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Swedish University of Agricultural Sciences

Faculty of Landscape Architecture,
Horticulture and Crop Production Science

Trapping social wasps, in apple orchards and vineyards with synthetic volatiles and live yeast cultures in Scania, Scandinavia

Malin Iris Dörre

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Trapping social wasps, in apple orchards and vineyards with synthetic volatiles and live yeast cultures in Scania, Scandinavia.

Fällfångst av sociala getingar, i äpple- och vinodling med syntetiska flyktiga ämnen och levande jästkulturer i Skåne, Skandinavien.

Malin Iris Dörre

Handledare/Supervisor: professor Marie Bengtsson, Department of Plant Protection Biology, SLU
Btr handledare/Assistant supervisor: professor Peter Witzgall, Department of Plant Protection Biology, SLU

Examinator/Examiner: professor Göran Birgersson, Department of Plant Protection Biology, SLU

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SLU
Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Landscape Architecture, Horticulture and Crop Production Science
Department of Biosystems and Technology

Abstract

Social wasps (Hymenoptera, Vespinae) foraging for carbohydrate nutrients are a major pest, particularly in berry and fruit orchards. The identification of volatile attractants for social wasp management is accordingly an urgent research challenge. Overripe fruit and fermenting sweet baits are since long known to attract wasps. Several synthetic compounds mimicking these food sources have been developed. However, yeast volatiles, signalling fermenting fruit are still incompletely known. For the first time five, synthetic compounds and five live yeast strains were evaluated as attractants for social wasps in orchards and vineyards in Scania, Scandinavia. When blended with acetic acid, either 2-methylpropan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol or 2-methylpropan-2-ol strongly attracted two social wasps, *Vespula germanica* and *V. vulgaris*. Single compounds attracted significantly fewer insects. Heptyl butanoate as a single test compound or blended with acetic acid was not attractive to social wasps. In addition to synthetic chemicals, five yeast strains known to be associated with or attractive to various insects were tested. Liquid medium with fermenting *Metschnikowia andauensis*, *M. chrysoperlae*, *M. pulcherrima, Saccharomyces* sp., or *Cryptococcus* sp. strongly attracted *V. germanica* and *V. vulgaris*.

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Introduction

Distribution of the subfamily Vespinae (social wasps)

The subfamily Vespinae (social wasps or vespine wasps) belongs to the family Vespidae (wasps), order Hymenoptera. It is divided into three groups comprising four genera, nocturnal hornets (*Provespa*), hornets (*Vespa*) and social wasps or yellow jackets (*Dolichovespula* and *Vespula*) and into 66 species (Archer 2012).

Vespinae species inhabit the northern hemisphere, 70°N is the northern limit for Vespinae except in Point Barrow; northern Alaska. In Indonesia, Vespinae inhabit areas south of the equator (Edwards 1980). *Provespa* only comprises three species, which are distributed in the tropics of eastern Asia. The genus *Vespa* mainly occurs in tropical southeastern Asia and consists of 22 species. Only two species, *V. crabro* and *V. orientalis* are found in Europe. *Dolichovespula* and *Vespula* consist of 42 species and are distributed throughout the Holarctic region (Archer 2012).

Several Vespinae species have accidently been spread and established outside their native range (Archer 2012). For example, *Vespula germanica* has successfully colonised North America, South America, South Africa, New Zealand and Australia (Spradbery & Dvorak 2010) and *Vespula vulgaris* has been established both in New Zealand and Australia (Donovan 1984; Matthews *et al*. 2000). The Asian hornet *Vespa velutina* has recently been introduced to Southern Europe and is likely to spread across the continent (Choi *et al*. 2012; Monceau *et al*. 2014; Jordbruksverket 2014).

Morphological characters of the subfamily Vespinae

The subfamily Vespinae, can be distinguished morphologically (Figure 1). Typical are their big kidney-shaped compound eyes and forewings that are folded along the body during rest. In addition the forewings have an elongated medial cell. The front cover of the thorax, the pronotum reaches the tegulae (sclerite in immediate connection with the base of the costal vein of the wing) and the section between the thorax (midsection of body) and the squarely cut gaster (main part of the abdomen behind the waist) is laced up. Their clypeus (part of the head just below the face) has a wide apical margin with two pointed edges (Edwards 1980; Matsuura & Yamane 1990).

Most vespine wasps, have brown and yellow or black and yellow striped patterned abdomens (the posterior segment of the body) except for some Asian species (Edwards 1980).

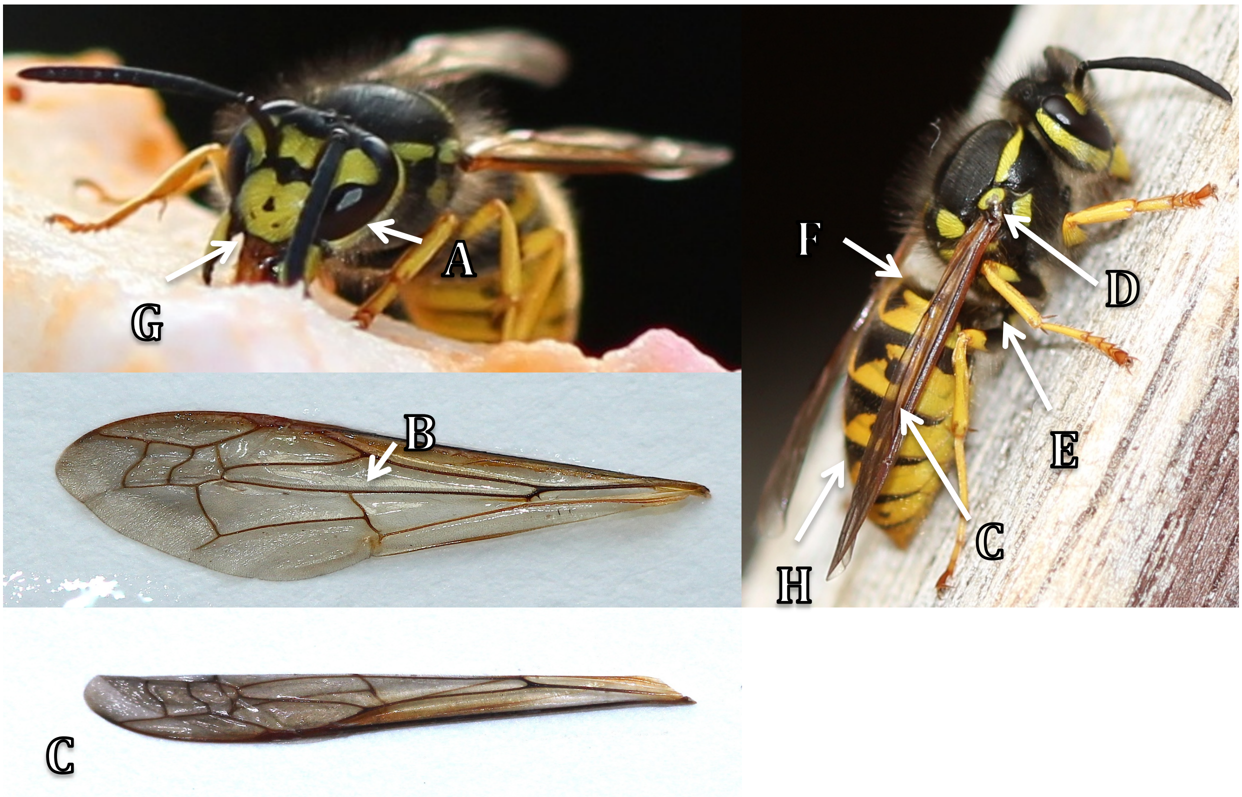


Figure 1: Morphological characters of the subfamily Vespinae: (A) Large compound eyes, (B) elongated medial cell of forewing, (C) folded forewing, (D) pronotum reaching tegulae, (E) laced up section between thorax and abdomen, (F) squarly cut gaster, (G) clypeus with wide apical margin and two pointed edges, (H) abdomen yellow and black. (Photos by author)

The Vespinae of Scandinavia

In Scandinavia 12 Vespinae species within the three genera *Vespa*, *Dolichovespula* and *Vespula* are found. Nine of these: *V. crabro* (Hornet), *D. media* (Median wasp), *D. sylvestris* (Tree wasp), *D. norwegica* (Norwegian wasp), *D. saxonica* (Saxon wasp), the parasitic *D. omissa*, *V. rufa* (Red wasp), *V. germanica* (German wasp) and *V. vulgaris* (common wasp) are present in Scania, Southern Sweden (Douwes *et al*. 2012).

Morphological characters of the Scandinavian Vespinae genera

The vespine genera are distinguished according to the upper surface shape of the head behind the compound eyes (vertex), the length of the oculo-malar space (space between compound eyes and mandibles) and their difference in body length (Table 1 and Figure 2). *V. crabro*, the sole *Vepsa* species present in Scandinavia, is the largest wasp species in this range, with a queens body length of up to thirty-five millimetres. The most distinguishing feature of *V. crabro* compared to *Dolichovespula* and *Vespula* is its distinctly swollen head behind the compound eyes.

Dolichovespula are categorized as long-cheeked wasps and the oculo-malar space between the bottom of the eye and the mandible attachment is used for identification. The oculo-malar space is compared with the width of the antennae basal scape, which in *Dolichovespula* at least equals to the basal scape width. Species in the genus Vespula are short-cheeked and the length of the oculo-malar space is shorter than the width of the antennae basal scape (Douwes *et al*. 2012; Kruse Pedersen 1975; Edwards 1980).

Table 1: Summary of morphological characters in the Vespinae genera; *Vespa*, *Dolichovespula* and *Vespula* (Douwes *et al*. 2012; Kruse Pedersen 1975; Edwards 1980)

	Vespa	***Dolichovespula***	***Vespula***
Queens, workers and males			
Vertex shape and color	Distinctly swollen, yellow and brownish red	Not conspicuously swollen, yellow and black	Not conspicuously swollen, yellow and black
Body size	18-35 mm	11-22 mm	11-19 mm
Oculo-malar space	Short (shorter than width of antennae basal scape)	Long (longer than width of antennae basal scape)	Short (shorter than width of antennae basal scape)

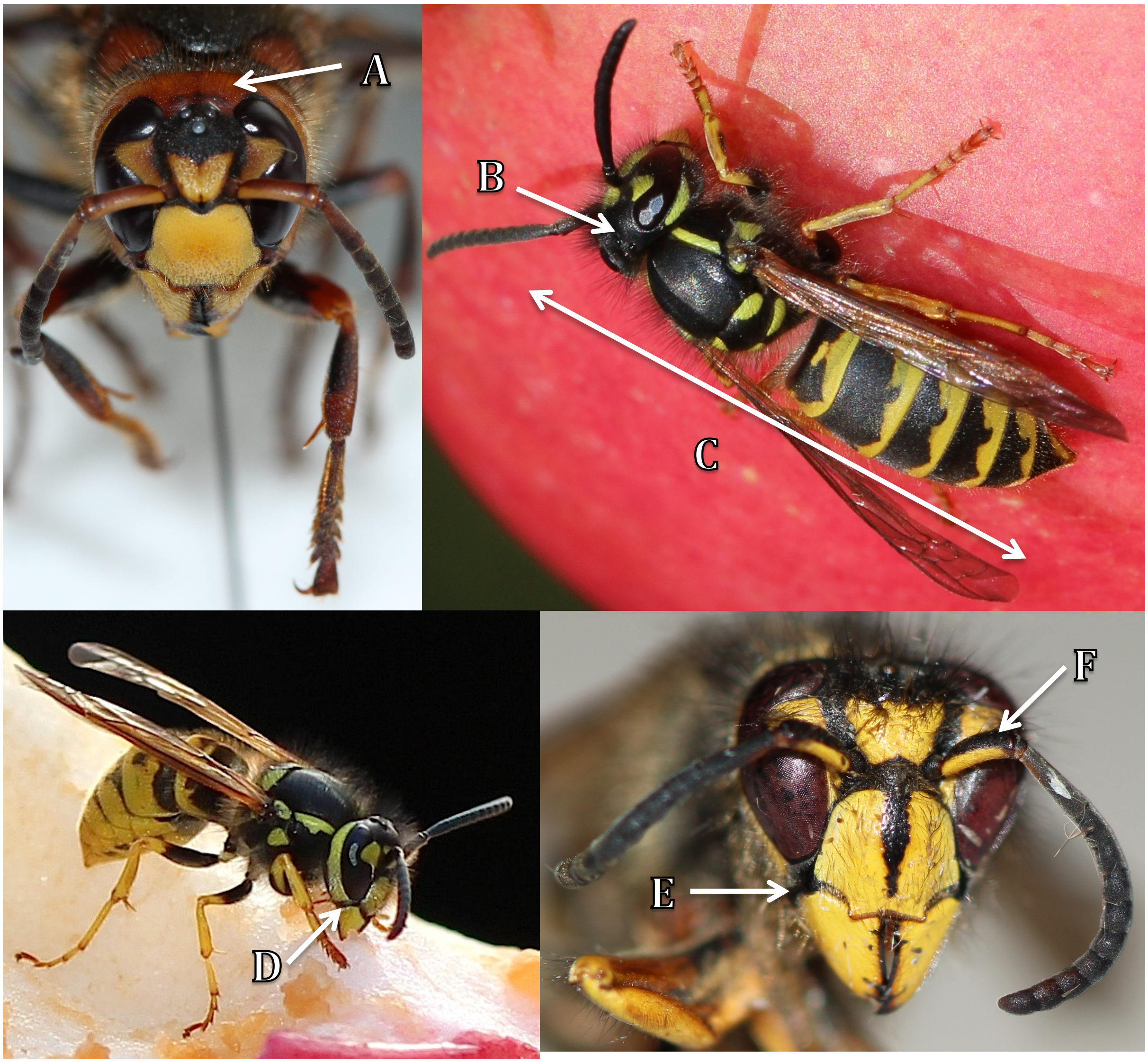


Figure 2: Distinguishing characteristics in the Vespinae genera. (A) *V. crabro*, brownish red and swollen shaped vertex, (B) *Vespula* (*V. vulgaris*), black and yellow narrow vertex, (C) *Vespula* body length ranges between 11 and 19 millimetres, (D) *Vespula* (*V. germanica*) with short oculo-malar space, (E) *Dolichovespula* (*D. media*) with a oculo-malar space longer than (F) the antennae basal scape. (Photos by author)

Biology of Vespinae

Wasps of the subfamily Vespinae live in social societies comprising three distinct castes: queens, workers (sterile females) and reproductive males (Matsuura & Yamane 1990). Few species, such as the previously mentioned *D. omissa,* parasitize such social societies, which means that the queen does not produce her own workers (Douwes *et al*. 2012).

In spring, between April and May in Scandinavia, inseminated overwintering queens emerge from their hibernating nooks and search for suitable nesting sites (Douwes *et al*. 2012). The queen initiates a small nest constructed from masticated woody plant fibres.

Species of the Vespinae inhabit biotopes of woodland areas, edges of heathland and banks where they build concealed spherical nests (Edwards 1980). *Vespula* build their nests preferably underground but also in protected cavities aboveground such in hollow trees, wall voids and attics of buildings. *Vespa* usually nest aboveground and prefer hollow trees but do also occur in buildings as described for *Vespula* above. Nests of *Dolichovespula* colonies more commonly are founded freely exposed aboveground. However, in populated areas nests are also built in protected locations as in attics of houses (Douwes *et al*. 2012; Akre & Davis 1978).

The nest consists of a number of round combs attached one below the other and is enveloped by layers of paper carton. Each comb consists of hexagonal cells in which the eggs are laid and were the entire larval development takes place. The queen manages the colony by herself until the first workers emerge. Throughout the rest of the season the primary activity of the queen is oviposition. The first offspring of the colony are females that become workers. As long as the queen of the colony is present the ovaries of these workers are suppressed and they forage for construction material (Figure 3), build new cells, forage for arthropod prey to feed the larvae and for carbohydrates to feed the queen (Douwes *et al*. 2012; Akre & Davis 1978).

Figure 3: *V. germanica* foraging for construction material. (Photo by author)

Division of labour, that is polyethism appears to be widespread amongst workers in the Vespinae (Jeanne 1980). Jeanne (1980) reviews that *Vespa* and *Vespula* workers begin their lives as nurses inside the nest. Eventually they shift to pulp and liquid foragers, later to prey foragers, and at the end of their lives their duty is to guard the colony.

A colony can consist of up to several thousand individuals. *Vespula* colonies can exceed ten thousand individuals (Douwes *et al*. 2012) and during almost the whole existence of the colony there will be a continuous production of new workers (Akre & Davis 1978). The worker-larva ratio increases and the colony becomes more efficient, more food is available for the larvae and the adult workers increase in size. It takes about four weeks for a worker to complete the immature development and its adult life lasts for about three weeks (Greene 1991). *Vespa* and *Dolichovespula* build smaller colonies with only a few hundred individuals in the Nordic countries (Douwes *et al*. 2012). Their colony life cycle, including that of *V. rufa* is also shorter, three to four months compared to *Vespula* which colonies last for approximately six months (Douwes *et al*. 2012; Archer 2012). When autumn approaches the number of workers starts to cease and males and virgin queens will develop instead (Akre & Davis 1978). Before these queens go into their solitary diapuse they mate with males from other colonies and seek a suitable place for hibernation. The annual cycle of the colony terminates with the death of the foundress queen, workers and males from the bygone season. However, colonies of *V. vulgaris*, and *V. germanica* frequently survive mild winters in places such as New Zealand, Australia, South Africa and South America. These colonies become perennial polygynous with several queens producing large populations of workers (Jeanne 1980).

Food sources and foraging behaviour

Vespine wasps not only hunt for protein rich aliments but also for liquids and plant fibres (Raveret Richter 2000). Vespine wasps are opportunistic generalists when hunting prey and searching for host plants (Raveret Richter 2000; D'Adamo & Lozada 2003). They easily exploit new habitats and eat a broad variety of foods (Raveret Richter 2000). Since these wasps are able to learn they often return to sites of previous food collecting success and individuals can therefore act as facultative specialists (Raveret Richter 2000; D'Adamo & Lozada 2003).

The larvae are mainly carnivorous and are fed chewed arthropods or vertebrate and invertebrate carrion. Insect prey eaten by the Vespinae brood includes moths (Lepidoptera), other wasps (Hymenoptera) and flies (Diptera) (Archer 2012). A foraging wasp has to locate the prey and distinguish it from non-eatable items; catch and kill the prey and either consume it or transport it back to the nest. The wasps quickly attack their live prey and kill them with their mandibles. If the catch is to be brought back to the nest it has to be prepared for the transport and protected from other predators. Insect prey is typically reduced to thorax and abdomen, by biting off the extremities. Sometimes the catch is too large to be transported all at once back to the nest and it needs to be hidden at a location safe from theft from other wasps or ants (Raveret Richter 2000).

Adult wasps mainly consume sugar-containing food from a wide range of sources to obtain energy. Host plants suitable for carbohydrate foraging *Vespa*, *Vespula* and *Dolichovespula* species are all kinds of fruits and berries with a fairly soft pericarp and high sugar content. Grapes are most attractive, but apple, pear and plum are also consumed (Matsuura & Yamane 1990; Alford 2007). *Vespula* and *Dolichovespula* visit flowers for nectar whereas *Vespa* are more dependent on tree sap as energy source. Sweet honeydew excreted from plant-feeding insects such as aphids, psyllids and coccids is another carbohydrate source especially for *Vespula* and *Dolichovespula* (Matsuura & Yamane 1990). Honeybees are robbed of their stored honey but are also prey for the vespine wasps. Vepula are well-known to scavange for carbohydrates and protein at anthropogenic sources, including sweet beverages and food (Raveret Richter 2000; Matsuura & Yamane 1990).

Location of food sources

When insects are foraging for food, visual, olfactory and mechanical stimuli mediate the location and selection of the food utilized (Gillot 1980). First the insect will orientate towards the food source by distinguishing attractive and repellent volatile cues. The insect usually flies upwind towards the attractive volatile odour where it will be arrested by additional olfactory and visual cues. Subsequently, the perceived cues will either induce landing or make the insect turn away. If landing is induced, the insect will make an assessment of the food source surface using olfactory, gustatory, visual and mechanical stimuli. If deterrents are present on the surface the insect will stop feeding and leave but if the insect recognizes stimulants it will stay to feed (Renwick 1989). The searching behaviour of wasps is elicited by different cues depending on the type of food they are exploiting. Visual cues are more important for wasps feeding on carbohydrate sources than for wasps feeding on proteins. But landing responses are mainly elicited by odour cues both in protein- and carbohydrate-rich food sources (Moreyra *et al*. 2006).

Species-specific information on forage distances for the vespine wasps is very limited. Smaller species, *Vespula* and *Dolichovespula* seem to forage within 300 m of the nest while *Vespa* have been observed to travel up to 8 km (Akre & Davis 1978; Edwards 1980). How far wasps are able to travel upwind towards food sources is so far unknown (Edwards 1980).

Vespine wasps normally return to food sources that are not depleted if they are attractive enough. When returning to food sources, visual landmarks are used for recognition of the area, and olfactory ones for identifying the source, at close range. Through specialised learning flights the wasp examines the location and stores necessary information of the place to which it will return (Moreyra *et al*. 2006). With the food source in focus, the wasps will fly back and forth in arcs while increasing the distance and eventually fly away from the feeding site (Raveret Richter 2000).

Social wasps as pests

Species of the subfamily Vespinae inhabit both natural and human environments as described previously. When human environments are selected as nesting and nutrient feeding sites these insects are regarded as harmful pests and may cause economic losses (Matsuura & Yamane 1990). Wasp stings are a serious threat to humans and domesticated animals (Edwards 1980). Anaphylactic reactions to wasp stings are described as traumatic and can have fatal consequences (Johansson *et al*. 1991). According to MSB 2014 (Swedish civil contingencies agency) 29 deaths caused by bee and wasp stings occurred in Sweden between year 1997 and 2012. In addition around 300 people are hospitalized every year due to bee and wasp stings (MSB 2014).

Foraging wasps move between different locations and can vector disease organisms. For example several food-poisoning bacteria are potentially transmitted from household garbage dumps, animal corpses or from animal faeces to human food when the wasps are hunting for arthropod prey. Wasps can accordingly be classified as hygienic pests that potentially cause food-poisoning (Edwards 1980; Matsuura & Yamane 1990).

Changes in world distribution of several vespine species have had invasive impacts on native fauna (Choi *et al*. 2012; El-Sayed *et al*. 2009). For example in New Zealand, *Vespula vulgaris* (L.) and *Vespula germanica* (L.) are a threat to native birds and invertebrates as they compete for honeydew as food (El-Sayed *et al*. 2009). The introduction of *V. germanica* to New Zealand and the fact that these wasps prey on honeybees and their honey has caused tremendous problems for beekeepers as well as fruit growers because of reduced pollination (Akre and Davis 1978; Raveret Richter 2000; Edwards 1980). The same observations have also been made in France were the introduced *V. velutina* attacks beehives more aggressively than native Vespinae species (Monceau *et al*. 2014).

Information about economic losses is incomplete, since damage by wasps is widespread and ranges from households, commercial orchards and entire ecosystems. Nevertheless, it is well established that wasps cause economical losses in horticultural fruit and grape production.

As already mentioned, wasps scavenge for carbohydrate rich sources in orchards and vineyards. In Nordic viticulture vespine wasps are serious pests of grapevines if not controlled. Due to short growing seasons, only early maturing grape varieties are cultivated in Scandinavia, which are specially exposed to wasps. Wasps feed on grape berries, which makes them susceptible to microbe secondary infections and spoil clusters of grapes (Akre *et al*. 1981; Barata *et al*. 2012; Sofrakis, pers. comm., Hagerman, pers. comm.). For example, acetic acid bacteria cause sour rot, initiating acidification of berries before harvest (Akre *et al*. 1981; Barata *et al*. 2012). Up to 95 % grape yield loss due to wasps, was observed in smaller vineyards in the New York area (Akre *et al*. 1981). In 2014, wasps destroyed more than 50% of the early grape variety Madeleine Angevine yield at a vineyard in Denmark (Hagerman, pers. comm.). Apples can also be damaged by feeding wasps but are attacked to a lesser degree (Edwards 1980; Matsuura & Yamane 1990).

Weather conditions and food source availability influence abundance of social wasps. Wasp outbreaks occur in warm and humid weather, and when sufficient food, in terms of arthropod prey and carbohydrate nutrients, are available (Edwards 1980). In July

2014, Anticimex performed twelve thousand wasp sanitations during one single month in Sweden (Anticimex 2014).

Several reports conclude that climate change has an impact on the abundance and distribution of insects (Sparks *et al*., 2007; Jamieson *et al*. 2012; Choi *et al*. 2012; Monceau *et al*. 2014). Tryjanowski *et al*. (2010) showed that the phenology of *V. crabro* and *V. germanica* advanced over the last three decades and concluded that climate change is likely to affect the life cycle of wasps. As mentioned previously *V. velutina* has invaded parts of southern Europe and Barbet-Massin *et al*. (2013) predict an increase in climatic suitability for *V. velutina* in the Northern Hemisphere.

Control of social wasps

Wasp control measures can be divided into four categories: (1) manual destruction and removal of colonies, (2) use of insecticide baits, (3) use of attractive synthetic compounds and (4) use of food lures (Edwards 1980; Matsuura & Yamane 1990; Landolt *et al*. 2000; Landolt *et al*. 2003).

Injecting insecticides into the nest or applying ether, chloroform or carbondioxide to anesthetize the wasps are methods used for manual destruction of colonies. After either of the treatments the nest is removed and destroyed.

Meat, fish and catfood prepared with slow-acting insecticides can be used as baits to exterminate wasp populations. When returning foragers feed the larvae with the poisoned bait the colony will eventually be exterminated (Matsuura & Yamane 1990; Edwards 1980).

Heptyl butanoate is attractive to the pestiferous *Vespula pensylvanica* in the western USA but is ineffective as a lure to other species of Vespinae wasps (Akre *et al*. 1981; Landolt *et al*. 2003). The method involves traps baited with the synthetic compound to which individual foraging wasps will be attracted, caught and eventually die. Since this method has been very efficient against *V. pensylvanica*, research continues (Landolt *et al*. 2007; El-Sayed *et al*. 2009).

Food lures of fruits or fermented beverages are frequently used for catching social wasps in traps (Edwards 1980). In Nordic viticulture growers solely depend on homemade traps usually made of PET bottles or big buckets baited with yam and honey as lures (Bäck, pers. comm.; Hagerman, pers. comm.; Sofrakis, pers. comm.).

Trapping social wasps

Odour-based pest management

The basis of trap catching insects is to use the innate attraction behaviour (Del Socorro *et al*. 2010). As described previously, attractive cues can be visual, olfactory or the combination of both (Moreyra *et al*. 2006). Semiochemicals are substances, which provide signals between individuals and comprise intraspecific pheromones and interspecific allelochemicals. Semiochemicals can hence be emitted from either the same species or other species, being an insect, a plant or a microorganism (Nordlund & Lewis 1976).

Semiochemicals can for example repel pest insects from the crop, attract beneficial insects to the field, prevent mate-finding or be used in traps. Within current entomological and chemical ecology research, identification of specific compounds, which act as behavioural cues, is ongoing (Khan *et al*. 2008; Witzgall *et al*. 2010). The aim is to identify and to use synthetic compounds specific to each pest insect, to reduce the number of non-target insects, to reduce the use of environmental hazardous insecticides and to increase longevity of the traps. Behaviourally active compounds identified from insects or plants can be synthesized and used for trapping (Witzgall *et al*. 2010; Del Socorro *et al*. 2010). In social wasps, semiochemicals eliciting the behaviour of finding food have been identified (see below).

Volatiles attractive to social wasps

The disaccharide sucrose and its constituent monosaccharides fructose and glucose are recognized by contact chemoreception with the mouthparts or tarsi and can first be detected by the insect when it is in direct contact with the food substrate. Volatile olfactory compounds emanating from the food source including those induced by colonising microorganisms mediate long- range attractance of the insect (Shoonhoven *et al*. 1998; Davis *et al*. 2013).

Several volatile organic compounds related to carbohydrate sources have been tested for social wasp attraction. Table 2 summarizes these substances and combinations of substances that have been reported as most effective for attracting social wasps in different regions (Carle *et al*. 1987; Landolt 1998; Landolt *et al*. 1999; Landolt *et al*. 2000; Reed & Landolt 2002; Landolt *et al*. 2005; Landolt *et al*. 2007; El-Sayed *et al*. 2009; Brown *et al*. 2014).

Acetic acid is a microbal product of fermented sugars and is weekly attractive to social wasp species in North America and Europe (Landolt *et al*. 1999; Reed & Landolt 2002; Landolt *et al*. 2005; Landolt *et al*. 2007). It has been tested in combination with different alcohols and especially the combination with 2-methylproapan-1-ol has been effective in social wasp attraction (Landolt 1998; Landolt *et al*. 1999; Reed & Landolt 2002; Landolt *et al*. 2005; Landolt *et al*. 2007).

Heptyl butanoate, an ester naturally produced in fresh apples (Carle *et al*. 1987) is used in combination with acetic acid as a commercial synthetic lure in North America and efficiently attracts *Vespula pensylvanica* (Landolt *et al*. 2003) but is only weakly attractive to other social wasps in this region (Landolt *et al*. 2005; Reed and Landolt 2002). Similar results were obtained in Hungary, Europe were the social wasp species *V. germanica*, *V. vulgaris*, *D. media* and *V.crabro* did not respond to heptyl butanoate (Landolt *et al*. 2007).

In New Zealand, El-Sayed *et al*. (2009) found that heptyl butanoate was attractive to *V*. *vulgaris* and *V*. *germanica* and that the addition of acetic acid as co-attractant decreased the attraction of wasps. In contrast to the North American and European experiments, social wasps in New Zealand were not attracted to 2-methylpropan-1-ol, neither alone nor in combination with acetic acid (Landolt 1998; Landolt *et al*. 1999; Reed & Landolt 2002; Landolt *et al*. 2005; Landolt *et al*. 2007; El-Sayed *et al*. 2009).

Table 2: Summary of synthetic compounds and blends attractive to Vespine wasps.

Compounds	Species	Source	Reference
Esters			
Butyl butanoate + acetic acid	*V. germanica*[a]	Fermented molasses	Landolt (1998)
Heptyl butanoate + acetic acid	*V. pensylvanica*[a]		Landolt (1998)
	V. atropilosa[a]		Landolt (1998)
	V. rufa species group*[ab]		Reed & Landolt (2002) Landolt *et al.* (2005)
Heptyl butanoate	*V. pensylvanica*[a]	Fresh apples	Landolt *et al.* (2003)
	V. atropilosa[a]		Landolt *et al.* (2003)
	V. vulgaris[c]		El-Sayed *et al.* (2009)
Octyl butanoate	*V. vulgaris*[c]		El-Sayed *et al.* (2009)
3-Methylbutyl acetate	*V. vulgaris*[c]	Fermented brown sugar	El-Sayed *et al.* (2009)
Ethyl hexanoate	*V. vulgaris*[c]	Fermented brown sugar	El-Sayed *et al.* (2009)
Alcohols			
2-Methylpropan-1-ol + acetic acid	*V. pensylvanica*[a]	Fermented molasses/ fermented sugar solution	Landolt (1998) Landolt *et al.* (2005)
	V. maculifrons[a]		Landolt *et al.* (1999)
	V. squamosa[a]		Landolt *et al.* (1999)
	V. flavopilosa[a]		Reed & Landolt (2002)
	V. germanica[ad]		Landolt *et al.* (1999) Landolt *et al.* (2007)
	V. vulgaris[abd]		Reed & Landolt (2002) Landolt *et al.* (2005) Landolt *et al.* (2007)
	D. maculata[ab]		Reed & Landolt (2002) Landolt *et al.* (2005)
	V. crabro[ad]		Landolt *et al.* (1999) Landolt *et al.* (2007)
2-Methylpropan-2-ol + acetic acid	*V. pensylvanica*[a]		Landolt *et al.* (2000)
	V. germanica[ad]		Landolt *et al.* (2000) Landolt *et al.* (2007)
	V. vulgaris[d]		Landolt *et al.* (2007)
2-Methylbutan-1-ol + acetic acid	*V. pensylvanica*[a]		Landolt *et al.* (2000)
	V. germanica[a]		Landolt *et al.* (2000)
3-Methylbutan-1-ol + acetic acid	*V. pensylvanica*[a]	Fermented brown sugar	Landolt *et al.* (2000)
	V. germanica[a]		Landolt *et al.* (2000)

Species captured in the regions: [a]USA, [b]Alaska, [c]New Zealand and [d]Europe.

Live yeast volatiles as semiochemicals

Numerous yeasts and yeast-like species inhabit the phyllosphere, aerial parts of plants (Last & Deighton 1965). Yeasts thrive for instance in habitats were fruits are present (Trindade *et al*. 2002). Vadkertiová (2012) investigated yeasts associated with apple, plum and pear. *Metschnikowia pulcherrima*, *Saccharomyces cervisiae* and *Cryptococcus* sp. where identified amongst those. These species have also been isolated from grape berry surfaces and grape must (Raspor *et al*. 2006; Nemcová *et al*. 2015).

Yeasts produce bioactive odours and the understanding of insect and phyllospherous microorganism interaction is a current research theme (Davis *et al.* 2013). Witzgall *et al*. (2012) showed that female *Cydia pomonella* (Tortricidae, Lepidoptera), a pest on apple, responded to yeast volatiles and layed more eggs on yeast-inoculated apples than on apples lacking yeast colonies. Both yeast and apple fruit are essential diets for the *C. pomonella* larvae development and a suitable ovipositon site for the female moth is not only signalled by the host plant itself but also by volatiles emitted from the yeast. This suggests that odours produced by yeast fungi in the phyllosphere alter insect behaviour (Davis *et al.* 2013).

Volatile emissions from the yeast-like epiphyte *Aureobasidium pullulans* attracted social wasps (Davis *et al*. 2012). Two of the identified *A*. *pullulans* headspace volatiles were 2-methylbutan-1-ol and 3-methylbutan-1-ol (Davis *et al*. 2012). 3-methylbutan-1-ol was identified from fermented un-refined sugar, which is attractive to social wasps (Brown *et al*. 2014). Davis *et al*. (2012) suggest that, "fungal emissions may signal suitable nutrient sources to foraging wasps".

Objectives and aim

The first objective of this project was to test if social wasps in Scania, Scandinavia are attracted to and can be captured in traps baited with synthetic odorants that mimic volatiles naturally emitted from carbohydrate food sources of social wasps. These compounds have previously been tested in North America and Hungary or New Zealand.

The second objective was to evaluate if social wasps in Scania, Scandinavia are attracted and can be captured in traps baited with live yeasts naturally occurring on ripe apples and grapes, which are food sources for wasps.

Aim
The aim of this study was to provide a basis for further investigation of odour-based control methods by attracting and trapping social wasps with synthetic compounds and live yeasts.

Materials and methods

Chemicals

Test compounds are shown in table 3.

Table 3: Synthetic compounds tested for social wasp attraction

No.	IUPAC name	Synonyme	CAS-number	Functionality	Molecular formula	Molar mass (g/mol)	Purity (%)
1	2-Methylpropan-1-ol[a]	Isobutanol	78-83-1	primary alcohol	$C_4H_{10}O$	74.122	99.0
2	2-Methylpropan-2-ol[a]	tert-Butanol	75-65-0	tertiary alcohol	$C_4H_{10}O$	74.12	99.7
3	2-Methylbutan-1-ol[a]	Active amyl alcohol	137-32-6	primary alcohol	$C_5H_{12}O$	88.148	98.0
4	3-Methylbutan-1-ol[b]	Isopentanol	123-51-3	primary alcohol	$C_5H_{12}O$	88.148	98.5
5	Heptyl butanoate[a]	Heptyl butyrate	5870-93-9	ester	$C_{11}H_{22}O_2$	186.29l	98.0
6	Ethanoic acid[a]	Acetic acid	64-19-7	carboxylic acid	$C_2H_4O_2$	60.05	99.0

Chemicals were purchased from [a]Aldrich and [b]ACROS Organics

Yeasts

Yeast cultures of *Metschnikowia andauensis*, *M. pulcherrima*, *M. chrysoperlae*, *Saccharomyces* sp. and *Cryptococcus* sp. were kindly provided by Dr. Maria Karlsson (department of Plant Protection Biology, SLU).

In 2013, these yeast strains had been isolated from apples in an organic apple orchard at Alnarp, Sweden and stored as frozen cultures at -80° C in 15% glycerol. Yeast cells were prepropagated for 7-14 days on YPD medium (Merico *et al*. 2007). One colony forming unit of each yeast was inocultaed in 50 ml liquid synthetic minimal media (Merico *et al*. 2007) on a shaking incubator (VWR, Incubating mini shaker) and fermented for 24 h at 26° C and with a stirrer rate of 260 rpm.

Traps

Ball traps, developed by Dr. Teun Dekker (department of Plant Protection Biology, SLU), produced by Resence (Netherlands) were used. These traps consisted of two semicircular parts with funnel-shaped openings at the top and bottom. The opaque top was designed to hold a 3 cm diameter receptacle divided into two compartments and could contain two different blends.

A receptacle lid prevented dilution of the attractants and forced the volatiles to move through small holes (7 x 5 mm) at the bottom of the receptacle, downwards through the trap entrance. The bottom part of the trap was yellow, had a funnel-shaped entrance opening and served as a basin for drowning solution (Figure 4).

Figure 4: Ball trap. Recaptacle with two compartments placed in the middle of opaque top. Yellow bottom with funnel shaped entrance. Receptacle lid at the bottom right-hand corner. (Photo by author)

Field trial areas

In Western Scania, traps baited with synthetic compounds, were placed at Klagshamn vineyard (55°32'8.0"N12°55’48.3”E) and in East Scania at Juleboda apple orchard (55°46'2.4"N14°11’32.1”E), between August 22 and September 20, 2014. The surroundings of Klagshamn vineyard are dominated by field and greenhouse vegetable cultivation and urban areas. Juleboda apple orchard is situated in a forest close to the Baltic sea.

The vineyard was managed without chemical pesticides. Social wasps were controlled by home-made sweet food baits. Juleboda apple orchard had been under low input management during the last years and has now been abandoned. Social wasps were not controlled.

Yeast isolates were tested in an experimental apple orchard at the campus of the Swedish University of Agricultural Sciences Alnarp, southwest Scania (Sweden) (55°39'35. 6"N13°4'41. 3"E) between September 5 and September 11, 2014. Experimental field and greenhouse areas as well as smaller woodland park areas surrounded the organically managed apple orchard.

At Klagshamn vineyard and Alnarp apple orchard plants were pruned manually while mechanical weeding was carried out underneath the vines and apple trees. Between crop rows grass was mowed. At Juleboda apple orchard no pruning, weeding or grassmowing was carried out. All trial areas were surrounded by tree rows as wind shelters.

Synthetic lures

At Klagshamn vineyard and Juleboda apple orchard traps baited with synthetic compounds were compared. Each compound was tested singly with sterile water as well as in combination with acetic acid (Table 4).

Table 4: Synthetic lure composition. 1.5 ml test compound was applied to a cotton wick.

No.	Compound	(ml)	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
1	2-Methylpropan-1-ol	1.5	•	•										
2	2-Methylbutan-1-ol	1.5			•	•								
3	3-Methylbutan-1-ol	1.5					•	•						
4	2-Methylpropan-2-ol	1.5							•	•				
5	Heptyl butanoate	1.5									•	•		
6	Acetic acid	1,5		•		•		•		•		•	•	
7	Sterile water	1,5	•		•		•		•		•		•	••

Bullet (•) mark shows synthetic compounds included in treatments.

Compounds were applied directly to dental cotton wicks (Top Dent, Ø 12 mm) and placed in the two compartments of the trap receptacle (Figure 5). Each compound was tested separately as well as in combination with acetic acid. Single compound treatments (I, III, V, VII, IX) were dispensed from single cotton wicks placed in one compartment. The other compartment contained a cotton wick with sterile water only.

With 2-component lures (II, IV, VI, VIII, X) test compounds were dispensed from a single cotton wick placed in one compartment and acetic acid was applied to one single cotton wick placed in the other compartment. One control containing acetic acid placed in one compartment and water in the other one was used as well as one blank with only water.

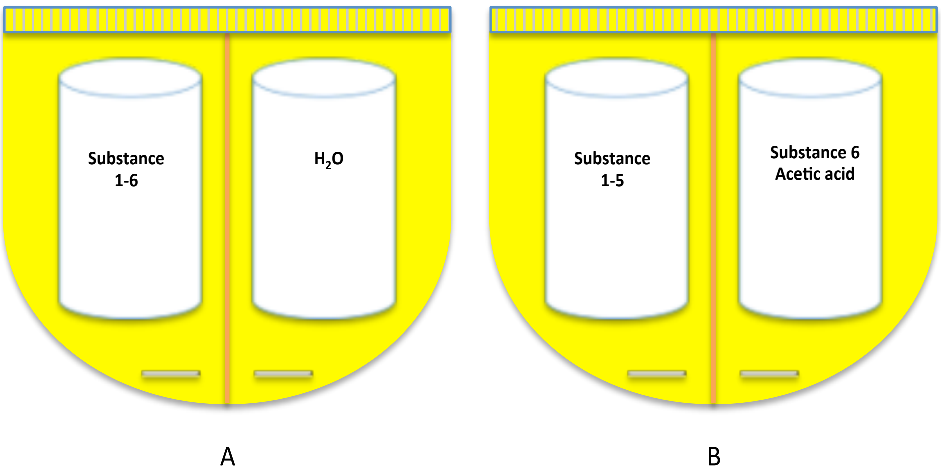


Figure 5: Placement of test compounds formulated on cotton wicks in trap receptacles (Figure 4). A) Single compound treatments; compounds were dispensed from the left-hand side. Sterile water was added to a cotton wick at the right-hand side. B) Two compound treatments; compounds 1 to 5 were dispensed from the left-hand side and acetic acid from the right-hand side.

Yeast lures

Five treatments of liquid medium with fermenting yeasts: (I) 50 ml *Metschnikowia andauensis*, (II) 50 ml *M. chrysoperlae*, (III) 50 ml *M. pulcherrima*, (IV) 50 ml *Saccharomyces* sp. and (V) 50 ml *Cryptococcus* sp. were compared in an organic apple orchard at Alnarp. One control containing only liquid minimal medium (VI) was used as well as one blank control with only water (VII). Fifty milliliters of each fermenting yeast strain was applied to 6 cotton wicks (Top Dent, Ø 12 mm) and placed in the two compartments of the trap (Figure 6).

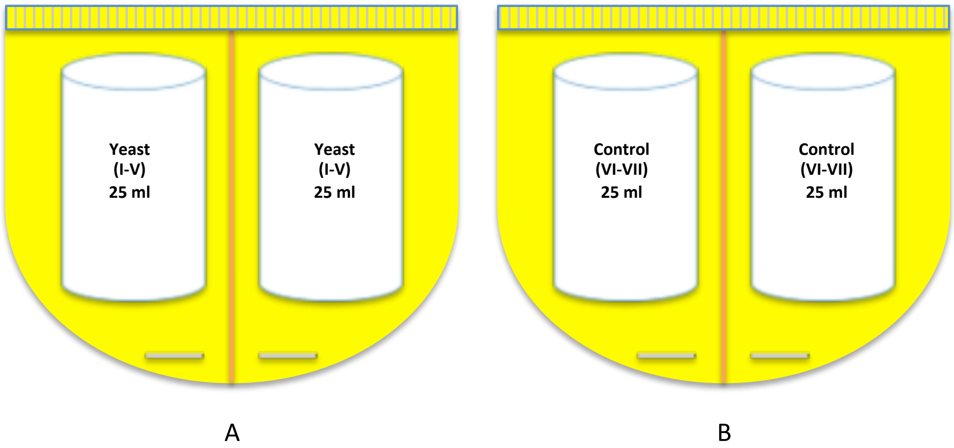


Figure 6: Placement of fermenting yeasts (I-V) and controls (VI-VII) formulated on cotton wicks. A) Yeast treatments and B) controls were dispensed from both sides of the trap receptacle.

Trap placement

Traps were placed in randomized block designs with a line of traps constituting one block. Three blocks at each site were set up. Traps were placed within crop rows at a distance of 7-10 m and at a height of 1.5 m above ground. Distances between blocks were at least 30 m at Klagshamn vineyard and Juleboda apple orchard. At Alnarp organic apple orchard the distance between blocks was 20 m.

Figure 7: Ball trap within crop row at Juleboda apple orchard. (Photo by author)

Trap captures

Traps baited with synthetic compounds, at Juleboda apple orchard and Klagshamn vineyard were read 4 times between August 22 and September 20, 2014. Trapped wasps were collected and stored at – 20°C. The chemical baits and controls where renewed every second week.

Between September 5 and September 11, 2014 fermenting yeast baits at Alnarp apple orchard were read every 48 h. At the time of trap reading baits were renewed. Trap catches were collected and stored at -20°C.

All traps contained 400 ml of drowning solution, water with a few drops of dishwashing detergent (Skona handdisk, clear and fragrance free). This solution was changed at each trap reading.

Taxonomic identification of wasps

Specimens of social wasps were separated from trapped samples and stored in 70% ethanol at -20°C. Social wasps were morphologically identified to species levelling according to the keys by Douwes *et al*. (2012) and Kruse Pedersen (1975) and counted. As a complement, the specimen reference collection of social wasps at the Biological museum, Lunds University Sweden was studied and a smaller reference material of random trap catches was established.

Non-target insects were morphologically identified and counted at least to order level (Appendix 1, 2 and 3). The entomological keys in Douwes (1997) were used for identification of insects others than social wasps.

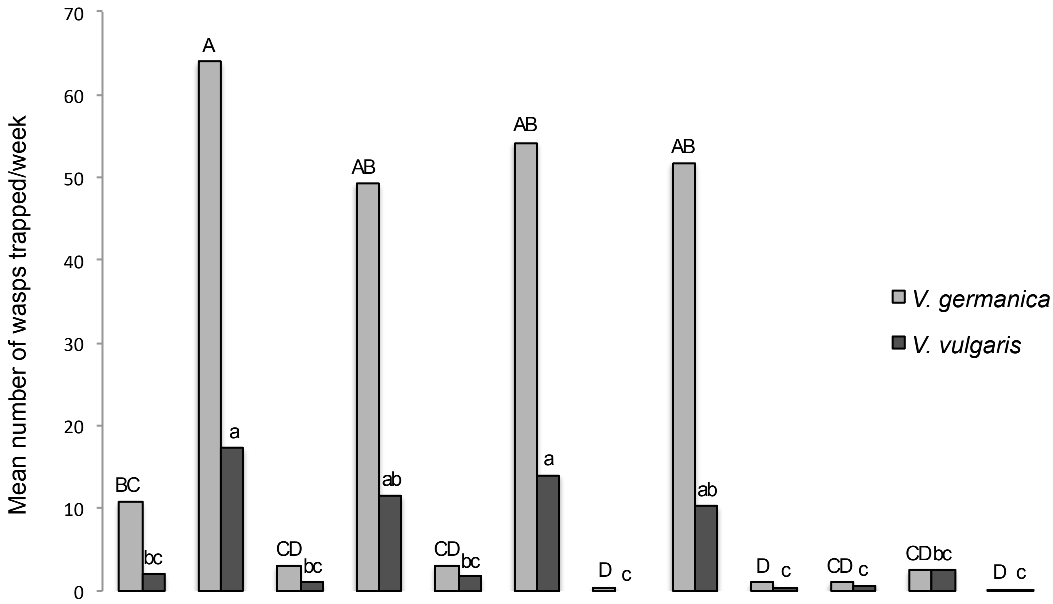
Statistical analysis

Data were log(x + 1) transformed to normalize the counted trap catches. For each species, means of captured wasps per trap, per week were compared between treatments using one-way ANOVA variance analysis followed by Tukey´s test to determine significant differences among treatment means.

Results

Synthetic feeding attractants

In the first field trial, conducted in Klagshamn and Juleboda, ten treatments of five synthetic compounds; 2-methylpropan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol, 2-methylpropan-2-ol and heptyl butanoate and their combination with acetic were compared (Figure 8). One control containing acetic acid as well as one unbaited control (sterile water) was used. The most abundant species of wasps captured were *V. germanica* and *V. vulgaris*. Numbers of *V. crabro*, *D. media* and *V. rufa* were too small for statistical comparison.



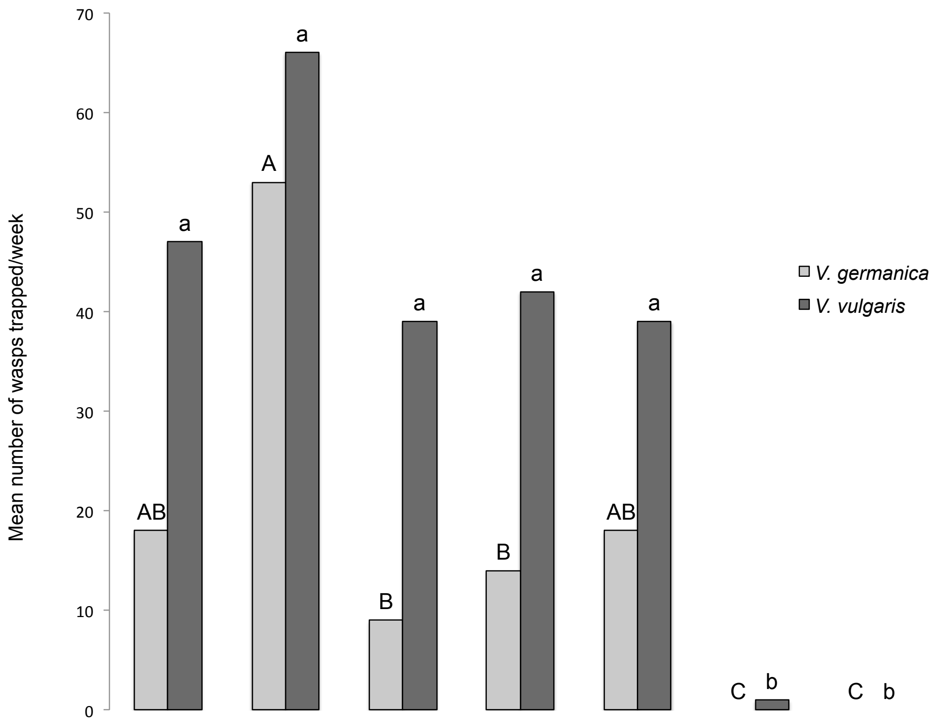
Treatment	(ml)	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2-Methylpropan-1-ol	1.5	•	•										
2-Methylbutan-1-ol	1.5			•	•								
3-Methylbutan-1-ol	1.5					•	•						
2-Methylpropan-2-ol	1.5							•	•				
Heptyl butanoate	1.5									•	•		
Acetic acid	1.5		•		•		•		•		•	•	
Sterile water	1.5	•		•		•		•		•		•	••
Wasps captured													
V.germanica		10.8^{BC}	64.0^{A}	3.0^{CD}	49.3^{AB}	3.0^{CD}	54.3^{AB}	0.5^{D}	51.8^{AB}	1.0^{D}	1.0^{CD}	2.5^{CD}	0.3^{D}
V. vulgaris		2.0^{bc}	17.3^{a}	1.0^{bc}	11.5^{ab}	1.8^{bc}	14.0^{a}	0.0^{c}	10.3^{ab}	0.5^{c}	0.8^{c}	2.5^{bc}	0.3^{c}
V. crabro		0.3	3.8	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
V. rufa		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.25	0.0	0.0	0.0	0.0
D. media		0.0	0.75	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0

Figure 8: Mean numbers of social wasps captured per week in traps baited with synthetic test compounds (I-X) and controls (XI and XII). For each species, means followed by the same letter are not significantly different (*V. germanica* F = (12, 55)= 4,83; P ≤ 0,05; N = 6, *V. vulgaris* F = (12, 55)= 4,83; P ≤ 0,05; N=6). Mean numbers of *V. crabro*, *D. media* and *V. rufa* captured per week (I-X) and in controls (XI and XII).

All test compounds, except heptyl butanoate were more attractive when blended with acetic acid (Figure 8). *V. germanica* was the most abundant species and individuals were most attracted to traps baited with acetic acid plus 2-methylpropan-1-ol. This blend caught significantly higher numbers of *V. germanica* than 2-methylpropan-1-ol as single bait, compared to remaining blends. *V. vulgaris* were less abundant but attracted to the same lures as *V. germanica*. No or few wasps were captured in unbaited traps and in traps baited with acetic acid. In total 20 individuals of *V. crabro*, 7 individuals of *D. media* and 1 *D. rufa* were captured (Figure 8). Non-target insects were captured from 6 orders, especially flies (Diptera) (see appendix 1 and 2).

Fermenting yeast cultures

In the second field trial, conducted in Alnarp, five treatments of fermenting yeast cultures were compared. One control with only medium and one unbaited control (sterile water) were used (Figure 9). The most abundant wasp species caught were *V. germanica* and *V. vulgaris*. Numbers of *V. crabro* were too small for any statistical comparison.



Treatment	(ml)	I	II	III	IV	V	VI	VII
M. andauensis	50	•						
M. chrysoperlae	50		•					
M. pulcherrima	50			•				
Saccharomyces sp.	50				•			
Cryptococcus sp.	50					•		
Control (medium)	50						•	
Control (sterile water)	50							•
Wasps captured								
V. germanica		18.0^{AB}	53.0^{A}	9.0^{B}	14.0^{B}	18.0^{AB}	0.0^{C}	0.0^{C}
V. vulgaris		47.0^{a}	66.0^{a}	39.0^{a}	42.0^{a}	39.0^{a}	1.0^{b}	0.0^{b}
V. crabro		0.0	3.0	2.0	1.0	2.0	0.0	0.0

Figure 9: Mean numbers of social wasps captured per week in traps baited with fermenting yeast cultures (I-V). For each species, means followed by the same letter are not significantly different (*V. germanica* F = (7, 12)= 4,95; P ≤ 0,05; N = 3, *V. vulgaris* F = (7, 12)= 4,95; P ≤ 0,05; N=3). Mean number of *V. crabro* captured per week (I-V) and in controls (VI and VII).

A significant higher number of both *V. germanica* and *V. vulgaris* wasps were captured in traps baited with the five treatments of fermenting yeast cultures (I-V) compared to either the media control or the unbaited control (sterile water) (Figure 9). Most *V. germanica* were captured in traps baited with *M. chrysoperlae* and this treatment differed significantly from traps baited with *Saccharomyces sp.* and *M. pulcherrima* but not significantly from *M. andauensis* and *Cryptococcus sp.*. *V. vulgaris* trap catches did not differ significantly between the five fermenting yeast culture treatments (I-V). In total 8 individuals of *V.* c*rabro* were captured. They were captured in traps baited with *M. chrysoperlae, Saccharomyces* sp., *M. pulcherrima* and *Cryptococcu*s sp. but in neither of the controls. Yeasts also attracted non-target insects from 5 orders, especially flies (Diptera) (see appendix 3).

Discussion

Synthetic food lures

The objective of this study was to determine if social wasps in Scandinavia are attracted to synthetic compounds. Lures based on these chemicals have been shown to be attractive in several field studies in North America, New Zealand and Europe (Landolt *et al*. 2000; Landolt *et al*. 2007; El-Sayed *et al*. 2009). The results of the experiment show that *V. germanica* and *V. vulgaris* are attracted to four volatile compounds, when these are blended with acetic acid. These compounds are barely attractive by themselves, but blending them with acetic acid produces strong synergistic effect in wasp attraction. Nor is the acetic acid attractive by itself (Figure 8). If an esterification reaction occurs between acetic acid and alcohol released from the synthetic blend treatments in these experiments is unknown. 3-Methylbutyl acetate, an esterification product of carboxylic acid and 3-Methylbutan-1-ol has shown to be attractive to *V. vulgaris* when blended with 3- Methylbutan-1-ol and ethyl hexanoate (Brown *et al*. 2014). Indicating that acetate esters could influence social wasp attraction. A headspace analysis of the blends tested in this study can, in further studies, identify if an esterification and thereby a spontaneous modification of the tested blends has occurred or not.

Insects use volatile compounds for communication with conspecifics (pheromones) and they use volatile compounds to find food sources (kairomones). Highly specific communication with other individuals of the same species and surrounding environment is achieved by tuning the olfactory response to blends of chemicals, in specific proportions, and more seldom as single compounds (Linn *et al*. 1986; Johansson & Jones 2007; Bruce & Picket 2001; El-Sayed 2015).

Blending the test compounds with acetic acid produced a very strong increase in trap captures. Single compounds, including acetic acid, did not significantly differ from the blank (Figure 8). However, not all compounds showed this synergistic effect. A blend of heptyl butanoate and acetic acid did not produce any attraction.

The lure that attracted most wasps of both species was a blend of 2-methylpropan-1-ol plus acetic acid. The result of this trapping study thus confirms work by Landolt *et al*. (2000, 2007) in North America and Europe. Heptyl butanoate, alone or in combination with acetic acid did not trap *V. germanica* and *V. vulgaris*, neither in North America or in Europe (Hungary) (Landolt 1998; Landolt *et al*. 2007), nor in Scania (Figure 8). Interestingly, heptyl butanoate is a commercial lure effective in trapping *Vespula pensylvanica*, a pestiferous wasp in North America (Akre *et al*. 1981; Landolt *et al*. 2007), demonstrating the species-specifity of the attraction to fermenting food volatiles. However, in contrast to the trapping results in Scania (Figure 8) and Hungary (Landolt *et al*. 2007), *V. vulgaris* is attracted to heptyl butanoate in New Zealand (El-Sayed *et al*. 1999). In addition, wasp attraction in New Zealand showed no apparent synergism, when the test compound heptyl butanoate or 2-methylpropan-1-ol were combined with acetic acid (El-Sayed *et al*. 2009).

The wasp response to odour stimuli may vary according to food availability, between locations (El-Sayed *et al*. 2009), and in differences of foraging behaviours over the season (Douwes *et al*. 2012). Geographical and seasonal differences in wasp attraction must be taken into account when developing effective, odour-based control of social wasps.

Vespa crabro, *D. media* and *V. rufa* were only captured in small numbers, which could be due to a weak response or locally low population densities. It would accordingly be necessary to place traps near colonies of these species to reliably compare their attraction response.

Fermenting yeast lures

Live yeast cultures have been tested for social wasp attraction in Scandinavia for the first time. Yeasts are found on fruits and berries (Madigan *et al*. 2003; Vadkertiová 2012) and emit blends of volatiles, signalling food for social wasps (Davis *et al*. 2012).

The objective of the experiment was to investigate if social wasps are attracted to and can be captured with live yeasts that naturally occur on apples and grapes. The result shows a strong attraction of both *V. germanica* and *V. vulgaris* to all yeast cultures tested. These findings confirm the study made by Davis *et al*. (2012), showing that social wasps were strongly attracted to colonies of *Aureobasidium pullulans* and clearly establish that yeast odours stimulate social wasp behaviour.

Large numbers of both *V. germanica* and *V. vulgaris* wasp were captured in traps baited with *M. chrysoperlae*. *Vespula germanica* was significantly more attracted to *M. chrysoperlae* compared with S*accharomyces* sp. and *M. pulcherrima*. The composition and proportion of volatiles emitted differ between yeast species (Witzgall et al. 2012) and stronger attraction to *M. chrysoperlae* is most likely due to a more attractive blend of volatiles emitted, either qualitatively or quantitatively, or both in comparison with the other yeasts tested. Another explanation could be that the growth medium composition used in the trap or environmental factors such as temperature and humidity, were more favourable for the growth of *M. chrysoperlae* than for the other yeasts tested.

Application of fermentation volatiles for wasp control

There is no efficient control measure apprehensible for suppressing social wasp populations at present time. Home-made food lures of fruits and beverages are the only option for vinegrowers. These lures are not efficient enough, lure life is limited and lures need to be exchanged frequently.

Identification of social wasp synthetic attractants, in comparison with live yeast, will accordingly lead to the development of efficient synthetic lure formulations and lay the ground for social wasp management.

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Dear *Mom* and dear *Sister*, I did it for our future and in memory of you *Dad*.
I know it would have made you happy.

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Supplementary information

Appendix 1:

Total amount of arthropods trapped in synthetic compound blend treatments, at Juleboda apple orchard between August 22 and September 20, 2014

Treatment	Order	Suborder[a]/Superfamily[b]	Family	Number captured
2-Methylpropan-1-ol + acetic acid	Diptera	Brachycera[a]	-	≈3000
		Syrphoidea[b]	Syrphidae	45
		Ephydroidea[b]	Drosophilidae	5
		Ephydroidea[b]	Chloropidae	200
		Nematocera[a]	-	520
	Neuroptera	-	Chrysopidae	9
	Homoptera	Cicadoidea[b]	Cicadellidae	2
	Hymenoptera	Apocrita[a]	-	1
		Chalcidoidea[b]	-	240
		Ichneumonoidea[b]	-	4
		Symphyta[a]	-	1
	Lepidoptera	-	-	186
2-Methylbutan-1-ol + acetic acid	Diptera	Brachycera[a]	-	364
		Syrphoidea[b]	Syrphidae	1
		Ephydroidea[b]	Drosophilidae	3
		Ephydroidea[b]	Chloropidae	2
		Nematocera[a]	-	195
	Neuroptera	-	Chrysopidae	4
		Psylloidea[b]	Psyllidae	1
		Chalcidoidea[b]	-	38
		Ichneumonoidea[b]	-	3
	Lepidoptera	-	-	73
		Sphecidae	-	1
	Coleoptera	Cucujoidea[b]	Coccinellidae	1
3-Methylbutan-1-ol + acetic acid	Diptera	Brachycera[a]	-	259
		Syrphoidea[b]	Syrphidae	4
		Ephydroidea[b]	Chloropidae	4
		Nematocera[a]	-	141
		Thephritoidea	Thephritidae	1
	Neuroptera	-	Chrysopidae	16
	Homoptera	Cicadoidea[b]	Cicadellidae	1
		Psylloidea[b]	Psyllidae	3
		Chalcidoidea[b]	-	14
		Ichneumonoidea[b]	-	3
	Lepidoptera	-	-	70
2-methylpropan-2-ol + acetic acid	Diptera	Brachycera[a]	-	≈2300
		Syrphoidea[b]	Syrphidae	40
		Ephydroidea[b]	Drosophilidae	7
		Ephydroidea[b]	Chloropidae	42
		Nematocera[a]	-	550
	Neuroptera	-	Chrysopidae	5
	Hymenoptera	Chalcidoidea[b]	-	392
		Ichneumonoidea[b]	-	17
		Symphyta[a]	-	5
	Lepidoptera	-	-	224
	Coleoptera	Cucujoidea[b]	Coccinellidae	2
Heptyl butanoate + acetic acid	Diptera	Brachycera[a]	-	29
		Syrphoidea[b]	Syrphidae	3
		Ephydroidea[b]	Chloropidae	469
		Nematocera[a]	-	11
	Homoptera	Cicadoidea[b]	Cicadellidae	1
		Psylloidea[b]	Psyllidae	1
	Hymenoptera	Chalcidoidea[b]	-	1
	Lepidoptera	-	-	1
	Coleoptera	Cucujoidea[b]	Coccinellidae	1

Appendix 2:

Total amount of arthropods trapped in synthetic compound blend treatments, at Klagshamn vineyard between August 22 and September 20, 2014

Treatment	Order	Suborder[a]/Superfamily[b]	Family	Number captured
2-Methylpropan-1-ol + acetic acid	Diptera	Brachycera[a]	-	434
		Syrphoidea[b]	Syrphidae	4
		Ephydroidea[b]	Chloropidae	4
		Nematocera[a]	-	77
		Thephritoidea	Thephritidae	1
	Neuroptera	-	Chrysopidae	9
	Hymenoptera	Ichneumonoidea [b]	-	1
	Lepidoptera	-	-	7
2-Methylbutan-1-ol + acetic acid	Diptera	Brachycera[a]	-	26
		Ephydroidea[b]	Drosophilidae	1
		Ephydroidea[b]	Chloropidae	19
		Nematocera[a]	-	9
	Homoptera	Cicadoidea[b]	Cicadellidae	2
	Hymenoptera	Chalcidoidea[b]	-	1
	Lepidoptera	-	-	6
3-Methylbutan-1-ol + acetic acid	Diptera	Brachycera[a]	-	93
		Syrphoidea[b]	Syrphidae	2
		Ephydroidea[b]	Chloropidae	17
		Nematocera[a]	-	21
	Homoptera	Cicadoidea[b]	Cicadellidae	1
	Lepidoptera	-	-	46
2-Methylpropan-2-ol + acetic acid	Diptera	Brachycera[a]	-	187
		Syrphoidea[b]	Syrphidae	1
		Ephydroidea[b]	Drosophilidae	2
		Ephydroidea[b]	Chloropidae	8
		Nematocera[a]	-	27
	Homoptera	Cicadoidea[b]	Cicadellidae	2
	Hymenoptera	Ichneumonoidea [b]	-	1
	Lepidoptera	-	-	3
Heptyl butanoate + acetic acid		Ephydroidea[b]	Chloropidae*	≈1200
		Nematocera[a]	-	1
	Lepidoptera	-	-	1

Tricimba cincta

Appendix 3:

Total amount of arthropods trapped in live yeast treatments at Alnarp apple orchard, 5-11 September 2014 in Alnarp

Treatment	Order	Suborder[a]/Superfamily[b]	Family	Number captured
M. andauensis	Diptera	Brachycera[a]	-	60
		Syrphoidea[b]	Syrphidae	3
		Ephydroidea[b]	Drosophilidae	8
		Ephydroidea[b]	Chloropidae	3
		Nematocera[a]	-	5
	Neuroptera	-	Chrysopidae	17
	Homoptera	Cicadoidea[b]	Cicadellidae	1
		Psylloidea[b]	Psyllidae	1
	Hymenoptera	Chalcidoidea[b]	-	3
		Proctotrupiodea [b]	-	1
		Ichneumonoidea [b]	-	1
	Lepidoptera	-	-	1
M. chrysoperlae	Diptera	Brachycera[a]	-	369
		Syrphoidea[b]	Syrphidae	6
		Ephydroidea[b]	Drosophilidae	3
		Ephydroidea[b]	Chloropidae	5
		Nematocera[a]	-	17
	Neuroptera	-	Chrysopidae	3
	Hymenoptera	Chalcidoidea[b]	-	38
		Proctotrupiodea[b]	-	1
		Apoidea[b]	Sphecidae	3
	Lepidoptera	-	-	7
M. pulcherrima	Diptera	Brachycera[a]	-	152
		Syrphoidea[b]	Syrphidae	4
		Ephydroidea[b]	Drosophilidae	8
		Ephydroidea[b]	Chloropidae	1
		Nematocera[a]	-	13
	Neuroptera	-	Chrysopidae	4
	Homoptera	-	-	1
	Hymenoptera	Ichneumonoidea[b]		1
		Apoidea[b]	Sphecidae	1
		Chalcidoidea[b]	-	15
	Lepidoptera	-	-	1
Saccharomyces sp.	Diptera	Brachycera[a]	-	207
		Syrphoidea[b]	Syrphidae	4
		Ephydroidea[b]	Drosophilidae	9
		Ephydroidea[b]	Chloropidae	2
		Nematocera[a]	-	15
	Neuroptera	-	Chrysopidae	5
	Homoptera	Psylloidea[b]	Psyllidae	2
	Hymenoptera	Chalcidoidea[b]	-	13
	Lepidoptera			1
Cryptococcus sp.	Diptera	Brachycera[a]	-	190
		Syrphoidea[b]	Syrphidae	3
		Ephydroidea[b]	Drosophilidae	3
		Ephydroidea[b]	Chloropidae	13
		Nematocera[a]	-	14
	Neuroptera	-	Chrysopidae	4
	Hymenoptera	Chalcidoidea[b]	-	15
	Lepidoptera	-	-	2