Spurr, 1995; Wood *et al*., 2006) are sometimes used to attract yellowjackets, they pose difficulties due to their short shelf life (Day & Jeanne, 2001) and potential to attract non-target species (Spurr, 1995, 1996). The most commonly used attractants are chemical lures, which often have a long shelf life and attract a narrow range of species (Day & Jeanne, 2001; Landolt & Zhang, 2016). Many esters and higher alcohols are known to be yellowjacket attractants, possibly due to their occurrence in fermenting fruit. Heptyl butyrate is a very effective yellowjacket attractant in North America; it is strongly attractive to *V. pensylvanica*, *V. squamosa*, *V. acadica*, *V. atropilosa*, *V. consobrina*, and *V. vidua* (MacDonald *et al.*, 1973; Landolt *et al.*, 2003, 2005; El-Sayed *et al*., 2009; Landolt & Zhang, 2016). Another widely used chemical attractant is 2-methyl-1-

butanol, which has been deployed either alone or in combination with acetic acid to attract *V. pensylvanica*, *V. germanica*, and *V. maculifrons* (Landolt *et al.*, 2000; Day & Jeanne, 2001). Most commercial yellowjacket lures contain either heptyl butyrate or 2-methyl-1-butanol (Landolt & Zhang, 2016). An alternative yellowjacket lure comprises 2-methyl-1-propanol (isobutanol) and acetic acid. Isobutanol is structurally similar to 2-methyl-1-butanol and is attractive to some populations of *V. germanica* and *V. vulgaris* as well as *V. pensylvanica* and *V. maculifrons* (Landolt, 1998; Day & Jeanne, 2001; Reed & Landolt, 2002; Landolt *et al.*, 2005).

### 1.3.3. Challenges

Although chemical attractants can be an effective tool for yellowjacket abatement, there is still room for improvement. Many lures are attractive only to a few yellowjacket species (Landolt *et al.*, 1999; Day & Jeanne, 2001), and may not be effective at controlling other species of yellowjackets. For example, although heptyl butyrate is a strong attractant for *V. pensylvanica*, it does not attract the two most commonly invasive and pestiferous yellowjacket species, *V. germanica* and *V. vulgaris* (Landolt & Zhang, 2016), nor does it attract *V. maculifrons* (Reed & Landolt, 2002), which is the major native pestiferous species in eastern North America. Other attractants are attractive only to certain populations of a targeted species and are unattractive to populations of the same yellowjacket species in different regions. This is true for a lure comprising isobutanol and acetic acid, which is a powerful attractant for *V. germanica* in Washington and Alaska (Landolt *et al.*, 1999, 2005) but not in New Zealand (El-Sayed *et al.*, 2009), Argentina, or British Columbia, Canada (Babcock & Borden, unpubl. obs.). Additional research is needed to identify attractants that can be used to trap those pestiferous yellowjacket species and populations which do not respond well to the above chemical lures.

## 1.4. Insect-yeast associations

### 1.4.1. Overview of interactions

Insect-yeast partnerships are widespread and, in many cases, are essential for the survival of one or both partners. Many insects receive nutritional benefits from their yeast

symbionts; for example, passalid beetles are unable to digest wood in their diet and rely on their yeast symbiont *Pichia stipitis* to break down xylose through fermentation (Suh *et al.*, 2003). Similarly, several species of rice planthoppers require certain sterols involved in moulting, which are provided by their *Candida* yeast symbionts (Eya *et al.*, 1989; Vega & Dowd, 2005). There is evidence that some yeast symbionts may play a role in detoxifying toxins within their insect host's diet; for example, yeasts present in the digestive tract of some cerambycid beetles are able to assimilate salicin, a toxin found in the leaves and bark of willow and popular trees (Meyer *et al.*, 1998; Vega & Dowd, 2005). Yeasts also play a role in the production of certain insect pheromones, such as the bark beetle anti-aggregation pheromone verbenone (Hunt & Borden, 1990). In turn, yeasts may benefit from their insect host, as the insect can vector them to new locations and provide a protected overwintering site within its gut (Vega & Dowd, 2005; Stefanini *et al.*, 2012). There is also evidence that the insect digestive tract provides a favourable environment for sexual reproduction by the yeasts (Stefanini *et al.*, 2016).

Many insects have been shown to respond to volatiles produced by their yeast symbionts (Davis *et al.*, 2013). This communication may be an important mechanism in facilitating an insect-yeast relationship, as it provides a way for the insect and yeast to signal to one another in a complex environment. It may also be exploited for pest management by trapping pestiferous insects using volatiles produced by their yeast symbiont as a trap bait. For example, the use of *Metschnikowia* yeast volatiles have been proposed as a bait for trapping codling moths, *Cydia pomonella*, the yeasts' host insect (Witzgall *et al.*, 2012).

### 1.4.2. Yellowjackets and fermentative yeasts

Recent research has found evidence that social wasps share a symbiotic relationship with yeasts within their digestive tract. In Italy, *Polistes* and *Vespa* wasps were found to internally harbour the yeast *Saccharomyces cerevisiae* (Stefanini *et al.,* 2012). A similar study found that a high proportion of *Vespula* and *Dolichovespula* yellowjackets in British Columbia, Canada, harboured the yeasts *Hanseniaspora uvarum* and *Lachancea thermotolerans* in their digestive tracts (Ibarra Jimenez *et al.,* 2017). Attraction of yellowjackets to fungal volatiles has also been demonstrated in that *V. pensylvanica* and

*V. germanica* respond to volatiles produced by the epiphytic fungus *Aureobasidium pullulans* isolated from the surface of apples (Davis *et al.,* 2012). Yellowjackets are also known to prefer aged, fermenting fruit over fresh fruit (Edwards, 1980; Matsuura & Yamane, 1990; Day & Jeanne, 2001), suggesting that fermentative yeasts may affect the attractiveness of fruit to foraging yellowjackets. To the best of our knowledge, however, attraction of yellowjackets to their yeast symbionts has not been investigated, and symbiotic yeasts have not yet been considered as a bait for trapping pestiferous yellowjackets.

## 1.5. Research objectives

My research aims to investigate the relationship between yellowjacket wasps and the symbiotic yeasts they harbour in their digestive tract, and to determine whether insect hosts and yeasts communicate. The overall goal of my research is to identify attractive volatiles from symbiotic yeasts that can be used to attract *Vespula* yellowjackets, particularly *V. germanica*, to a trap so that they can be controlled in areas where they become a pest problem. My specific objectives were to:

1. Determine whether the addition of brewer's yeast enhances attraction of yellowjackets to dried fruit and fruit powder baits (Chapter 2);

2. Investigate whether yeasts from the digestive tract of yellowjackets produce volatiles that attract yellowjackets (Chapter 3); and

3. Determine whether a commercially available strain of symbiotic yeast combined with fruit powder is an attractive bait for yellowjackets (Chapter 4).

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# Chapter 2.

# Brewer's yeast, *Saccharomyces cerevisiae*, enhances attraction of two invasive yellowjackets (Hymenoptera: Vespidae) to dried fruit and fruit powder[1]

[1]The corresponding manuscript is published in the *Journal of Insect Science* (2017, Volume 17(5): 91) with the following authors: Babcock, T., Gries, R., Borden, J.H., Palmero, L., Mattiacci, A., Masciocchi, M., Corley, J. and Gries, G. For this chapter, I conceived the study with feedback from G. Gries and J.H. Borden, prepared all baits, conducted aerations and identified volatiles with assistance from R. Gries, led the expedition to Argentina, designed all experiments, conducted experiments with assistance from L. Palmero, A. Mattiacci, and M. Masciocchi, identified and counted captured wasps with assistance from L. Palmero, analyzed all data statistically, and wrote the first draft of the manuscript.

## 2.1. Abstract

The German yellowjacket, *Vespula germanica* F., and common yellowjacket, *Vespula vulgaris* L. (Hymenoptera: Vespidae), are pests of significant economic, environmental, and medical importance in many countries. There is a need for the development and improvement of attractive baits that can be deployed in traps to capture and kill these wasps in areas where they are a problem. Yellowjackets are known to feed on fermenting fruit, but this resource is seldom considered as a bait due to its ephemeral nature and its potential attractiveness to nontarget species. We analyzed the headspace volatiles of dried fruit and fruit powder baits with and without Brewer's yeast, *Saccharomyces cerevisiae*, using gas chromatography–mass spectrometry, and we field tested these baits for their attractiveness to yellowjackets in Argentina. The addition of yeast to dried fruit and fruit powder changed the volatile compositions, increasing the number of alcohols and acids and decreasing the number of aldehydes. Dried fruit and fruit powder baits on their own had low attractiveness to yellowjackets, but the addition of

yeast improved their attractiveness by 9- to 50-fold and surpassed the attractiveness of a commercial heptyl butyrate-based wasp lure. We suggest that further research be done to test additional varieties and species of yeasts. A dried fruit or fruit powder bait in combination with yeast could become a useful tool in the management of yellowjackets.

**Keywords:** *Vespula;* Brewer's yeast; *Saccharomyces*; fermenting fruit; trap bait

## 2.2. Introduction

Yellowjackets in the genus *Vespula* (Hymenoptera: Vespidae) are significant nuisance pests due to their aggressive nest-defense behavior, their painful sting, and their tendency to frequent urban and agricultural areas (Akre *et al.*, 1980; de Jong, 1990; Beggs *et al*., 2011). They are also pests of the beekeeping industry, as they can attack honey bee hives to feed on honey and bee larvae (Clapperton *et al*., 1989; de Jong, 1990). They frequently sting humans, causing thousands of hospital visits every year (Langley *et al*., 2014), and they also cause life-threatening anaphylactic shock in some people (Bonay *et al*., 1997; Faux *et al*., 1997; Vetter *et al*., 1999). Yellowjackets build cryptic underground or aerial nests and, in late summer, build up high population levels. Their presence, especially in invaded areas, has adversely impacted logging and agricultural productivity and has resulted in school and recreational park closures in some instances (Akre *et al*., 1980).

The common yellowjacket, *Vespula vulgaris* L., and German yellowjacket, *Vespula germanica* F., are native to Europe but have become invasive in many countries, including some with no native yellowjackets such as New Zealand and Argentina (Beggs *et al*., 2011). In New Zealand, *V. vulgaris* competes with endemic bird species for honeydew resources in beech forests and has also greatly reduced populations of several native arthropods (Beggs, 2001). In Argentina, both *V. vulgaris* and *V. germanica* are invasive (Willink, 1980; Masciocchi *et al*., 2010), have rapidly spread across the country (Masciocchi & Corley, 2013), and have become prevalent nuisance pests.

Many synthetic chemical lures have been developed and deployed in traps to capture and kill social wasps (MacDonald *et al*., 1973; Landolt, 1998; Landolt *et al.*, 2000; Day & Jeanne, 2001; El-Sayed *et al*., 2009; Rust & Su, 2012). These traps can alleviate

the impact of yellowjacket pests by reducing local populations and by diverting foraging wasps away from humans (Davis *et al*., 1973; Rust & Su, 2012). However, these lures are not equally attractive to all species, and no operational synthetic lure has been developed specifically for *V. germanica* and *V. vulgaris*. Although a blend of isobutanol and acetic acid attracts *V. germanica* (Landolt, 1998; Day & Jeanne, 2001), this lure does not appear to work for all populations (El-Sayed *et al*., 2009; Babcock & Borden, unpubl. obs.), suggesting that yellowjackets may respond to lures differently in different geographical locations. Yellowjackets are generalist scavengers of both carbohydrate and protein resources and are known to feed on overripe fruit (Akre *et al*., 1980; Edwards, 1980; Matsuura & Yamane, 1990). While yellowjackets utilize both visual and olfactory cues to locate carbohydrate resources, olfaction is the more important sensory mode (Hendrichs *et al*., 1994; Moreyra *et al*., 2006). Several studies have considered volatiles from fruit and sugar resources as attractants for yellowjackets (McGovern *et al*., 1970; Landolt, 1998; Day & Jeanne, 2001; Dvořák & Landolt, 2006; Brown *et al*., 2014; Landolt & Zhang, 2016), but many of these lures are attractive only to a few species (Landolt *et al*., 2005; El-Sayed *et al*., 2009). This suggests that synthetic chemical lures may not accurately represent the full spectrum and dynamics of volatile production in ripening fruit. Fresh fruit as an attractant is limited by its rapid spoilage (Day & Jeanne, 2001) and its potential to attract nontarget species (Spurr, 1996); however, dried fruit and freeze-dried fruit powders may produce the same volatiles as fresh fruit and can be stored for long periods without spoiling. Prior to this study, these materials have never been considered for use as operational wasp attractants.

Yellowjackets are typically observed feeding on overripe and fermenting fruits (Edwards, 1980; Matsuura & Yamane, 1990). Fruits such as pear may become more attractive as they age (Day & Jeanne, 2001), and one study suggests that fermented apple pieces in syrup is attractive to wasps (Dvořák & Landolt, 2006). Fermentation involves the breakdown of fruits by microorganisms such as bacteria and yeasts, which metabolize fruit constituents and alter the chemical composition (Ubeda & Briones, 2000; Pino *et al*., 2010; Pielech-Przybylska *et al*., 2016). Microorganisms can produce volatiles that play a role in attracting insects to food resources (Davis *et al*., 2013). For example, vinegar flies, *Drosophila melanogaster* (Meigen), exploit volatiles from the yeast *Saccharomyces cerevisiae* to locate suitable fermenting fruits (Becher *et al*., 2012). In a recent study, *V.*

*germanica* and the western yellowjacket, *Vespula pensylvanica* (Saussure), were attracted to an epiphytic fungus isolated from the surface of apples (Davis *et al*., 2012), providing evidence that microbial cues affect foraging decisions.

Our objectives were to test the hypotheses that dried fruit and fruit powders are attractive to *V. vulgaris* and *V. germanica* and that adding Brewer's yeast, *S. cerevisiae*, to these fruit sources will change their headspace volatiles and enhance their attractiveness.

## 2.3. Materials and methods

### 2.3.1. Fruit baits

Dried fruit baits were made from dried apples (Real Canadian Superstore, Coquitlam, BC, Canada) and dried bananas (Thrifty Foods, Coquitlam, BC, Canada) ground into small pieces (approximately 0.5 $cm^2$) using a food processor. The two fruits were mixed together in equal proportions by weight, and 25 g of this mixture were placed into a teabag (6 × 8 cm; Finum Slim Tea Filter, Riensch & Held GmbH & Co. KG, Hamburg, Germany) to which sodium lauryl sulfate (2.5 g) was added to reduce water surface tension. Dried fruit plus yeast baits were made by adding 2.5 g of Brewer's yeast (Danstar Belle Saison Beer Yeast, Lallemand Inc., Montreal, QC, Canada) to the above mixture. Teabags were folded shut and secured using a single metal staple.

Fruit powder baits were made from freeze-dried apple powder (Drum Dried Northern Spy Apple Powder, Firehouse Pantry, Brookville, OH) and freeze-dried banana, strawberry, and raspberry powder (Just Tomatoes, Etc., Westley, CA) mixed together in equal proportions by weight. Aliquots of this mixture (25 g) were placed in teabags as above, and 2.5 g of sodium lauryl sulfate were added. Fruit powder plus yeast baits were made by adding 2.5 g of Brewer's yeast to the above mixture. Teabags were folded shut and secured as above.

### 2.3.2. Headspace volatile analyses

For each of the four teabag baits, headspace volatiles were captured and analyzed. A single teabag bait was submerged in 500 mL of water in a 600-mL beaker, which was placed into a clean Pyrex glass aeration chamber (340 mm high × 125 mm wide). An air pump (A.O. Smith, Tipp City, OH) drew charcoal-filtered air at 0.5 liter/min through the aeration chamber and then through a glass tube containing 0.2 g of Porapak-Q (50–80 mesh). Aerations were run for 24 h, after which volatiles were desorbed from Porapak-Q with 2 mL of a 50:50 mixture of pentane and ether. The extracts were then concentrated to a volume of 500 µL.

Aliquots (2 µL) of Porapak-Q extracts were analyzed using a Varian 3800 gas chromatograph (GC) coupled to a Saturn 2000 Ion Trap mass spectrometer (MS) (Agilent Technologies Inc., Santa Clara, CA). The GC-MS was fitted with a DB-5 GC-MS column (30 m × 0.25 mm internal diameter) and operated in full-scan electron impact mode. Helium was used as the carrier gas at a flow rate of 35 cm/s, with the following temperature program: 50°C (held for 5 min), then 10°C/min until 280°C (held for 36 min). The injector was set at 250°C and the transfer line at 280°C. The foreline pressure was 52.4 kPa. Sample volatiles were identified and quantified by comparing their retention times and mass spectra with those of authentic standards.

### 2.3.3. Field experiments

Two field experiments were run concurrently at different sites near San Carlos de Bariloche, Argentina from 2–4 March, 2016. Experiment 1 (N = 12 replicates) was conducted at a sheep and cattle farm, and Experiment 2 (N = 8 replicates) was conducted in an urban nature reserve. Both experiments were set up in a randomized complete block design, with the blocking factor as different sections of the field site. Experimental baits were immersed in 500 mL of water inside plastic bag-style wasp traps (Scotts Canada Ltd., Delta, BC, Canada), with two offset entry ports to discourage escape. There was no incubation period for any bait prior to the onset of Experiments 1 and 2. Traps were hung ≥5 m apart approximately 1 m above ground from bush and tree branches using white cotton string. Traps were left in the field for 48 h before being collected. The contents of each trap were poured through a strainer and captured yellowjackets were counted and

identified to species using characteristic markings on their head and abdomen (Akre *et al*., 1980; Masciocchi *et al*., 2010).

Experiment 1 tested the attractiveness of dried fruit with and without Brewer's yeast. The treatments were: 1) dried fruit teabag bait, 2) dried fruit plus yeast teabag bait, 3) heptyl butyrate-based lure (50-mL emulsifiable concentrate, 77.4% = 0.81 g/lure heptyl butyrate in 450-mL water; Scotts Canada Ltd., Delta, BC, Canada), and 4) water control. Because heptyl butyrate is attractive to several yellowjacket species (MacDonald *et al*., 1973; Landolt *et al*., 2005; El-Sayed *et al*., 2009), treatment 3) was used as a positive control. Treatment 4) consisted of 500-mL water and 2.5 g of sodium lauryl sulfate and was used as a negative control.

Experiment 2 tested the attractiveness of fruit powder with and without Brewer's yeast using methodology as described for Experiment 1. The treatments were: 1) fruit powder teabag bait, 2) fruit powder plus yeast teabag bait, 3) heptyl butyrate-based lure, and 4) water control.

## 2.4. Results

### 2.4.1. Headspace volatile analyses

The headspace volatiles identified by GC-MS and their relative abundance in blends emanating from dried fruit teabags with and without yeast (Figure 2.1) and fruit powder teabags with and without yeast (Figure 2.2) are summarized in Table 2.1. Volatile compositions of all four baits differed. In general, the presence of yeast increased the number of alcohols and carboxylic acids. 2-Methylbutyric acid, isobutyric acid, and 2-phenylethyl alcohol emanated only from baits containing yeast. The presence of yeast also appeared to reduce the number of aldehydes and to increase the relative abundance of isoamyl acetate (1.33 and 1.43 times in the dried fruit bait and fruit powder bait, respectively). Dodecyl alcohol emanated from all the baits and was likely a derivative of the sodium lauryl sulfate surfactant.

### 2.4.2. Field experiments

In Experiment 1, there was a significant difference among treatment means for both *V. vulgaris* ($F_{3,33} = 79.47$; $P < 0.0001$) and *V. germanica* ($F_{3,33} = 43.76$; $P < 0.0001$) (Figure 2.3). For both species, traps baited with dried fruit plus yeast captured significantly more yellowjackets than traps baited with dried fruit and water controls. For *V. vulgaris* (but not *V. germanica*), traps baited with dried fruit plus yeast captured significantly fewer yellowjackets than traps with the heptyl butyrate lure ($P_{V. vulgaris} = 0.005$; $P_{V. germanica} = 0.984$). For both species, captures in traps baited with dried fruit alone did not differ from those in water control traps ($P_{V. vulgaris} = 0.740$; $P_{V. germanica} = 0.577$) and were significantly lower than in traps baited with the heptyl butyrate lure ($P < 0.0001$ for both species).

In Experiment 2, there was also a significant difference among the treatment means for both *V. vulgaris* ($F_{3,21} = 46.16$; $P < 0.0001$) and *V. germanica* ($F_{3,21} = 45.37$; $P < 0.0001$) (Figure 2.4). For both species, traps baited with fruit powder plus yeast captured significantly more yellowjackets than traps baited with fruit powder, the heptyl butyrate–based lure or water ($P < 0.0001$ for each of the six pairwise comparisons). Traps baited with fruit powder alone captured significantly more *V. germanica* than water control traps ($P < 0.001$), but these treatments did not differ significantly in the number of *V. vulgaris* captured ($P = 0.069$). For both species, captures in traps baited with fruit powder did not differ from those in traps baited with the heptyl butyrate-based lure ($P_{V. vulgaris} = 0.279$; $P_{V. germanica} = 0.962$).

Traps baited with dried fruit and fruit powder teabags with and without yeast captured low numbers of vinegar flies and earwigs. Traps baited with fruit powder plus yeast also captured a total of three *Polistes* paper wasps. No honey bees or other hymenopteran insects were captured in either of the two experiments.

## 2.5. Discussion

The addition of yeast had a significant effect on the volatile blends emanating from dried fruit and fruit powder. Isobutyric acid, 2-methylbutyric acid, and 2-phenylethyl alcohol were present in headspace volatile blends of dried fruit and fruit powder only when yeast

was added, and furfuryl alcohol was produced when yeast was added to fruit powder. This is expected because yeast metabolism produces higher alcohols during fruit fermentation (Palanca *et al*., 2013). Surprisingly, the esters in headspace volatile blends of fruit compositions did not change when yeast was added. Yeast fermentation typically produces esters from the metabolism of carboxylic acids and alcohols (Palanca *et al*., 2013; Golonka *et al*., 2014). It is possible that the dried fruit and fruit powder, unlike fresh fruit, do not contain the specific precursors necessary for ester formation. *S. cerevisiae* possesses several aldehyde dehydrogenases and reductases, which convert aldehydes to carboxylic acids such as acetic acid (Liu & Moon, 2009; Datta *et al*., 2017). This explains the smaller number of aldehydes and the larger number of carboxylic acids in headspace volatile blends of dried fruit mixtures containing yeast. In particular, furfural is a known inhibitor of microbial fermentation, as it damages cell membranes and DNA, inhibits enzymatic activity, and prevents DNA and RNA synthesis (Liu & Moon, 2009). Furfural is present in the fruit powder volatile blend but is absent when yeast is added; this is indicative of aldehyde reductase activity.

Neither dried fruit nor fruit powder was very attractive on its own to yellowjackets. Fruit powder alone attracted only a few yellowjackets, but it performed on par with the heptyl butyrate-based lure for both species (Figure 2.4). In comparison, the dried fruit bait attracted significantly fewer yellowjackets than the heptyl butyrate lure (Figure 2.3). These data in combination suggest that fruit powder is more attractive to yellowjackets than dried fruit. This phenomenon could be explained by the greater number of esters emanating from fruit powder than from dried fruit. Esters are fruity or floral fruit odorants, and many are known as yellowjacket attractants (Davis *et al*., 1967, 1968; McGovern *et al*., 1970; Landolt, 1998). Butyl butyrate, an ester present in our fruit powder bait, but not in our dried fruit bait, is a known attractant for *V. vulgaris* (El-Sayed *et al*., 2009) and other species such as the western yellowjacket, *V. pensylvanica* (Landolt, 1998). Two additional esters, ethyl hexanoate and isoamyl acetate, are reported as attractants for *V. vulgaris* (Brown *et al*., 2014) and both were present in the fruit powder volatile blend. Isoamyl acetate was also found in dried fruit volatiles but in much lower concentrations. The fruit powder volatile blend contained isobutyl acetate, which is formed by the esterification of isobutanol and acetic acid. These two latter odorants are strong attractants for *V. germanica* (Landolt, 1998; Day & Jeanne, 2001), suggesting that the corresponding ester may also be

attractive. Furthermore, our fruit powder volatile blend included raspberries and strawberries, which were not present in the dried fruit composition. Raspberries emit esters such as butyl acetate and ethyl hexanoate (Aprea *et al*., 2015) that we observed only in the fruit powder volatile blend, and strawberries contain 4-hydroxy-2,5-dimethyl-3-furanone (Williams *et al*., 2005); some of these odorants may have played a role in the differential attractiveness of dried fruit and fruit powder.

The presence of Brewer's yeast in the dried fruit and fruit powder compositions resulted in a 9- to 50-fold increase in bait attractiveness relative to the dried fruit and fruit powder alone. For both species of yellowjackets, the fruit powder plus yeast bait was also significantly more attractive than the heptyl butyrate-based lure. Heptyl butyrate is a widely used attractant for yellowjackets (MacDonald *et al*., 1973; Landolt *et al*., 2005; El-Sayed *et al*., 2009; Landolt & Zhang, 2016).

By-catches of nontarget insects were minimal. Small numbers of vinegar flies and earwigs were captured in traps with dried fruit and fruit powder, regardless of the presence of yeast, and the eight traps baited with fruit powder plus yeast captured a total of three *Polistes* paper wasps. No bees or any other Hymenoptera were captured, even though they were present in both field sites. Therefore, there is apparently minimal potential for traps baited with dried fruit or fruit powder plus yeast to have an adverse effect on beneficial species in this geographical area.

The distinctively different headspace volatile blends originating from dried fruit and fruit powder with and without yeast are consistent with reports that fruit fermentation by yeasts results in the production of higher alcohols and esters (Palanca *et al*., 2013; Golonka *et al*., 2014) and the reduction of aldehydes to carboxylic acids (Liu & Moon, 2009; Datta *et al*., 2017). One higher alcohol, 2-phenylethyl alcohol, originated only from baits containing yeast. This alcohol alone is weakly attractive to yellowjackets (Davis *et al*., 2012) and may have contributed to the attractiveness of fruit plus yeast baits. Several carboxylic acids such as isobutyric acid, 2-methylbutyric acid, and 3-methylbutyric acid were present in headspace volatile blends only when yeast was part of the fruit composition. These acids may be attractive semiochemicals for yellowjackets. Fewer aldehydes were generated from compositions containing yeast, suggesting that aldehydes

may be yellowjacket repellants that are removed by the yeast. The role of Brewer's yeast in attracting yellowjackets suggests that, like other insects (Becher *et al*., 2012; Davis *et al*., 2013), yellowjackets may use microbe-produced volatiles to locate fermenting fruit. It is also possible that yeasts may be mutualistic symbionts of yellowjackets. Recent studies indicate that *S. cerevisiae* uses social wasps as vectors, overwintering sites (Stefanini *et al*., 2012), and sexual reproduction sites (Stefanini *et al*., 2016). In turn, yellowjackets may receive nutrients in the form of metabolic by-products, e.g., amino acids (Hansen *et al*., 2011), from endosymbiotic yeasts, as shown in many insect–microbe associations (Douglas, 1989). Thus, orientation to yeast-produced volatiles may be an adaptive trait for yellowjackets. The recent discovery (Ibarra Jimenez *et al*., 2017) that the digestive tracts of five species of North American yellowjackets harbor several species of yeasts (particularly species in the genera *Lachancea* and *Hanseniaspora* but not *S. cerevisiae*) suggests that these potential symbionts may produce a composition of volatiles that is even more attractive than those produced by *S. cerevisiae* on a fruit powder substrate.

In conclusion, our data suggest that fruit powder plus yeast shows potential for use as an operational yellowjacket bait. The bait may be improved further by using different fruits or varieties and species of yeasts.

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## 2.7. References

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**Table 2.1. Compositions of headspace volatile blends captured from teabags containing dried fruit or fruit powder baits, with and without Brewer's yeast. The percentage of each volatile identified by GC-MS in a specific blend is shown. Numbers correspond with compounds identified in Figures 1 and 2. Total percentages of all odorants may be less than 100% due to GC column bleed compounds and to several unknown odorants (all less than 2%).**

| Compounds | No. | Dried Fruit | Dried Fruit + Yeast | Fruit Powder | Fruit Powder + Yeast |
|---|---|---|---|---|---|
| **Carboxylic acids** | | | | | |
| butyric acid | 1 | | 5.3 | 1.8 | 3.9 |
| isobutyric acid | 2 | | 12.8 | | 7.2 |
| 2-methyl butyric acid | 3 | | 8.4 | | 9.8 |
| 3-methyl butyric acid | 4 | 1.0 | 17.1 | | 14.4 |
| hexanoic acid | 5 | | 5.0 | 1.3 | 0.9 |
| octanoic acid | 6 | 1.0 | 5.1 | 1.6 | |
| nonanoic acid | 7 | 0.5 | | | |
| benzoic acid | 8 | 2.7 | 1.6 | | |
| **Alcohols** | | | | | |
| 2-heptanol | 9 | | | 1.2 | 0.4 |
| dodecyl alcohol | 10 | 66.7 | 30.2 | 25.5 | 8.8 |
| linalool | 11 | | | 0.5 | |
| furfuryl alcohol | 12 | | | | 5.6 |
| 2-phenylethyl alcohol | 13 | | 4.3 | | 2.4 |
| eugenol | 14 | | | 2.1 | 1.0 |
| **Esters** | | | | | |
| butyl acetate | 15 | | | 4.1 | 1.2 |
| isobutyl acetate | 16 | | | 6.6 | 3.7 |

**Table 2.1 continued**

| Compounds | No. | Dried Fruit | Dried Fruit + Yeast | Fruit Powder | Fruit Powder + Yeast |
|---|---|---|---|---|---|
| isoamyl acetate | 17 | 1.8 | 2.4 | 12.4 | 17.7 |
| ethyl butyrate | 18 | | | 7.5 | 4.1 |
| butyl butyrate | 19 | | | 1.7 | 0.3 |
| butyl isobutyrate | 20 | | | 1.5 | 0.8 |
| ethyl hexanoate | 21 | | | 1.1 | 0.4 |
| **Aldehydes** | | | | | |
| hexanal | 22 | 1.8 | 1.8 | | |
| (*E*)-2-hexenal | 23 | | | 3.9 | |
| heptanal | 24 | 0.8 | | | |
| (*E*)-2-heptenal | 25 | 0.5 | | | |
| octanal | 26 | 1.7 | | 1.3 | |
| nonanal | 27 | 7.6 | 3.8 | 3.4 | 1.6 |
| decanal | 28 | 3.5 | 2.2 | 1.4 | 1.6 |
| undecanal | 29 | 0.8 | | | |
| furfural | 30 | | | 6.4 | |
| **Ketones** | | | | | |
| sulcatone | 31 | 0.3 | | | 0.7 |
| geranyl acetone | 32 | 0.6 | | | |
| 2-undecanone | 33 | 0.9 | | | |
| α-ionone | 34 | | | 0.8 | |
| β-ionone | 35 | | | 0.8 | |
| 4-hydroxy-2,5-dimethyl-3-furanone | 36 | | | 8.6 | 5.8 |

**Table 2.1 continued**

| Compounds | No. | Dried Fruit | Dried Fruit + Yeast | Fruit Powder | Fruit Powder + Yeast |
|---|---|---|---|---|---|
| **Others** | | | | | |
| hydrogen peroxide | 37 | 1.1 | | | |
| styrene | 38 | 1.0 | | | 6.7 |

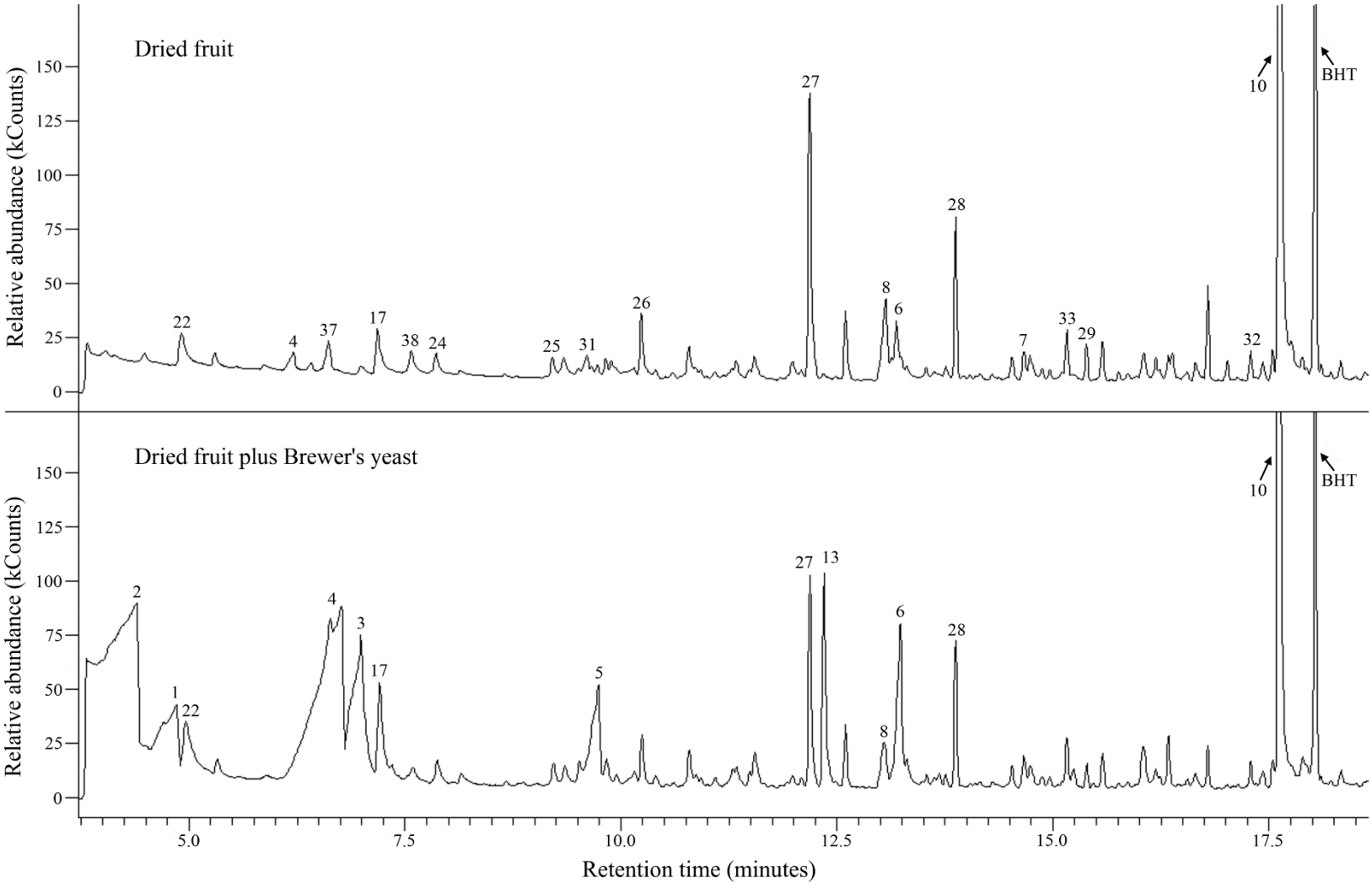


**Figure 2.1. Total ion chromatograms of odorants originating from dried fruit baits with and without Brewer's yeast. Numbers above or next to odorants (peaks) correspond to those listed in Table 1. BHT = butylated hydroxytoluene (an antioxidant in the solvent). Note particularly the increase in relative abundance of poorly chromatographing acids (numbers 1, 2, 3, 4, and 5; see Table 1) when yeast is present.**

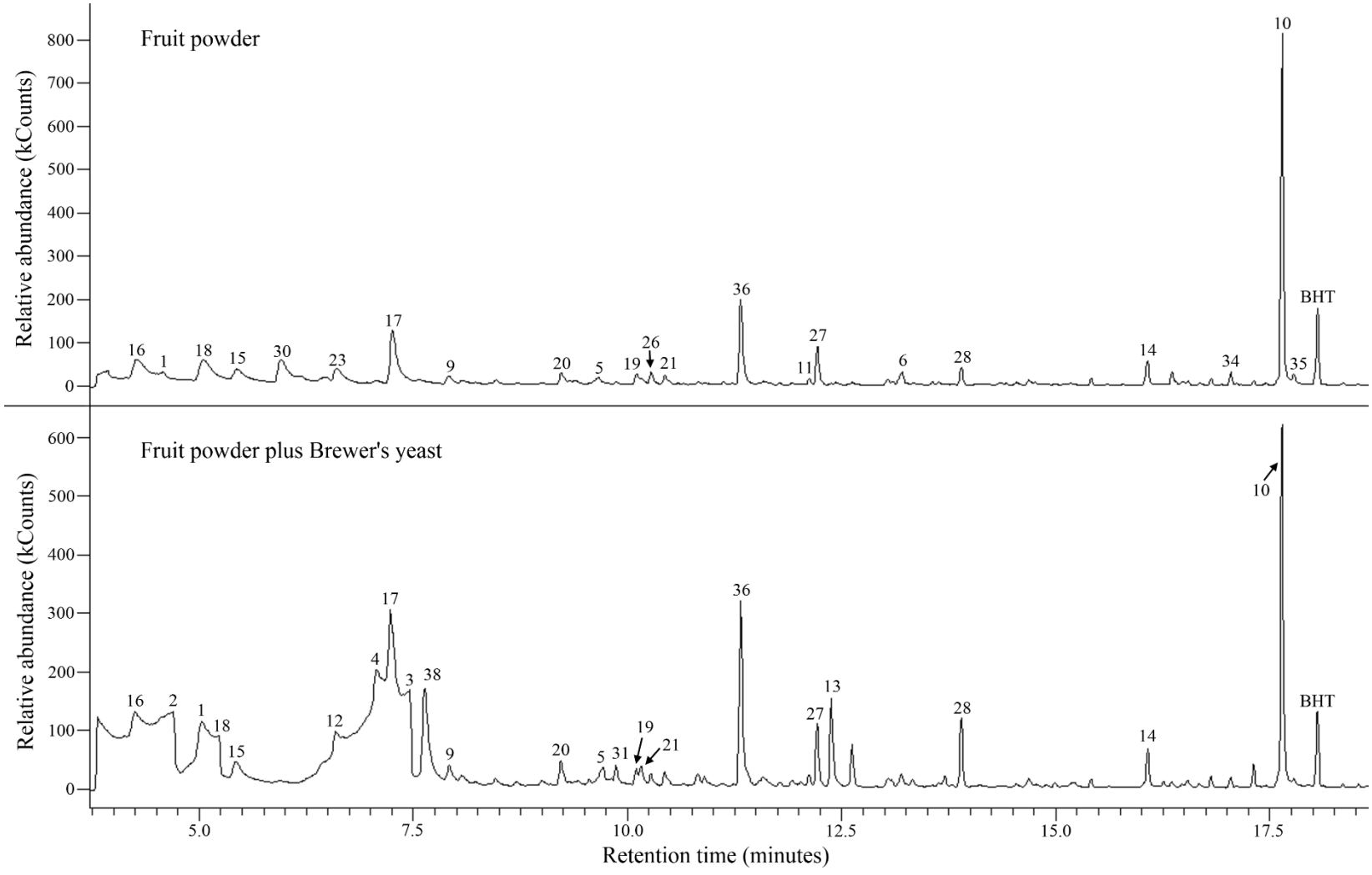


**Figure 2.2. Total ion chromatograms of odorants originating from fruit powder baits with and without Brewer's yeast. Numbers above or next to odorants (peaks) correspond to those listed in Table 1. BHT = butylated hydroxytoluene (an antioxidant in the solvent). Note particularly the increase in relative abundance of poorly chromatographing acids (numbers 1, 2, 3, and 4; see Table 1) when yeast is present.**

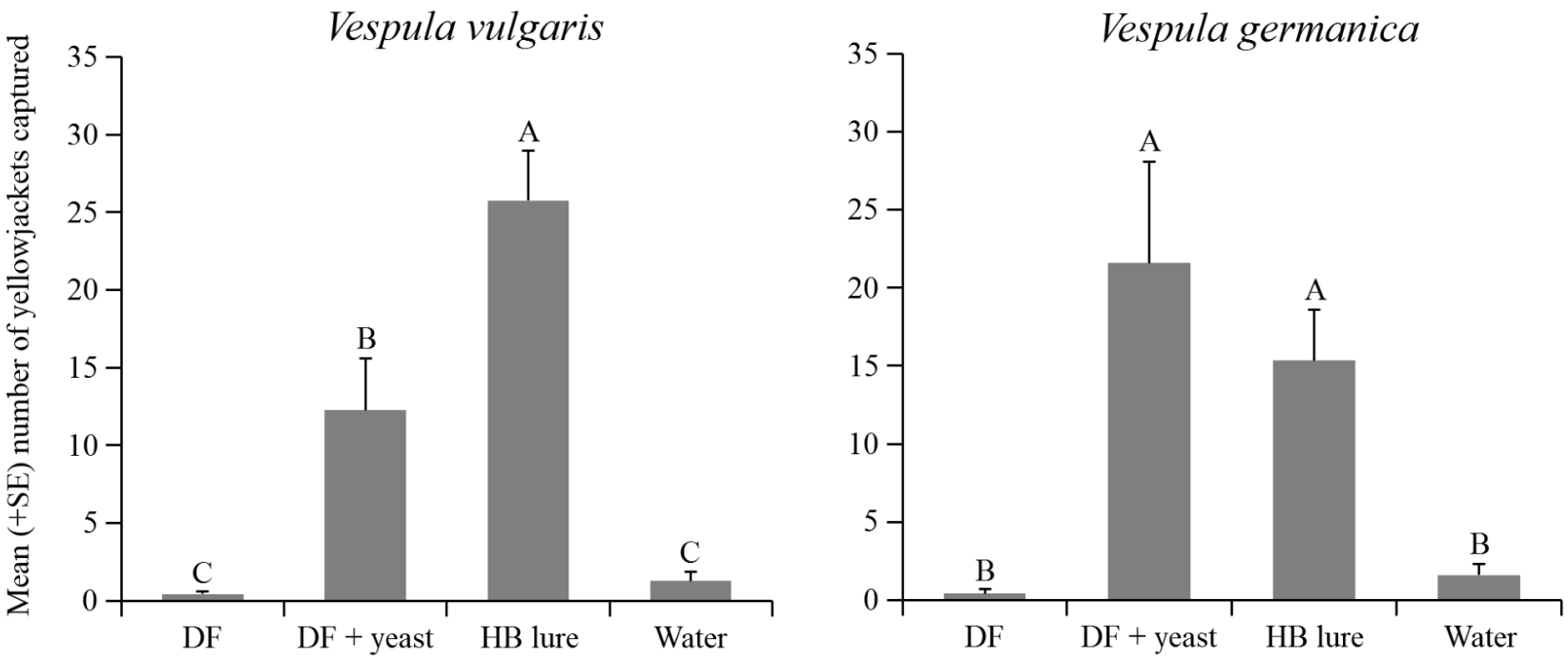


**Figure 2.3.** **Mean numbers of *Vespula vulgaris* and *Vespula germanica* captured per trap in Experiment 1 (N = 12) that was run at a sheep and cattle farm near San Carlos de Bariloche (Argentina) and tested teabags containing dried fruit (DF) with and without Brewer's yeast. Traps baited with a heptyl butyrate (HB)-based lure or filled with water were used as positive and negative controls, respectively. Bars labelled with the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).**

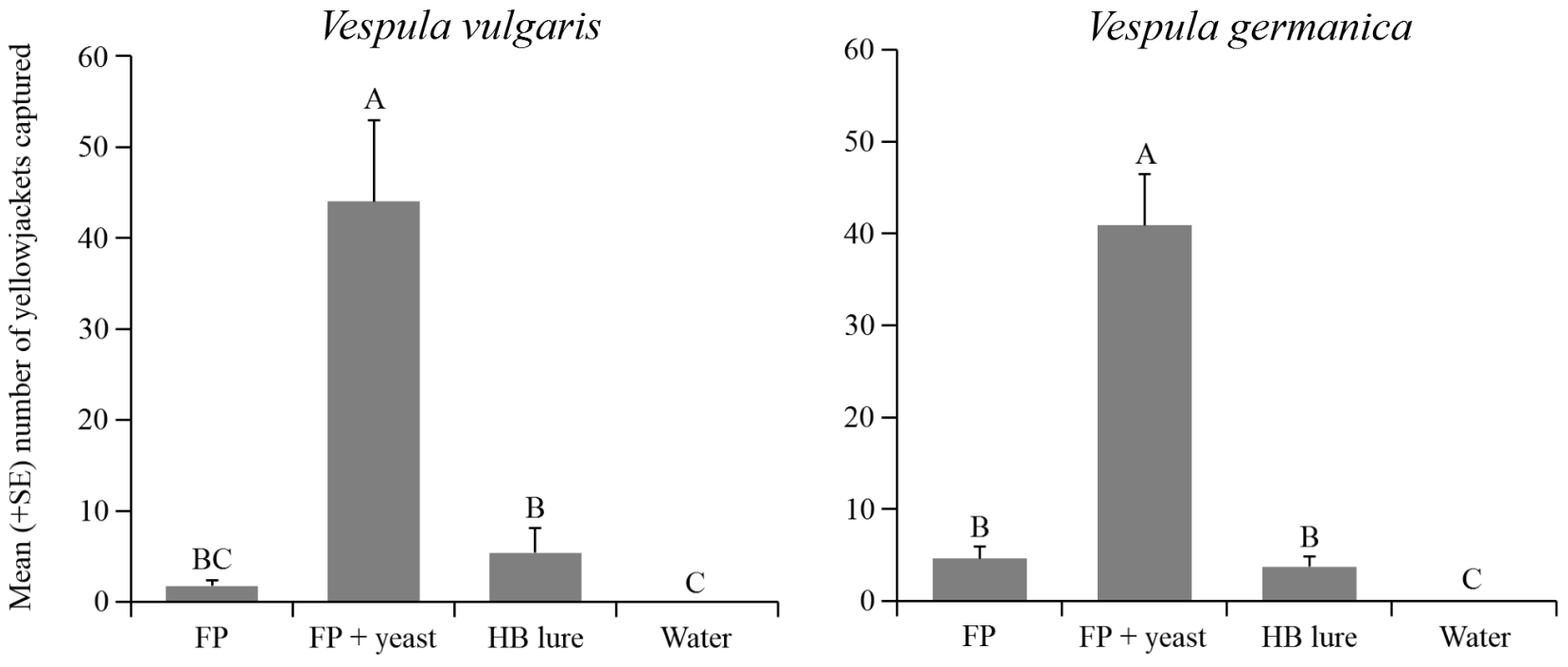


**Figure 2.4.** **Mean numbers of *Vespula vulgaris* and *Vespula germanica* captured per trap in Experiment 2 (N = 8) that was run at an urban nature reserve near San Carlos de Bariloche (Argentina) and tested fruit powder teabags (FP) with and without Brewer's yeast. Traps baited with a heptyl butyrate (HB)-based lure or filled with water were used as positive and negative controls, respectively. Bars labelled with the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).**

# Chapter 3.

# Inter-kingdom signaling – symbiotic yeasts produce semiochemicals that attract their yellowjacket hosts[1]

[1]The corresponding manuscript has been submitted for peer review to *Entomologia Experimentalis et Applicata* with the following authors: Babcock, T., Borden, J.H., Gries, R., Carroll, C., Lafontaine, J.P., Moore, M., and Gries, G. For this chapter, I conceived the study with feedback from G. Gries, M. Moore, and J.H. Borden, prepared yeast cultures with assistance from C. Carroll, conducted aerations and identified volatiles with assistance from R. Gries, formulated emulsifiable concentrates of synthetic volatile blends with feedback from J.P. Lafontaine, designed all experiments, conducted experiments with assistance from J.H. Borden, identified and counted captured wasps with assistance from J.H. Borden, analyzed all data statistically, and wrote the first draft of the manuscript.

## 3.1. Abstract

The yeast species *Hanseniaspora uvarum* and *Lachancea thermotolerans* were isolated from the digestive tract of four North American yellowjacket species. In a separate study, we demonstrated attraction of yellowjackets in Argentina to brewer's yeast, *Saccharomyces cerevisiae*, growing on fruit powder. We tested the hypothesis that *Vespula* spp. are attracted to cultures of *H. uvarum* and *L. thermotolerans* and their respective volatiles. In field experiments, we found that *H. uvarum* and *L. thermotolerans* are attractive to three species of yellowjackets, but only when grown on grape juice-infused YPD agar. Using gas chromatography–mass spectrometry, we analyzed the headspace volatiles produced by these yeasts, and field tested an 18-component yeast synthetic semiochemical blend. This synthetic blend attracted western yellowjackets, *Vespula pensylvanica* (Saussure), but no other yellowjacket species. Acetic acid or ethanol added to the synthetic blend at biologically relevant doses either had no effect or

significantly lowered trap captures. Our results demonstrate that yeast symbionts isolated from the digestive tract of yellowjackets are attractive to their hosts. Further research is needed to identify the volatiles mediating attraction of species other than *V. pensylvanica* to the yeast cultures.

**Keywords:** *Vespula*; yeast symbiosis; *Hanseniaspora uvarum*; *Lachancea thermotolerans*; yellowjacket semiochemicals

## 3.2. Introduction

Yellowjackets are worldwide pests of significant economic, environmental, and medical importance. Members of the genus *Vespula* (Hymenoptera: Vespidae) build nests under- or above-ground and aggressively defend these nests when disturbed (Akre *et al*., 1980; Matsuura & Yamane, 1990). Their stings are painful and can cause anaphylactic shock or even death in individuals with venom hypersensitivity (Bonay *et al*., 1997; Faux *et al*., 1997; Vetter *et al*., 1999). They are also apicultural pests, attacking honey bee hives to feed on honey and bee larvae (Clapperton *et al*., 1989; de Jong, 1990). Several species of yellowjackets are invasive in many countries around the world (Beggs *et al*., 2011); in some instances, introduced yellowjackets consume copious amounts of endemic arthropods and out-compete native bird species for food resources, resulting in a severe ecological impact (Beggs, 2001). In their native range, yellowjackets may play a beneficial role by consuming forest defoliators or other arthropod pests (Edwards, 1980; Donovan, 2003), or by pollinating plants such as common ivy (Jacobs *et al*., 2009) and certain orchid species (Cheng *et al*., 2009). However, both native and invasive yellowjackets can become pests when they nest in agricultural fields, parks, or school playgrounds (Akre *et al*., 1980). A single nest can have thousands of nest mates that may pose a serious hazard to people in the area (Akre *et al*., 1980; Edwards, 1980).

Many different lures (baits) have been developed for trapping yellowjackets (MacDonald *et al*., 1973; Landolt, 1998; Landolt *et al*., 2000; Day & Jeanne, 2001; El-Sayed *et al*., 2009; Rust & Su, 2012; Babcock *et al*., 2017). However, these lures do not effectively attract all species of yellowjackets (Landolt *et al*., 1999; Day & Jeanne, 2001). There is also evidence that some lures do not work equally well in all geographic areas. For example, isobutanol and acetic acid work very well for attracting German

yellowjackets, *Vespula germanica* (F.), in some areas (Landolt *et al*., 1999) but not in others (El-Sayed *et al*., 2009; Babcock & Borden, unpubl. obs.).

In Europe, vespine wasps share a symbiotic relationship with the yeast *Saccharomyces cerevisiae* that resides in their gastrointestinal tract (Stefanini *et al*., 2012). In British Columbia, Canada, the yeasts *Hanseniaspora uvarum* and *Lachancea thermotolerans* were the species most frequently isolated from the digestive tract of multiple *Vespula* and *Dolichovespula* spp. (Ibarra Jimenez *et al*., 2017). Insect-microbe symbioses are widespread, and many are mutualistic (Vega & Dowd, 2005; Douglas, 2009). In some of these symbioses, the insects and microbes signal to one another via chemical communication. For example, apple maggots, *Rhagoletis pomonella* (Walsh), preferentially orient to the odour of *Enterobacter* spp. bacteria, which are common gut symbionts (Lauzon *et al*., 1998). Response to microbes has also been demonstrated with yellowjackets. Both the German yellowjacket and the western yellowjacket, *Vespula pensylvanica* (Saussure), are attracted to volatiles produced by the epiphytic fungus *Aureobasidium pullulans* (Davis *et al*., 2012), and German yellowjackets and common yellowjackets, *Vespula vulgaris* (L.), are attracted to brewer's yeast (Babcock *et al*. 2017).

We hypothesized that *Vespula* spp. are attracted to volatiles produced by yeasts found in their digestive tract. Our objectives were (1) to determine whether previously isolated *H. uvarum* and *L. thermotolerans* strains are attractive to *Vespula* spp., (2) to identify and quantify the headspace volatiles produced by these yeasts, and (3) to test whether a synthetic blend of the identified compounds is attractive to *Vespula* species.

## 3.3. Materials and methods

### 3.3.1. Field experiments

Field experiments were conducted at the following agricultural sites in British Columbia, Canada: Experiments 1, 2, 3, and 8 at a raspberry farm (28025 Smith Avenue, Abbotsford, V4X 1C7), Experiment 4 at a vineyard with an apiary (Campbell's Gold Honey Farm and Meadery, 2595 Lefeuvre Road, Abbotsford, V4X 1H5), and Experiments 5, 6, and 7 at a blueberry and raspberry farm (1400 272nd Street, Langley, V4W 2P9). All

experiments had 12 replicates except for Experiment 7 which had 15 replicates. Experiments were set up in a randomized complete block design, with the blocking factor as different sections of the field site. Baits or lures were placed into plastic cylindrical wasp traps [PheroTech Inc, Delta, BC V4G 1E9, Canada (no longer in operation)] with a horizontal entry tube that allowed access from either side, and a cut-out portion at mid-span of the tube that gave yellowjackets entry into the trap chamber, while discouraging escape. Agar baits were hung underneath the lid of the trap, and 350 mL of water mixed with 0.1% Sparkleen-1 detergent (Fisherbrand™, Fisher Scientific Co., Pittsburgh, PA 15219, USA) was poured into the bottom of the trap to serve as the drowning solution for captured wasps. The detergent decreased the surface tension of the water, causing captured wasps to sink and drown. Synthetic blend lures, formulated as emulsifiable concentrates in water, were poured directly into the bottom of the trap in place of the drowning solution. The surfactant in the concentrates eliminated the need to add detergent. Traps were hung ≥ 10 m apart, 1 – 1.5 m above ground from fence posts or wires in crop fields using 18-gauge steel wire (Rona, Coquitlam, BC V3B 1B9, Canada). Traps were left in the field for 3 – 6 days before being collected. The contents of each trap were poured through a strainer, and captured yellowjackets were counted and identified to species using characteristic markings on their head and abdomen (Akre *et al*., 1980).

### 3.3.2. Testing yeast cultures

Pure stock cultures of *H. uvarum* and *L. thermotolerans*, obtained from the digestive tract of North American yellowjackets (Ibarra Jimenez *et al*., 2017), were maintained on Yeast Peptone Dextrose (YPD) agar media (10 g/L yeast extract, 20 g/L bacteriological peptone, 20 g/L dextrose, and 20 g/L agar) at 4°C.

Experiments with cultured yeasts grown on agar in petri dishes were conducted in August and September 2016. Experiment 1 tested the attractiveness of *H. uvarum* and *L. thermotolerans* cultures to yellowjackets. The treatments were: a) *H. uvarum* on YPD agar, b) *L. thermotolerans* on YPD agar, and c) YPD agar control. Yeast cultures were made by pouring approximately 25 mL of autoclaved YPD agar into sterile plastic petri dishes (60 mm × 15 mm) and inoculating the surface of the cooled agar with yeast cells from the stock cultures. Plates were incubated at 30°C for 24 h prior to the experiment. The lid of

each petri dish was removed at the field site immediately prior to the experiment, and a piece of 18-gauge steel wire was threaded through a small hole in the side of the dish. This wire was then inserted through the lid of a wasp trap such that the open petri dish was suspended inside the trap.

Experiment 2 tested whether the volatiles from *H. uvarum* and *L. thermotolerans* are synergistically attractive with volatiles from grapes, without allowing contact of the grapes and yeast. The treatments were: a) *H. uvarum* on YPD agar plus grapes, b) *L. thermotolerans* on YPD agar plus grapes, and c) YPD agar plus grapes control. Yeast cultures were made and secured inside traps as above. Three red seedless grapes (Nester's Market, Burnaby, BC V5A 4X6, Canada) were pierced and threaded onto the wire suspending the petri dish from the lid of the trap.

Experiment 3 tested the attractiveness of *H. uvarum* and *L. thermotolerans* cultures growing on YPD agar infused with grape juice. The treatments were: a) *H. uvarum* on "grape juice agar", b) *L. thermotolerans* on grape juice agar, and c) grape juice agar control. Yeast cultures were prepared as above, except that the YPD agar was supplemented with grape juice by replacing half of the water in the media with filter-sterilized grape juice (Western Family™, Vancouver, BC V6B 4E4, Canada), which was added after autoclaving the media. Each petri dish contained 12 mL of grape juice, or approximately three grape equivalents. Cultures were secured inside wasp traps as above.

### 3.3.3. Analyses of headspace volatiles

Grape juice agar was prepared as above and poured into sterile plastic petri dishes (100 × 15 mm). The plates were then inoculated by transferring yeast cells from a stock plate to each grape juice agar plate. Yeast cultures were incubated at 30°C for 24 h prior to volatile captures. The agar was then removed from each of five petri dishes and placed into a clean Pyrex® glass aeration chamber (34 cm high × 12.5 cm wide). An air pump (A.O. Smith, Tipp City, OH 45371, USA) drew charcoal-filtered air at 0.5 L/min through the aeration chamber and then through a glass tube containing 0.2 g of Porapak-Q adsorbent

(50-80 mesh). After 8 h of aerations, volatiles were desorbed from the Porapak-Q with 2 mL of pentane/ether (1/1).

Aliquots (2 µL) of Porapak-Q extracts were analyzed using a Varian 3800 gas chromatograph (GC) coupled to a Saturn 2000 Ion Trap mass spectrometer (MS) (Agilent Technologies Inc., Santa Clara, CA 95051, USA). The GC–MS was fitted with a DB-5 GC–MS column (30 m × 0.25 mm internal diameter) and operated in full-scan electron impact mode. Helium was used as the carrier gas at a flow rate of 35 cm/s, with the following temperature program: 50°C for 5 min, then 10°C/min until 280°C. The injector was set at 250°C and the transfer line at 280°C. Sample volatiles were identified by comparing their retention times and mass spectra with those of authentic standards.

### 3.3.4. Field testing of a synthetic volatile blend

Yeast headspace volatiles identified by GC–MS (Table 3.1) were purchased or synthesized and prepared as a synthetic blend (Table 3.2), which was field tested from July – September, 2017. Because *H. uvarum* and *L. thermotolerans* produced comparable volatile blends (Table 3.1), only the 18-component *H. uvarum* volatile blend was used as the template for synthetic blend preparation. The candidate synthetic blend was formulated into an emulsifiable concentrate by combining 15 g of the blend with 153 g of CO-630 nonionic surfactant (polyoxyethylene nonylphenylether; Norman, Fox, & Co., City of Industry, CA 91744, USA) and 618 g of water. Immediately prior to the start of each experiment, the emulsifiable concentrate was diluted to a volume of approximately 350 mL per trap with the amounts and percentages of volatiles as shown in Table 3.2. For experiments that tested the effect of ethanol or acetic acid in admixture with the synthetic blend, these additional components were added directly to the diluted emulsion immediately prior to the experiment. In Experiment 8, some chemicals were deleted from treatments b) through e) and replaced by water; the weights and percentages of the remaining components remained the same as in Table 3.2.

Experiment 4 tested the response of yellowjackets to the candidate synthetic blend (SB) of volatiles produced by *H. uvarum* yeast growing on grape juice agar. The treatments were: a) SB, b) SB plus 5% ethanol and 0.5% acetic acid, and c) water control. Ethanol

and acetic acid were both added to treatment b) because both are produced by *H. uvarum* during grape must fermentation (Ciani & Picciotti, 1995; Moreira *et al*., 2008). There is also evidence that acetic acid enhances the attraction of yellowjackets to certain lures (Landolt *et al*., 2000). We estimated the percentage of ethanol to add based on the amounts reported during grape fermentation by *H. uvarum* (Ciani & Picciotti, 1995), as well as anecdotal reports that beer (containing approximately 5% alcohol) is attractive to yellowjackets. Similarly, we estimated the percentage of acetic acid to add to the blend based on reports that 0.5% is most effective for enhancing yellowjacket attraction to esters and alcohols (Landolt, 1998).

Experiments 5 – 7 tested the response of yellowjackets to the candidate SB plus various dosage combinations of ethanol and acetic acid. The treatments for Experiment 5 were: a) SB, b) SB plus 1% ethanol, c) SB plus 5% ethanol, and d) water control. The treatments for Experiment 6 were a) SB, b) SB plus 0.1% acetic acid, c) SB plus 0.5% acetic acid, and d) water control. The treatments for Experiment 7 were: a) SB, b) SB plus 1% ethanol and 0.1% acetic acid, c) SB plus 1% ethanol and 0.5% acetic acid, d) SB plus 5% ethanol and 0.1% acetic acid, and e) SB plus 5% ethanol and 0.5% acetic acid.

Experiment 8 tested the response of yellowjackets to the yeast synthetic blend with certain chemical groups removed from the blend, one or two at a time. The treatments were: a) SB, b) SB minus all esters, c) SB minus all alcohols, d) SB minus all carboxylic acids and aldehydes, and e) SB minus all ketones and pyrazines.

### 3.3.5. Statistical analyses

Data of field experiments were transformed by $\log_{10}(x + 1)$ and analyzed using JMP 12® (SAS Institute Inc., Cary, NC 27513, USA). For all experiments, the mean numbers of yellowjackets captured per trap were compared among treatments using a one-way randomized block ANOVA and Tukey's HSD test for pairwise comparisons of means. In all cases $\alpha = 0.05$.

## 3.4. Results

### 3.4.1. Testing yeast cultures

There was no difference in the number of yellowjackets responding to each of the treatments in Experiment 1 (effect of yeast cultures on YPD agar) ($F_{2,22} = 1.00$, $P = 0.384$) and Experiment 2 (effect of grapes and yeast cultures on YPD agar) ($F_{2,22} = 2.02$, $P = 0.156$). Experiment 1 captured only three yellowjackets, all *V. germanica*, with two in the *H. uvarum* treatment and one in the *L. thermotolerans* treatment. Experiment 2 captured five yellowjackets; a single *V. germanica* was captured to *L. thermotolerans*, while the control traps contained one *V. pensylvanica* and three *Vespula alascensis* (Packard) [previously misidentified in North America as *V. vulgaris* (Carpenter & Glare, 2010)].

In Experiment 3 (effect of yeast cultures on grape juice-infused YPD agar), trap captures differed among treatment means ($F_{2,22} = 11.37$, $P = 0.0004$), with traps containing *H. uvarum* or *L. thermotolerans* cultures capturing significantly more yellowjackets than traps containing grape juice agar controls ($P = 0.0006$ and $P = 0.003$, respectively). Traps containing *H. uvarum* or *L. thermotolerans* cultures as baits captured similar numbers of yellowjackets ($P = 0.783$) (Figure 3.1) and similar numbers of *V. pensylvanica*, *V. germanica*, and *V. alascensis*.

### 3.4.2. Analyses of headspace volatiles

The headspace volatiles produced by *H. uvarum* and *L. thermotolerans* growing on grape juice YPD agar are shown in Table 3.1, with the respective blends containing 18 and 22 compounds. The two blends had 15 compounds in common, and all compounds unique to either blend were present at < 1%. Relatively abundant components of the *H. uvarum* blend were isoamyl alcohol (36.5%), ethyl acetate (23.8%), isoamyl acetate (16.5%), 2-methyl-1-butanol (14.0%), 2-phenylethyl alcohol (3.0%), and phenylethyl acetate (1.82%). Similarly, relatively abundant components of the *L. thermotolerans* blend were isoamyl alcohol (74.7%), 2-phenylethyl alcohol (11.5%), 2-methyl-1-butanol (8.04%), and isoamyl acetate (2.27%).

### 3.4.3. Field testing of a synthetic volatile blend

In Experiment 4 (effect of the SB with or without ethanol and acetic acid), there was a significant difference among the treatment means ($F_{2,22} = 3.65$, $P = 0.0428$) (Figure 3.2). SB-baited traps captured significantly more yellowjackets than traps containing the water control ($P = 0.0463$). Captures in traps baited with the SB plus ethanol and acetic acid were not significantly different from those in traps baited with either the SB alone ($P = 0.124$) or the water control ($P = 0.872$). The yellowjackets captured in Experiment 4 were almost exclusively *V. pensylvanica*, with only three *V. germanica* (one per treatment) and no *V. alascensis*.

The treatment means also differed significantly from one another in Experiment 5 ($F_{3,33} = 7.28$, $P = 0.0007$) and Experiment 6 ($F_{3,33} = 4.13$, $P = 0.0136$) (Figure 3.3) that each tested the SB plus various dosage combinations of ethanol and acetic acid. In Experiment 5, all variations of the SB (without ethanol, with 1% ethanol, and with 5% ethanol) attracted significantly more yellowjackets than the water control ($P = 0.0013$, $P = 0.0021$, and $P = 0.0255$); however, addition of either 1% or 5% ethanol to the SB did not increase its attractiveness ($P = 0.681$ and $P = 0.999$). In Experiment 6, the SB alone and with 0.5% acetic acid attracted significantly more yellowjackets than the water control ($P = 0.0224$ and $P = 0.0224$), but the 0.1% acetic acid treatment did not ($P = 0.259$). Addition of either 0.1% or 0.5% acetic acid to the SB did not increase its attractiveness ($P = 0.648$ and $P = 1.000$). In both experiments, almost all yellowjackets captured were *V. pensylvanica*, with a single *V. germanica* responding to the SB plus 0.5% acetic acid treatment and no *V. alascensis* captured.

In Experiment 7 (further effects of the SB plus various dosage combinations of ethanol and acetic acid), there was a significant difference among the treatment means ($F_{4,56} = 8.25$, $P < 0.0001$), with the SB alone capturing more yellowjackets than the SB plus 1% ethanol and 0.1% acetic acid ($P = 0.0004$), 1% ethanol and 0.5% acetic acid ($P = 0.0186$), 5% ethanol and 0.1% acetic acid ($P < 0.0001$), or 5% ethanol and 0.5% acetic acid ($P = 0.0136$) (Figure 3.4). Captures to treatments with varying doses of ethanol and acetic acid did not differ significantly from one another. Almost all yellowjackets captured in this experiment were *V. pensylvanica*, with only five *V. germanica* and two *V. alascensis* captured to the various treatments.

In Experiment 8 (effects of complete and partial SBs), there was a significant difference among the treatment means ($F_{4,44}$ = 3.96, P = 0.0078) (Figure 3.5). The SB lacking alcohols attracted significantly more yellowjackets than the SB lacking esters (P = 0.0097), or the SB lacking both ketones and pyrazines (P = 0.0256). However, traps baited with the complete SB captured as many yellowjackets as traps baited with SBs lacking esters (P = 0.499), alcohols (P = 0.351), carboxylic acids and aldehydes (P = 0.978), or ketones and pyrazines (P = 0.723). Except for one *V. germanica* responding to the SB without alcohols, only *V. pensylvanica* were captured in this experiment.

## 3.5. Discussion

Our experiments show that the yeasts *H. uvarum* and *L. thermotolerans* are attractive to yellowjackets when grown on YPD agar infused with grape juice (Figure 3.1), but not when these yeasts are grown on YPD agar deployed alone or with grapes. We conclude that these yeasts produce semiochemicals (message bearing chemicals) when metabolizing grape juice (and probably other fruit-based substrates), but not when metabolizing YPD agar. Thus, as has been observed with other microbes, these yeasts utilize specific metabolic pathways to metabolize diverse substrates that alter the volatiles they produce (Fiddaman & Rossall, 1994; Kiviranta *et al*., 1998; Van Lancker *et al*., 2008; Plugge *et al*., 2011).

Both *H. uvarum* and *L. thermotolerans* are ubiquitous in the environment, but are frequently isolated from various fruits (Pitt & Hocking, 2009; Hranilovic *et al*., 2017). They are particularly common in grapes and grape must (Paraggio, 2004; Barata *et al*., 2012; Balikci *et al*., 2016). YPD medium contains dextrose, peptone, yeast extract, and agar, which provide limited substrates for synthesis of complex volatile blends. In contrast, fruits such as grapes contain a diverse array of sugars and amino acids (Shiraishi, 2000), which would provide substrates for biosynthesizing higher alcohols (Palanca *et al*., 2013). Fruits also contain carboxylic acids (Shiraishi, 2000), which – when combined with alcohols – form esters (Palanca *et al*., 2013; Golonka *et al*., 2014). Higher alcohols and esters are abundant constituents of the volatiles produced by *H. uvarum* and *L. thermotolerans* grown on grape juice agar (Table 3.1) and are also common constituents of synthetic lures

for yellowjackets (Davis *et al*., 1968; McGovern *et al*., 1970; Landolt, 1998; Landolt *et al*., 2000).

The similarity of headspace volatiles produced by *H. uvarum* and *L. thermotolerans* (Table 3.1) is expected, as both yeasts occupy similar ecological niches and share the same metabolic pathways (Barata *et al*., 2012). Several of the most abundant compounds produced by these yeasts have the potential to attract yellowjackets. For example, isoamyl alcohol is a common product of yeast fermentation (Viana *et al*., 2008; González-Robles *et al*., 2015), and is moderately attractive to yellowjackets (Landolt *et al*., 2000; Brown *et al*., 2014). Its corresponding ester, isoamyl acetate, is a known attractant for yellowjackets (Brown *et al*., 2014), and 2-methyl-1-butanol is a potent attractant for many yellowjacket species (Landolt & Zhang, 2016).

Comparing the compounds produced by *H. uvarum* and *L. thermotolerans* to those produced by brewer's yeast, *S. cerevisiae*, grown on freeze-dried fruit powder in water (Babcock *et al*., 2017), reveals that the three yeast species share few volatiles. Isoamyl acetate, 2-phenylethyl alcohol, and butyric acid are produced by all three yeasts, and isobutyric acid, 2-methylbutanoic acid, and 3-methylbutanoic acid are produced by *L. thermotolerans* and *S. cerevisiae*, but not *H. uvarum*. Because *S. cerevisiae* is attractive to yellowjackets in Argentina (Babcock *et al*., 2017), the volatiles shared between multiple yeast species may play a role in yellowjacket attraction to yeasts in general. However, the same brewer's yeast bait that we tested in Argentina (Babcock *et al*., 2017) was only weakly attractive to *V. alascensis* and not attractive to western yellowjackets in British Columbia (Babcock & Borden, unpubl. obs.). This could indicate that yellowjackets in different regions may be more selective about or prefer different yeast species. Volatiles that are not shared among multiple species of yeasts may inform yellowjackets about the presence of specific species inhabiting an ecological niche.

The indifferent or adverse effects of ethanol and acetic acid when added to the SB (Figures 3 – 5) were unexpected, given that both compounds are produced by *H. uvarum* (Ciani & Piciotti, 1995). Many insect semiochemicals are known to be attractive at a low dose, but repellant at a higher dose (Finch, 1978; Hao *et al*., 2013). Thus, although care was taken to test ethanol and acetic acid at biologically realistic levels, the doses may still

have been too high. These data suggest that yellowjackets may be most strongly attracted to yeasts in the early stages of fermentation, when ethanol and acetic acid are produced at very low concentrations. This concept is supported by findings that yellowjackets responded more strongly to pears aged for 24-h than to fresh or 48-h aged pears, possibly due to optimal concentrations of energy-yielding sugars in the early stages of aging (Day & Jeanne, 2001), with sugar concentrations diminishing in later stages of aging (Chanprasartsuk *et al*., 2010). Therefore, higher concentrations of ethanol and acetic acid released during later stages of fruit fermentation (Romano *et al*., 2003; Chanprasartsuk *et al*., 2010) could inform yellowjackets that the fruit is no longer suitable for consumption.

To determine the key constituent(s) of the *H. uvarum* semiochemical blend, we tested partial blends lacking certain functional groups of chemicals. The results show that no single group makes the complete attractiveness of blend, but that esters make a more significant contribution than other chemical groups. Esters are associated with floral or fruity odours, and many are known attractants for yellowjackets (Davis *et al*., 1967, 1968; McGovern *et al*., 1970; Landolt, 1998).

Transitioning from a yeast culture bait (Figure 3.2) to a SB (Figures 3.3 – 3.6) essentially terminated attraction of *V. germanica* and *V. alascensis*. Possible explanations are that the yeast culture bait, unlike the SB, produced some highly volatile components, including gases, that were either not captured on the Porapak Q or that remained below detection threshold of the GC–MS. One such component might be $CO_2$ which serves as an attractant and behavioral activator for many insect species, including cotton bollworms, hawkmoths, wireworms, phytophagous beetles, bed bugs and kissing bugs, fleas, and various phytophagous and hematophagous dipterans (Johnson & Gregory, 2006; Jones, 2013; Gries 2018). Other such components may be ammonia, acetone and dimethyldisulfide, which are attractants or behavioral activators for hematophagous dipterans (Hassanali *et al*., 1986; Braks *et al*., 2001; Bernier *et al*., 2003; Mathew *et al*., 2013). Some yeasts have been shown to produce ammonia (Palková *et al*., 1997; Zikánová *et al*., 2002) and dimethyldisulfide (Cholet *et al*., 2008), and the storage mold *Penicillum brevicompactum* produces acetone (Börjesson *et al*., 1992).

Yeasts may accrue substantial benefits from symbiosis with yellowjackets. Wasps vector yeasts to new locations and provide sites for overwintering (Stefanini *et al*., 2012) and sexual reproduction (Stefanini *et al*., 2016) within their gut. The wasps too may accrue benefits. Many insects depend on yeasts or other microbial symbionts for production of nutritional by-products that insects then use as a source of nitrogen, amino acids, vitamins, or sterols (Potrikus & Breznak, 1981; Vega & Dowd, 2005; Douglas, 2009). Honey bees depend on their gut microbiome which enables digestion of polysaccharides and polypeptides in their diet (Lee *et al*., 2015). Yeasts dwelling within the yellowjacket gut may provide a similar metabolic service to their hosts. Alternatively, yeast semiochemicals may guide yellowjackets to suitable food resources. For example, semiochemicals produced by *Saccharomyces cerevisiae* guide the vinegar fly, *Drosophila melanogaster* (Meigen), to fermenting fruit (Becher *et al*., 2012). This phenomenon could explain why yellowjackets are attracted to yeasts only when growing on grape juice-supplemented YPD substrate compared to YPD media alone.

In conclusion, we show that the yeasts *H. uvarum* and *L. thermotolerans* growing on a fruit substrate are attractive to their yellowjacket hosts. A yeast synthetic semiochemical blend is also attractive, but only to western yellowjackets, posing a challenge to future researchers to identify the missing components that impart more general attractiveness. The yeast synthetic semiochemical blend developed in this work might then have potential for use as an operational yellowjacket trap lure.

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**Table 3.1. Compositions of headspace volatile blends emanating from *H. uvarum* and *L. thermotolerans* yeast grown on grape-juice infused YPD agar. The percentage of each volatile identified by GC-MS in each blend is shown.**

| Compounds | *Hanseniaspora uvarum* | *Lachancea thermotolerans* |
|---|---|---|
| **Esters** | | |
| ethyl acetate | 23.8 | 0.31 |
| ethyl propionate | 0.50 | |
| phenylethyl acetate | 1.82 | 0.10 |
| ethylphenyl acetate | | 0.17 |
| isoamyl acetate | 16.5 | 2.27 |
| isoamyl propionate | 0.21 | |
| furfuryl acetate | 0.05 | |
| ethyl butyrate | | 0.12 |
| ethyl hexanoate | 0.21 | 0.28 |
| ethyl octanoate | 0.70 | 0.21 |
| ethyl decanoate | 0.80 | 0.03 |
| ethyl dodecanoate | 0.16 | 0.05 |
| methyl anthranilate | | 0.10 |
| **Alcohols** | | |
| 2-phenylethyl alcohol | 3.00 | 11.5 |
| isoamyl alcohol | 36.5 | 74.7 |
| 2-methyl butanol | 14.0 | 8.04 |
| methionol | 0.16 | 0.08 |
| **Carboxylic Acids** | | |
| butyric acid | 0.12 | 0.17 |

**Table 3.1 continued**

| Compounds | *Hanseniaspora uvarum* | *Lachancea thermotolerans* |
|---|---|---|
| isobutyric acid | | 0.40 |
| 2-methylbutanoic acid | | 0.05 |
| 3-methylbutanoic acid | | 0.09 |
| **Aldehydes** | | |
| acetal | 0.39 | 0.50 |
| **Ketones** | | |
| acetoin | 0.98 | 0.73 |
| **Others** | | |
| 2,5-dimethyl pyrazine | 0.10 | 0.08 |
| styrene | | 0.06 |

**Table 3.2. Weights and proportions of synthetic blend components in the emulsifiable concentrate lure and per 350 mL diluted emulsion for baiting one trap.**

| Component | mg/trap | %/trap | Purity (%) | Supplier |
|---|---|---|---|---|
| ethyl acetate | 208 | 0.059 | 99 | Caledon[1] |
| ethyl propionate | 4.55 | 0.0013 | 99 | Sigma-Aldrich[2] |
| phenylethyl acetate | 15.9 | 0.0045 | 95 | Gries-lab[3] |
| isoamyl acetate | 144 | 0.041 | 97 | Sigma-Aldrich[2] |
| isoamyl propionate | 1.84 | 0.00053 | 95 | Gries-lab[3] |
| furfuryl acetate | 0.44 | 0.00013 | 95 | Gries-lab[3] |
| ethyl hexanoate | 1.84 | 0.00053 | 95 | Gries-lab[3] |
| ethyl octanoate | 6.13 | 0.0018 | 95 | Gries-lab[3] |
| ethyl decanoate | 7.00 | 0.0020 | 95 | Gries-lab[3] |
| ethyl dodecanoate | 1.40 | 0.00040 | 95 | Gries-lab[3] |
| 2-phenylethyl alcohol | 26.3 | 0.0075 | 99 | Fluka[4] |
| isoamyl alcohol | 319 | 0.091 | >95 | Fisher Chemical[5] |
| 2-methyl-butanol | 123 | 0.035 | 98 | Sigma-Aldrich[2] |
| methionol | 1.40 | 0.00040 | 98 | Sigma-Aldrich[2] |
| acetal | 3.41 | 0.00097 | 99 | Sigma-Aldrich[2] |
| butyric acid | 1.05 | 0.00030 | 99 | Sigma-Aldrich[2] |
| acetoin | 8.58 | 0.0025 | >95 | Sigma-Aldrich[2] |
| 2,5-dimethyl pyrazine | 0.88 | 0.00025 | 98 | Sigma-Aldrich[2] |
| CO-630 | 8925 | 2.55 | | Norman, Fox, & Co.[6] |
| Water | 340 200 | 97.2 | | |
| TOTALS | 350 000 | 100.00 | | |

[1] Caledon Laboratory Chemicals, Georgetown, ON L7G 4R9, Canada
[2] Sigma-Aldrich, St. Louis, MO 631903, USA
[3] Synthesized from corresponding alcohols and acids by standard procedures
[4] Fluka Chemie GMBH, Buchs, CH-9471, Switzerland
[5] Fisher Scientific, Fair Lawn, NJ 07410, USA
[6] Norman, Fox, & Co., City of Industry, CA 91744, USA

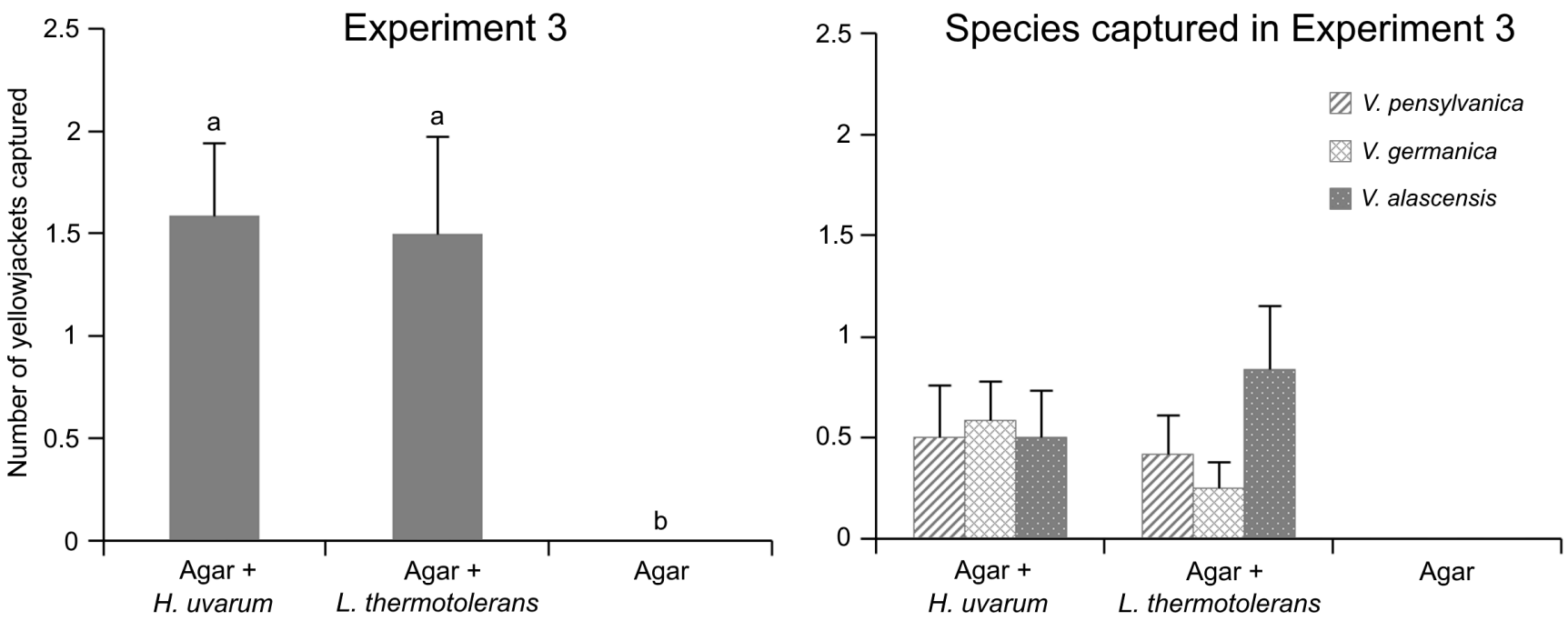


**Figure 3.1.** **Mean (+ SE) numbers of yellowjackets captured per trap in Experiment 3 (N = 12), testing cultures of *H. uvarum* and *L. thermotolerans* growing on grape juice-infused YPD agar. The left graph shows the means for all yellowjackets, and the right graph shows the means for individual yellowjacket species. Traps baited with uninoculated agar were used as controls. Bars labelled with the same letter are not significantly different (Tukey's HSD test, P < 0.05).**

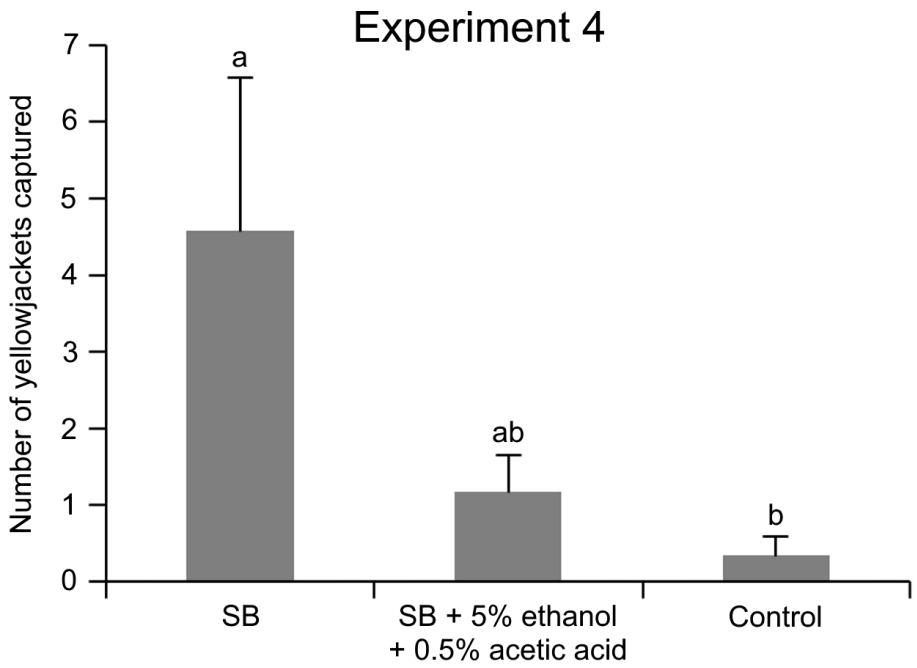


**Figure 3.2.** **Mean (+ SE) numbers of yellowjackets captured per trap in Experiment 4 (N = 12), testing a synthetic blend (SB) of headspace volatiles, with and without ethanol and acetic acid, produced by *H. uvarum* growing on grape juice-infused YPD agar. Traps baited with water were used as controls. Bars labelled with the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).**

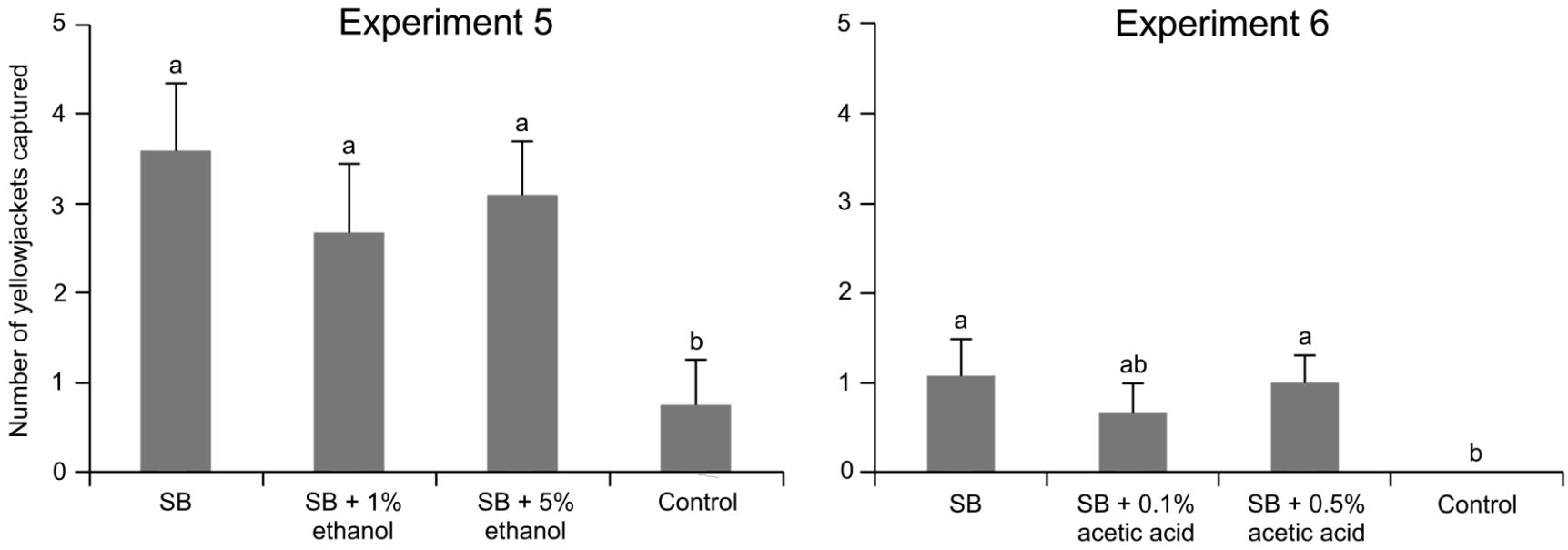


**Figure 3.3.** **Mean (+ SE) numbers of yellowjackets captured per trap in Experiment 5 (N = 12), testing a yeast synthetic volatile blend (SB; see Table 2) alone, with 1% or 5% ethanol, and in Experiment 6 (N = 12), testing a yeast synthetic volatile blend (SB) alone, with 0.1% or 0.5% acetic acid. Traps baited with water were used as controls. Bars labelled with the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).**

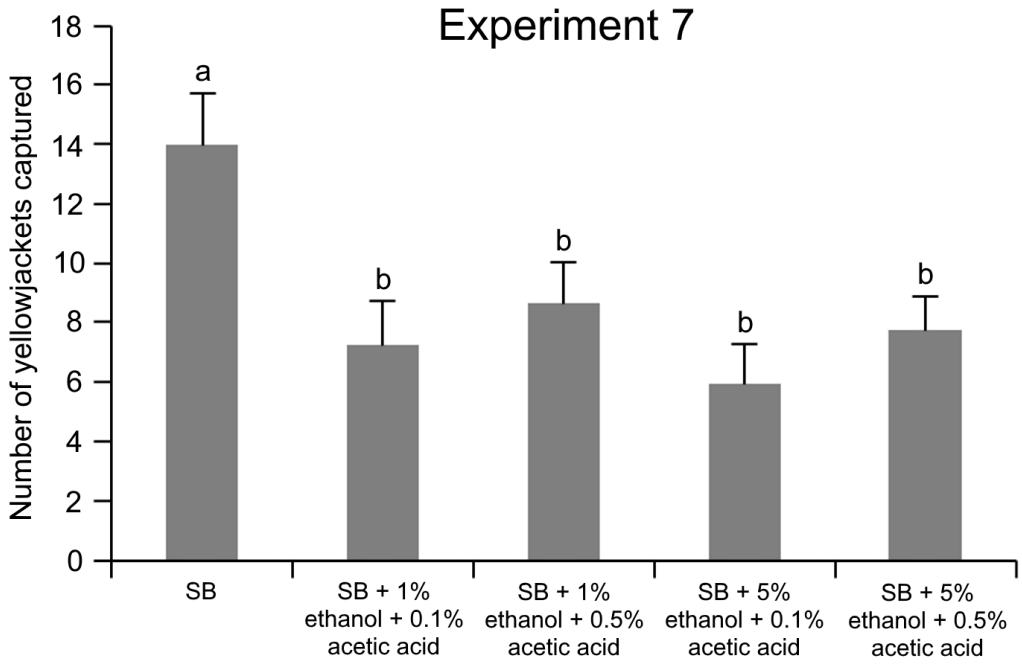


**Figure 3.4.** **Mean (+ SE) numbers of yellowjackets captured per trap in Experiment 7 (N = 15), testing a yeast volatile synthetic blend (SB; see Table 2) alone or with various combinations of high and low doses of ethanol and acetic acid. Bars labelled with the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).**

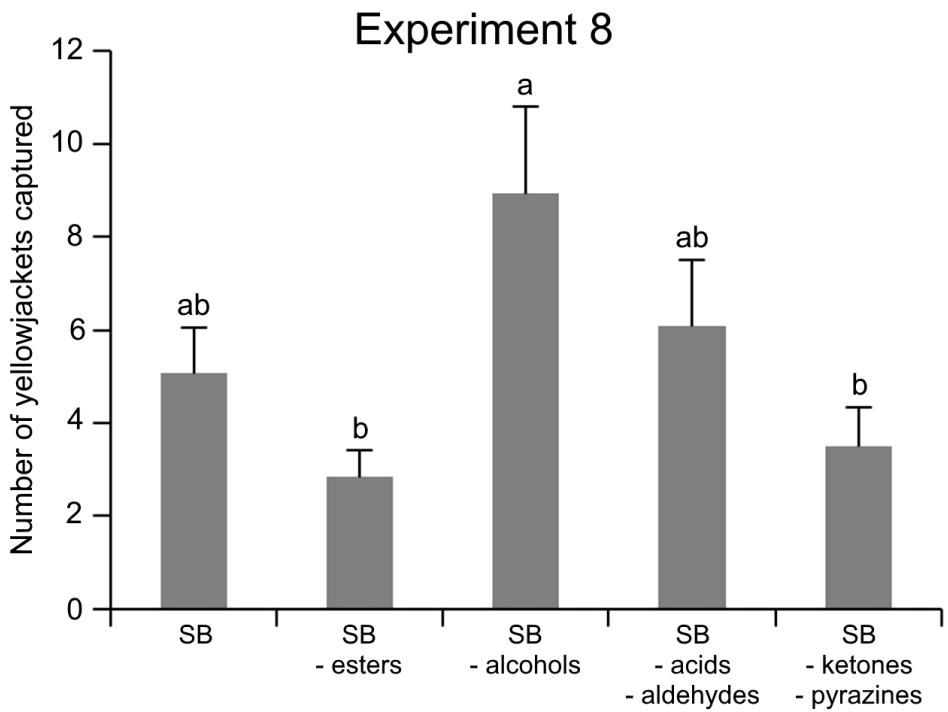


**Figure 3.5.** **Mean (+ SE) numbers of yellowjackets captured per trap in Experiment 8 (N = 12), testing a complete yeast synthetic volatile blend (SB; see Table 2) and partial blends lacking one or two chemical groups. Bars labelled with the same letter are not significantly different (Tukey's HSD test, P < 0.05)**

# Chapter 4.

# *Lachancea thermotolerans*, a yeast symbiont of yellowjackets, enhances attraction of three yellowjacket species (Hymenoptera: Vespidae) to fruit powder[1]

[1]The corresponding manuscript has been submitted for peer review to *Environmental Entomology* with the following authors: Babcock, T., Borden, J.H., Gries, R., Carroll, C., Moore, M., and Gries, G. For this chapter, I conceived the study with feedback from G. Gries, M. Moore, and J.H. Borden, prepared yeast cultures with assistance from C. Carroll, extracted and analyzed DNA with assistance from C. Carroll, conducted aerations and identified volatiles with assistance from R. Gries, prepared all baits, formulated the synthetic volatile blend, designed all experiments, conducted experiments with assistance from J.H. Borden, identified and counted captured wasps with assistance from J.H. Borden, analyzed all data statistically, and wrote the first draft of the manuscript.

## 4.1. Abstract

Previously, we showed that the symbiotic yeast *Lachancea thermotolerans* is attractive to its *Vespula* (Hymenoptera: Vespidae) yellowjacket hosts when grown on media supplemented with grape juice. We hypothesized that 'Concerto™', a commercial strain of this yeast, could be combined with fruit powder to form a shelf-stable bait for trapping yellowjackets. Using molecular techniques, we first confirmed that Concerto yeast is indeed the species *L. thermotolerans*. We then tested whether: 1) Concerto yeast produces volatiles similar to those produced by *L. thermotolerans* isolated from yellowjackets, 2) Concerto yeast enhances attraction of *Vespula* spp. to fruit powder, 3) a Concerto yeast/fruit powder bait interacts synergistically with a yellowjacket semiochemical lure, and 4) a synthetic analog blend of Concerto-produced volatiles

attracts yellowjackets. Using gas chromatography–mass spectrometry, we demonstrated that Concerto-produced volatiles closely resemble those produced by a yellowjacket-isolated strain of *L. thermotolerans.* In field experiments, addition of Concerto to fruit powder enhanced its attractiveness to multiple yellowjacket species. Addition of the Concerto/fruit powder bait to a heptyl butyrate-based wasp lure revealed a weak additive effect. A three-component synthetic analog blend of volatiles identified from the Concerto/fruit powder bait attracted *Vespula pensylvanica* (Saussure), but no other yellowjacket species. Our results suggest that commercial *L. thermotolerans* in combination with fruit powder could be used as a yellowjacket bait, and that addition of yeast-produced volatiles to a commercial wasp lure may improve its attractiveness to *V. pensylvanica*. Further research should determine why the synthetic volatile blend failed to attract *Vespula* species other than *V. pensylvanica*.

**Keywords:** yellowjackets; *Lachancea thermotolerans*; semiochemical attractant; fruit bait; symbiotic yeast

## 4.2. Introduction

Many insects depend on symbiotic yeasts for the breakdown of food and the production of nitrogen, amino acids, vitamins, sterols, and pheromones (Vega and Dowd 2005, Douglas 2009, Hoang et al. 2015, Zhao et al. 2015). For example, rice planthoppers require a *Candida* yeast symbiont to produce sterols necessary for successful molting (Eya et al. 1989, Vega and Dowd 2005), wood-ingesting passalid beetles rely on *Pichia stipitis* and related symbionts to aid in the digestion of xylose (Suh et al. 2003), and symbiotic yeasts of the bark beetle *Dendroctonus ponderosae* (Hopkins) convert a component of the beetle's aggregation pheromone to an anti-aggregation pheromone (Hunt & Borden 1990). In many cases, insects respond to volatiles produced by their yeast symbionts (Davis et al. 2013). Such communication could potentially be exploited by using yeast symbionts, or the volatiles they produce, as a trap bait or lure. Traps baited with baker's yeast (*Saccharomyces cerevisiae*) have already been utilized for trapping and monitoring *Drosophila* vinegar flies (Birmingham et al. 2011; Hamby and Becher 2016), and attract-and-kill or mass trapping strategies have been proposed for control of the codling moth, *Cydia pomonella* (L.), using volatiles produced by its *Metschnikowia* yeast symbionts (Witzgall et al. 2012).

*Saccharomyces cerevisiae* is a gut symbiont of *Polistes* and *Vespa* wasps in Italy (Stefanini et al. 2012), whereas the yeasts *Hanseniaspora uvarum* and *Lachancea thermotolerans* are gut symbionts of yellowjackets in British Columbia, Canada (Ibarra Jimenez et al. 2017). Attraction of yellowjackets to yeasts and yeast-produced volatiles has also been demonstrated; for example, Western yellowjackets, *Vespula pensylvanica* (Saussure), and German yellowjackets, *Vespula germanica* (F.), are attracted to volatiles produced by the yeast-like fungus *Aureobasidium pullulans* (Davis et al. 2012). Yellowjackets in Argentina were attracted to *S. cerevisiae* growing on a fruit powder substrate (Babcock et al. 2017), and North American yellowjackets responded to volatiles produced by their symbionts *H. uvarum* and *L. thermotolerans* growing on grape juice-infused agar (Babcock et al. 2018).

Yellowjackets are well known as a nuisance pest. When their nest is disturbed, many yellowjacket species inflict painful stings (Akre et al. 1980, Matsuura and Yamane 1990) that may cause anaphylactic shock and death in people with hypersensitivity to wasp venom (Bonay et al. 1997, Faux et al. 1997, Vetter et al. 1999). Yellowjackets have been responsible for school and park closures, delays in logging and farming (Akre et al. 1980), and loss of commercial bee hives, as they will attack hives to feed on the honey and bee larvae (Clapperton et al. 1989, de Jong 1990). Certain species such as *V. germanica* are invasive in many countries (Beggs et al. 2011). For example, in New Zealand, *V. germanica* has significantly reduced populations of native arthropods and has had adverse effects on endemic bird species through competition for food resources (Beggs 2001).

Many lures have been developed for trapping yellowjackets (Landolt 1998, Rust and Su 2012), including heptyl butyrate (MacDonald et al. 1973, El-Sayed et al. 2009), 2-methyl-1-butanol (Landolt et al. 2000, Day and Jeanne 2001), and a blend of isobutanol and acetic acid (Landolt et al. 1999). However, lures have not worked equally well for all species and populations. For example, *Vespula* spp. responded strongly to isobutanol and acetic acid in Washington and Alaska, USA (Landolt et al. 1999, 2005), but did not respond at all in New Zealand (El-Sayed et al. 2009), British Columbia, or Argentina (Babcock and Borden unpubl. obs.). We hypothesized that a composition of freeze-dried fruit powder and ‘Concerto™’, a commercially available yeast containing a Mediterranean strain of *L.*

*thermotolerans*, is attractive to *Vespula* yellowjackets. Our objectives were to 1) confirm the identity of Concerto commercial yeast as *L. thermotolerans*, 2) compare the headspace volatiles of Concerto yeast to those produced by *L. thermotolerans* isolated from North American yellowjackets, 3) determine whether Concerto yeast growing on fruit powder is attractive to yellowjackets, 4) determine whether Concerto yeast enhances the attractiveness of existing yellowjacket lures, and 5) test synthetic analogs of volatiles produced by Concerto yeast growing on fruit powder as a trap lure.

## 4.3. Materials and methods

### 4.3.1. DNA sequencing of Concerto yeast

To confirm the identity of Concerto as *L. thermotolerans*, DNA from purchased Concerto yeast cells (Chr. Hansen Viniflora, Hørsholm, Denmark) was extracted and sequenced. Under sterile conditions, approximately 1 g dry Concerto was added to 10 mL of autoclaved YPD broth (10 g/L yeast extract, 20 g/L bacteriological peptone, and 20 g/L dextrose in $H_2O$). This mixture was vortexed, and 1 mL was spread-plated onto a sterile YPD agar plate (10 g/L yeast extract, 20 g/L bacteriological peptone, 20 g/L dextrose, and 20 g/L agar in $H_2O$). The yeast plate was incubated at 30 °C for 24 h. Cells from a single colony on the plate were transferred to a flask containing 10 mL of YPD broth and incubated on a shaker at 30 °C overnight. DNA was extracted from the broth culture according to Lõoke et al. (2011). A NanoDrop UV/Vis 2000 spectrophotometer was used to determine the DNA concentration.

The Concerto DNA was identified by amplifying and sequencing the D1/D2 domain of large subunit (26S) ribosomal DNA using the primers NL1 (GCATATCAATAAGCGGAGGAAAAG) and NL4 (GGTCCGTGTTTCAAGACGG) (Kurtzman and Robnett, 1998). Kodaq DNA polymerase (Biological Materials, Richmond, BC V6V 2J5, Canada) was used for polymerase chain reaction (PCR). The PCR product was concentrated and purified using a NucleoSpin Gel and PCR Cleanup kit (Macherey-Nagel, Bethlehem, PA 18020, USA). Amplicons were sequenced (Genewiz, South Plainfield, NJ 07080, USA) and compared to known NL sequences in the BLASTn database.

### 4.3.2. Analysis of headspace volatiles produced by Concerto growing on grape juice-infused agar

Volatiles produced by Concerto were captured and compared with those previously identified from isolated *L. thermotolerans* (Ibarra Jimenez et al. 2017) on grape juice-infused YPD agar (Babcock et al. 2018). Grape juice agar was prepared by combining 10 g/L yeast extract, 20 g/L bacteriological peptone, 20 g/L dextrose, and 20 g/L agar with equal parts distilled water and filter-sterilized grape juice (Western Family™, Vancouver, BC V6B 4E4, Canada). All components except the grape juice were mixed together and autoclaved; filter-sterilized grape juice was added to the medium after autoclaving. The agar was poured into 100 mm × 15 mm sterile plastic petri dishes. Plates were inoculated with Concerto as above, and incubated at 30 °C for 24 h. Five agar discs (100 mm diameter) were then removed from the petri dishes and placed into a clean Pyrex® glass aeration chamber (340 mm high × 125 mm wide). An air pump (A.O. Smith, Tipp City, OH 45371, USA) drew charcoal-filtered air at 0.5 L/min through the aeration chamber and then through a glass tube containing 0.2 g of Porapak-Q (50-80 mesh). The aeration ran for 8 h, after which volatiles were desorbed from the Porapak-Q with 2 mL of a 50:50 mixture of pentane and ether. The extract was then concentrated to a volume of 500 µL.

Aliquots of the concentrated Porapak-Q extract were analyzed using a Varian 3800 gas chromatograph (GC) coupled to a Saturn 2000 Ion Trap mass spectrometer (MS) (Agilent Technologies Inc., Santa Clara, CA 95051, USA). The GC-MS was fitted with a DB-5 GC-MS column (30 m × 0.25 mm internal diameter) and operated in full-scan electron impact mode. Helium was used as the carrier gas at a flow rate of 35 cm/s, with the following temperature program: 50 °C for 5 min, then 10 °C/min until 280 °C. The injector was set at 250 °C and the transfer line at 280 °C. Sample volatiles were identified and quantified by comparing their retention times and mass spectra with those of authentic standards.

### 4.3.3. General design of field experiments

Field experiments were conducted at the following agricultural sites in BC, Canada: Experiments 1 (N = 8) and 3 (N = 12) at a blueberry and raspberry farm (1400 272$^{nd}$ Street,

Langley, V4W 2P9), and Experiment 2 (N = 11) at a vineyard with an apiary (Campbell's Gold Honey Farm and Meadery, 2595 Lefeuvre Road, Abbotsford, V4X 1H5). Experiments were set up in a randomized complete block design, with the blocking factor as different sections of the site. Baits or lures in Experiments 1 and 3 were placed into plastic bag wasp traps (Scotts Canada Ltd., Delta, BC V4G 1E9, Canada), with two offset entry ports to discourage escape. Baits and lures in Experiment 2 were placed into wide jar-style wasp traps [19 cm high × 14 cm wide; PheroTech Inc, Delta, BC V4G 1E9, Canada (no longer in operation)] with a horizontal entry tube that allowed access from either side, and a cut-out portion at mid-span that gave yellowjackets entry into the trap chamber, while discouraging escape. Teabag baits (see below) were immersed in water at the bottom of the trap, with no incubation period prior to the experiment. Sodium lauryl sulfate (SLS) was added to teabag baits and water controls as a surfactant, decreasing water surface tension and thus making sure captured wasps sank and drowned. Synthetic blend lures, formulated as emulsifiable concentrates in water, were poured directly into the bottom of the trap. The surfactant in the concentrates eliminated the need for SLS. Traps were hung ≥ 10 m apart 1 – 1.5 m above ground from fence posts or wire supports in crop fields using 18-gauge steel wire (Rona, Coquitlam, BC V3B 1B9, Canada). Traps were left in the field for 3–5 days before being collected. The contents of each trap were poured through a strainer and captured yellowjackets were counted and identified to species using characteristic markings on their head and abdomen (Akre et al. 1980).

### 4.3.4. Preparation and field testing of yeast/fruit powder baits

Fruit powder baits were prepared by combining spray-dried grape powder (HiActives Grape Powder N49, FutureCeuticals, Momence, IL 60954, USA), freeze dried mango powder, freeze-dried peach powder, and freeze-dried pomegranate powder (all from Van Drunen Farms, Momence, IL 60954, USA) in equal proportions by weight. Aliquots of this mixture (20 g) were placed into teabags (6 × 8 cm; Finum Slim Tea Filter, Riensch & Held GmbH & Co. KG, Hamburg, Germany) along with 2 g of SLS. Baits comprising both yeast and fruit powder (henceforth yeast/fruit powder baits) were prepared by adding 2 g of either brewer's yeast (Danstar Belle Saison Beer Yeast, Lallemand Inc., Montreal, QC H1N 2C4, Canada) or Concerto to the above mixture. Teabags were folded shut and secured using a single metal staple.

Experiment 1 tested the attractiveness of fruit powder teabag baits without yeast or with either brewer's or Concerto yeast. The treatments were: *a*) fruit powder bait, *b*) brewer's yeast/fruit powder bait, *c*) Concerto/fruit powder bait, and *d*) water control. Teabag baits were immersed in 500 mL of water. Treatment *d* consisted of 500 mL of water and 2 g of SLS and served as a negative control.

Experiment 2 compared the attractiveness of the Concerto/fruit powder bait and that of a heptyl butyrate-based yellowjacket lure (50 mL of emulsifable concentrate, 77.4% = 0.81 g/lure heptyl butyrate in 450 mL of water; Scotts Canada Ltd., Delta, BC V4G 1E9, Canada), alone and in combination. The treatments were: *a*) Concerto/fruit powder bait, *b*) heptyl butyrate-based lure, and *c*) both the bait and the lure. Teabags were immersed in 100 mL water inside plastic solo cups (Dollarama, Coquitlam, BC V3J 3X5, Canada) with the sides of the cup trimmed to 5.1 cm. The cups were covered with a square of organdy cloth (Fanny's Fabrics, Burnaby, BC V5B 4Y5, Canada) secured with an elastic band. For Treatment *b*, a teabag containing 2 g of SLS was used in place of a fruit powder bait. Solo cups were placed in the bottom of a trap, and the heptyl butyrate-based lure was poured into the bottom of the trap around the cup. The solo cup prevented contact of the teabag with the chemical lure. For Treatment *a*, water mixed with 2 g of SLS was used in place of the heptyl butyrate lure.

### 4.3.5. Analysis of headspace volatiles emanating from the Concerto/fruit powder bait

Because the Concerto/fruit powder teabag bait was attractive to yellowjackets (see results), its headspace volatiles were captured and analyzed. A single teabag bait was submerged in 500 mL of water in a 600-mL beaker, which was placed into a clean Pyrex glass aeration chamber (340 mm high × 125 mm wide). Aerations were run for 24 h using the parameters and equipment as above. Captured volatiles were desorbed from Porapak-Q using 2 mL of a 50:50 mixture of pentane and ether, and the resulting extract was concentrated to 500 µL as described above.

Aliquots of the concentrated extract were analyzed using GC-MS as above. Sample volatiles were identified and quantified by comparing their retention times and mass spectra with those of authentic standards.

### 4.3.6. Preparation and testing of a synthetic volatile blend

A synthetic blend (SB) of Concerto-produced headspace volatiles was prepared (Table 4.2) and tested in September 2017. The SB was formulated into an emulsifiable concentrate by combining 12 g of the SB with 123 g of CO-630 nonionic surfactant (polyoxyethylene nonylphenylether; Norman, Fox, & Co., City of Industry, CA 91744, USA) and 493 g water. Immediately prior to the start of each experiment, emulsifiable concentrates were diluted to a volume of 500 mL per trap with the amounts and percentages as shown in Table 4.2.

Experiment 3 compared captures of yellowjackets in traps baited with the SB or Concerto growing on fruit powder. The treatments were: *a*) Concerto/fruit powder teabag bait, *b*) SB, and *c*) water control. The water control was prepared as in Experiment 1.

### 4.3.7. Statistical analyses

Data from all field experiments were transformed by $\log_{10}(x + 1)$ and analyzed using JMP 12® (SAS Institute Inc., Cary, NC 27513, USA). The mean numbers of yellowjackets captured per trap were compared among treatments using a one-way randomized block ANOVA and Tukey's HSD test for pairwise comparisons of means. In all cases $\alpha = 0.05$.

## 4.4. Results

### 4.4.1. DNA sequencing of Concerto yeast

Concerto yeast was confirmed as *L. thermotolerans* by comparing the sequenced DNA region to known sequences in the BLASTn data base. Our sequenced strain showed 99% identity to the D1/D2 large subunit ribosomal DNA of *L. thermotolerans* (accession number JX129903.1).

### 4.4.2. Analysis of headspace volatiles produced by Concerto growing on grape juice-infused agar

The headspace volatile blend produced by Concerto growing on grape juice-infused agar closely resembled that produced by *L. thermotolerans* isolated from North American yellowjackets (Babcock et al. 2018) (Table 4.1). Each strain produced a blend of 22 compounds, 20 of which were shared between the two blends. The four compounds that were unique to either blend were each present in low amounts (≤ 0.2%). The Concerto volatile blend contained less isoamyl acetate and isoamyl alcohol, but more 2-phenylethyl alcohol and acetoin than the volatile blend of *L. thermotolerans* isolated from yellowjackets.

### 4.4.3. Field testing of yeast/fruit powder baits

In Experiment 1 (effect of yeast on fruit powder attractiveness), there was a difference among treatment means for *V. germanica* ($F_{3,21}$ = 19.60; $P$ < 0.0001), but not for *V. pensylvanica* ($F_{3,21}$ = 2.80; $P$ = 0.0650) (Figure 4.1). Traps baited with either brewer's yeast or Concerto growing on fruit powder captured significantly more *V. germanica* than either water control traps ($P$ = 0.0001 and $P$ < 0.0001) or traps with fruit powder alone ($P$ = 0.0003 and $P$ = 0.0002) and captures in traps containing fruit powder alone did not differ from those in control traps ($P$ = 0.9645). Only three *Vespula alascensis* (Packard) were captured in this experiment; one in a trap with the brewer's yeast/fruit powder bait, and two in traps with the Concerto/fruit powder bait.

In Experiment 2 (interaction between the Concerto/fruit powder bait and a semiochemical lure), there was a significant difference among treatment means ($F_{2,20}$ = 186.35; $P$ < 0.0001) (Figure 4.2). The heptyl butyrate-based lure, with or without the Concerto/fruit powder teabag bait, attracted substantially more yellowjackets than the Concerto teabag bait alone ($P$ < 0.0001 for both comparisons). Although the addition of the teabag bait to the heptyl butyrate-based lure did not significantly improve trap captures relative to the lure alone ($P$ = 0.5690), the combination treatment attracted a mean of 47 more yellowjackets per trap than the heptyl butyrate lure alone. Over 99% of the yellowjackets captured in this experiment were *V. pensylvanica.*

### 4.4.4. Analysis of headspace volatiles produced by the Concerto/fruit powder baits

Only three compounds were present in headspace volatiles produced by the Concerto/fruit powder teabag bait. These compounds, and their relative amounts, were: ethyl acetate (50.0%), isoamyl alcohol (35.7%), and 2-methyl-1-butanol (14.3%).

### 4.4.5. Testing a synthetic blend of volatiles

In Experiment 3 (comparative attractiveness of the Concerto/fruit powder bait and a corresponding SB), there was a significant difference among the treatment means for *V. pensylvanica* ($F_{2,22}$ = 28.34; $P$ < 0.0001), *V. germanica* ($F_{2,22}$ = 120.59; $P$ < 0.0001), and *V. alascensis* ($F_{2,22}$ = 24.12; $P$ < 0.0001) (Figure 4.3). Traps baited with the Concerto bait captured more yellowjackets than the water control ($P$ < 0.0001 for all species). For *V. pensylvanica*, the SB was also more attractive than the water control ($P$ < 0.0001), and as effective as the Concerto bait ($P$ = 0.9510). Traps baited with the SB did not capture more *V. germanica or V. alascensis* than water control traps ($P$ = 0.2675 and $P$ = 0.2045), and the Concerto bait outperformed the SB for both of these species ($P$ < 0.0001 and $P$ = 0.0002).

## 4.5. Discussion

Both DNA sequencing and headspace volatile analysis confirmed that the commercially-available 'Concerto' yeast from the Mediterranean region is *L. thermotolerans*, and that it produces a volatile blend closely resembling that of *L. thermotolerans* isolated from North American yellowjackets (Table 4.1) when grown on the same substrate (Babcock et al. 2018). Such convergent volatile blends are not obvious, as different strains of the same wine yeast species can produce divergent volatile profiles during fruit fermentation (Lurton et al. 1995, Li et al. 2012). The similarity in the volatile profiles of Mediterranean and North American *L. thermotolerans* strains may reflect occupation of congruent ecological niches in their respective geographic regions.

Traps baited with fruit powder alone were as ineffective as water control traps in attracting *V. germanica*, but the addition of either brewer's yeast or Concerto to fruit powder significantly increased captures of *V. germanica* relative to the control (Figure 4.1). These results support those of Babcock et al. (2018) in that *Vespula* spp. in British Columbia responded positively to odorants of isolated *L. thermotolerans* grown on grape juice-infused agar, but not to grapes or agar alone. We conclude that addition of yeast to a fruit powder bait enhances the bait's attractiveness for *V. germanica*. This supports our previous findings that brewer's yeast added to a fruit powder mixture of apple, banana, strawberry, and raspberry was attractive to both *V. germanica* and *V. alascensis* in Argentina (Babcock et al. 2017). However, the same brewer's yeast/fruit powder bait effective in Argentina was only weakly attractive to *V. alascensis* and not attractive at all to other *Vespula* yellowjackets in British Columbia (Babcock and Borden unpubl. obs.). The attraction of yellowjackets to the brewer's yeast/fruit powder bait in Experiment 1 may be attributed to the bait composition comprising yeast, mango, peach, pomegranate, and grape powder. This composition produced isoamyl acetate and 2-methyl-1-butanol (Table 4.1), which were not produced by the brewer's yeast/fruit powder bait used in Argentina (Babcock et al. 2017), possibly due to the absence of certain biosynthetic precursors.

Although the addition of a Concerto/fruit powder bait to the heptyl butyrate lure did not significantly affect lure attractiveness, it did result in a mean increase of 47 yellowjackets captured per trap (Figure 4.2). This combination treatment also captured more yellowjackets on average than the sum of the mean captures to each treatment alone, suggesting a marginal additive interaction between the Concerto/fruit powder bait and the heptyl butyrate lure.

Surprisingly, only three compounds were present in captured headspace volatiles from Concerto/fruit powder baits, significantly fewer than the 22 odorants comprising the headspace volatile blend of Concerto yeast growing on grape juice-infused agar. Two of the most abundant compounds present in the latter blend, isoamyl alcohol and 2-methyl-1-butanol, also originated from the Concerto/fruit powder bait. Notably, the Concerto/fruit powder bait produced large amounts of ethyl acetate, which was only a trace constituent in headspace volatiles of *L. thermotolerans* growing on grape juice-infused agar. This large amount of ethyl acetate may have had an inhibitory effect on the growth of yeasts

(Urit et al. 2013) and thus on the odorants they produced. Moreover, ethyl acetate could signal the presence of ethanol, which forms an ester derivative with acetic acid (Kruis et al. 2017). Ethanol slows the growth of *L. thermotolerans* at concentrations of > 3% with complete growth inhibition at 9% ethanol (Kapsopoulou et al. 2005). Alternatively, the less complex volatile composition produced by Concerto growing on fruit powder could be attributed to differential nutrient compositions of fruit powder and grape juice-infused agar; the YPD agar contains yeast extract, many essential nutrients and vitamins, a sugar source and growth factors, as well as bacteriological peptone (Hahn-Hägerdal et al. 2005).

Two of the three odorants present in headspace volatiles of Concerto/fruit powder baits, isoamyl alcohol and 2-methyl-1-butanol, are common products of yeast fermentation (Lurton et al. 1995, Viana et al. 2008, González-Robles et al. 2015) and are known yellowjacket attractants (Landolt et al. 2000, Brown et al. 2014, Landolt and Zhang 2016).

The three-component synthetic blend was as attractive to *V. pensylvanica* as the Concerto/fruit powder bait, but not at all attractive to *V. germanica* or *V. alascensis* (Figure 4.3), likely because essential attractants for the latter species were absent. However, neither *V. germanica* nor *V. alascensis* responded to a more complex 18-component blend of volatiles produced by *H. uvarum* and *L. thermotolerans* on grape juice-infused agar (Babcock et al. 2018). Thus, it is conceivable that the yeasts produce attractive gases or highly volatile, low-molecular weight compounds that we could not detect by GC-MS in headspace volatile analyses. Such attractants may include $CO_2$, ammonia, acetone, or dimethyl disulfide, all of which are known to attract insects. For example, $CO_2$ attracts or activates insects of diverse taxa (Johnson and Gregory 2006, Jones 2013, Gries 2018), and ammonia, acetone, and dimethyl disulfide are attractants to mosquitoes (Braks et al. 2001, Bernier et al. 2003, Mathew et al. 2013).

In conclusion, we demonstrate that Concerto™ yeast is a strain of *Lachancea thermotolerans* and that when grown on the same substrate, it produces a volatile blend similar to that produced by *L. thermotolerans* isolated from North American yellowjackets. Growing on a fruit-based substrate, each of the two *L. thermotolerans* strains attracts *Vespula* spp. The headspace volatile blend of the Concerto/fruit powder bait contains only three components which – when prepared as synthetic analogs – attract *V. pensylvanica,*

but no other yellowjacket species. Further research might aim to determine why attraction of *V. germanica* and *V. alascensis* is lost when transitioning from a yeast bait to a synthetic blend.

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**Table 4.1. Compositions of headspace volatiles identified by GC-MS produced by Concerto™ yeast and a strain of *L. thermotolerans* isolated from North American yellowjackets, both grown on grape juice-infused YPD agar.**

| | Percentage of each volatile in headspace blend | |
|---|---|---|
| **Compounds** | **Concerto yeast** | ***Lachancea thermotolerans*[1]** |
| **Esters** | | |
| ethyl acetate | 0.25 | 0.31 |
| phenylethyl acetate | 0.12 | 0.10 |
| ethylphenyl acetate | 0.22 | 0.17 |
| isoamyl acetate | 1.84 | 2.27 |
| ethyl butyrate | | 0.12 |
| ethyl hexanoate | 0.41 | 0.28 |
| ethyl octanoate | 2.55 | 0.21 |
| ethyl decanoate | 0.53 | 0.03 |
| ethyl dodecanoate | 0.08 | 0.05 |
| methyl anthranilate | | 0.10 |
| **Alcohols** | | |
| 2-phenylethyl alcohol | 20.1 | 11.5 |
| isoamyl alcohol | 54.6 | 74.7 |
| 2-methyl butanol | 10.8 | 8.04 |
| methionol | 0.13 | 0.08 |
| furfuryl alcohol | 0.14 | |
| **Carboxylic acids** | | |
| butyric acid | 1.40 | 0.17 |
| isobutyric acid | 0.12 | 0.40 |
| 2-methylbutanoic acid | 0.14 | 0.05 |
| 3-methylbutanoic acid | 0.50 | 0.09 |
| **Aldehydes** | | |
| acetal | 1.54 | 0.50 |
| **Ketones** | | |
| acetoin | 4.10 | 0.73 |
| 2-acetylfuran | 0.11 | |

**Table 4.1 continued**

| Compounds | Percentage of each volatile in headspace blend | |
|---|---|---|
| | Concerto yeast | *Lachancea thermotolerans*[1] |
| **Others** | | |
| 2,5-dimethyl pyrazine | 0.05 | 0.08 |
| styrene | 0.27 | 0.06 |

[1]Babcock et al. 2018

**Table 4.2. Weights and proportions of synthetic blend components in the emulsifiable concentrate lure and per 350 mL diluted emulsion for baiting one trap.**

| Component | mg/trap | %/trap | Purity (%) | Supplier |
|---|---|---|---|---|
| ethyl acetate | 500 | 0.10 | 99 | Caledon[1] |
| isoamyl alcohol | 357 | 0.071 | >95 | Fisher Chemical[2] |
| 2-methyl-butanol | 143 | 0.029 | 98 | Sigma-Aldrich[3] |
| CO-630 | 10 250 | 2.05 | | Norman, Fox & Co.[4] |
| Water | 488 750 | 97.75 | | |
| TOTALS | 500 000 | 100.00 | | |

[1] Caledon Laboratory Chemicals, Georgetown, ON L7G 4R9, Canada
[2] Fisher Scientific, Fair Lawn, NJ 07410, USA
[3] Sigma-Aldrich, St. Louis, MO 631903, USA
[4] Norman, Fox, & Co., City of Industry, CA 91744, USA

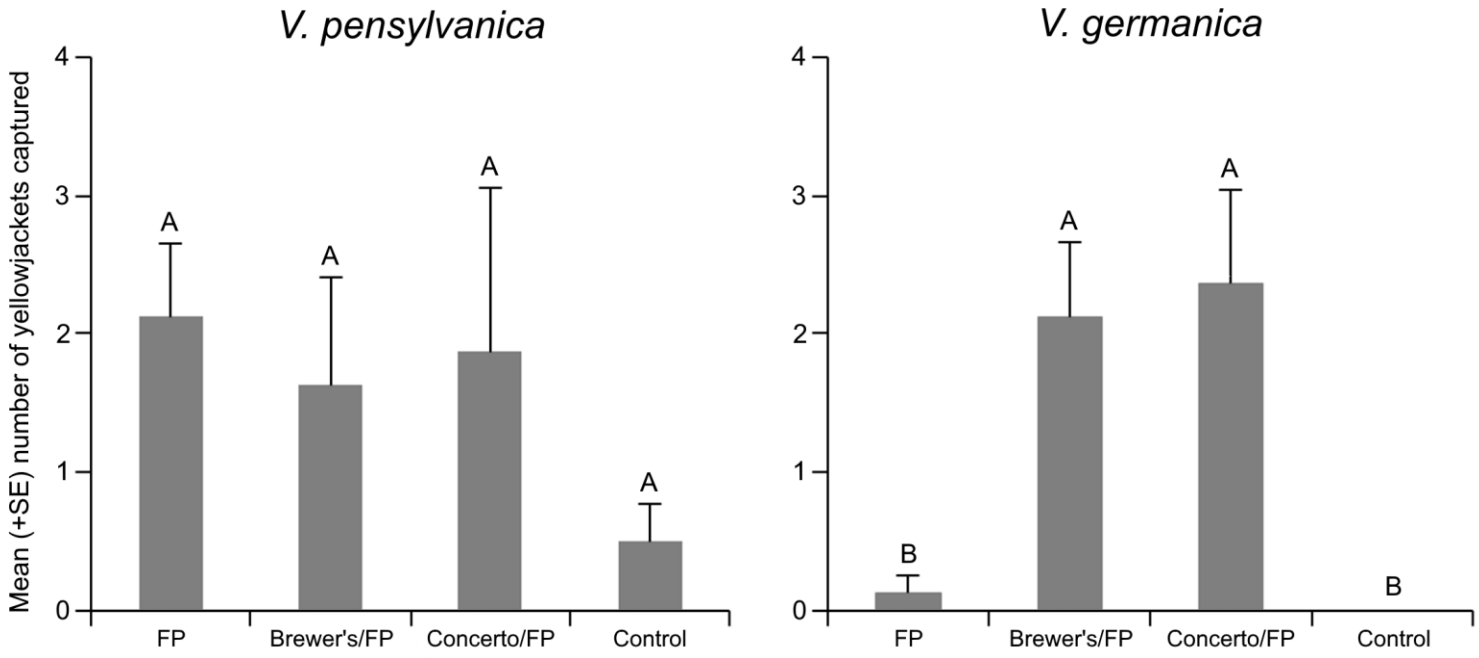


**Figure 4.1.** **Mean numbers of *Vespula* (*V.*) yellowjackets captured per trap in Experiment 1 (N = 8), testing teabag baits of fruit powder (FP) alone, or FP mixed with either brewer's yeast (Brewer's/FP) or Concerto™ yeast (Concerto/FP). Separate graphs for *V. pensylvanica* and *V. germanica* are shown. Traps baited with water were used as controls. Bars labelled with the same letter are not significantly different (Tukey's HSD test, P < 0.05).**

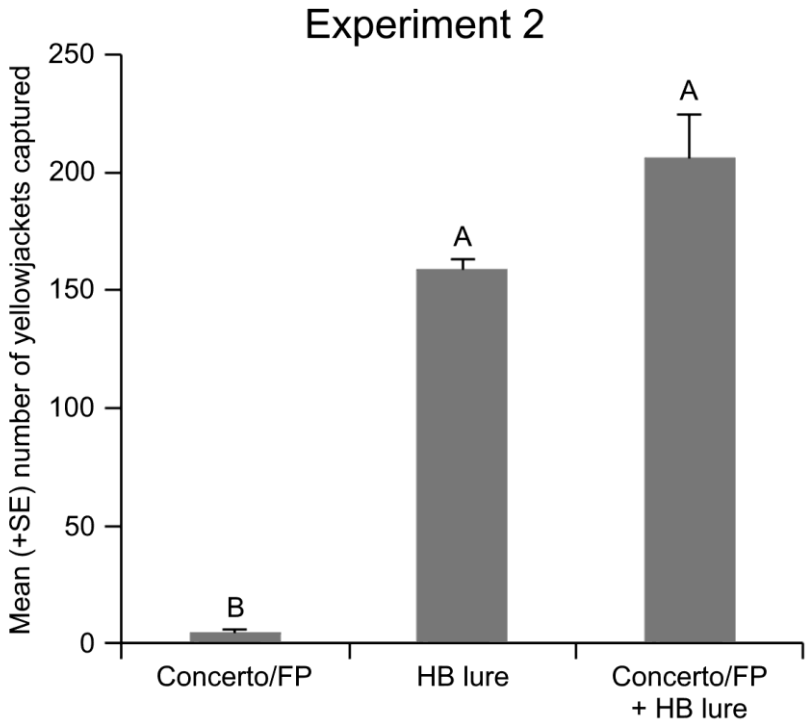


**Figure 4.2.** **Mean numbers of yellowjackets captured per trap in Experiment 2 (N = 11), testing teabag baits of fruit powder mixed with Concerto™ yeast (Concerto/FP), heptyl butyrate-based (HB) yellowjacket lures, or both in combination. Bars labelled with the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).**

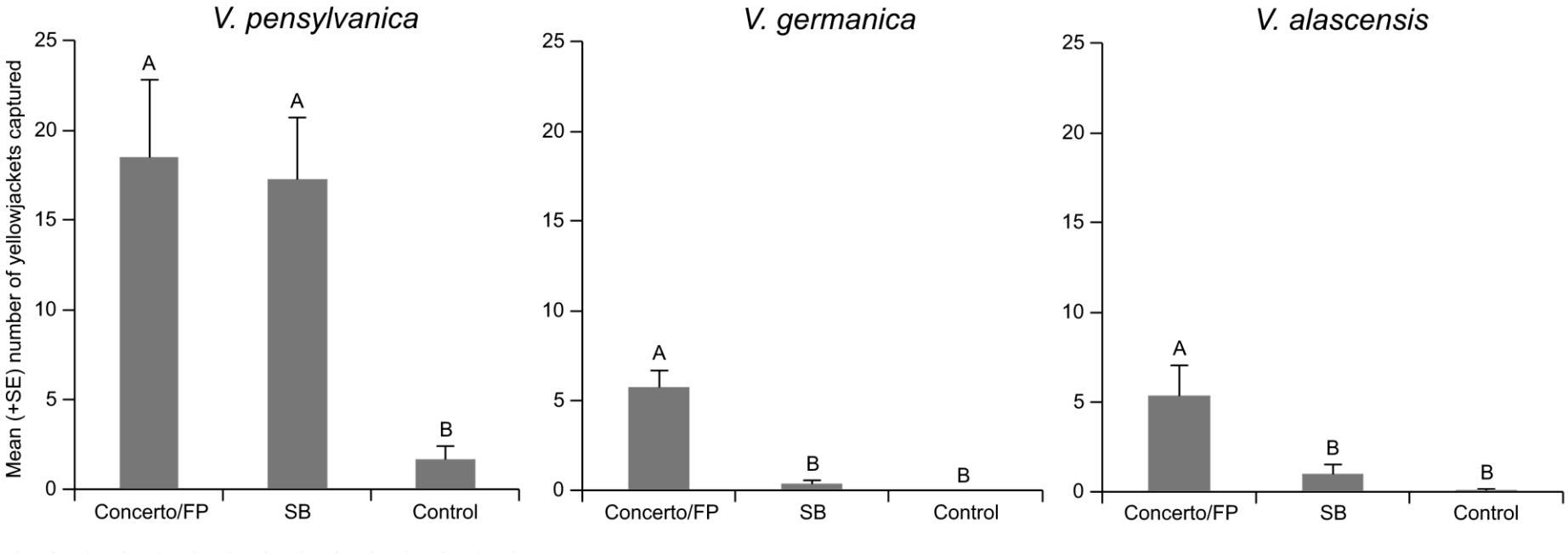


**Figure 4.3.** **Mean numbers of *Vespula* (*V.*) yellowjackets captured per trap in Experiment 3 (N = 12), testing teabag baits of fruit powder mixed with Concerto™ yeast (Concerto/FP) and a synthetic blend (SB) of the three headspace volatiles emitted by the Concerto/FP teabag bait. A separate graph for each yellowjacket species captured is shown. Traps containing water served as controls. Bars labelled with the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).**

# Chapter 5.

# Concluding Summary

Yellowjackets are significant pests worldwide (Akre *et al.*, 1980; Edwards, 1980; Beggs *et al.*, 2011), and effective methods for abatement of pestiferous populations are very much needed. Although baits and lures have already been developed for deployment in yellowjacket trapping programs (MacDonald *et al*., 1973; Rust & Su, 2012; Landolt & Zhang, 2016), they do not work consistently for all species and populations of yellowjackets (Landolt *et al*., 1999; Day & Jeanne, 2001). There is a need for the development of more effective lures which can target a broader range of species and geographic populations. Throughout my research, I have made contributions to this field by investigating the relationship and communication between yellowjackets and the internal yeast symbionts they harbour. My major findings are summarized as follows:

- Weak attraction of *Vespula germanica* and *Vespula vulgaris* in Argentina to dried fruit or fruit powder baits is greatly enhanced by the addition of brewer's yeast to these baits.

- Brewer's yeast substantially alters the volatile composition emitted by dried fruit and fruit powder baits.

- The yeasts *Hanseniaspora uvarum* and *Lachancea thermotolerans* isolated from North American yellowjackets are attractive to two species of native North American yellowjackets (*Vespula pensylvanica*, and *V. alascensis*) and one exotic species (*V. germanica*) when grown on media supplemented with grape juice, but are not attractive when grown on unsupplemented media or when grown on media adjacent to grapes.

- A synthetic analog blend of volatiles produced by *H. uvarum* growing on grape juice-infused media is attractive to *V. pensylvanica*, but is not attractive to *V. germanica* and *V. alascensis.*

- Addition of the fermentation by-products ethanol and acetic acid at biologically realistic levels either had no effect on, or reduced, the attractiveness of the *H. uvarum* synthetic analog blend.

- Subtracting chemical groups, one or two at a time, from the *H. uvarum* synthetic analog blend did not significantly affect the blend's attractiveness, suggesting that no single chemical group is responsible for the blend's attractiveness and that there is redundancy among synthetic blend components; however, subtle differences in the attractiveness of incomplete blends suggests that alcohols may be slightly inhibitory, and that esters may contribute more than other groups to the blend's attractiveness.

- Weak attraction of North American yellowjackets to a fruit powder bait was greatly enhanced by the addition of either brewer's yeast or a commercial strain of *L. thermotolerans* yeast ("Concerto"); this phenomenon was particularly strong for *V. germanica*.

- The combination of a heptyl butyrate-based commercial yellowjacket lure and a fruit powder/Concerto bait captured more yellowjackets on average than the sum of the captures to the lure and bait alone, suggesting a slight additive effect.

- A synthetic blend of the volatiles produced by the fruit powder/Concerto bait is attractive to *V. pensylvanica* but not to any other yellowjacket species.

I conclude that yeast-produced volatiles are important in nature and can act as semiochemicals for yellowjackets. My research findings provide the foundation for the development of an effective yeast volatile lure which can be used in improved yellowjacket management. In this work, I have demonstrated that yeast-derived volatiles are effective attractants for *V. pensylvanica.* Further research should aim to determine the as yet unknown yeast-produced volatiles (possibly evanescent gases) that mediate attraction of other species of yellowjackets.

## 5.1. References

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