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ATTRACTION OF A FLYING NITIDULID (*Carpophilus humeralis*) TO VOLATILES PRODUCED BY YEASTS GROWN ON SWEET CORN AND A CORN-BASED MEDIUM

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Abstract—Attraction to microbial volatiles was examined for the sap beetle, *Carpophilus humeralis*, which is a pest of maize. Using 54 pure yeast and bacterial cultures, we evaluated differences in volatile emissions among species of microorganisms and whether these differences were associated with insect attraction. On a sterile corn-based medium, both yeasts and bacteria generally multiplied well and produced detectable volatile metabolites. The yeasts ranged from inactive to highly attractive, but no bacterial cultures attracted beetles above control levels. A variety of alcohols, esters, ketones, acids, and phenolic compounds were identified from the headspace above yeast cultures. Growth, volatile production, and, ultimately, attractiveness to beetles depend strongly on the ability of the yeasts to assimilate and/or ferment the carbohydrates present. Abundant volatile production on sweet corn was observed only with yeasts that are able to ferment sucrose and/or maltose. *Saccharomyces cerevisiae* (ferments glucose, sucrose, and maltose) and *Candida shehatae* (ferments glucose and maltose) produced considerably more attractive volatiles than *Candida guilliermondii*, which only ferments glucose. Yeast volatiles important for beetle attraction included typical fermentation-associated substances (ethanol, acetaldehyde, 2-methyl-1-propanol, 1-propanol, ethyl acetate, 3-methyl-1-butanol and 2-methyl-1-butanol), and also 3-hydroxy-2-butanone, whose presence was not correlated with the occurrence of fermentation. Using aqueous mixtures of synthetic components that pro-

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duced headspace compositions simulating those of attractive yeasts, it was shown that the typical fermentation volatiles are attractive but not essential for attractiveness. 3-Hydroxy-2-butanone is sufficient but not necessary, although its attractiveness is enhanced by the presence of fermentation volatiles such as ethanol and 2-methyl-1-propanol. In nature, the beetles could take advantage of a variety of different microbial metabolic processes to locate hosts. The laboratory bioassays in this study involved flight and therefore were particularly relevant to host-finding behavior in the field.

Key Words—Sap beetle, attraction, yeast, volatile, headspace, fermentation, glucose, maltose, sucrose, maize, sweet corn.

INTRODUCTION

Sap beetles (Coleoptera: Nitidulidae) are pests of a wide variety of crops and commodities and are known to be especially attracted to plant materials that are damaged, fermenting, or otherwise decomposing. One example of such attraction is the ability of sap beetles to locate maize (*Zea mays* L.) ears that have been wounded by lepidopterous larvae or by birds. *Glischrochilus quadrisignatus* (Say) and *G. fasciatus* (Olivier) are two nitidulids that respond to damaged corn kernels (Alm et al., 1985). Beetles of the genus *Carpophilus* are also attracted to wounded ears and are particularly important because they are capable of introducing mycotoxin-producing fungi at the wound sites (Lussenhop and Wicklow, 1990). A likely factor contributing to the initial attraction of these beetles is that yeasts present on the ears proliferate in the wounds and emit attractive volatiles.

Attractive microbial and fruit-derived volatiles have been identified and have even been used to bait field traps for sap beetles. Smilanick et al. (1978) used a mixture of ethanol, acetaldehyde, and ethyl acetate for *Carpophilus* spp. Alm et al. (1985, 1989) employed butyl acetate and other esters to attract *Glischrochilus* spp. Lin and Phelan (1991a, b, 1992) and Phelan and Lin (1991) analyzed the volatiles from a number of representative yeasts and fungi grown on natural and prepared substrates, and they studied their attractiveness to nitidulid beetles. Their work was especially germane to understanding natural systems because their synthetic volatile blends closely simulated attractive microbial volatiles both qualitatively and quantitatively. Their insect bioassays monitored activity primarily by observing walking behavior. Active compounds included ethanol, acetaldehyde, ethyl acetate, 2-methyl-1-propanol, and other alcohols, esters, and ketones. Having appropriate mixtures of compounds was crucial for maximum activity, but the interactions of component volatiles were complex, and it was not always necessary to include all natural components to achieve full attractiveness.

Attraction of insects by microbial volatiles was examined further in this

study, using the nitidulid *Carpophilus humeralis* (F), which is a pest of maize. A wide range of yeast species and strains found on maize (Nout et al., 1997) and other plants (Starmer et al., 1992) was studied for their production of volatiles. One objective was to evaluate, using pure cultures, any differences in volatile emissions among species of microorganisms and to determine whether these differences were associated with the degree of insect attraction. The results of Lin and Phelan (1991a) for the yeast *Saccharomyces cerevisiae* provided valuable guidance for recognizing volatile compounds of potential behavioral importance. For several yeasts, the performance on sterile simulation media was compared to that on fresh sweet corn over time. Growth, volatile production, and beetle attraction are discussed in relation to yeast physiology (ability to assimilate and/or ferment various carbohydrates). The laboratory bioassays in this study involved flight, and therefore were particularly relevant to host-finding behavior in the field. Solid-phase microextraction (SPME) was used to sample volatiles for GC and GC-MS analysis; this greatly simplified the analytical procedures and offered particularly good sensitivity for volatiles of higher molecular weight.

METHODS AND MATERIALS

Microbial Strains. Tables 1 and 3 below list all strains of yeasts and bacteria tested. Yeasts isolated from cacti, fruits, insects, and other natural habitats were supplied by W. T. Starmer, whereas some killer yeasts were provided by L. Polonelli and Wageningen Agricultural University, and flavor producing yeasts and bacteria by the National Center of Agricultural Utilization Research. Other strains of yeasts and bacteria were isolated by us from maize (Nout et al., 1997).

Yeasts were grown on yeast malt agar (YM, containing 3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g glucose, and 15 g agar per liter of distilled water) at 28°C, and maintained at 4°C. Bacteria were grown on Plate Count Agar (PCA, Difco 0479 containing 5 g tryptone, 2.5 g yeast extract, 1 g glucose, and 15 g agar per liter of distilled water) or on De Man, Rogosa, and Sharpe agar (MRS, containing 10 g peptone, 10 g meat extract, 5 g yeast extract, 20 g glucose, 1 g Tween 80, 2 g K₂HPO₄, 5 g Na acetate, 2 g (NH₄)₃ citrate, 0.2 g MgSO₄ · 7H₂O, 0.05 g MnSO₄ · 4H₂O, and 15 g agar per liter of distilled water) at 30°C, and maintained at 4°C.

Tests for Assimilation and Fermentation Abilities. Assimilation of glucose and maltose was assessed at 25°C after 7, 21, and 28 days in broth, and fermentation of glucose, sucrose, and maltose was assessed at 28°C after 1, 2, 4, 6, 8, 12, 16, 20, and 24 days in broth, according to Wickerham (1951) and Kreger-Van Rij (1984). Ability to ferment was assessed by measuring production of carbon dioxide.

Corn Medium. A mixture containing 1 g (NH₄)₂HPO₄, 0.2 g KCl, 0.2 g

MgSO₄ · 7H₂O, 0.12 g MnSO₄, 0.034 g FeSO₄, 0.2 g yeast extract, 20 g of the carbohydrate to be tested (glucose, sucrose, or maltose), 150 g sifted corn meal (Quaker), and 1 liter of tapwater was cooked in a microwave oven. The paste was stirred and dispensed into glass wide-neck screw-capped jars, and sterilized for 30 min at 121°C.

Sweet Corn Chunks. Freshly purchased cobs of sweet corn were sliced and quartered. Damaged kernels were removed. Until inoculation, the chunks were kept in sterile Petri dishes in a laminar flow hood under UV for 1 hr. The inoculation of a chunk of sweet corn was carried out by jabbing 10 kernels on each chunk with a straight inoculation needle carrying the designated microorganism. Previously, glass jars (2-oz size), which were covered with a layer of textile gauze kept in place by a copper wire, had been sterilized for 15 min at 121°C. Each inoculated chunk was transferred into a sterile jar, which was covered with aluminum foil to prevent excessive loss of volatiles. Prepared jars were placed in a high humidity box to avoid dehydration and were incubated at 28°C.

Monitoring of Growth of Yeasts and Bacteria. For sweet corn chunks, the entire chunk was weighed in a sterilized beaker of a Stein mill (Seedboro Equipment Co., Chicago, IL). A ninefold weight of sterile physiological salt solution (PPS, containing 1.0 g peptone and 8.5 g NaCl per liter of distilled water) was added and the mixture was homogenized (giving a 10-fold dilution of the sample). For corn medium, a representative subsample of about 10 g was weighed and treated similarly. Consecutive decimal dilutions were prepared using PPS as diluant.

Appropriate decimal dilutions were pour-plated in YM agar duplicate plates with tetracycline (Sigma T-3383, 37.5 mg/liter) for yeast counts. Bacteria were counted in duplicate pour-plates of PCA with nystatin (Sigma N-3503, 50 mg/l). Microbial colonies were counted in plates containing 20–250 colonies. Numbers of colony forming units (CFU, N/g = number of colony forming units per gram of sample) were calculated by multiplying average numbers of counted colonies by dilution factors used and were reported as $\log_{10} N/g$.

Insects. The *C. humeralis* culture was originally collected near Fresno, California. The beetles were reared on the artificial diet described by Dowd (1987), except that the pinto beans were replaced with additional brewers yeast. The species is prolific and easily reared.

Bioassays to Measure Insect Attraction. The wind tunnel used for bioassays was as described previously (Bartelt et al., 1990). For bioassays, about 500–1000 adult beetles (less than 1 week old) were removed from rearing cups and released into the wind tunnel. The inlet air was heated to $29 \pm 1^\circ\text{C}$. After 2–3 hr, the beetles became active and began to fly readily, so that about 100 were in the air at any instant. Test samples were suspended from a wire stretched between

the sides of the wind tunnel near the upwind end, 40 cm above the wind tunnel floor, and perpendicular to the air flow. Samples were normally tested in pairs, separated by 30 cm. Bioassays were of 1.5 or 2 min duration, with the time being constant within any experiment. Total counts of beetles alighting on the samples during the test period were recorded. The initial study that screened cultures for attractiveness was not analyzed statistically, but subsequent studies (with replicated treatments) were analyzed in the variance-stabilizing $\log(x + 1)$ scale as described previously (Bartelt et al., 1990).

The 30-ml vials containing the microbial cultures on prepared medium were covered with thin cloth to prevent beetle entry; this was held in place by a rubber band. The cloth was white in color to provide contrast for counting beetles. A wire hook, slid under the rubber band, allowed the bottles to be suspended in the wind tunnel. The relatively bulky sweet corn samples (about 10 g) were held in larger bottles (70 ml). Between tests the bottles were covered with aluminum foil to reduce diffusion of volatiles.

Collection of Headspace Volatiles, Chromatography, and Mass Spectrometry. Solid-phase microextraction (SPME) was used for collection of headspace samples. The fiber type was 100- μ m polydimethylsiloxane (Supelco, Bellefonte, Pennsylvania). The fiber-needle assembly was inserted through the cotton plug (or through the aluminum foil cover) into the vial to be sampled. The fiber was extended and left in place for about 30 min. Sample desorption in the injector of the gas chromatograph (GC) was for 0.5 min, and the fiber was conditioned for an additional 2 min at 200°C in another GC injector before collecting the next sample.

Sample analysis was by GC on a Hewlett-Packard 5890 Series II instrument, equipped with cool on-column injector, flame ionization detector, electronic pressure control, and Hewlett-Packard 3396A integrator. The column was a 30-m DB-1 capillary (J&W Scientific, Folsom, California), with an inside diameter of 0.32 mm and a film thickness of 5 μ m. Carrier gas was helium and injector head pressure was 16.5 psig. Oven temperature program began with a 1-min hold at 50°C, increased at 10°C/min to 250°C, and ended with a final 6-min hold at 250°C. Injector temperature was 200°C, and detector temperature was 250°C.

A Hewlett-Packard 5970 MSD was used to obtain mass spectra. Scanning range was normally 40–300 amu, but additional runs under different conditions were sometimes beneficial for compound identification (e.g., starting mass of 20 amu). Parameters for the GC inlet were as above except that the injector was of the split/splitless type, operated in splitless mode, and the head pressure was 6 psig. Again, samples were introduced by SPME.

Tentative compound identifications were based on searches of the mass spectral library. Most identifications were confirmed by demonstrating that

authentic standards had the same GC retentions and mass spectra as the unknown compounds. Quantitation of headspace concentration by SPME and GC was as described by Bartelt (1997).

SPME offers significant advantages over the thermal desorption procedure described by Phelan and Lin (1991). First, it is not necessary to handle the volatile sample with a gas-tight syringe, so that there is no chance of polar or less volatile components being lost to adsorption onto syringe surfaces. Second, a single sampling scheme is appropriate for analyzing all volatiles, regardless of quantity or volatility, and the very volatile and abundant compounds such as ethanol and acetaldehyde do not have to be sampled in a special way (Phelan and Lin, 1991). Third, SPME analyses are extremely simple to conduct and require no special sampling valves or injector hardware on the GC. Fourth, the sensitivity of SPME is sufficient to detect the concentrations and compounds reported by Lin and Phelan (1991a), and SPME has superior sensitivity for compounds of still higher molecular weight (by as much as three or four orders of magnitude) (Bartelt, 1997).

The measured headspace concentrations represent a balance between diffusion through the cotton plug (or around the foil covering) and volatile production by the microbial culture. While we use the values for comparing cultures, they do not represent an absolute rate of chemical emission by the cultures.

Screening, Attractiveness, and Volatile Production Tests. An initial screening of pure cultures of yeasts and bacteria for their attractiveness towards *C. humeralis* was performed. Pure cultures were inoculated on sterile corn medium with glucose (CM-G), incubated for two days at 28°C and tested for attractiveness by wind-tunnel bioassay. Subsequently, the volatiles produced by selected attractive as well as nonattractive cultures on CM-G were compared after a two-day incubation at 28°C.

Relations between the ability to assimilate or ferment glucose and maltose and the production of volatiles were tested using CM-G and CM-M (maltose) after two days of incubation at 28°C.

Using a representative attractive yeast (*C. guilliermondii* Y17861), growth and volatile production were compared when inoculated on CM-G and sweet corn.

Test of Hypothesis Relating Carbohydrate Fermentation, Production of Volatiles, and Attraction of Beetles. To test the hypothesis that the ability of yeasts to ferment the predominant sugars in sweet corn will result in significant headspace volatiles and attraction of *C. humeralis*, we inoculated chunks of sweet corn with yeast cultures able to ferment glucose, sucrose, and maltose (*Sacch. cerevisiae* Y17898); glucose and maltose (*C. shehatae* Y17880); and glucose only (*C. guilliermondii* Y17861). Volatile production, attractiveness to beetles, and the number of colony-forming units present were measured for the corn chunks. All three measurements were made on the same, specific sweet

corn chunks (rather than on replicates) so that variation among chunks would not affect correlations among these measurements. This was done in two consecutive experiments: in experiment 1, Y17861 was grown on sweet corn and compared with uninoculated sweet corn. Volatiles were analyzed every day (0–7); bioassays and cell counts were conducted on days 0, 2, 4, and 6. For the bioassays, Y17861 grown on CM-G at peak fermentation level served as sample with maximum attraction (a high control). In experiment 2, Y17861, Y17898, and Y17880 were grown on sweet corn and compared with uninoculated sweet corn. Volatile analyses, bioassays, and cell counts were conducted on each of days 0, 1, 2, 3, and 4. For these bioassays, baker's yeast on bread dough served as the high control sample with which to verify beetle responsiveness.

Test of Synthetic Mixtures. The attractiveness of a few mixtures of volatile synthetic compounds was assessed. Mixtures of synthetic volatiles were prepared by dissolving small amounts in water. Headspace concentrations were measured by GC-SPME and adjustments were made to solutions until the desired headspace concentrations were achieved. Ten milliliters of the solutions in 70-ml wide-mouth bottles were covered with cloth and bioassayed in the wind tunnel in the same way as the chunks of sweet corn. The mixtures were chosen to simulate two natural samples that were attractive in the wind tunnel (Y17861 and Y17898, both on sweet corn on day 3). One sample was composed primarily of typical fermentation volatiles, and the other contained a compound (3-hydroxy-2-butanone) that was not correlated to fermentation.

RESULTS AND DISCUSSION

An initial screening of microbial cultures grown on corn medium with glucose (CM-G) for two days at 28°C for their attractiveness to *C. humeralis* (Table 1) revealed that the uninoculated corn medium remained sterile ($\log_{10}N/g < 1.7$) and had a very low attraction level. There was a wide range of attractiveness among microbial cultures, ranging from none to very attractive; none of the bacteria were attractive, but they grew well and also developed a distinct odor (as noted by human olfaction; no objective data available). All yeasts grew well in CM-G as confirmed visually and by smelling for typical yeastly volatiles. Typically, yeast cultures achieved $\log_{10}N/g$ ranging from 6.8 to 7.2 (data not shown). We considered a culture attractive if it showed >5% of the attractiveness of the reference strain Y17861. All of the attractive yeasts are able to ferment glucose. On the other hand, 17 of the 25 nonattractive strains are not able to ferment glucose. These findings indicate that CM-G is a suitable medium for studies of beetle attraction because its baseline attractiveness is very low. The data also suggest that the unattractiveness of some yeasts is not caused by an inability to grow on CM-G, but by their inability to ferment glucose.

TABLE 1. FERMENTATIVE PROPERTIES OF YEASTS AND BACTERIA, LISTED IN DECREASING ORDER OF ATTRACTIVENESS TO *C. humeralis* WHEN GROWN ON CORN MEDIUM WITH GLUCOSE (CM-G) AT 28°C FOR 2 DAYS

Strain	Supplied as		Corresponding collection No.	Ferment ^d				Wind tunnel ^e		
	Collection and No. ^a	Origin ^b		G	S	M		Attr. 1	Attr. 2	Attr. 3
<i>Pichia kluyveri</i> var. <i>kluyveri</i>	WTS; 88-330.1	F	NRRL Y17716	+ ^f	-	-				124 ^g (185%) ^h
<i>Kluyveromyces lactis</i> var. <i>drasophilatum</i>	WTS; 85-272.1	T	NRRL Y17756	+	ND	ND				120 (179%)
<i>Williopsis californica</i>	WTS; 85-756.1	C	NRRL Y17720	+	ND	ND				108 (161%)
<i>Pichia pastoris</i>	WTS; 85-263.1	NS	NRRL Y17741	+	-	-				103 (154%)
<i>Saccharomyces cerevisiae</i>	WAU; LU340	NS	NRRL Y17898	+	+	+		125 (145%)		
<i>Candida shehatae</i> A	NPW; NRRL Y17869	M	LU024	+	-	-		123 (143%)		
<i>Pichia kluyveri</i> var. <i>cephalocereana</i>	WTS; 82-555B-13	NS	NRRL Y17739	±	-	-				92 (137%)
<i>Debaryomyces hansenii</i>	NPW; NRRL Y17873	M	LU182	+	-	-		107 (124%)		
<i>Candida zeylanoides</i> B	NPW; NRRL Y17863	M	LU027	+	-	-		99 (115%)		
<i>Candida guilliermondii</i> C	NPW; NRRL Y17881	M	LU031	+	-	-		96 (112%)		
<i>Pichia anomala</i>	LP; K3 UM(2)	NS	NRRL Y17901	ND	ND	ND			143 (104%)	
<i>Candida guilliermondii</i> A	NPW; NRRL Y17861	M	LU021	+	-	-		86 (100%)		67 (100%)
<i>Pichia kluyveri</i> var. <i>cephalocereana</i>	WTS; 82-556D-30	C	NRRL Y17738	±	-	-				63 (94%)
<i>Kluyveromyces lactis</i> var. <i>lactis</i>	WAU; LU270	NS	NRRL Y17889	ND	ND	ND			113 (82%)	

<i>Saccharomycopsis fibuligera</i>	NCAUR; NRRL Y2388 WTS; 85-256.2	FF		ND	ND	ND	69 (80%)	
<i>Pichia anomala</i>		T	NRRL Y1777	+	ND	ND		108 (79%)
<i>Williopsis mucosca</i>	NCAUR; NRRL YB1344 WTS; 86-927.3	NS		ND	ND	ND	58 (67%)	
<i>Pichia kluyveri</i> var. <i>kluyveri</i>	LP; K22 UCSC	C	NRRL Y17750	+	-	-		37 (55%)
<i>Candida guilliermondii</i>	WTS; 83-379.2	NS	NRRL Y17887	+	-	-	43 (50%)	
<i>Pichia fermentans</i>	WTS; 88-387.2	F	NRRL Y17755	+	-	-		23 (34%)
<i>Pichia fermentans</i>		F	NRRL Y17717	+	-	-		20 (30%)
<i>Williopsis saturnus</i> var. <i>mrakii</i>	NCAUR; NRRL Y1364 NPW; NRRL Y17886	NS	ATCC 10743	ND	ND	ND	19 (22%)	
<i>Candida zeylanoides</i> E		M	LU029	±	-	-	5.5 (6%)	
<i>Candida zeylanoides</i> B	NPW; NRRL Y17878	M		±	-	-	3.5 (4%)	
<i>Pichia kluyveri</i>	WTS; 87-498.2	NS	NRRL Y17743	+	-	-		2 (3%)
<i>Pichia thermotolerans</i>	WTS; 83-510.1	IN	NRRL Y17745	-	-	-		2 (3%)
<i>Pichia membranifaciens</i>	WTS; 85-753.4	C	NRRL Y17736	-	-	-		1.5 (2.2%)
<i>Saccharomycopsis crataegensis</i>	NCAUR; NRRL Y5902	FF		ND	ND	ND	1.5 (1.7%)	
<i>Bacillus natto</i>	NCAUR; NRRL B14206 WTS; 84-860.2	FF		±	-	-	1.5 (1.7%)	
<i>Pichia opuntiae</i> var. <i>opuntiae</i> "hem"	WTS; 85-201.2	C	NRRL Y17721	-	-	-		1 (1.5%)
<i>Pichia cactophila</i>		C	NRRL Y17722	-	-	-		1 (1.5%)

TABLE 1. Continued

Strain	Supplied as		Corresponding collection No.	Ferment ^d			Wind tunnel ^e		
	Collection and No. ^a	Origin ^b		G	S	M	Attr. 1	Attr. 2	Attr. 3
<i>Pichia kluyveri</i> var. <i>kluyveri</i>	WTS; 84-670.2B	C	NRRL Y17734	+	—	—			1 (1.5%)
<i>Pichia antillensis</i>	WTS; 82-550A	C	NRRL Y17719	—	—	—			1 (1.5%)
<i>Pichia antillensis</i>	WTS; 82-604B	NS	NRRL Y17740	—	—	—			1 (1.5%)
<i>Pichia thermotolerans</i>	WTS; 83-505.1	IN	NRRL Y17744	—	—	—			1 (1.5%)
<i>Pichia opuntiae</i> var. <i>opuntiae</i>	WTS; 84-677.2	C	NRRL Y17746	—	—	—			1 (1.5%)
<i>Pichia opuntiae</i> var. "hem"	WTS; 84-853.3	C	NRRL Y17748	—	—	—		2 (1.5%)	
<i>Trichosporon cutaneum</i>	NPW; NRRL Y17874	M	LU696	—	—	—		1 (0.7%)	
<i>Pichia kluyveri</i>	WTS; 88-370.2F	F	NRRL Y17715	+	—	—			0.5 (0.7%)
<i>Pichia kluyveri</i> var. <i>eremophila</i>	WTS; 83-466.1B	C	NRRL Y17735	±	—	—			0.5 (0.7%)
<i>Pichia opuntiae</i> var. <i>opuntiae</i>	WTS; 84-821.2	C	NRRL Y17737	—	—	—			0.5 (0.7%)
<i>Pichia anethionina</i> var. <i>pachycereana</i>	WTS; 80-314.1	IN	NRRL Y17779	—	—	—			0.5 (0.7%)
<i>Trichosporon cutaneum</i>	NPW; NRRL Y17868	M	LU697	—	—	—	0.5 (0.6%)		
<i>Trichosporon cutaneum</i>	NPW; NRRL Y17875	M		—	—	—	0.5 (0.6%)		
<i>Pichia kluyveri</i> var. <i>eremophila</i>	WTS; 86-541.5A	C	NRRL Y17751	±	—	—	0.5 (0.6%)		

<i>Bacillus subtilis</i>	NPW; NRRL B23190	M	±	—	—	0.5 (0.4%)
<i>Bacillus subtilis</i>	NPW; NRRL B23189	M	±	—	—	0.2 (0.15%)
<i>Pichia kluyveri</i> var. <i>cephalocerana</i>	WTS; 82-555B- 17	C	±	—	—	0 (0%)
<i>Pichia opuntiae</i> var. "hem"	WTS; 84-833.1	C	—	—	—	0 (0%)
<i>Pichia fermentans</i>	WTS; 88-379.2	F	+	—	—	0 (0%)
<i>Lactobacillus</i> <i>malvaromicus</i>	NCAUR; NRRL B14852	NS	+	+	+	0 (0%)
<i>Bacillus natto</i>	NCAUR; NRRL B14204	FF	±	—	—	0 (0%)
<i>Candida mucilagina</i>	WTS; 84-201.7	C	—	—	—	0 (0%)
<i>Cryptococcus cereanus</i>	WTS; 83- 1112.2	C	—	—	—	0 (0%)
Control CM-G						0 (0%) 0.13 (0.1%) 0.4 (0.6%)

^aLP = L. Polonelli, NCAUR = National Center for Agricultural Utilization Research, NPW = Nout et al. (1997), WAU = Wageningen Agricultural University, WTS = W. T. Stamer.

^bC = cacti, F = fruits, FF = fermented food, IN = insects, M = maize, NS = not specified, T = trees.

^cATCC = American Type Culture Collection, DSM = Deutsche Sammlung fuer Mikroorganismen, LU = Department of Food Technology and Nutritional Sciences, Wageningen Agricultural University, NRRL = collection at NCAUR.

^dAbility to ferment glucose (G), sucrose (S), maltose (M).

^eThree independent single wind-tunnel bioassays.

^f+, able; ±, weakly able; —, unable; ND, not determined.

^gAverage number of landings per 1.5 min.

^hRelative to *C. guilliermondii* NRRL Y17861.

Headspace volatiles of representative attractive and nonattractive cultures (grown on CM-G at 28°C for two days) were separated and identified by GC and MS. Table 2 shows that the less-attractive yeasts produced considerably less volatile matter. Specific volatiles that occurred only in attractive samples

TABLE 2. HEADSPACE VOLATILES FORMED ON CM-G AT 28°C^a

Identity	Volatile (ng/ml gas)					
	<i>P.</i> <i>kluyveri</i> Y17716	<i>C.</i> <i>shehatae</i> Y17869	<i>D.</i> <i>hansenii</i> Y17873	<i>C.</i> <i>zeyla-</i> <i>noides</i> B Y17863	<i>C.</i> <i>guillier-</i> <i>mondii</i> C Y17881	<i>C.</i> <i>guillier-</i> <i>mondii</i> A Y17861
Relative attractivity index (<i>C.</i> <i>guilliermondii</i> A Y17861 = 100%)	185	143	124	115	112	100
Acetaldehyde	6.2	5.2	3.9	0.55	1.2	11.5
Ethanol	790	120	200	120	210	790
Formic acid	16	4.7	1.7	0	1.9	14
1-Propanol	0	0	0	0	0	2.0
Acetic acid	3.2	0	0	3.3	0	0
Ethyl acetate	1000	0	2.2	0	0	1.1
2-Methyl-1-Propanol	7.9	2.9	7.1	16	8.9	13
3-Hydroxy-2-Butanone	0	0.54	0.72	2.4	0	0.41
Ethyl propanoate	2.4	0.19	0	0	0	0.15
Propyl Acetate	0	0	0	0	0	0
3-Methyl-1-Butanol	4.7	6.0	6.7	42	5.7	6.9
2-Methyl-1-Butanol	1.9	1.5	2.3	6.4	3.1	3.1
2,3-Butanediol (chiral)	0	0	0.079	0	0.05	0.05
2-Methylpropyl acetate	2.6	0	0	0	0	0
2,3-Butanediol (<i>meso</i>)	0	0.066	0.015	0	0	0.04
Butyl acetate	0.20	0	0	0	0	0
3-Methylbutanoic acid	0.025	0.018	0.060	0.034	0.033	0
2-Methylbutanoic acid	0	0.036	0.062	0.11	0.074	0.04
3-Methylbutyl acetate	110	0	0.093	0	0	0
Pentyl acetate	0.25	0	0	0	0	0
2-Octanone	0.055	0	0	0	0	0
Ethyl hexanoate	0.0090	0	0	0	0	0
Hexyl acetate	0.51	0	0	0	0	0
Heptyl acetate	0.074	0	0	0	0	0
2-Phenylethanol	0.0077	0.040	0.054	0.094	0.055	0.037
4-Ethylphenol	0.0017	0	0	0	0.022	0.020
Benzyl acetate	0	0	0	0	0	0
Ethyl octanoate	0.011	0	0	0	0	0
2-Phenylethyl acetate	1.62	0	0	0.0034	0	0
4-Ethyl-2-methoxyphenol	0	0	0	0	0.032	0.035
2-Phenylethyl propanoate	0	0	0	0	0	0
Ethyl decanoate	0	0	0	0	0	0

^aHeadspace concentration of 0 indicates that the analyte was not detected.

were alcohols [ethanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 2,3-butanediol (chiral and *meso*)], ketones (3-hydroxy-2-butanone), esters (ethyl acetate, ethyl propanoate), and phenolic derivatives (2-phenylethanol, 4-ethylphenol, 2-phenylethyl acetate, 4-ethyl-2-methoxyphenol). Some

TABLE 2. Continued

Volatile (ng/ml gas) Continued							
<i>P.</i> <i>kluyveri</i> <i>kluyveri</i> Y17750	<i>C.</i> <i>zeyla-</i> <i>noides</i> E Y17886	<i>C.</i> <i>zeyla-</i> <i>noides</i> B Y17878	<i>T.</i> <i>cutaneum</i> Y17874	<i>T.</i> <i>cutaneum</i> Y17875	<i>T.</i> <i>cutaneum</i> Y17868	<i>P.</i> <i>kluyveri</i> <i>cephalo-</i> <i>cereana</i> Y17718	CM-G uninocu- lated control
55	6	4	0.7	0.6	0.6	0	
1.8	9.4	0	1.7	0.48	1.1	0	0
420	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
4.8	0	0	0	0	2.2	0	0
340	0	0	0	0	0	0	0
1.4	4.2	0.90	0	0	0	0	0
0	8.5	0.88	0	0	0	0	0
0	0	0	0	0	0	0	0
2.1	0	0	0	0	0	0	0
0.17	32	13	0	0	0	0	0
0.53	4.2	2.0	0	0	0	0	0
0	0.16	0	0	0	0	0	0
5.7	0	0	0	0	0	0	0
0	0.70	0.027	0	0	0	0	0
0.49	0	0	0	0	0	0	0
0	2.8	2.9	0	0	0.11	0	0
0	0.50	0.44	0	0	0	0	0
44	0	0	0	0	0	0	0
0.32	0	0	0	0	0	0	0
0.041	0	0	0	0	0	0	0
0.0072	0	0	0	0	0	0	0
0.45	0	0	0	0	0	0	0
0.056		0	0	0	0	0	0
0.0054	0.13	0.081	0	0	0	0	0
0	0	0	0	0	0	0	0
0.015	0	0	0	0	0	0	0
0.0068	0	0	0	0	0	0	0
0.44	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0.0012	0	0	0	0	0	0	0
0.0032	0	0	0	0	0	0	0

volatiles such as acetaldehyde and acetic acid, as well as minute amounts of other, unidentified compounds occurred throughout, including less attractive samples. This would suggest that if a culture is not able to ferment a carbon source, low levels of volatile production result. It appears that such nonfermenting cultures would only produce some volatiles such as acetaldehyde and acetic acid. On the other hand, fermentation yields much larger quantities of volatiles, especially alcohols (predominantly ethanol), ketones, esters, and phenolic derivatives and also higher amounts of acetaldehyde. The phenolic compounds presumably originate from metabolism of aromatic amino acids.

The effect of carbohydrate assimilation and fermentation on volatile production by selected yeasts was measured using CM-G or CM-M (maltose) (incubated at 28°C, for two to four days) and analysis by GC and MS (Table 3).

TABLE 3. MAJOR HEADSPACE VOLATILES FORMED ON CM-G AND CM-M AT 28°C
(MAXIMUM VALUES ACHIEVED AT 2-4 DAYS)^a

Identity	<i>Candida shehatae</i> B NPW; NRRL Y17880; M		<i>D. hansenii</i> Y17873		<i>C. guilliermondii</i> Y17861	
	G	M	G	M	G	M
Assimilation	+	+	+	+	+	+
Fermentation	+	+	+	—	+	—
Acetaldehyde	2.9	5.9	6.8	0 ^b	4.5	0
Ethanol	365	214	410	0	410	0
Formic acid	1.5	0	4.9	0	5.3	0
1-Propanol	11.6	0	1.5	0	2.1	0
Acetic acid	24.4	49.4	3.7	5.8	3.5	6.5
Ethyl acetate	0	0	0.9	0	0	0
2-Methyl-1-Propanol	3.2	1.3	4.9	0.3	8.4	0.2
3-Hydroxy-2-Butanone	6.7	3.2	0.6	0	0.7	0
Ethyl propanoate	0	0	0	0	0	0
3-Methyl-1-Butanol	3.2	2.4	1.7	0.3	2.9	0.5
2-Methyl-1-Butanol	1.4	0.8	0.7	0.3	1.2	1.0
2-Methylpropyl acetate	0	0	0	0	0	0
2,3-Butanediol (meso)	0.5	0.2	0	0	0	0
2-Phenylethanol	0.02	0.01	0.02	0.01	0.01	0.008
4-Ethylphenol	0.009	0.009	0	0.007	0.01	0.02
2-Phenylethyl acetate	0	0.008	0	0	0	0
4-Ethyl-2-methoxyphenol	0	0	0	0	0.007	0.03

^aFor abbreviations see Table 1. G, corn medium with glucose; M, corn medium with maltose. Assimilation, ability to assimilate; Fermentation, ability to ferment +, able; ±, weakly able; — unable.

^bHeadspace concentration of 0 indicates that the analyte was not detected.

The data indicate that the production of volatiles is determined by two factors: (1) the ability to assimilate a carbon source (compare Y17873 and Y17861 with Y17871 on CM-M) is responsible for some (trace) volatiles such as acetic acid, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, and 2-phenyl-ethanol; and (2) the ability to ferment a carbon source (compare Y17880 with Y17873 and Y17861 on CM-M; compare Y17880, Y17873, Y17861, Y17886 and Y17871 with Y17876 and Y17874 on CM-G) is responsible for abundant amounts of ethanol and acetaldehyde and increased amounts of the volatiles associated with assimilation, such as 2-methyl-1-propanol, 3-methyl-1-butanol, and 2-methyl-1-butanol. In addition, 3-hydroxy-2-butanone, *meso*-2,3-butanediol, and several esters occurred sporadically in the cultures displaying fermentation.

TABLE 3. Continued

<i>C. zeylanoides</i> Y17886		<i>Pichia</i> <i>membranifaciens</i> NPW; NRRL Y17871; M		<i>Cryptococcus</i> <i>albidus</i> var. <i>aerius</i> NPW; NRRL Y17876; M		<i>T. cutaneum</i> Y17874	
G	M	G	M	G	M	G	M
+	±	+	—	+	+	+	+
±	—	±	—	—	—	—	—
5.5	0	0.9	0	0	0	0	0
569	0	269	0	1.8	2.7	0	0
0.5	0	2.0	0	0	0	0	0
3.2	0	0	0	0	0	0	0
5.1	3.7	6.7	0	0	3.3	0	0
0	0	1528	0	0	0	0	0
6.1	0	6.0	0	0	0	0	0
1.5	0	0	0	0	0	0	0
0	0	6.6	0	0	0	0	0
8.5	0.3	12.7	0.1	0	0	0	0
2.1	0.2	2.2	0.3	0	0	0	0
0	0	0.3	0	0	0	0	0
0	0	1.6	0	0	0	0	0
0.02	0.005	3.7	0	0	0	0	0
0	0.01	0.2	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0.01	0	0	0	0	0	0

To translate results obtained with the CM-medium to a more practical situation, the performance of *Candida guilliermondii* Y17861 on sweet corn and CM-G was tested. Table 4 summarizes volatile production (as indicated by ethanol) and growth, as a function of incubation time. The data indicate that ethanol production on CM-G was much stronger than on sweet corn; the fermentation on CM-G lasted longer as well (up to eight days, data not shown). No ethanol formation was observed on sterile uninoculated CM-G. From the uninoculated (nonsterile) sweet corn, a small amount of ethanol was detected, especially shortly after wounding. It is not known whether this is from corn itself or from other microbes present. In any case, the volatile production from *C. guilliermondii* Y17861 on sweet corn was relatively poor, and this supports a conclusion that even though it grows well on corn, this yeast can not ferment corn sugars (primarily maltose and sucrose) very well.

The studies with sweet corn were expanded to include yeasts that can ferment corn sugars and also to monitor attractiveness to *C. humeralis*. *Sacch. cerevisiae* Y17898 (which ferments glucose, maltose, and sucrose), *C. shehatae* Y17880 (which ferments glucose and maltose but not sucrose), and *C. guilliermondii* Y17861 (which ferments only glucose) all grew comparably well on sweet corn (Table 5). However, the ability to ferment the corn sugars dramat-

TABLE 4. CONCENTRATION OF ETHANOL (ng/ml OF HEADSPACE $\pm$ SD) ABOVE *C. guilliermondii* A (Y17861) CULTURES AND UNINOCULATED SUBSTRATES^a

Volatile source	Days after inoculation				
	0	1	2	3	4
CM-G with Y17861					
Ethanol (ng/ml headspace)	0.5 $\pm$ 0.7	1.0 $\pm$ 1.4	760 $\pm$ 890	1130 $\pm$ 40	740 $\pm$ 360
Biomass (Log ₁₀ N/g)	3.0	4.68	8.86	8.3	8.89
CM-G sterile, uninoculated					
Ethanol (ng/ml headspace)	ND	ND	ND	ND	ND
Sweet corn with Y 17861					
Ethanol (ng/ml headspace)	5.3 $\pm$ 4.0	7.1 $\pm$ 6.4	53 $\pm$ 50	10 $\pm$ 11	7.0 $\pm$ 9.1
Biomass (Log ₁₀ N/g)	6.47		7.7		8.48
Sweet corn non-sterile, uninoculated					
Ethanol (ng/ml headspace)	6.2 $\pm$ 4.0	7.2 $\pm$ 8.9	2.3 $\pm$ 3.2	2.5 $\pm$ 4.2	0.9 $\pm$ 1.1

^aEthanol concentration is reported here as an indicator for amount of fermentation. There were two replications for CM-G (corn medium with glucose) and four for sweet corn. ND indicates that ethanol was not detected.

TABLE 5. NUMBERS OF YEAST AND BACTERIA COLONY FORMING UNITS PRESENT ON SWEET CORN INOCULATED WITH THREE YEAST STRAINS AT VARIOUS TIMES AFTER INOCULATION

Inoculum	Exp ^a	Days after inoculation					
		0	1	2	3	4	6
Yeasts (Log ₁₀ N/g)							
Uninoculated	1	1.74	ND ^b	5.76	ND	6.48	4.95
sweet corn	2	ND	5.26	5.26	6.85	6.70	ND
<i>C. guilliermondii</i> A	1	6.47	ND	7.70	ND	8.48	8.51
Y17861	2	5.43	8.13	8.26	8.68	8.38	ND
<i>S. cerevisiae</i> Y17898	2	4.13	8.02	8.09	8.25	8.22	ND
<i>C. shehatae</i> B	2	4.73	8.27	8.49	8.44	8.66	ND
Y17880							
Bacteria (Log ₁₀ N/g)							
Uninoculated	1	3.40	ND	7.53	ND	8.28	7.76
sweet corn	2	4.02	8.89	8.93	9.20	9.06	ND
<i>C. guilliermondii</i> A	1	3.93	ND	8.72	ND	8.71	7.82
Y17861	2	4.31	9.06	8.35	8.93	9.15	ND
<i>S. cerevisiae</i>	2	4.67	8.58	8.61	7.91	9.05	ND
Y17898							
<i>C. shehatae</i> B	2	4.51	7.13	7.99	9.59	9.03	ND
Y17880							

^aIn experiment 1, only Y17861 and uninoculated corn were analyzed. Experiment 1 corresponds to Figure 1, and experiment 2 corresponds to Figure 2.

^bND = not determined.

ically affected volatile production (Figures 1 and 2, Table 6) and attraction of *C. humeralis* (Figures 1 and 2).

The seven compounds reported from the headspace above yeast cultures by Lin and Phelan (1991a) (acetaldehyde, ethanol, 1-propanol, ethyl acetate, 2-methyl-1-propanol, 3-methyl-1-butanol, and 2-methyl-1-butanol) were detected above most of the attractive yeast cultures (Table 2), and for a given yeast strain and culture substrate, ratios of headspace concentrations among these compounds tended to be stable. Strains Y17861, Y17898, and Y17880 all had similar relative profiles (if greatly different absolute amounts) for these compounds when grown on sweet corn (Table 6). The correlations among the concentrations were used to simplify the graphs (Figures 1 and 2): only the most abundant of these seven volatiles, ethanol, was included in the graphs, but it served as an approximate indicator for trends in the other six. It is recognized that mixtures of the volatiles are important for insect attraction (Lin and Phelan, 1991a). However, the importance of individual components among the seven was not assessed for

TABLE 6. RELATIVE ABUNDANCE OF EIGHT VOLATILES FROM SWEET CORN INOCULATED WITH YEASTS AND CORRELATIONS AMONG CONCENTRATIONS OF THESE VOLATILES^a

Compound	Relative abundance ^b							SD
Ethanol (EtOH)	100							
Acetaldehyde (AcALD)	19							26
2-Methyl-1-propanol (2MePrOH)	2.5							3.1
1-Propanol (PrOH)	0.07							0.1
Ethyl acetate (EtAC)	0.05							0.1
3-Methyl-1-butanol (3MeBuOH)	0.47							1.0
2-Methyl-1-butanol (2MeBuOH)	0.31							0.5
3-Hydroxy-2-butanone (OHBuKET)	5.2							15
Correlations: ^c								
	EtOH	AcALD	2MePrOH	PrOH	EtAC	3MeBuOH	2MeBuOH	
AcALD	0.93***							
2MePrOH	0.83***	0.75***						
PrOH	0.90***	0.85***	0.80***					
EtAC	0.72***	0.80***	0.70***	0.82***				
3MeBuOH	0.50**	0.42*	0.71***	0.49**	0.58**			
2MeBuOH	0.76***	0.67***	0.95***	0.75***	0.71***	0.86***		
OHBuKET	-0.23	-0.30	-0.06	-0.18	-0.08	0.04	-0.03	

^aData combined for yeast strains *C. guilliermondii* A Y17861, *S. cerevisiae* Y17898, and *C. shehatae* B Y17880.

^bRelative abundance expressed as percentage of headspace concentration of ethanol (26 observations, for which total concentration of the eight compounds exceeded 10 ng/ml). Standard deviation (SD) is in the same scale.

^cBased on 26 observations for which total headspace concentration > 10 ng/ml. Significance of correlation coefficients at the 0.05, 0.01, and 0.001 levels denoted by *, **, and ***, respectively.

C. humeralis. Rather, this seven-compound blend was considered primarily as a single unit when evaluating attractiveness.

On the other hand, 3-hydroxy-2-butanone, which was detected from many of the sweet corn samples, was not correlated with ethanol or the other fermentation-related volatiles (Table 6). Therefore, 3-hydroxy-2-butanone is graphed separately in Figure 2. (The compound was not detected in the experiment shown in Figure 1).

The results of the first experiment (Table 5, Figure 1) indicate that the growth of Y17861 on sweet corn was good and clearly different from the uninoculated corn. Values in Table 5 are logarithms; numbers of yeasts on uninoculated corn were only about 1% of those on inoculated corn. The bioassay beetles were responsive: there were, overall, 35 landings per test on the attractive, high control, but for Y17861 (which cannot ferment the major corn sugars)

FIG. 1. Insect attraction and ethanol production for pieces of sweet corn inoculated with YM17861 and nonsterile, uninoculated wounded sweet corn. Within each day, bioassay values (landings) accompanied by the same letter were not significantly different (LSD, 0.05, $N = 7$). Means ($N = 7$) for samples with maximum attraction (high controls) on days 0, 2, 4, and 6 were 38.2, 25.2, 56.5, and 26.1, respectively.

and uninoculated corn, landings and ethanol production were low (essentially background), and there was no difference between Y17861 and uninoculated corn.

The results of the second experiment (Table 5, Figure 2) indicate that all yeasts grew equally well compared with the uninoculated corn; in addition, there was a consistent and equal background of bacterial growth in all treatments including the uninoculated corn. The attractiveness to *C. humeralis* was suitably low in the uninoculated corn, indicating (and supporting our earlier findings) that bacteria do not play a significant role in attraction of this particular insect.

Yeasts that can ferment sucrose and/or maltose (*S. cerevisiae* Y17898 and *C. shehatae* Y17880) were significantly more attractive, and this supports the hypothesis that it is the ability to ferment the naturally occurring C source that favors the attractiveness of a particular yeast. Although Y17861 was a little more attractive than previously, it was still not as active as the yeasts that can ferment corn sugars. As a result of using a different batch of corn, production

FIG. 2. Insect attraction and production of ethanol and 3-hydroxy-2-butanone from sweet corn pieces inoculated with three yeast strains, and nonsterile, uninoculated wounded sweet corn. Within each day, bioassay values (landings) accompanied by the same letter were not significantly different (LSD, 0.05, $N = 4$). Means ($N = 4$) for the sample with maximum attraction (high control) on days 0–4 were 166.0, 295.0, 171.5, 216.0, and 210.0, respectively.

of ethanol by Y17861 during experiment 1 was lower than in experiment 2. The relatively low levels of ethanol and other volatiles produced by Y17861 at day 2 may have originated from limited levels of glucose, the only carbohydrates that can be fermented by this strain.

Attraction measured by bioassay generally followed the amounts of fermentation volatiles (indicated in Figure 2 by ethanol), but several data points were higher than expected (the ethanol group could not account for the attraction). These were Y17861 on days 3 and 4, Y17898 on day 4, and control on day 2. This extra attraction coincided with presence of a small amount of 3-hydroxy-2-butanone, whose concentration did not correlate with the ethanol group. 3-Hydroxy-2-butanone was not detected in the first experiment with Y17861 on sweet corn. From these data, we conclude that the ability to ferment maltose and/or sucrose increases the potential attraction significantly as these are the main fermentable C sources in sweet corn. Low levels of glucose can cause low levels of attraction that by themselves can be sufficient to initiate insect visits.

We attempted to confirm the attractiveness of a few mixtures of synthetic volatiles (Table 7) and to demonstrate the attraction by 3-hydroxy-2-butanone.

TABLE 7. INSECT ATTRACTION BY MIXTURES OF VOLATILE SYNTHETIC COMPOUNDS DISSOLVED IN WATER^a

Attractiveness ^b	Mixtures				Control
	A	B	C	D	
Experiment 1 (N = 4)	264.0a				1.7b
Experiment 2 (N = 4)		203.5a			1.5b
Experiment 3 (N = 3)		157.2a	5.8b	75.0a	3.6b
Composition (ng/ml of headspace)					
Ethanol	390	24	21	0	0
Acetaldehyde	39	0	0	0	0
2-Methyl-1-propanol	2.8	0.57	0.41	0	0
1-Propanol	2.0	0	0	0	0
Ethyl acetate	1.2	0	0	0	0
2-Methyl-1-butanol	0.33	0	0	0	0
3-Hydroxy-2-butanone	0	1.1	0	1.0	0

^aMixture A simulated the *S. cerevisiae* Y17898 inoculated fermentation of sweet corn on day 3, and mixture B simulated the *C. guilliermondii* Y17861 inoculated fermentation of sweet corn on day 3 (Figure 2). Mixtures C and D were subsets of B. The control was water only. Within a row, treatments accompanied by the same letter were not significantly different (LSD, 0.05).

^bAverage of number of landings per 2 min.

The mixtures were chosen to simulate two natural samples that were attractive in the wind tunnel (Y17861 and Y17898, both on sweet corn on day 3). One sample was composed primarily of typical fermentation volatiles, and the other contained a compound (3-hydroxy-2-butanone) that was not correlated with fermentation.

Bioassays were conducted to determine whether the mixtures (or mixtures minus certain components) were sufficient to attract the beetles. We did not attempt to compare synthetic mixtures with live yeast cultures because of their dynamic nature; by the time we analyzed them and made a good synthetic version, the culture volatile pattern would have changed, making the comparison invalid. The data in Table 7 indicate that the six-volatile mix (mixture A) was sufficient to elicit excellent bioassay response and that 3-hydroxy-2-butanone was not required for attraction. The mixture of ethanol, 2-methyl-1-propanol, and 3-hydroxy-2-butanone (mixture B) was also sufficient for attraction. In fact, the ethanol/2-methyl-1-propanol part of the mix (mixture C) was not attractive, whereas 3-hydroxy-2-butanone alone (mixture D) was, but the attractiveness is enhanced by the presence of ethanol/2-methyl-1-butanol.

It is important to note that the typical fermentation volatiles can account for attraction of the beetles, but it appears that these compounds are not essential. We therefore presume the existence of other attractants not related to fermentation. The beetles, being polyphagous, are very flexible in what they can use to direct them to a new food source.

We found 3-hydroxy-2-butanone at very low levels in many yeast cultures grown in the model medium CM-G and also on sweet corn when fermentation could take place (data not shown). 3-Hydroxy-2-butanone was noted earlier in the volatiles from *S. cerevisiae* by Phelan and Lin (1991), but this compound did not make much difference in the responses of *C. hemipterus* (L.); this may have been because of low amounts of the 3-hydroxy-2-butanone or because complex blends were being tested that could have masked the effect of the compound, or perhaps *C. hemipterus* responds in a different manner than *C. humeralis*.

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