Yeast volatomes differentially effect larval feeding in an insect herbivore

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ABSTRACT Yeasts form mutualistic interactions with insects. Hallmarks of this interaction include provision of essential nutrients, while insects facilitate yeast dispersal and growth on plant substrates. A phylogenetically ancient, chemical dialogue coordinates this interaction, where the vocabulary, the volatile chemicals that mediate the insect response, remains largely unknown. Here, we employed gas chromatography-mass spectrometry (GC-MS), followed by hierarchical cluster (HCA) and orthogonal partial least square discriminant analysis (OPLS-DA), to profile the volatomes of six Metschnikowia spp., Cryptococcus nemorosus and brewer’s yeast Saccharomyces cerevisiae. The yeasts, which are all found in association with insects feeding on foliage or fruit, emit characteristic, species-specific volatile blends that reflect the phylogenetic context. Species-specificity of these volatome profiles aligned with differential feeding of cotton leafworm larvae Spodoptera littoralis on these yeasts. Bioactivity correlates with yeast ecology; phylloplane species elicited a stronger response than fruit yeasts, and larval discrimination may provide a mechanism for establishment of insect-yeast associations. The yeast volatomes contained a suite of insect attractants known from plant and especially floral headspace, including (Z)-hexenyl acetate, ethyl (2E,4Z)-deca-2,4-dienoate (pear ester), (3E)-4,8-dimethylnona-1,3,7-triene (DMNT), linalool, α-terpineol, β-myrcene or (E,E)-a-farnesene. A wide overlap of yeast and plant volatiles, notably floral scents further emphasizes the prominent role of yeasts in plant-microbe-insect relationships including pollination. The knowledge of insect-
yeast interactions can be readily brought to practical application, live yeasts or yeast metabolites mediating insect attraction provide an ample toolbox for the development of sustainable insect management.

**IMPORTANCE** Yeasts interface insect herbivores with their food plants. Communication depends on volatile metabolites, and decoding this chemical dialogue is key to understanding the ecology of insect-yeast interactions. This study explores the volatomes of eight yeast species which have been isolated from foliage, flowers or fruit, and from plant-feeding insects. They each release a rich bouquet of volatile metabolites, including a suite of known insect attractants from plant and floral scent. This overlap underlines the phylogenetic dimension of insect-yeast associations, which according to the fossil record, long predate the appearance of flowering plants. Volatome composition is characteristic for each species, aligns with yeast taxonomy, and is further reflected by a differential behavioural response of cotton leafworm larvae, which naturally feed on foliage of a wide spectrum of broad-leaved plants. Larval discrimination may establish and maintain associations with yeasts and is also a substrate for designing sustainable insect management techniques.

**KEYWORDS** Yeast volatome, metabolomic profile, floral odorants, chemical signals, larval attraction, olfaction, Noctuidae, Lepidoptera

Microorganisms essentially interface plants and insects (1-4). Invisibly, microorganisms broadcast a rich bouquet of volatile metabolites to mediate communication with other microorganisms, plants and associated animals (5-7).
Plants also release volatile compounds in abundance, but there is mounting evidence that the production of volatiles by bacteria (8, 9), fungi (10-12) and yeasts (13-17) is equally prolific. A wide overlap in compounds released by plants (18) and microbes (19) suggests furthermore that plant headspace includes volatiles that are produced by plant-associated epiphytic and endophytic microbes. A striking example is floral scent, where bacteria and especially yeasts metabolize pollen, nectar and other floral compounds and thus become a prominent source of volatiles (20-25).

Yeasts are widely associated with insects since they require vectors for dispersal and outbreeding. In addition, larval feeding facilitates yeast growth on plant substrate. Yeasts provide, on the other hand, nutritional services to insects (26-32). This mutualistic interaction is facilitated by communication with volatile metabolites.

Yeasts have apparently evolved the capacity to synthesize aroma compounds to attract insects (33-35) and insects, on the other hand, possess dedicated olfactory receptors tuned to yeast fermentation metabolites that signal suitable substrates for adult and larval feeding (36-39). Consequently, yeast volatiles, in addition to plant volatiles, play a part in host-plant and food finding in insect herbivores, including flies and moths (5, 40-44).

Investigations of the volatile metabolites of insect-associated yeasts serve a dual purpose. Plants and their insect herbivores are fundamental to many ecosystems. Olfactory recognition of food plants by insects is key to their interactions (45-47), and it is, accordingly, of fundamental interest to identify the microbial component of plant-insect communication. Furthermore, yeast metabolites or live yeasts facilitate insect management. Lack of environmentally benign, yet efficient control methods is an increasingly pressing issue in times of global change and increasing food
insecurity (48-52). Yeast attraction of insects and their larvae for feeding (33, 44, 53-56) can be exploited for population control of herbivores (57, 58), as well as for improved crop pollination (59).

We asked whether taxonomically related yeasts, isolated from different insects and habitats, differ with respect to their volatile metabolomes, and whether cotton leafworm larvae behave differently towards them. We selected a Cryptococcus and several Metschnikowia yeasts, which have been isolated from insect larvae feeding on fruit or foliage, including the cotton leafworm, Spodoptera littoralis, a polyphagous noctuid moth (60). We have investigated the volatomes of these yeasts by gas chromatography-mass spectrometry (GC-MS) and comparative multivariate discriminant analysis, affording unique volatile fingerprints. Differential larval attraction reveals the behavioural relevance of characteristic differences in yeast volatome composition.

RESULTS

Yeast headspace analysis. A phylogenetic tree of the yeasts investigated, Cryptococcus nemorosus, six Metschnikowia spp. and baker’s yeast Saccharomyces cerevisiae is shown in Figure 1. These yeasts have all been found to occur in association with insects. The volatiles released during fermentation were investigated by GC-MS, and shown to contain a range of compounds, including largely methyl and ethyl esters but also terpenoids, straight and branched alcohols, aldehydes, ketones, acids, five sulfur and three nitrogen-containing volatiles (Figure 2, Supplemental Table S1). Twenty-six of the 192 compounds found are not yet listed in databases of volatiles from yeasts, fungi and bacteria, and 33 compounds are new
for yeasts (17, 19). Many of these yeast-produced compounds, including terpenoids such as linaool and farnesenes, and esters, such as pear ester, have also been found in plant headspace (18).

Figure 2 compares the volatomes of these eight yeasts. Variation across replicates is substantial, despite rigorous protocols employed for yeast growing and headspace collection. However, species could still be separated according to headspace composition by a discriminant analysis (OPLS-DA M1, $R^2_X(\text{cum}) = 0.768$; $R^2_Y(\text{cum}) = 0.918$; $Q^2(\text{cum}) = 0.756$) resulting in 7 predictive components that explain 63% of the entire variation. Especially after grouping compounds into 14 groups by hierarchical cluster analysis (HCA) using the M1 loadings, Figure 2 further visualises that headspace composition is characteristic for each yeast. Headspace composition further reflects taxonomic position. *M. andauensis*, *M. fructicola* and *M. pulcherrima* share morphological and physiological characters, and the D1/D2 domain differs only with respect to few nucleotides (61-63). The close relation between these three species (Figure 1) is in line with their volatome composition, in comparison with the more distantly related *Metschnikowia* species (Figures 2-3).

Headspace composition also helped to clarify the taxonomic status of a yeast collected from apple, which had been tentatively and incorrectly determined as *Cryptococcus tephrensis*, according to morphological criteria. Visual inspection of its volatome fingerprint suggested this yeast to be closely related to *M. fructicola* (replicates 7 to 12 of *M. fructicola*, Figure 2). This was then confirmed by
sequencing the D1/D2 LSU rRNA gene, showing 99% similarity with the sequence obtained from *M. fructicola* (NCBI accession number KC411961, Figure 1).

A three-dimensional score plot of the first three predictive components of M1 show that *M. hawaiiensis* and *M. saccharicola* clearly separate according to the first three dimensions and that the other species are clustering with little overlap (Figure 3). The remaining species diverged in the remaining dimensions and by HCA of M1 scores (data not shown). Internal model robustness validation was performed by randomly excluding three observations for each yeast. So as to keep at least three observations, only one replicate was removed for *M. andauensis* and *S. cerevisiae* was removed completely. Excluded observations were thereafter used as prediction set in a new model made of the remaining observations [OPLS-DA M2, $R^2_X$(cum) = 0.686; $R^2_Y$(cum) = 0.879; $Q^2$(cum) = 0.596]. A zero misclassification error (Fishers probability = $2.8 \times 10^{-10}$) further corroborated the robust ability of OPLS-DA to distinguish between yeast headspace profiles.

*M. hawaiiensis, M. saccharicola and M. lopburiensis*. *M. hawaiiensis* has been isolated from morning glory flowers and is associated with drosophilid species (64), and *M. saccharicola* and *M. lopburiensis* have been found on sugarcane and rice leaves (65).

*M. hawaiiensis* and *M. saccharicola* were the most prolific producers of volatiles, several of which were not present in the other yeasts studied (Figure 2). They separated clearly, according to OPLS-DA, from each other and the other yeasts (Figure 3). The headspace of these two species has not yet been studied and contains a number of compounds which are new to databases of yeast volatiles (Supplemental...
They also contained several yet unknown compounds, which were not found in commercial or our own libraries, and which did not match commercially available standards.

Characteristic compounds for *M. saccharicola* were a range of putative sesquiterpenes and also pear ester, a characteristic odorant of pear (66, 67) which is also a strong bisexual attractant for codling moth *Cydia pomonella* (68-70). Indole, a nitrous compound, was released by both *M. lopburiensis* and *M. pulcherrima*.

Methanol, ethyl (E)-2-methylbut-2-enoate and ethyl furan-3-carboxylate are the primary class separators for *M. lopburiensis*. Methanol was also consistently found in *M. saccharicola* samples (Figure 2, Supplemental Table S1).

The volatome of *M. hawaiiensis* was clearly separated from the other Metschnikowia spp. (Figures 2, 3), methyl esters were key compounds in headspace class separation (Supplemental Table S1). Perhaps coincidentally, two sulphur-containing compounds, methyl 3-methylthio-propanoate and 3-methylsulfanylpropan-1-ol, which are typical for *M. hawaiiensis*, are associated with the aroma of pineapple (71, 72).

**M. andauensis**, **M. fructicola** and **M. pulcherrima**. These very closely related species (Figure 1; 63) are morphologically and ecologically similar. They have all been found in larval frass of lepidopteran larvae (41, 62). Three compounds that differentiate *M. fructicola* from other yeasts were 2-phenyl ethanal, 3-ethoxy-propan-1-ol and 3-methylbutan-1-ol. The top four discriminating compounds for *M. pulcherrima* were ethyl 3-methylbutanoate, ethyl propanoate, nonan-2-ol and sulcatone, which showed a high correlation with butyl butanoate. Two methyl-
branched short chain carboxylic acids, heptan-4-ol and unknown 147 were highly characteristic for *M. andauensis* (Figure 2, Supplemental Table S1).

**Cryptococcus nemorosus.** *Cryptococcus* is polyphyletic and several species such as *C. nemorosus* have been isolated from the plant phyllosphere and soil (73, 74). Ethyl 
(E)-2-methylbut-2-enoate, aliphatic ketones, and aliphatic methyl-branched primary alcohols were the main volatiles that separate *C. nemorosus* from the other species.

Strong correlations were observed for 6,10-dimethyl-5,9-undecadien-2-ol (fuscumol) and its respective ketone, 6,10-dimethyl-5,9-undecadien-2-one (geranyl acetone).

Shared structures for classifying other species were also observed, namely 2-pentylthiophene was shown to be highly correlating with class membership of *M. andauensis* (Supplemental Table S1).

**Larval feeding on live yeasts.** We next investigated attraction and feeding of *S. littoralis* larvae in a choice test. Larval feeding was assayed in a petri dish with two drops of liquid medium, one with live yeast and the other without. Three yeasts, *M. andauensis* (*p* < 0.05), *M. pulcherrima* (*p* = 0.031) and *S. cerevisiae* (*p* = 0.002) deterred feeding, more larvae fed on blank medium in their presence. Two species, *M. fructicola* (*p* = 0.06) and *M. saccharicola* (*p* = 0.096) had no significant effect, but *M. hawaiiensis* (*p* < 0.05), *M. lopburiensis* (*p* = 0.012) and *C. nemorosus* (*p* = 0.002) elicited larval attraction and feeding (Figure 4a).

An orthogonal partial least squares discriminant analysis (OPLS-DA) was used to explore the correlation of yeast volatiles from different species with respect to behavioural activity. Three classes, shown in Figure 4b, were used which resulted in a model with 2 predictive and 5 orthogonal components [OPLS-DA M3, \( R^2_{X{(cum)}} = \)]
0.68; $R^2_{Y\text{(cum)}} = 0.947; Q^2_{\text{(cum)}} = 0.812)$. Model M3 showed excellent classification performance (Fishers probability = $2.5 \times 10^{-19}$). When plotting the two OPLS-DA predictive components, three groups separate clearly, showing that these yeasts can be distinguished with respect to their behavioural effect (Figure 4b).

Among the eight yeast species, *M. andauensis* and *C. nemorosus* exhibited the strongest activity, resulting in larval avoidance and feeding, respectively (Figure 4a). The volatile profiles of these yeasts show considerable overlap with the other species (Figure 1) and we hypothesized that volatiles released by the other species could be used for sifting inactive volatile constituents and thus facilitate the search for bioactive candidate compounds. We constructed two models to this purpose.

Volatile of all species except *M. andauensis* were modelled against *C. nemorosus*, eliciting the highest rate of feeding which resulted in a model with 2 predictive and 4 orthogonal components [OPLS-DA M4, $R^2_{X\text{(cum)}} = 0.608; R^2_{Y\text{(cum)}} = 0.984; Q^2_{\text{(cum)}} = 0.933$]. *Metschnikowia andauensis*, which strongly deterred larvae from feeding, was likewise modelled against all other species except *C. nemorosus* [OPLS-DA M5, $R^2_{X\text{(cum)}} = 0.69; R^2_{Y\text{(cum)}} = 0.983; Q^2_{\text{(cum)}} = 0.572$]. Using M4 and M5, a shared and unique structure (SUS) plot was made to illustrate key compounds that are, compared to the other species, released in smaller or larger amounts by *M. andauensis* and *C. nemorosus*. The SUS-plot (Figure 5) assigns two acids, several methyl branched esters, camphene and two unknown compounds 132 and 147 to *M. andauensis*, and geranyl acetone, cyclohexanone, 2-ethyl-1-benzofuran and 1,3,5-undecatriene to *C. nemorosus*. 
DISCUSSION

The yeasts studied here occur on plants, and in connection with insect larvae feeding on these plants. A rich volatome found in all eight species may serve interactions within and between microbial taxa. Presence of many odor-active compounds also supports the idea that yeasts require animal vectors for dispersal and outbreeding.

Unlike fungal spores, yeast spores are not adapted for wind-borne transmission.

Needle-shaped ascospores, which are frequently found in the Metschnikowia clade, promote dispersal by flower-visiting flies, beetles and bees. Yeasts, on the other hand, provide nutritional services to insect larvae and adults (26, 27, 30, 31, 33, 40, 75-78).

Larvae of cotton leafworm S. littoralis that naturally feed on foliage of a broad range of annual plants (79, 80) were attracted to volatiles of three yeasts (Figure 4). Figure 2 illustrates that yeast headspace is at least as rich and complex as headspace of their food plants (91, 92), and that also many yeast compounds, including terpenoids, are shared by these plants. This raises the question whether larvae of insect herbivores become attracted to plant or to yeast odour for feeding, or both. Especially for insect species found on a range of plant species, such as S. littoralis, volatiles from plant-associated yeasts may be sufficiently reliable signals, especially when these yeasts are part of the larval diet.

The question to which extent yeast vs plant volatiles contribute to oviposition and larval feeding has been formally addressed in Drosophila melanogaster, where brewer's yeast headspace alone elicits attraction and oviposition. Drosophila larvae complete their entire development on yeast growing on minimal medium, which
supports the conclusion that the fruit merely serves as a substrate for yeast growth (40). In comparison, strict dissection of plant and microbial components is experimentally difficult in insects that require foliage for feeding. For example, grape moth *Paralobesia viteana* was attracted to grape leaves after washing off microbial colonies, but the participating role of microbes remaining on foliage or endophytes is yet unresolved (93).

A closer look at attractants identified from plant hosts produces a surprising insight: typical plant volatiles such as (Z)-3-hexenyl acetate, linalool, nonanal or even (3E)-4,8-dimethyl-1,3,7-triene (DMNT), which play an important role in attraction of *P. viteana* or the grape berry moth *Lobesia botrana*, are all produced by several yeasts (Figure 2, 93, 94). Likewise, compounds from cotton headspace that elicit antennal or behavioral responses in cotton leafworm *S. littoralis* are also produced by yeasts (Figure 2, 91, 95). Among these is again DMNT. Induced release of DMNT from plants following herbivore damage attracts natural enemies and deters some insect herbivores. In cotton leafworm, upwind flight to sex pheromone and cotton volatiles is suppressed by large amounts of DMNT, due to its prominent effect on central olfactory circuits (47, 92, 96).

DMNT is also a floral scent component, across a wide range of plants (97) and an attractant of flies and moths (94, 98-100). Yeasts obviously contribute to DMNT release from flowers, since DMNT was found in all yeasts studied here (Figure 2). Besides DMNT, a wide range of volatiles co-occurs in yeasts and angiosperm flowers, for example the typical *Drosophila* attractants acetoin, ethanol, ethyl acetate, 2-phenylethyl acetate, 2-phenylethanol (Figure 2; 101). 2-phenylethanol, a typical yeast odorant (e.g. 102), is also produced by green plants (e.g. 103). Let alone
an overlap of compounds produced by both plants and yeasts, fumigation of elderberry flowers with broad-spectrum antibiotics revealed that floral phyllospheric microbiota are unique producers of key floral terpenes (20). The yeasts investigated here all produce a range of terpenes (Figure 2, Supplemental Table S1).

Insect-yeast chemical communication has evolved long before the emergence of flowering plants. Fungivory and herbivory on plants was initiated during the Early Devonian ~400 Ma, concurrent with the appearance of budding yeasts and prior to angiosperm pollination syndromes during the Cretaceous ~100 Ma (104-107). This lends support to the idea that a sensory bias for yeast-produced compounds, together with ubiquitous presence of yeasts in flowers, has contributed to the evolution of floral scent and insect-mediated pollination (23, 24, 101, 108, 109).

While the ecological and evolutionary consequences of chemical dialogue between plants, microbes and insects are unequivocal, it is yet largely unclear which of the many volatiles released by yeasts encode this interaction. A comprehensive analysis of yeast volatomes is a first and necessary step towards identifying the active compounds. Thirty-three of the 192 volatiles (Figure 2, Supplemental Table S1) are new for yeasts. Most of them were released by *M. hawaiiensis* *M. lopburiensis* and *M. saccharicola*, which are the most recently discovered species (64, 65). The database of yeast volatiles creates a basis for future studies, aimed at functional characterization of insect olfactory receptors and attraction bioassays, towards the identification of the behaviourally active compounds (110-112).

The overall species-specific volatome patterns showed variation between replicates, even though growth conditions were strictly controlled and sampling intervals...
adjusted to cancel out growth stage variations (Figure 2). A general assumption in metabolomics is that identical genotypes produce the same steady state metabolite concentrations under stringent conditions, while metabolic snapshots often show considerable biological variability. Metabolite-metabolite correlations derived from enzymatic reaction network activity may nonetheless be robust, despite considerable intrinsic, stochastic variation of metabolite concentrations obtained at momentary peeks into the state of an organism (113-115).

In spite of inherent variation among volatile samples, numerical headspace analysis by OPLS-DA followed by HCA revealed characteristic volatile fingerprints for each of the eight yeasts (Figures 2, 3) which align with the phylogenetic analysis based on sequences from the D1/D2 region (Figure 1) and yeast taxonomy and ecology (Figure 1; 63, 76). Moreover, we found that OPLS-DA exhibited a robust yeast-species assignment headspace samples and is therefore a useful tool for studying and classifying unknown species with regard to their volatiles (Figure 3).

Volatile fingerprinting or chemotyping has previously been shown to differentiate between ectomycorrhizal, pathogenic and saprophytic fungi, as a complement to genotyping (116, 117). This was confirmed by comparing *M. fructicola* and *M. andauensis*, which are taxonomically close and difficult to discriminate according to genotyping (Figure 1; 118). They quantitatively and clearly separated according to headspace proportions, in addition to production of methanol by *M. fructicola* (Figures 2, 3). Further support for the use of volatomes in species discrimination comes from an isolate from codling moth larvae, which had been misidentified as *C. tephrrens*. Comparison of headspace data with *M. fructicola* evidenced overlap of 101 compounds with significant coefficient values and non-zero confidence interval
for the whole dataset. Subsequent DNA analysis identified this yeast as *M. fructicola* (Figure 1).

The species-specific volatome differences are corroborated by a selective larval feeding response (Figure 4), where cotton leafworm larvae, which are typical foliage feeders, prefer phyllosphere yeasts over the yeasts associated with fruit and frugivorous insects. Larvae avoided baker's yeast commonly found with *D. melanogaster* and *M. andauensis* and *M. pulcherrima* which have been isolated from codling moth, *Cydia pomonella*, feeding in apple (41). It is yet unknown whether cotton leafworm forms yeast associations with yeasts in natural habitats, but a consistent larval response to yeasts may establish and sustain such associations.

Identifying behaviorally active metabolites is key to understanding the ecology of insect-yeast interactions. Geranyl acetone, an aggregation pheromone component of *C. pomonella* larvae (119) is a distinctive compound for *C. nemorosus* (Figures 2, 5), which elicited the strongest larval feeding response (Figure 4). Among the cotton leafworm olfactory receptors which have been functionally characterized, several are tuned to compounds produced by yeasts, and some even elicited larval attraction as single compounds, such as benzyl alcohol, benzaldehyde or indole (120). For a more complete behavioral identification, it would probably be necessary to test compound blends, including candidate compounds from the headspace of *M. hawaiiensis*, *M. lopburiensis* or *C. nemorosus* (Figures 2, 4, 5).

At the same time, our study reveals potential antifeedants. Camphene has indeed already been reported as a repellant in *S. littoralis* (121) and its acute larval toxicity has been shown in the sister species *S. litura* (122). In addition, *S. littoralis* females...
detect camphene, as well as 3-methylbutyl acetate (123), both of which are sign
compounds for *M. andauensis* (Figures 2, 4, 5). Discriminant analysis points towards
presence of attractive and antagonistic yeast volatiles and highlights compounds for
future screening assays.

Cotton leafworm is polyphagous on a variety of crops including vegetables in the
Afrotropical and western Palearctic. Its sister species *S. litura* is found over Asia,
Australasia, and Oceania and the South-American species *S. frugiperda* has recently
invaded Africa (52, 60, 79, 124). Global change and increasing food insecurity
render insect control an ever more challenging and urgent task (51, 125-127).
Detrimental environmental and health effects warrant downregulation of
conventional pesticides and accentuate the further development of biological insect
control, comprising natural antagonists, insect pathogens or semiochemicals.
Semiochemicals and pathogens are widely and successfully used as stand-alone
techniques (128, 129), but semiochemicals could be combined with pathogens into
lure-and-kill strategies (57, 58, 130). Current use of insect semiochemicals is based
on controlled release formulations of synthetic chemicals, while yeasts could be used
for live production of insect attractants (5, 131)
That yeasts would make suitable producers of insect attractants is supported by
establishment of biofilms with strong survival ability, which enables postharvest
control of fungal diseases in fruit (132-134). Combination of attractant yeasts for
targeted ingestion of an insect baculovirus or a biological insecticide has been
successful in laboratory and first field experiments, against codling moth and spotted
wing *Drosophila* (130, 135, 136). For further improvement, identification of key
compounds mediating insect attraction will facilitate selection of yeast species and
strains.

Yeast volatiles are also antifungal (132, 133) and may directly, or through other
members of the plant microbiome, impact on plant fitness. A critical component of
functional interlinkages between plants and an ensemble of associated microbiota is
that the plant immune system reliably differentiates between synergistic and
antagonistic microbes (137, 138). Odorants are essential in regulating mutual and
detrimental colonizers in plant microbial networks (6, 7, 139) and yeast volatiles are
obviously involved in this chemical dialogue, since compounds such as farnesol or 2-
phenylethanol participate in quorum sensing and interspecies interactions (Figure 2,
Supplemental Table 1; 16, 140). Integrating plant microbiomes in crop protection
concepts, for insect control, enhanced stress tolerance and disease resistance is a
future challenge in agriculture (141).

MATERIALS AND METHODS

Yeast. Metschnikowia yeasts were purchased from the CBS-KNAW collection
(Utrecht, Netherlands), except M. fructicola, which was isolated from apple (Alnarp,
Sweden) infested with larvae of codling moth Cydia pomonella (Lepidoptera,
Tortricidae), Cryptococcus nemorosus was isolated from cotton leafworm
Spodoptera littoralis (Lepidoptera, Noctuidae) larvae (laboratory rearing, Alnarp,
Sweden), and Saccharomyces cerevisiae was obtained from Jästbolaget AB
(Sollentuna, Sweden).

M. andauensis, M. fructicola and M. pulcherrima were found in guts and larval feces
of caterpillars feeding on maize, corn earworm Helicoverpa armigera (Lepidoptera,
Noctuidae) and European corn borer *Ostrinia nubilalis* (Lepidoptera, Crambidae) 
(62). *M. andauensis* and *M. pulcherrima*, and occasionally *M. fructicola* were found 
in apple and larval feces of codling moth *Cydia pomonella* (Lepidoptera, Tortricidae) 
(41). *M. saccharicola*, *M. lopburiensis* were isolated from foliage in Thailand (65) 
and *M. hawaiiensis* from fruit flies (Drosophilidae, Diptera) (64).

**DNA isolation and yeast identification.** Genomic DNA was isolated from 
overnight yeast cultures grown in liquid yeast extract peptone dextrose (YPD) 
medium. 2 mL of the overnight cell cultures were pelleted (13,000 rpm for 1 min) 
and washed with sterile ddH2O. The pellets were resuspended in 200 μL lysis buffer 
(2% Triton X-100, 1% SDS, 0.1 M NaCl, 10 mM Tris, 1 mM EDTA, pH 8), and 200 
μL phenol:chloroform:isoamyl alcohol (25:24:1) mixture. The glass beads (200 μL) 
were then added to the tubes and vortexed thoroughly for 3 min. To this mixture, 200 
μL TE buffer (10 mM Tris-Cl, 1 mM EDTA; pH 8.0) was added and centrifuged at 
13,000 rpm for 10 min. The upper aqueous phase was collected and 10 μL of 
RNaseA (10 mg/mL) was added and incubated at 37°C during 45 min. The DNA 
was precipitated using 300 mM sodium acetate, three volumes of cold absolute 
ethanol (99.9%) and centrifuged at 13,000 rpm for 10 min at 4°C. The DNA pellet 
was then washed in 70% cold ethanol (13,000 rpm for 10 min, 4°C), air-dried, and 
re-suspended in 30 μL TE buffer and stored at -20°C until use.

The D1/D2 domains of the 26S ribosomal RNA gene was amplified with the 
universal primer pair NL-1 and NL-4 and the internally transcribed spacer (ITS) 
region with the primer combination ITS-1 and ITS-4 (142, 143). The PCR reaction 
and product visualisation on agarose gel was performed as previously described 
(101). PCR-products were purified using ExoStar-IT (USB Corporation, USA)
following the manufacturer’s protocol sequenced using BigDye v.1.1 terminator sequencing kit in an ABI PRISM 3130 genetic analyser (Applied Biosystems, New Jersey, US).

All sequences were aligned in the program Bioedit 7.0.9.0 Sequence Alignment Editor (144). Each sequence was tested for identity and similarity against sequences deposited in the National Center for Biotechnology Information (NCBI) using Megablast-search (accession number KF839191, KC411961). Only similarities over 95% were considered. The phylogenetic tree of the yeast species investigated, based on nucleotide sequences of the D1/D2 domain of 26S rDNA, was constructed using the neighbor-joining (NJ) method in MEGA 7.0 (145). The NJ method, based on the evolutionary distance data that minimizes the total branch length during clustering of operational taxonomic units (OTUs), is efficient and reliable for phylogenetic reconstructions (146). The evolutionary distances were calculated according to the Jukes and Cantor (JC) substitution model to minimize the bias due to nucleotide substitution during divergence. The JC substitution model considers the rate of substitution frequencies of all pairs of four nucleotides (A, T, G, C) to be equal (147). The bootstrap values for the phylogenetic tree reconstruction were determined from 1,000 replications and are given next to the branch (Figure 1).

**Headspace collection and chemical analysis.** Yeasts were grown in 100 mL liquid minimal medium (148) in 250-mL culture flasks, during 24 h in a shaking incubator (25°C, 260 rpm). Yeast headspace was collected by drawing charcoal-filtered air (0.125 L/min), through a 1-L gas wash bottle containing the yeast broth, over a 35-mg Super Q trap (80/100 mesh; Alltech, Deerfield, IL, USA), which was held between plugs of glass-wool in a 4 x 40-mm glass tube. Collections were done for
ca. 24 h, at 20 to 22 °C and 10 to 30 Lux. The charcoal filter (50 g activated charcoal) for incoming air and the Super Q trap were connected with glass fittings to the wash bottle. All glassware was heated to 375°C for 10 h before use (149).

Following volatile collections, the trap was extracted with 0.5 mL of redistilled hexane. Sample volumes were reduced to ca. 50 µL, at ambient temperature in Francke-vials with an elongated tip (5 cm x 2 mm i.d.). Samples were stored in sealed glass capillary tubes at -19°C. The Super Q trap was wrapped in aluminium foil for protection from light. Before use, it was rinsed sequentially with 3 mL of methanol (redistilled >99.9% purity, Merck, Darmstadt, Germany) and hexane (redistilled >99.9% purity; Labscan, Malmö, Sweden).

Yeast headspace collections were analysed on a coupled gas chromatograph–mass spectrometer (GC–MS; 6890 GC and 5975 MS; Agilent Technologies, Palo Alto, CA, USA), operated in the electron impact ionization mode at 70 eV. The GC was equipped with fused silica capillary columns (30 m x 0.25 mm, d.f. = 0.25 µm), DB-Wax (J&W Scientific, Folsom, CA, USA) or HP-5MS (Agilent Technologies). Helium was used as the mobile phase at an average linear flow rate of 35 cm/s. Two µL of each sample were injected (splitless mode, 30 s, injector temperature 225°C).

The GC oven temperature for both columns was programmed from 30°C (3 min hold) at 8°C/min to 225°C (5 min hold).

Data were exported in NetCDF file format and deconvoluted into compound spectra, elution profile and peak area. We used MS-Omics software (Vedbaek, Denmark) and the PARAFAC2 model (150), and the non-commercial package HDA (version 0.910, P. Johansson, Umeå University, Sweden), based on the H-MCR method (151). Both
methods utilize covariation between samples to separate co-eluting components, and to pool mass spectra across samples affording unambiguous spectra even at low signal to noise ratios. Approx. 70% and 30% of the peaks were separated by PARAFAC2 and H-MCR, respectively. However, neither deconvolution method produced satisfactory results for all compounds, which necessitated manual selection of the most feasible method in some cases.

Compounds were identified according to their retention times (Kovat’s indices) and mass spectra, in comparison with the National Institute of Standards and Technology (NIST, version 14) mass spectral library and authentic standards, on two columns. Extra care was taken to verify the identity of compounds showing high variation in abundance between yeast species. Compounds present in blank recordings of the growth medium were subtracted.

**Insects.** A cotton leafworm *Spodoptera littoralis* (Lepidoptera, Noctuidae) laboratory colony was established using field-collected insects from Alexandria (Egypt) in 2010. This colony was interbred with wild insects from Egypt every year. Insects were raised on a semisynthetic agar-based diet (152) under a 16L:8D photoperiod, at 24°C and 50 to 60% RH.

**Larval feeding assay.** Yeasts (*M. andauensis, M. fructicola, M. hawaiensis, M. lopburiensis, M. pulcherrima, M. saccharicola, S. cerevisiae* and *C. nemorosus*) were grown in 125-mL culture flasks in 50 mL of liquid minimal medium (148) for 20 h in a shaking incubator (25 °C, 260 rpm). Optical density at 595 nm was between 1.5 and 1.8, and cell counts were adjusted to 1.5 x 10^7 cells/mL.
A two-choice bioassay was conducted to determine neonate larval yeast attraction and feeding. Two 50 µL drops of a 20-h old yeast culture and blank minimal medium were pipetted opposite from each other, approx 1 cm from the edge of a plastic petri dish (92 mm ø, No.82.1472, Sarstedt AG & Co., Nümbrecht, DE). Colorants, blue and green, (Dr. Oetker Sverige AB, Göteborg, SE) were used at 1:10 dilution to color yeast and minimal medium, in order to distinguish between the larvae that fed on yeast medium, blank medium or both. Preliminary tests did not show a bias in larval attraction to the different colorants (N=30; F=1.529; p=0.222; ANOVA).

Ten starved neonate larvae were collected 24 to 36 h after hatching and were placed in the centre of the petri dish with a fine brush. The dish was covered with a lid to prevent the larvae from escaping, and larvae were left to feed for 2 h. They were then checked under a microscope for their gut coloration. Ten independent replicates with 10 neonate larvae were done for each yeast. The larvae response index (LRI) was calculated from the number of larvae feeding on the yeast treatment (nY) and control (nC): LRI = (nY-nC)/(nY+nC).

**Statistical Analysis.** All yeast species and volatile compounds were collated into a matrix, containing 56 yeast headspace collections and the integrated areas of the 192 compounds found. Two pre-treatment methods were selected before building the model. A logarithmic transformation of x-variables served to minimize skewness of the variables. Since the abundance of compounds does not necessarily correlate with biological activity, pareto scaling was used to augment the impact of compounds present in very small amounts. Hierarchical cluster analysis (HCA), orthogonal partial least square discriminant analysis (OPLS-DA) and partial least square discriminant analysis (PLS-DA) and its corresponding validation tests were
Three methods were used to validate the multivariate models. Internal cross validation (CV) was done with eight CV-groups of dissimilar observations. A permutation test, by randomization of the class vector, was used to test for the presence of spurious correlations between volatile profiles and class membership by fitting each permutation to a new PLS-DA model and observing the resulting explained variance and predictive power. Finally, an internal validation set of observations that spanned the multivariate space for each model was inspected for any large deviations.

Larval feeding of *S. littoralis* on yeasts was compared using Student’s *t*-test, with the level of significance set to *p*=0.05.

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at …

**SUPPLEMENTAL TABLE S1**, PDF file, 0.15 MB.

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**REFERENCES**

1. Janson EM, Stireman JO, Singer MS, Abbot P. 2008. Phytophagous insect-microbe mutualisms and adaptive evolutionary diversification. Evolution 62:997-1012.

2. Biere A, Bennett AE. 2013. Three-way interactions between plants, microbes and insects. Funct Ecol 27:567-573.

3. Hansen AK, Moran NA. 2014. The impact of microbial symbionts on host plant utilization by herbivorous insects. Molec Ecol 23:1473-1496.

4. Shikano I, Rosa C, Tan CW, Felton GW. 2017. Tritrophic interactions: microbe-mediated plant effects on insect herbivores. Annu Rev Phytopathol 55:313-331.

5. Davis TS, Crippen TL, Hofstetter RW, Tomberlin JK. 2013. Microbial volatile emissions as insect semiochemicals. J chem Ecol 39:840-859.

6. Leach JE, Triplett LR, Argueso CT, Trivedi P. 2017. Communication in the phytobiome. Cell 169:587-596.

7. Carthey AJ, Gillings MR, Blumstein DT. 2018. The extended genotype: microbially mediated olfactory communication. Tr Ecol Evol 33:885-894.

8. Schulz S, Dickschat JS. 2007. Bacterial volatiles: the smell of small organisms. Nat Prod Rep 24:814-842.

9. Citron CA, Rabe P, Dickschat JS. 2012. The scent of bacteria: headspace analysis for the discovery of natural products. J Nat Prod 75:1765-1776.

10. Kramer R, Abraham WR. 2012. Volatile sesquiterpenes from fungi: what are they good for? Phytochem Rev 11:15-37.

11. Heddergott C, Calvo AM, Latgé JP. 2014. The volatome of *Aspergillus fumigatus*. Eukaryotic Cell 13:1014-1025.

12. Li N, Alfiky A, Vaughan MM, Kang S. 2016. Stop and smell the fungi: fungal volatile metabolites are overlooked signals involved in fungal interaction with plants. Fungal Biol Rev 30:134-144.

13. Swiegers JH, Bartowsky EJ, Henschke PA, Pretorius IS. 2005. Yeast and bacterial modulation of wine aroma and flavour. Aust J Grape Wine Res 11:139-173.

14. Hernandez-Orte P, Cersosimo M, Loscos N, Cacho J, Garcia-Moruno E, Ferreira V. 2008. The development of varietal aroma from non-floral grapes
by yeasts of different genera. Food Chem 107:1064-1077.

15. Weldegergis BT, Crouch AM, Górecki T, De Villiers A. 2011. Solid phase extraction in combination with comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry for the detailed investigation of volatiles in South African red wines. Analytica Chimica Acta 701:98-111.

16. Dzialo MC, Park R, Steensels J, Lievens B, Verstrepen KJ. 2017. Physiology, ecology and industrial applications of aroma formation in yeast. FEMS Microbiol Rev 41:flux031, S95–S128.

17. Ramirez-Gaona M, Marcu A, Pon A, Guo AC, Sajed T, Wishart NA, Karu N, Feunang YD, Arndt D and Wishart DS. 2017. YMDB 2.0: a significantly expanded version of the yeast metabolome database. Nucleic Acids Res 45(D1):D440-D445.

18. Knudsen JT, Tollsten L, Bergström LG. 1993. Floral scents—a checklist of volatile compounds isolated by head-space techniques. Phytochemistry 33:253-280.

19. Lemfack MC, Gohlke BO, Toguem SMT, Preissner S, Piechulla B, Preissner R. 2018. mVOC 2.0: a database of microbial volatiles. Nucleic Acids Res 46(D1):D1261-D1265.

20. Penuelas J, Farré-Armengol G, Llusia J, Gargallo-Garriga A, Rico L, Sardans J, Terradas J, Filella I. 2014. Removal of floral microbiota reduces floral terpene emissions. Sc Rep 4:6727.

21. Raguso RA. 2004. Why are some floral nectars scented? Ecology 85:1486-1494.

22. Helletsgruber C, Dötterl S, Ruprecht U, Junker RR. 2017. Epiphytic bacteria alter floral scent emissions. J Chem Ecol 43:1073-1077.

23. Schaeffer RN, Mei YZ, Andicoechea J, Manson JS, Irwin RE. 2017. Consequences of a nectar yeast for pollinator preference and performance. Funct Ecol 31:613-621.

24. Rering CC, Beck JJ, Hall GW, McCartney MM, Vannette RL. 2018. Nectar-inhabiting microorganisms influence nectar volatile composition and attractiveness to a generalist pollinator. New Phytologist 220:750-759.

25. Sobhy IS, Baets D, Goelen T, Herrera-Malaver B, Bosmans L, Van den Ende W, Verstrepen KJ, Wäckers F, Jacquemyn H, Lievens B. 2018. Sweet scents: nectar specialist yeasts enhance nectar attraction of a generalist aphid parasitoid without affecting survival. Front Plant Sc 9:1009.

26. Brysch-Herzberg M. 2004. Ecology of yeasts in plant–bumblebee mutualism in Central Europe. FEMS Microbiol Ecol 50:pp.87-100.

27. Ganter PF. 2006. Yeast and invertebrate associations, p 303–370. In Rosa C, Péter G (ed), Biodiversity and ecophysiology of yeasts. Springer, Berlin,
Stefanini I, Dapporto L, Legras JL, Calabretta A, Di Paola M, De Filippo C, Viola R, Capretti P, Polsinelli M, Turillazzi S, Cavaliere, D. 2012. Role of social wasps in *Saccharomyces cerevisiae* ecology and evolution. Proc Natl Acad Sc USA 109:13398-13403.

Stefanini I. 2018. Yeast-insect associations: it takes guts. Yeast 35:315-330.

Guzman B, Lachance MA, Herrera CM. 2013. Phylogenetic analysis of the angiosperm-floricolous insect-yeast association: have yeast and angiosperm lineages co-diversified? Molec Phylogen Evol 68:161-175.

Blackwell M. 2017. Made for each other: ascomycete yeasts and insects. Microbiol Spectrum 5(3):FUNK-0081-2016.

Blackwell M. 2017. Yeasts in insects and other invertebrates, p 397-433. In Buzzini P, Lachance MA, Yurkov A (ed), Yeasts in natural ecosystems: diversity. Springer, Cham.

Palanca L, Gaskett AC, Günther CS, Newcomb RD, Goddard MR. 2013. Quantifying variation in the ability of yeasts to attract *Drosophila melanogaster*. PLoS One 8:e75332.

Christiaens JF, Franco LM, Cools TL, De Meester L, Michiels J, Wenseleers T, Hassan BA, Yaksi E, Verstrepen KJ. 2014. The fungal aroma gene ATF1 promotes dispersal of yeast cells through insect vectors. Cell Rep 9:425-432.

Babcock T, Borden JH, Gries R, Carroll C, Lafontaine JP, Moore M, Gries G. 2019. Inter-kingdom signaling - symbiotic yeasts produce semiochemicals that attract their yellowjacket hosts. Entomol Experiment Appl 167:220-230.

Stensmyr MC, Giordano E, Balloi A, Angioy AM, Hansson BS. 2003. Novel natural ligands for *Drosophila melanogaster* olfactory receptor neurones. J Exp Biol 206:715-724.

Arguello JR, Sellanes C, Lou YR, Raguso RA. 2013. Can yeast (*S. cerevisiae*) metabolic volatiles provide polymorphic signaling? PloS One 8:e70219.

Münch D, Galizia CG. 2016. DoOR 2.0-comprehensive mapping of *Drosophila melanogaster* odorant responses. Sc Rep 6:21841.

Elya C, Quan AS, Schiabor KM, Eisen M. 2017. Or22 allelic variation alone does not explain differences in discrimination of yeast-produced volatiles by *D. melanogaster*. bioRxiv https://doi.org/10.1101/186064.

Becher PG, Flick G, Rozpedowska E, Schmidt A, Hagman A, Lebreton S, Larsson MC, Hansson BS, Piskur J, Witzgall P, Bengtsson M. 2012. Yeast, not fruit volatiles mediate attraction and development of the fruit fly *Drosophila melanogaster*. Funct Ecol 26:822-828.

Witzgall P, Proffit M, Rozpedowska E, Becher PG, Andreadis S, Coracini M,
42. Scheidler NH, Liu C, Hamby KA, Zalom FG, Syed Z. 2015. Volatile codes: correlation of olfactory signals and reception in Drosophila–yeast chemical communication. Sc Rep 5:14059.

43. Cha DH, Mieles AE, Lahuatte PF, Cahuana A, Lincango MP, Causton CE, Tebbich S, Cimadom A, Teale SA. 2016. Identification and optimization of microbial attractants for Philornis downsi, an invasive fly parasitic on galapagos birds. J Chem Ecol 42:1101–1111.

44. Babcock T, Borden J, Gries R, Carroll C, Moore M, Gries G. 2018. Lachancea thermotolerans, a yeast symbiont of yellowjackets, enhances attraction of three yellowjacket species (Hymenoptera: Vespidae) to fruit powder. Environm Entomol 47:1553–1559.

45. Berlocher SH, Feder JL. 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? Annu Rev Entomol 47:773–785.

46. Bruce TJ, Pickett JA. 2011. Perception of plant volatile blends by herbivorous insects—finding the right mix. Phytochemistry 72:1605–1611.

47. Borrero-Echeverry F, Bengtsson M, Nakamuta K, Witzgall P. 2018. Plant odour and sex pheromone are integral elements of specific mate recognition in an insect herbivore. Evolution 72:2225–2233.

48. Birch ANE, Begg GS, Squire GR. 2011. How agro-ecological research helps to address food security issues under new IPM and pesticide reduction policies for global crop production systems. J Exp Botany 62:3251–3261.

49. Verger PJ, Boobis AR. 2013. Reevaluate pesticides for food security and safety. Science 341:717–718.

50. Hallmann CA, Foppen RP, van Turnhout CA, de Kroon H, Jongejans E. 2014. Declines in insectivorous birds are associated with high neonicotinoid concentrations. Nature 511:341.

51. Deutsch CA, Tewksbury JJ, Tichelaar M, Battisti DS, Merrill SC, Huey RB, Naylor RL. 2018. Increase in crop losses to insect pests in a warming climate. Science 361:916–919.

52. Onyutha C. 2018. African crop production trends are insufficient to guarantee food security in the sub-Saharan region by 2050 owing to persistent poverty. Food Security 10:1203–1219.

53. Nout MJR, Bartelt RJ. 1998. Attraction of a flying nitidulid (Carpophilus humeralis) to volatiles produced by yeasts grown on sweet corn and a corn-based medium. J Chem Ecol 24:1217–1239.

54. Torto B, Boucias DG, Arbogast RT, Tumlinson JH, Teal PE. 2007. Multitrophic interaction facilitates parasite–host relationship between an invasive beetle and the honey bee. Proc Nat Acad Sc USA 104:8374–8378.
55. Choi HS, Kim GJ, Shin HJ. 2011. Biocontrol of moth pests in apple orchards: preliminary field study of application potential for mass trapping. Biotechnol Bioproc Eng 16:153-157.

56. Davis TS, Landolt PJ. 2013. A survey of insect assemblages responding to volatiles from a ubiquitous fungus in an agricultural landscape. J Chem Ecol 39:860-868.

57. El-Sayed AM, Suckling DM, Byers JA, Jang EB, Wearing CH. 2009. Potential of “lure and kill” in long-term pest management and eradication of invasive species. J Econ Entomol 102:815-835.

58. Gregg PC, Del Socorro AP, Landolt PJ. 2018. Advances in attract-and-kill for agricultural pests: beyond pheromones. Annu Rev Entomol 63:453-470.

59. Bailes EJ, Ollerton J, Pattrick JG, Glover BJ. 2015. How can an understanding of plant–pollinator interactions contribute to global food security? Curr Op Plant Biol 26:72-79.

60. Kergoat GJ, Prowell DP, Le Ru BP, Mitchell A, Dumas P, Clamens AL, Condamine FL, Silvain JF. 2012. Disentangling dispersal, vicariance and adaptive radiation patterns: a case study using armyworms in the pest genus Spodoptera (Lepidoptera: Noctuidae). Mol Phylogen Evol 65:855-870.

61. Kurtzman CP, Droby S. 2001. Metschnikowia fructicola, a new ascosporic yeast with potential for biocontrol of postharvest fruit rots. System Appl Microbiol 24:395-399.

62. Molnar O, Prillinger H. 2005. Analysis of yeast isolates related to Metschnikowia pulcherrima using the partial sequences of the large subunit rDNA and the actin gene; description of Metschnikowia andauensis sp. nov. Syst Appl Microbiol 28:717-726.

63. Lachance MA. 2011. Metschnikowia kamieski (1899). p 575-620. In Kurtzman CP, Fell JW, Boekhout T (ed), The yeasts (5th edition). Amsterdam, Elsevier.

64. Lachance MA, Starmer WT, Phaff HJ. 1990. Metschnikowia hawaiiensis sp. nov., a heterothallic haploid yeast from Hawaiian morning glory and associated drosophilids. Int J System Evol Microbiol 40:415-420.

65. Kaewwichian R, Yongmanitchai W, Kawasaki H, Limtong S. 2012. Metschnikowia saccharicola sp. nov. and Metschnikowia lopburiensis sp. nov., two novel yeast species isolated from phylloplane in Thailand. Antonie van Leeuwenhoek 102:743-751.

66. Jennings WG, Heinz DE, Creveling RK. 1964. Volatile esters of Bartlett pear. IV. Esters of trans-2-cis-4-decadienoic acid. J Food Sci 29:730-734.

67. Miller RL, Bills DD, Buttery RG. 1989. Volatile components from Bartlett and Bradford pear leaves. J Agric Food Chem 37:1476-1479.

68. Light DM, Knight AL, Henrick CA, Rajapaska D, Lingren B, Dickens JC, Reynolds KM, Buttery RG, Merrill G, Roitman J, Campbell BC. 2001. A
pear-derived kairomone with pheromonal potency that attracts male and female codling moth, *Cydia pomonella* (L.). Naturwiss 88:333-338.

Light DM, Knight A. 2005. Specificity of codling moth (Lepidoptera: Tortricidae) for the host plant kairomone, ethyl (2E,4Z)-2,4-decadienoate: field bioassays with pome fruit volatiles, analogue, and isomeric compounds. J Agric Food Chem 53:4046-53.

Bengtsson JM, Gonzalez F, Cattaneo AM, Montagné N, Walker WB, Bengtsson M, Anfora G, Ignell R, Jacquin-Joly E, Witzgall P. 2014. A predicted sex pheromone receptor of codling moth *Cydia pomonella* detects the plant volatile pear ester. Front Ecol Evol 2:33.

Takeoka GR, Buttery RG, Teranishi R, Flath RA, Guentert M. 1991. Identification of additional pineapple volatiles. J Agric Food Chem 39:1848-1851.

Li Y, Qi H, Jin Y, Tian X, Sui L, Qiu Y. 2016. Role of ethylene in biosynthetic pathway of related-aroma volatiles derived from amino acids in oriental sweet melons (*Cucumis melo* var. *makuwa* Makino). Scientia Horticult 201:24-35.

Fell JW, Boekhout T, Fonseca A, Scorzetti G, Statzell-Tallman A. 2000. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. Int J System Evol Microbiol 50:1351-1371.

Golubev WI, Gadanho M, Sampaio JP, Golubev NW. 2003. *Cryptococcus nemorosus* sp. nov. and *Cryptococcus perniciosus* sp. nov., related to *Papiliotrema* Sampaio et al. (Tremellales). Int J System Evol Microbiol 53:905-911.

Lachance MA, Starmer WT, Rosa CA, Bowles JM, Barker JSF, Janzen DH. 2001. Biogeography of the yeasts of ephemeral flowers and their insects. FEMS Yeast Research 1:1-8.

Lachance MA. 2016. *Metschnikowia*: half tetrads, a regicide and the fountain of youth. Yeast 33:563-574.

Batista MR, Uno F, Chaves RD, Tidon R, Rosa CA, Klaczko LB. 2017. Differential attraction of drosophilids to banana baits inoculated with *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* within a neotropical forest remnant. PeerJ 5:e3063.

Vitanovic E, Aldrich JR, Winterton SL, Boundy-Mills K, Lopez JM, Zalom FG. 2019. Attraction of the green lacewing *Chrysoperla comanche* (Neuroptera: Chrysopidae) to yeast. J Chem Ecol 45:388-391.

Pogue MG. 2002. A world revision of the genus *Spodoptera* Guenee (Lepidoptera: Noctuidae). Mem Am Entomol Soc 43:1-202.

Thöming G, Larsson MC, Hansson BS, Anderson P. 2013. Comparison of plant preference hierarchies of male and female moths and the impact of
larval rearing hosts. Ecology 94:1744-1752.

91. Saveer AM, Kromann S, Birgerson G, Bengtsson M, Lindblom T, Balkenius A, Hansson BS, Witzgall P, Becher PG, Ignell R. 2012. Floral to green: mating switches moth olfactory coding and preference. Proc R Soc B 279:2314-2322.

92. Zakir A, Bengtsson M, Sadek MM, Hansson BS, Witzgall P, Anderson P. 2013. Specific response to herbivore-induced de novo synthesized plant volatiles provides reliable information for host plant selection in a moth. J Exp Biol 216:3257-3263.

93. Wolfin MS, Volo SL, Chilson RR, Liu Y, Cha DH, Cox KD, Loeb GM, Linn CE. 2019. Plants, microbes, and odorsants involved in host plant location by a specialist moth: who's making the message? Entomol Exp Appl 167:313-322.

94. Tasin M, Bäckman A-C, Bengtsson M, Ioriatti C, Witzgall P. 2006. Essential host plant cues in the grapevine moth. Naturwissenschaften 93:141-144.

95. Borrero-Echeverry F, Becher PG, Birgersson GÅO, Bengtsson M, Witzgall P, Saveer AM. 2015. Flight attraction of Spodoptera littoralis (Lepidoptera, Noctuidae) to cotton headspace and synthetic volatile blends. Front Ecol Evol 3:56.

96. Hatano E, Saveer AM, Borrero-Echeverry F, Strauch M, Zakir A, Bengtsson M, Iggel R, Anderson P, Becher PG, Witzgall P, Dekker T. 2015. A herbivore-induced plant volatile interferes with host plant and mate location in moths through suppression of olfactory signalling pathways. BMC Biology 13:75.

97. Tholl D, Sohrabi R, Huh JH, Lee S. 2011. The biochemistry of homoterpenes–common constituents of floral and herbivore-induced plant volatile bouquets. Phytochemistry 72:1635-1646.

98. Cha DH, Nojima S, Hesler SP, Zhang A, Linn CE, Roelofs WL, Loeb GM. 2008. Identification and field evaluation of grape shoot volatiles attractive to female grape berry moth (Paralobesia viteana). J Chem Ecol 34:1180-1189.

99. Cha DH, Yee WL, Goughnour RB, Sim SB, Powell TH, Feder JL, Linn CE. 2012. Identification of host fruit volatiles from domestic apple (Malus domestica), native black hawthorn (Crataegus douglasii) and introduced ornamental hawthorn (C. monogyna) attractive to Rhagoletis pomonella flies from the Western United States. J Chem Ecol 38:319-329.

100. Knight AL, Light DM, Trimble RM. 2011. Identifying (E)-4, 8-dimethyl-1,3,7-nonatriene plus acetic acid as a new lure for male and female coding moth (Lepidoptera: Tortricidae). Environm Entomol 40:420-430.

101. Becher PG, Hagman A, Verschut V, Chakraborty A, Rozpedowska E, Lebreton S, Bengtsson M, Flick G, Witzgall P, Piskur J. 2018. Chemical signaling and insect attraction is a conserved trait in yeasts. Ecol Evol 8:2962-2974.
102. Holt S, Miks MH, de Carvalho BT, Foulquié-Moreno MR, Thevelein JM. 2019. The molecular biology of fruity and floral aromas in beer and other alcoholic beverages. FEMS Microbiol Rev 43:193-222.

103. Dhandapani S, Jin J, Sridhar V, Chua NH, Jang IC. 2019. CYP79D73 participates in biosynthesis of floral scent compound 2-phenylethanol in Plumeria rubra. Plant Physiol 180:171-184.

104. Hedges SB, Blair JE, Venturi ML, Shoel JL. 2004. A molecular timescale of eukaryote evolution and the rise of complex multicellular life. BMC Evol Biol 4:2.

105. Taylor JW, Berbee ML. 2006. Dating divergence in the fungal tree of life: review and new analyses. Mycologia 98:838–849.

106. Labandeira CC. 2013. A paleobiologic perspective on plant–insect interactions. Curr Op Plant Biol 16:414-421.

107. Labandeira CC, Currano ED. 2013. The fossil record of plant-insect dynamics. Annu Rev Earth Planet Sc 41:287-311.

108. Pozo MJ, de Vega C, Canto A, Herrera CM. 2009. Presence of yeasts in floral nectar is consistent with the hypothesis of microbial-mediated signaling in plant-pollinator interactions. Plant Signal Behav 4:1102–1104.

109. Aleklett K, Hart M, Shade A. 2014. The microbial ecology of flowers: an emerging frontier in phyllosphere research. Botany 92:253-266.

110. Becher PG, Bengtsson M, Hansson BS, Witzgall P. 2010. Flying the fly: long-range flight behavior of Drosophila melanogaster to attractive odors. J Chem Ecol 36:599-607.

111. Gonzalez F, Witzgall P, Walker WB. 2016. Protocol for heterologous expression of insect odourant receptors in Drosophila. Front Ecol Evol 4:24

112. Cattaneo AM, Gonzalez F, Bengtsson JM, Corey EA, Jacquin-Joly E, Montagné N, Salvagnin U, Walker WB, Witzgall P, Anfora G, Bobkov YV. 2017. Candidate pheromone receptors from the insect pest Cydia pomonella respond to pheromone and kairomone components. Sc Rep 7:4110.

113. Steuer R, Kurths J, Fiehn O, Weckwerth W. 2003. Observing and interpreting correlations in metabolomic networks. Bioinformatics 19:1019-1026.

114. Weckwerth W. 2003. Metabolomics in systems biology. Annu Rev Plant Biol 54:669-689.

115. Thorn RMS, Reynolds DM, Greenman J. 2011. Multivariate analysis of bacterial volatile compound profiles for discrimination between selected species and strains in vitro. J Microbiol Meth 84:258-264.

116. Müller A, Faubert P, Hagen M, zu Castell W, Polle A, Schnitzler JP, Rosenkranz M. 2013. Volatile profiles of fungi–chemotyping of species and ecological functions. Fungal Gen Biol 54:25-33.
117. Bean HD, Dimandja JD, Hill JE. 2012. Bacterial volatile discovery using solid phase microextraction and comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry. J Chromatogr B 901:41-46.

118. Sipiczki M, Horvath E, Pflegler WP. 2018. Birth-and-death evolution and reticulation of ITS segments of Metschnikowia andauensis and Metschnikowia fructicola rDNA repeats. Front Microbiol 9:1193.

119. Jumean Z, Gries R, Unruh T, Rowland E, Gries G. 2005. Identification of the larval aggregation pheromone of codling moth, Cydia pomonella. J Chem Ecol 31:911-924.

120. De Fouchier A, Sun X, Caballero-Vidal G, Travaillard S, Jacquin-Joly E, Montagne N. 2018. Behavioral effect of plant volatiles binding to Spodoptera littoralis larval odorant receptors. Front Behav Neurosci 12:264.

121. Rharrabe K, Jacquin-Joly E, Marion-Poll F. 2014. Electrophysiological and behavioral responses of Spodoptera littoralis caterpillars to attractive and repellent plant volatiles. Front Ecol Evol 2:5.

122. Benelli G, Govindarajan M, Alsalhi MS, Devanesan S, Maggi F. 2018. High toxicity of camphene and γ-elemene from Wedelia prostrata essential oil against larvae of Spodoptera litura (Lepidoptera: Noctuidae). Environm Sc Pollution Res 25:10383-10391.

123. Binyameen M, Anderson P, Iglrell N, Birgersson G, Razaq M, Shad SA, Hansson BS, Schlyter F. 2014. Identification of plant semiochemicals and characterization of new olfactory sensory neuron types in a polyphagous pest moth, Spodoptera littoralis. Chem Senses 39:719-733.

124. Goergen G, Kumar PL, Sankung SB, Togola A, Tamo M. 2016. First report of outbreaks of the fall armyworm Spodoptera frugiperda (JE Smith)(Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PloS One 11:e0165632.

125. Battisti DS, Naylor RL. 2009. Historical warnings of future food insecurity with unprecedented seasonal heat. Science 323:240-244.

126. Wheeler T, von Braun J. 2013. Climate change impacts on global food security. Science 341:508-513.

127. Ehrlich PR, Harte J. 2015. Opinion: to feed the world in 2050 will require a global revolution. Proc Natl Acad Sc USA 112:14743-14744.

128. Witzgall P, Kirsch P, Cork A. 2010. Sex pheromones and their impact on pest management. J chem Ecol 36:80-100

129. Lacey LA. 2017. Microbial control of insect and mite pests: from theory to practice. Academic Press, London.

130. Knight AL, Witzgall P. 2013. Combining mutualistic yeast and pathogenic virus - a novel method for codling moth control. J Chem Ecol 39:1019-1026.
131. Beck JJ, Vannette RL. 2016. Harnessing insect–microbe chemical communications to control insect pests of agricultural systems. J Agric Food Chem 65:23-28.

132. Droby S, Wisniewski M, Macarisin D, Wilson C. 2009. Twenty years of postharvest biocontrol research: is it time for a new paradigm? Postharv Biol Technol 52:137-145.

133. Sharma RR, Singh D, Singh R. 2009. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: a review. Biol Control 50:205-221.

134. Manso T, Nunes C. 2011. Metschnikowia andauensis as a new biocontrol agent of fruit postharvest diseases. Postharv Biol Technol 61:64-71.

135. Knight AK, Basoalto E, Witzgall P. 2015. Improving the performance of the granulosis virus of codling moth (Lepidoptera: Tortricidae) by adding the yeast Saccharomyces cerevisiae with sugar. Environ Entomol 44:252-259.

136. Mori BA, Whitener AB, Leinweber Y, Revadi S, Beers EH, Witzgall P, Becher PG. 2017. Enhanced yeast feeding following mating facilitates control of the invasive fruit pest Drosophila suzukii. J appl Ecol 54:170–177.

137. Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. 2015. The importance of the microbiome of the plant holobiont. New Phytologist 206:1196-1206.

138. Müller DB, Vogel C, Bai Y, Vorholt JA. 2016. The plant microbiota: systems-level insights and perspectives. Annu Rev Genet 50:211-234.

139. Junker RR, Tholl D. 2013. Volatile organic compound mediated interactions at the plant-microbe interface. J Chem Ecol 39:810-825.

140. Leeder AC, Palma-Guerrero J, Glass NL. 2011. The social network: deciphering fungal language. Nature Rev Microbiol 9:440.

141. Busby PE, Soman C, Wagner MR, Friesen ML, Kremer J, Bennett A, Morsy M, Eisen JA, Leach JE, Dangl JL. 2017. Research priorities for harnessing plant microbiomes in sustainable agriculture. PLoS Biol 15:e2001793.

142. White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p 315-322. In PCR protocols: a guide to methods and applications, Innis MA, Gelfand DH, Sninsky JJ, White TJ (ed). Academic Press, New York.

143. Kurtzman CP, Robnett CJ. 2003. Phylogenetic relationships among yeasts of the ‘Saccharomyces complex’ determined from multigene sequence analyses. FEMS Yeast Res 3: 417-432.

144. Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, p 95-98. In Nucleic Acids Symposium Series 41:41.

145. Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: molecular
evolutionary genetics analysis (MEGA) software version 4.0. Molec Biol Evol 24:1596-1599.

146. Saitou N., Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molec Biol Evol 4:406-425.

147. Jukes TH, Cantor CR. 1969. Evolution of protein molecules, p 21-132. In Munro HN (ed), Mammalian protein metabolism, Vol. 3. Academic Press, New York, London.

148. Merico A, Sulo P, Piskur J, Compagno C. 2007. Fermentative lifestyle in yeasts belonging to the *Saccharomyces* complex. FEBS J 274:976-989.

149. Bengtsson M, Bäckman A-C, Liblikas I, Ramirez MI, Borg-Karlson A-K, Ansebo L, Anderson P, Löfqvist J, Witzgall P. 2001. Plant odor analysis of apple: antennal response of codling moth females to apple volatiles during phenological development. J Agric Food Chem 49:3736-3741.

150. Amigo JM, Skov T, Bro R, Coello J, Maspoch S. 2008. Solving GC-MS problems with PARAFAC2. Trends Anal Chem 27:714-725.

151. Jonsson P, Johansson AI, Gullberg J, Trygg J, Grung B, Marklund S, Sjöström M, Antti H, Moritz T. 2005. High-throughput data analysis for detecting and identifying differences between samples in GC/MS-based metabolomic analyses. Anal Chem 77:5635-5642.

152. Hinks CF, Byers JR. 1976. Biosystematics of the genus *Euxoa* (Lepidoptera: Noctuidae): V. Rearing procedures, and life cycles of 36 species. Can Entomol 108:1345-1357.
Figure legends

**FIG 1** Phylogenetic tree of the yeasts used for volatile analysis (bold-faced) and sequences deposited in the NCBI database, based on the nucleotide sequences of the D1/D2 domain of the 26S rDNA, constructed according to the neighbor-joining method with bootstrap values >50%. Asterisks denote the two isolates of *M. fructicola* corresponding to replicates 1 to 6 and 7 to 12 in Figure 2.

**FIG 2** Volatile compounds identified from headspace collections of fermenting yeasts by GC-MS (see also Supplemental Table S1). Compounds were grouped according to hierarchical cluster analysis (HCA), circles depict relative abundance of compounds after normalization by abundance across species (rows) and observations (columns). Yeast species listed according to the phylogenetic tree (Figure 1). Abbreviations see Figure 1.

**FIG 3** Groups of yeast volatiles according to orthogonal partial least square discriminant analysis (OPLS-DA). The first three principal components represent 22.9 %, 14.7 % and 9.6 % of the total variation in the dataset of eight yeast species. Abbreviations see Figure 1.

**FIG 4** Larval feeding assay and class separation, according to orthogonal partial least square discriminant analysis (OPLS-DA). (a) Bars show the response index RI for larval attraction and feeding (RI>0) and avoidance (RI<0) in response to eight yeasts. Asterisks show significance according to Student’s t-test (P<0.05 and P<0.01, respectively); (b) OPLS-DA score plot of M3 with yeasts classified according to larvae response. Component 1 and 2 are predictive. Yeasts separate according to
behavioral effect, repellency, feeding and no effect. The outline ellipse shows Hotelling’s $T^2$ (95%) limit.

**FIG 5** Shared and unique structures plot (SUS-plot) featuring metabolites of the most and least preferred yeasts for cotton leafform larval feeding, *C. nemorosus* and *M. andauensis* (Figure 4). Correlation from the predictive components of the two models, $\text{Corr}(t_p,X)$, are plotted against each other. Unique volatiles (top left and bottom right quadrants) are oppositely affected for both *M. andauensis* and *C. nemorosus*. Compounds similarly affected in all yeasts are located along the diagonal running through the shared effect quadrants. Unique, significant volatiles are shown for *M. andauensis* (blue circles) and *C. nemorosus* (red circles).
Metschnikowia fructicola **
M. fructicola *
M. andauensis (MA)
M. pulcherrima (MP)
M. saccharicola (MS)
M. lopburiensis (ML)
M. hawaiiensis (MH)
M. andauensis AJ745110
M. fructicola EU373454
M. pulcherrima KY108497
M. saccharicola AB697752
M. lopburiensis AB697756
M. hawaiiensis KY108477
C. nemorosus (CN)
C. nemorosus KF830191
Cryptococcus tephrensis JQ688991
Saccharomyces cerevisiae JQ914745 (SC)
Avoidance (RI<0) Feeding (RI>0)

Component 1 (Q2 = 0.43)
Component 2 (Q2 = 0.38)
Feeding Avoidance
n.s.

-6
-4
-2
0
2
4
6
-8 -6 -4 -2 0 2 4 6 8
A B
C. nemorosus vs. other species except M. anduaensis

Shared +/+

C. nemorosus

Unique

M. anduaensis

Unique

Cyclohexaneone

Geranyl acetone

1,3,5-Undecatriene

2-Ethyl-1-benzofuran

Geranyl acetone

Nonan-2-ol

2-Phenyl ethanal

Methanol

2-Phenylethyl acetate

3-Methylbutyl propanoate

3-Methylpentan-1-ol

Isobutanoic acid

Unknown 147

Unknown 159

2-Methylbutyl 2-methyl-butanoate

2-Phenyl-2-methylpropanoate

1,3,5-Undecatriene

Cyclohexaneone

Geranyl acetone

1,3,5-Undecatriene