AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology®

INVERTEBRATE MICROBIOLOGY

Yeast Volatomes Differentially Affect Larval Feeding in an Insect Herbivore

Joel Ljunggren,[a] Felipe Borrero-Echeverry,[b*] Amrita Chakraborty,[b*] Tobias U. T. Lindblom,[b*] Erik Hedenström,[a] Maria Karlsson,[c] Peter Witzgall,[b] Marie Bengtsson[b]

[a]Department of Chemical Engineering, Mid Sweden University, Sundsvall, Sweden

[b]Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden

[c]Department of Horticulture, Swedish University of Agricultural Sciences, Alnarp, Sweden

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 used gas chromatography-mass spectrometry, followed by hierarchical cluster and orthogonal partial least-squares discriminant analyses, 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 (*Spodoptera littoralis*) larvae 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 (2*E*,4*Z*)-deca-2,4-dienoate (pear ester), (3*E*)-4,8-dimethylnona-1,3,7-triene (DMNT), linalool, α-terpineol, β-myrcene, or (*E*,*E*)-α-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, as 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, from flowers or fruit, and from plant-feeding insects. These yeasts 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 behavioral 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

Citation Ljunggren J, Borrero-Echeverry F, Chakraborty A, Lindblom TUT, Hedenström E, Karlsson M, Witzgall P, Bengtsson M. 2019. Yeast volatomes differentially affect larval feeding in an insect herbivore. Appl Environ Microbiol 85:e01761-19. https://doi.org/10.1128/AEM.01761-19.

Editor Irina S. Druzhinina, Nanjing Agricultural University

Address correspondence to Peter Witzgall, peter.witzgall@slu.se, or Marie Bengtsson, marie.bengtsson@slu.se.

* Present address: Felipe Borrero-Echeverry, Corporación Colombiana de Investigación Agropecuaria–AGROSAVIA, Mosquera, Colombia; Amrita Chakraborty, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Prague, Czech Republic; Tobias U. T. Lindblom, Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Ultuna, Sweden.

J.L. and F.B.-E. contributed equally to this article.

Received 31 July 2019
Accepted 18 August 2019
Accepted manuscript posted online 23 August 2019
Published 16 October 2019

Microorganisms enable insect feeding and development on plants (1–4). Invisibly, they 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. The wide overlap in compounds released by plants (18) and microbes (19) further suggests that plant headspace includes volatiles that are produced by plant-associated epiphytic and endophytic microbes. A striking example is floral scent: 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, correspondingly, 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 sought to determine whether taxonomically related yeasts, isolated from different insects and habitats, differ with respect to their volatile metabolomes and whether cotton leafworm larvae behave differently toward 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 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 behavioral 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 brewer's yeast (*Saccharomyces cerevisiae*)—is shown in Fig. 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, and five sulfur- and three nitrogen-containing volatiles (Fig. 2; see also Table S1 in the supplemental material). Of the 192 compounds found, 26 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 used for yeast growing and headspace collection. However, species could still be separated according to headspace composition by

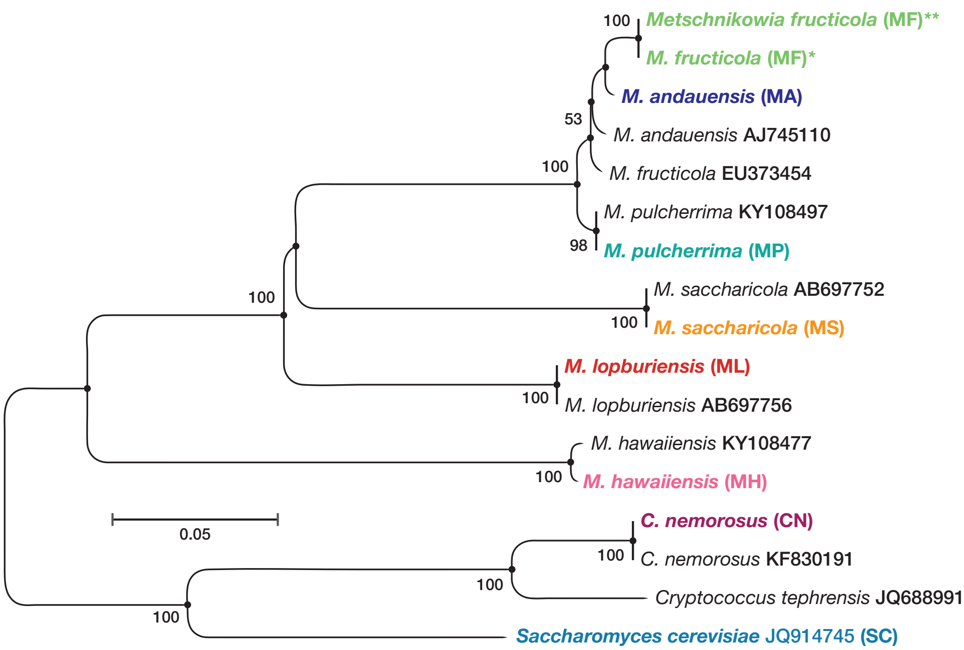

FIG 1 Phylogenetic tree of the yeasts used for volatile analysis (boldfaced text) and sequences deposited in the NCBI database, based on the nucleotide sequences of the D1/D2 domain of the 26S rDNA, constructed according to the NJ method with bootstrap values of >50%. Asterisks denote the two isolates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

a discriminant analysis (OPLS-DA M1, $R^2X_{(cum)} = 0.768$; $R^2Y_{(cum)} = 0.918$; $Q^2_{(cum)} = 0.756$) resulting in seven 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, Fig. 2 further visualizes 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 a few nucleotides (61–63). The close relation between these three species (Fig. 1) is in line with their volatome composition in comparison to the more distantly related *Metschnikowia* species (Fig. 2 and 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*; Fig. 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; Fig. 1).

A three-dimensional score plot of the first three predictive components of M1 shows that *M. hawaiiensis* and *M. saccharicola* clearly separate according to the first three dimensions and that the other species are clustering with little overlap (Fig. 3). The other species diverged in the remaining dimensions and by HCA of the M1 scores (data not shown). Internal model robustness validation was performed by randomly excluding three observations for each yeast. In order 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 a prediction set in a new model made of the remaining observations [OPLS-DA M2, $R^2X_{(cum)} = 0.686$; $R^2Y_{(cum)} = 0.879$; $Q^2_{(cum)} = 0.596$]. A zero misclassification error (Fisher probability $= 2.8 \times 10^{-10}$) further corroborated the robust ability of OPLS-DA to distinguish between yeast headspace profiles.

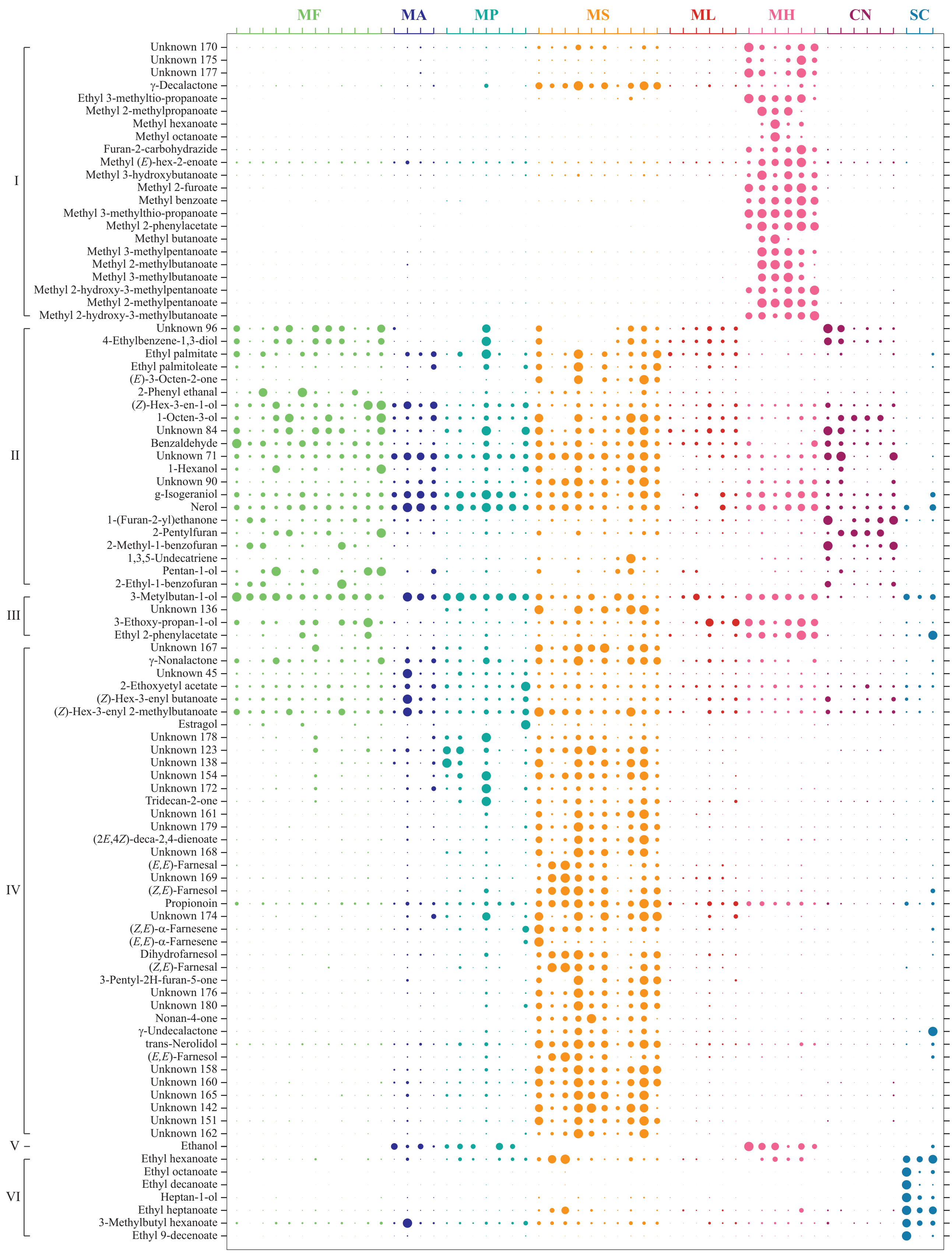

FIG 2 Volatile compounds identified from headspace collections of fermenting yeasts by GC-MS (see also Table S1 in the supplemental material). Compounds were grouped according to hierarchical cluster analysis. Circles depict the relative abundance of compounds after normalization by abundance across species (rows) and observations (columns). Yeast species are listed according to the phylogenetic tree (Fig. 1). For abbreviations, see Fig. 1.

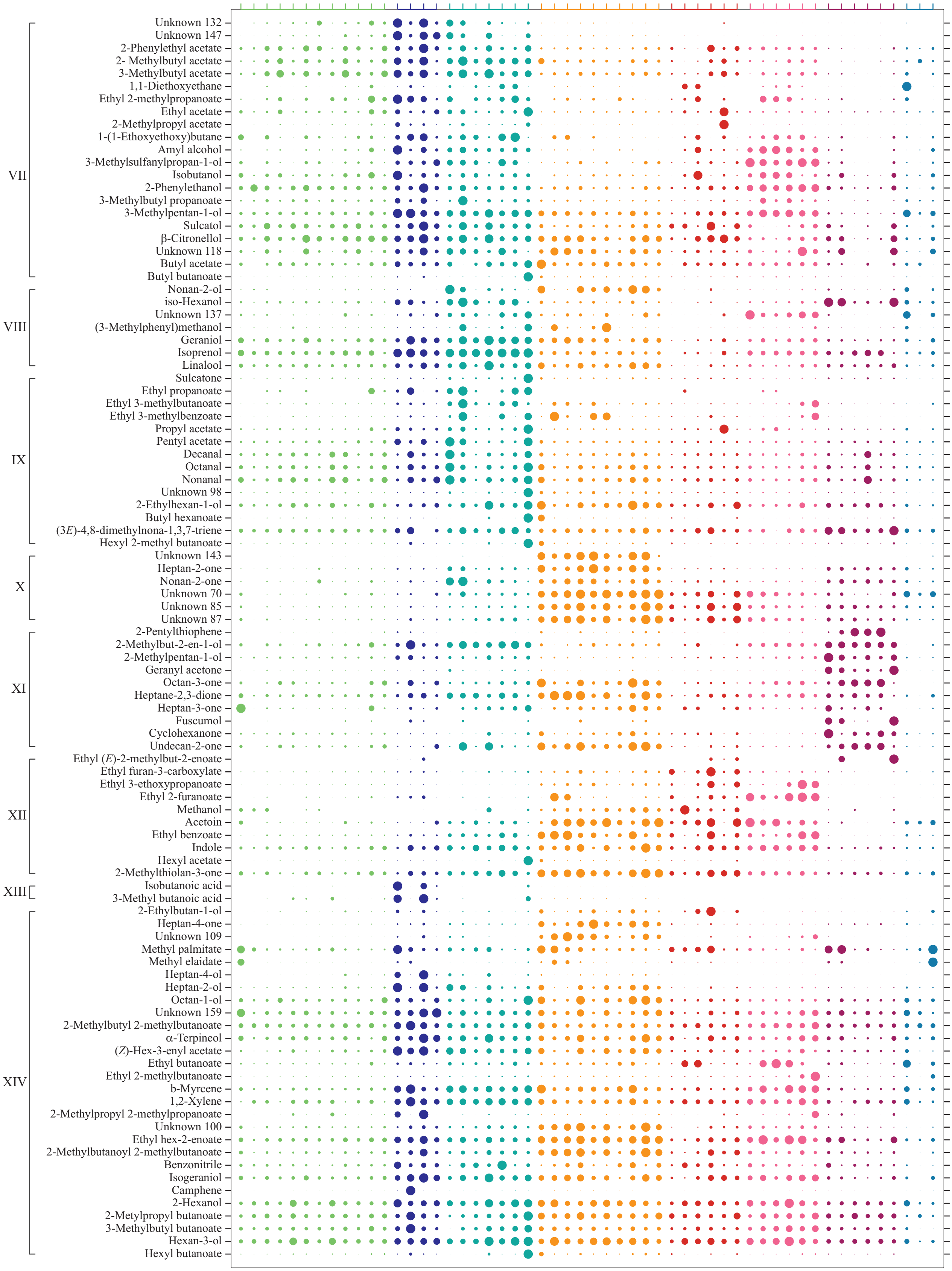

FIG 2 (Continued)

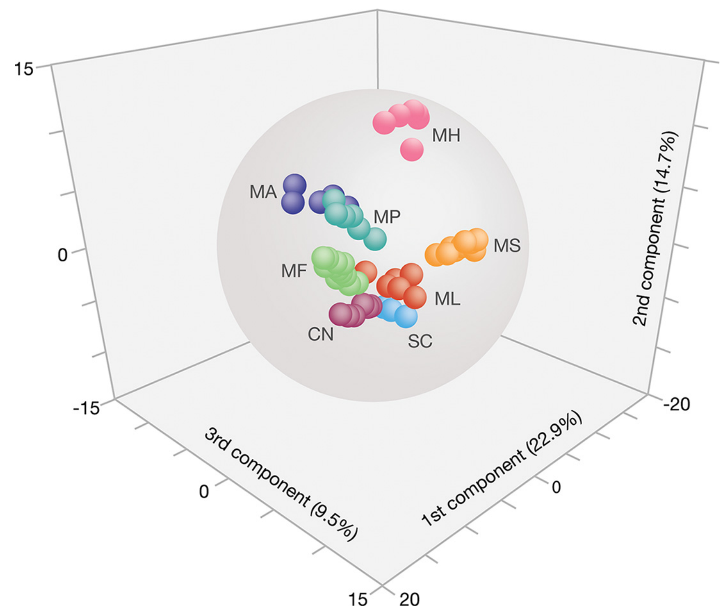

FIG 3 Groups of yeast volatiles according to OPLS-DA. The first three principal components represent 22.9, 14.7, and 9.5% of the total variation in the data set of eight yeast species. For abbreviations, see Fig. 1.

***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 (Fig. 2). They separated clearly, according to OPLS-DA, from each other and the other yeasts (Fig. 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 (Table S1) (17, 19). 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 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 (Fig. 2).

The volatome of *M. hawaiiensis* was clearly separated from the other *Metschnikowia* spp. (Fig. 2 and 3), and methyl esters were key compounds in headspace class separation (Table S2). Perhaps coincidentally, two sulfur-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*.** Three very closely related species (Fig. 1) (63)—*M. andauensis*, *M. fructicola*, and *M. pulcherrima*—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-metylbutan-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* (Fig. 2; Table S2).

***Cryptococcus nemorosus*.** *Cryptococcus* is polyphyletic, and several species, such as *C. nemorosus*, have been isolated from the plant phyllosphere and soil (73, 74).

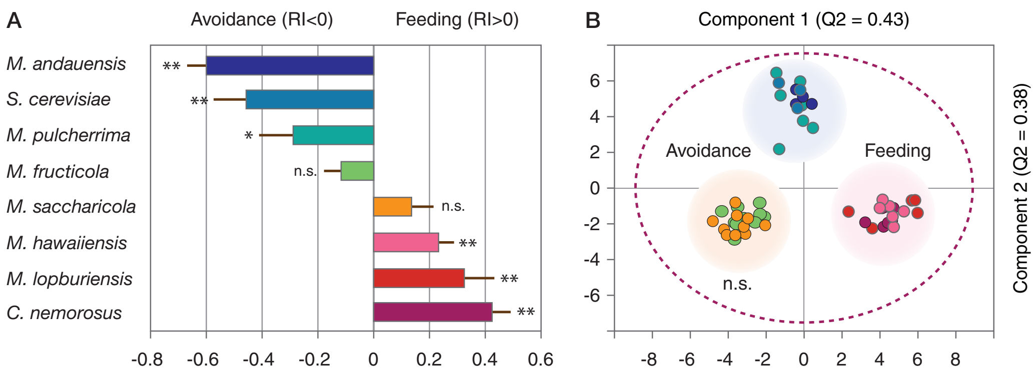

FIG 4 Larval feeding assay and class separation according to OPLS-DA. (A) Bars show the response index (RI) for larval attraction and feeding (RI $>$ 0) and avoidance (RI $<$ 0) in response to eight yeasts. * and ** indicate the significance according to a Student *t* test ($P < 0.05$ and $P < 0.01$, respectively). (B) OPLS-DA score plot of M3, with yeasts classified according to larval response. Components 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.

Ethyl (*E*)-2-methylbut-2-enoate, aliphatic ketones, and aliphatic methyl-branched primary alcohols were the main volatiles that separated *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 highly correlate with the class membership of *M. andauensis* (Table S2).

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 (Fig. 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 behavioral activity. Three classes (shown in Fig. 4B) were used, which resulted in a model with two predictive and five orthogonal components [OPLS-DA M3, $R^2X_{(cum)} = 0.68$; $R^2Y_{(cum)} = 0.947$; $Q^2_{(cum)} = 0.812$]. Model M3 showed excellent classification performance (Fisher probability = 2.5×10^{-19}). When plotting the two OPLS-DA predictive components, three groups separate clearly, showing that these yeasts can be distinguished with respect to their behavioral effect (Fig. 4B).

Among the eight yeast species, *M. andauensis* and *C. nemorosus* exhibited the strongest activity, resulting in larval avoidance and feeding, respectively (Fig. 4A). The volatile profiles of these yeasts show considerable overlap with the other species (Fig. 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 for this purpose. Volatiles of all species except *M. andauensis* were modeled against *C. nemorosus*, eliciting the highest rate of feeding which resulted in a model with two predictive and four orthogonal components [OPLS-DA M4, $R^2X_{(cum)} = 0.608$; $R^2Y_{(cum)} = 0.984$; $Q^2_{(cum)} = 0.933$]. *Metschnikowia andauensis*, which strongly deterred larvae from feeding, was likewise modeled against all other species except *C. nemorosus* [OPLS-DA M5, $R^2X_{(cum)} = 0.69$; $R^2Y_{(cum)} = 0.983$; $Q^2_{(cum)} = 0.572$]. 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 (Fig. 5) assigns two acids, several methyl branched esters, camphene, and the two unknown compounds

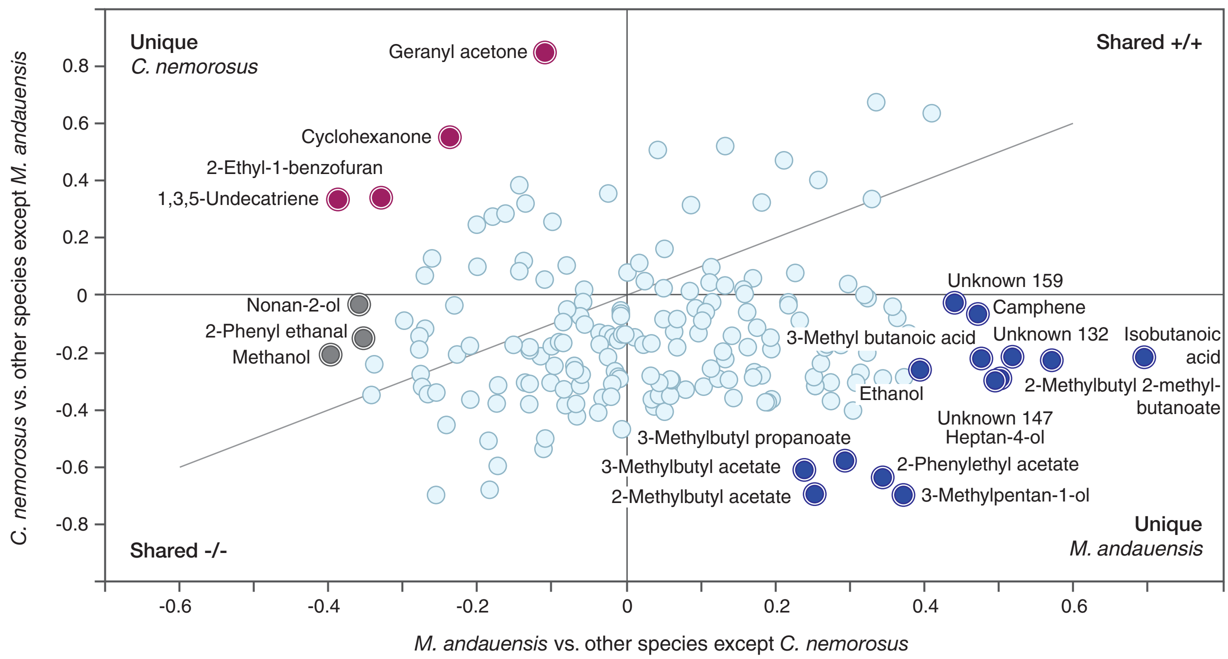

FIG 5 Shared and unique structures (SUS) plot featuring metabolites of the most and least preferred yeasts for cotton leafworm larval feeding, *C. nemorosus* and *M. andauensis* (Fig. 4). Correlations from the predictive components of the two models, Corr(t_p,X), are plotted against each other. Uniquely affected volatiles in *C. nemorosus* (red circles) and *M. andauensis* (blue circles) are shown in the top left and bottom right quadrants, respectively. Compounds similarly affected in all yeasts are located along the diagonal running through the shared effect quadrants.

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. The 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 (Fig. 4). Figure 2 illustrates that yeast headspace is at least as rich and complex as headspace of their food plants (81, 82) and that also many yeast compounds, including terpenoids, are shared by these plants. This raises the question of whether the larvae of insect herbivores become attracted to plant or to yeast odor 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 of to which extent yeast versus 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, the grape moth *Paralobesia viteana* was attracted to grape leaves after microbial colonies were washed off, but the participating role of microbes remaining on foliage or endophytes is yet unresolved (83).

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 (3*E*)-4,8-dimethylnona-1,3,7-triene (DMNT), which play an important role in the attraction of *P. viteana* or the grape berry moth *Lobesia botrana*, are all produced by several yeasts (Fig. 2) (83, 84). Likewise, compounds from cotton headspace that elicit antennal or behavioral responses in cotton leafworm *S. littoralis* are also produced by yeasts (Fig. 2) (81, 85). 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, 82, 86).

DMNT is also a floral scent component across a wide range of plants (87) and an attractant of flies and moths (84, 88–90). Yeasts obviously contribute to DMNT release from flowers, since DMNT was found in all of the yeasts studied here (Fig. 2). In addition to DMNT, a wide range of volatiles cooccurs in yeasts and angiosperm flowers, for example, the typical *Drosophila* attractants acetoin, ethanol, ethyl acetate, 2-phenylethyl acetate, and 2-phenylethanol (Fig. 2) (91). 2-Phenylethanol, a typical yeast odorant (see, for example, reference 92), is also produced by green plants (see, for example, reference 93). In addition to 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 (Fig. 2; Table S1).

Insect-yeast chemical communication evolved long before the emergence of flowering plants. Fungivory and herbivory on plants was initiated during the Early Devonian (~400 million years ago [Ma]) concurrent with the appearance of budding yeasts and prior to angiosperm pollination syndromes during the Cretaceous period (∼100 Ma) (94–97). 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, 91, 98, 99).

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 toward identifying the active compounds. Of the 192 volatiles (Fig. 2; Table S1), 33 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, toward the identification of the behaviorally active compounds (100–102).

The overall species-specific volatome patterns showed variation between replicates, even though growth conditions were strictly controlled and sampling intervals were adjusted to cancel out growth-stage variations (Fig. 2). A general assumption in metabolomics is that identical genotypes produce the same steady-state metabolite concentrations under stringent conditions, whereas 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 (103–105).

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 (Fig. 2 and 3) which align with the phylogenetic analysis based on sequences from the D1/D2 region (Fig. 1) and yeast taxonomy and ecology (Fig. 1) (63, 76). Moreover, we found that OPLS-DA exhibited a robust yeast-species assignment of headspace samples and is therefore a useful tool for studying and classifying unknown species with regard to their volatiles (Fig. 3).

Volatile fingerprinting or chemotyping has previously been shown to differentiate between ectomycorrhizal, pathogenic, and saprophytic fungi, as a complement to genotyping (106, 107). This was confirmed by comparing *M. fructicola* and *M. andauensis*, which are taxonomically close and difficult to discriminate according to genotyping (Fig. 1) (108). They quantitatively and clearly separated according to headspace proportions, in addition to the production of methanol by *M. fructicola* (Fig. 2 and 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. tephrensis*. Comparison of headspace data with *M. fructicola* evidenced overlap of 101 compounds with significant coefficient values and nonzero confidence intervals for the whole data set. Subsequent DNA analysis identified this yeast as *M. fructicola* (Fig. 1).

The species-specific volatome differences are corroborated by a selective larval feeding response (Fig. 4), where cotton leafworm larvae, which are typical foliage feeders, prefer phyllosphere yeasts over the yeasts associated with fruit and frugivorous insects. Larvae avoided brewer'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 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 (109), is a distinctive compound for *C. nemorosus* (Fig. 2 and 5), which elicited the strongest larval feeding response (Fig. 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 (110). 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* (Fig. 2, 4, and 5).

At the same time, our study reveals potential antifeedants. Camphene has indeed already been reported as a repellant in *S. littoralis* (111), and its acute larval toxicity has been shown in the sister species *S. litura* (112). In addition, *S. littoralis* females detect camphene, as well as 3-methylbutyl acetate (113), both of which are sign compounds for *M. andauensis* (Fig. 2, 4, and 5). Discriminant analysis points toward 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, 114). Global change and increasing food insecurity render insect control an ever more challenging and urgent task (51, 115–117). Detrimental environmental and health effects warrant the 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 (118, 119), but semiochemicals could be combined with pathogens into lure-and-kill strategies (57, 58, 120). The 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, 121).

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 (122–124). The 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 *Drosoph-*

ila (120, 125, 126). For further improvement, the identification of key compounds mediating insect attraction will facilitate the selection of yeast species and strains.

Yeast volatiles are also antifungal (122, 123) and may directly, or through other members of the plant microbiome, impact 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 (127, 128). Odorants are essential in regulating mutual and detrimental colonizers in plant microbial networks (6, 7, 129) 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 (Fig. 2; Table S1) (16, 130). Integrating plant microbiomes in crop protection concepts for insect control, enhanced stress tolerance, and disease resistance is a future challenge in agriculture (131).

MATERIALS AND METHODS

Yeasts. *Metschnikowia* yeasts were purchased from the CBS-KNAW collection (Utrecht, Netherlands), except *M. fructicola*, which was isolated from apples (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*, as well as occasionally *M. fructicola*, were found in apple and larval feces of codling moth *Cydia pomonella* (Lepidoptera, Tortricidae) (41). *M. saccharicola* and *M. lopburiensis* were isolated from foliage in Thailand (65), and *M. hawaiiensis* was obtained 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 medium. Then, 2-ml portions of the overnight cell cultures were pelleted (13,000 rpm for 1 min) and washed with sterile double-distilled water (ddH_2O). The pellets were resuspended in 200 μl of lysis buffer (2% Triton X-100, 1% sodium dodecyl sulfate, 0.1 M NaCl, 10 mM Tris, 1 mM EDTA; pH 8), and 200 μl of phenol-chloroform-isoamyl alcohol (25:24:1) mixture. Glass beads (200 μl) were then added to the tubes, and the samples were vortexed thoroughly for 3 min. To this mixture, 200 μl of TE buffer (10 mM Tris-Cl, 1 mM EDTA; pH 8.0) was added, followed by centrifugation at 13,000 rpm for 10 min. The upper aqueous phase was collected, 10 μl of RNase A (10 mg/ml) was added, and the mixtures were incubated at 37°C during 45 min. The DNA was precipitated using 300 mM sodium acetate and 3 volumes of cold absolute ethanol (99.9%), followed by centrifugation 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, resuspended in 30 μl of TE buffer, and stored at −20°C until use.

The D1/D2 domain of the 26S rRNA gene was amplified with the universal primer pair NL-1 and NL-4 and the internal transcribed spacer (ITS) region with the primer combination ITS-1 and ITS-4 (132, 133). The PCR and product visualization on agarose gel were performed as previously described (91). PCR products were purified using ExoStar-IT (USB Corp.) according to the manufacturer's protocol and sequenced using a BigDye v.1.1 terminator sequencing kit in an ABI Prism 3130 genetic analyzer (Applied Biosystems, Fair Lawn, NJ).

All sequences were aligned in the program Bioedit 7.0.9.0 Sequence Alignment Editor (134). Each sequence was tested for identity and similarity against sequences deposited in the National Center for Biotechnology Information (NCBI) using Megablast search (accession numbers KF830191 and KC411961). Only similarities of >95% were considered. The phylogenetic tree of the yeast species investigated, based on nucleotide sequences of the D1/D2 domain of 26S ribosomal DNA (rDNA), was constructed using the neighbor-joining (NJ) method in MEGA 7.0 (135). The NJ method, based on the evolutionary distance data that minimize the total branch length during clustering of operational taxonomic units, is efficient and reliable for phylogenetic reconstructions (136). The evolutionary distances were calculated according to the Jukes-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, and C) to be equal (137). The bootstrap values for the phylogenetic tree reconstruction were determined from 1,000 replications and are given next to the branch (Fig. 1).

Headspace collection and chemical analysis. Yeasts were grown in 100 ml of liquid minimal medium (138) in 250-ml culture flasks for 24 h in a shaking incubator (25°C, 260 rpm). Yeast headspace was collected by drawing charcoal-filtered air (0.125 liter/min) through a 1-liter gas wash bottle containing the yeast broth over a 35-mg SuperQ trap (80/100 mesh; Alltech, Deerfield, IL), which was held between plugs of glass wool in a 4-by-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 of activated charcoal) for incoming air and the SuperQ trap were connected with glass fittings to the wash bottle. All glassware was heated to 375°C for 10 h before use (139).

After volatile collections, the trap was extracted with 0.5 ml of redistilled hexane. Sample volumes were reduced to ca. 50 μl at an ambient temperature in Francke vials with an elongated tip (5 cm by 2 mm, inner diameter). Samples were stored in sealed glass capillary tubes at −19°C. The SuperQ trap was wrapped in aluminum 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 analyzed on a coupled gas chromatograph-mass spectrometer (GC-MS; 6890 GC and 5975 MS; Agilent Technologies, Palo Alto, CA), operated in the electron impact ionization mode at 70 eV. The GC was equipped with fused silica capillary columns (30 m by 0.25 mm; df = 0.25 μm), DB-Wax (J&W Scientific, Folsom, CA) or HP-5MS (Agilent Technologies). Helium was used as the mobile phase at an average linear flow rate of 35 cm/s. Two microliters 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), the PARAFAC2 model (140), and the noncommercial package HDA (v0.910; P. Johansson, Umeå University, Umeå, Sweden), based on the H-MCR method (141). Both methods utilize covariation between samples to separate coeluting components and to pool mass spectra across samples, affording unambiguous spectra even at low signal-to-noise ratios. Approximately 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 to the National Institute of Standards and Technology (NIST; v14) 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 (142) under a 16-h light/8-h dark photoperiod at 24°C and 50 to 60% relative humidity.

Larval feeding assay. Yeasts (*M. andauensis*, *M. fructicola*, *M. hawaiiensis*, *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 (138) for 20 h in a shaking incubator (25°C, 260 rpm). The optical density at 595 nm was between 1.5 and 1.8, and cell counts were adjusted to 1.5×10^7 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, ~1 cm from the edge of a plastic petri dish (92-mm diameter, no. 82.1472, Sarstedt AG & Co., Nümbrecht, Germany). Colorants, blue and green (Oetker Sverige AB, Göteborg, Sweden), 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; $P = 0.222$ [analysis of variance]).

Ten starved neonate larvae were collected 24 to 36 h after hatching and were placed in the center 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 performed for each yeast. The larval 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 pretreatment 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. HCA, OPLS-DA, and PLS-DA and its corresponding validation tests were calculated with SIMCA 13.0.3 (Sartorius Stedim Data Analytics AB, Umeå, Sweden).

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 using randomization of the class vector was applied 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 a Student *t* test, with the level of significance set to $P = 0.05$.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.01761-19.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

This study was supported by the Linnaeus environment Insect Chemical Ecology, Ethology, and Evolution (IC-E3; Formas, SLU); the Västernorrland County Administrative

Board; the European Regional Development Fund of the European Union; and the Faculty of Landscape Architecture, Horticulture, and Crop Production Science (SLU, Alnarp, Sweden).

We gratefully acknowledge the Swedish Metabolomics Centre, Umeå, Sweden, for access to GC-MS evaluation software and helpful discussions. An anonymous reviewer and Cyrus Mahmoudi (comgraphix, Germany) provided constructive comments.

REFERENCES

1. Janson EM, Stireman JO, Singer MS, Abbot P. 2008. Phytophagous insect-microbe mutualisms and adaptive evolutionary diversification. Evolution 62:997–1012. https://doi.org/10.1111/j.1558-5646.2008.00348.x.
2. Biere A, Bennett AE. 2013. Three-way interactions between plants, microbes, and insects. Funct Ecol 27:567–573. https://doi.org/10.1111/1365-2435.12100.
3. Hansen AK, Moran NA. 2014. The impact of microbial symbionts on host plant utilization by herbivorous insects. Mol Ecol 23:1473–1496. https://doi.org/10.1111/mec.12421.
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. https://doi.org/10.1146/annurev-phyto-080516-035319.
5. Davis TS, Crippen TL, Hofstetter RW, Tomberlin JK. 2013. Microbial volatile emissions as insect semiochemicals. J Chem Ecol 39:840–859. https://doi.org/10.1007/s10886-013-0306-z.
6. Leach JE, Triplett LR, Argueso CT, Trivedi P. 2017. Communication in the phytobiome. Cell 169:587–596. https://doi.org/10.1016/j.cell.2017.04.025.
7. Carthey AJ, Gillings MR, Blumstein DT. 2018. The extended genotype: microbially mediated olfactory communication. Tr Ecol Evol 33:885–894. https://doi.org/10.1016/j.tree.2018.08.010.
8. Schulz S, Dickschat JS. 2007. Bacterial volatiles: the smell of small organisms. Nat Prod Rep 24:814–842. https://doi.org/10.1039/b507392h.
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. https://doi.org/10.1021/np300468h.
10. Kramer R, Abraham WR. 2012. Volatile sesquiterpenes from fungi: what are they good for? Phytochem Rev 11:15–37. https://doi.org/10.1007/s11101-011-9216-2.
11. Heddergott C, Calvo AM, Latgé JP. 2014. The volatome of *Aspergillus fumigatus*. Eukaryot Cell 13:1014–1025. https://doi.org/10.1128/EC.00074-14.
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. https://doi.org/10.1016/j.fbr.2016.06.004.
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. https://doi.org/10.1111/j.1755-0238.2005.tb00285.x.
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. https://doi.org/10.1016/j.foodchem.2007.09.032.
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. Anal Chim Acta 701:98–111. https://doi.org/10.1016/j.aca.2011.06.006.
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:S95–S128. https://doi.org/10.1093/femsre/fux031.
17. Ramirez-Gaona M, Marcu A, Pon A, Guo AC, Sajed T, Wishart NA, Karu N, Feunang YD, Arndt D, Wishart DS. 2017. YMDB 2.0: a significantly expanded version of the yeast metabolome database. Nucleic Acids Res 45:D440–D445. https://doi.org/10.1093/nar/gkw1058.
18. Knudsen JT, Tollsten L, Bergström LG. 1993. Floral scents: a checklist of volatile compounds isolated by head-space techniques. Phytochemistry 33:253–280. https://doi.org/10.1016/0031-9422(93)85502-I.
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:D1261–D1265. https://doi.org/10.1093/nar/gkx1016.
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. Sci Rep 4:6727. https://doi.org/10.1038/srep06727.
21. Raguso RA. 2004. Why are some floral nectars scented? Ecology 85:1486–1494. https://doi.org/10.1890/03-0410.
22. Helletsgruber C, Dötterl S, Ruprecht U, Junker RR. 2017. Epiphytic bacteria alter floral scent emissions. J Chem Ecol 43:1073–1077. https://doi.org/10.1007/s10886-017-0898-9.
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. https://doi.org/10.1111/1365-2435.12762.
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 Phytol 220:750–759. https://doi.org/10.1111/nph.14809.
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 Sci 9:1009. https://doi.org/10.3389/fpls.2018.01009.
26. Brysch-Herzberg M. 2004. Ecology of yeasts in plant–bumblebee mutualism in Central Europe. FEMS Microbiol Ecol 50:87–100. https://doi.org/10.1016/j.femsec.2004.06.003.
27. Ganter PF. 2006. Yeast and invertebrate associations, p 303–370. *In* Rosa C, Péter G (ed), Biodiversity and ecophysiology of yeasts. Springer, Berlin, Germany.
28. Stefanini I, Dapporto L, Legras JL, Calabretta A, Di Paola M, De Filippo C, Viola R, Capretti P, Polsinelli M, Turillazzi S, Cavalieri D. 2012. Role of social wasps in *Saccharomyces cerevisiae* ecology and evolution. Proc Natl Acad Sc U S A 109:13398–13403. https://doi.org/10.1073/pnas.1208362109.
29. Stefanini I. 2018. Yeast-insect associations: it takes guts. Yeast 35:315–330. https://doi.org/10.1002/yea.3309.
30. Guzman B, Lachance MA, Herrera CM. 2013. Phylogenetic analysis of the angiosperm-floricolous insect-yeast association: have yeast and angiosperm lineages co-diversified? Mol Phylogen Evol 68:161–175. https://doi.org/10.1016/j.ympev.2013.04.003.
31. Blackwell M. 2017. Made for each other: ascomycete yeasts and insects. Microbiol Spectrum 5:FUNK-0081-2016.
32. 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, Switzerland.
33. 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. https://doi.org/10.1371/journal.pone.0075332.
34. 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. https://doi.org/10.1016/j.celrep.2014.09.009.
35. 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 Exp Appl 167:220–230. https://doi.org/10.1111/eea.12752.
36. Stensmyr MC, Giordano E, Balloi A, Angioy AM, Hansson BS. 2003. Novel natural ligands for *Drosophila* olfactory receptor neurones. J Exp Biol 206:715–724. https://doi.org/10.1242/jeb.00143.
37. Arguello JR, Sellanes C, Lou YR, Raguso RA. 2013. Can yeast (*S. cerevisiae*) metabolic volatiles provide polymorphic signaling? PLoS One 8:e70219. https://doi.org/10.1371/journal.pone.0070219.

38. Münch D, Galizia CG. 2016. DoOR 2.0: comprehensive mapping of *Drosophila melanogaster* odorant responses. Sci Rep 6:21841. https://doi.org/10.1038/srep21841.
39. 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.
40. Becher PG, Flick G, Rozpędowska E, Schmidt A, Hagman A, Lebreton S, Larsson MC, Hansson BS, Piškur 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. https://doi.org/10.1111/j.1365-2435.2012.02006.x.
41. Witzgall P, Proffit M, Rozpedowska E, Becher PG, Andreadis S, Coracini M, Lindblom TUT, Ream LJ, Hagman A, Bengtsson M, Kurtzman CP, Piskur J, Knight A. 2012. "This is not an apple": yeast mutualism in codling moth. J Chem Ecol 38:949–957. https://doi.org/10.1007/s10886-012-0158-y.
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. Sci Rep 5:14059. https://doi.org/10.1038/srep14059.
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. https://doi.org/10.1007/s10886-016-0780-1.
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. Environ Entomol 47:1553–1559. https://doi.org/10.1093/ee/nvy139.
45. Berlocher SH, Feder JL. 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? Annu Rev Entomol 47:773–815. https://doi.org/10.1146/annurev.ento.47.091201.145312.
46. Bruce TJ, Pickett JA. 2011. Perception of plant volatile blends by herbivorous insects–finding the right mix. Phytochemistry 72:1605–1611. https://doi.org/10.1016/j.phytochem.2011.04.011.
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. https://doi.org/10.1111/evo.13571.
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 Bot 62:3251–3261. https://doi.org/10.1093/jxb/err064.
49. Verger PJ, Boobis AR. 2013. Reevaluate pesticides for food security and safety. Science 341:717–718. https://doi.org/10.1126/science.1241572.
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. https://doi.org/10.1038/nature13531.
51. Deutsch CA, Tewksbury JJ, Tigchelaar 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. https://doi.org/10.1126/science.aat3466.
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 Sec 10:1203–1219. https://doi.org/10.1007/s12571-018-0839-7.
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. https://doi.org/10.1023/A:1022451020013.
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 Natl Acad Sci U S A 104:8374–8378. https://doi.org/10.1073/pnas.0702813104.
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 16:153–157. https://doi.org/10.1007/s12257-010-0127-7.
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. https://doi.org/10.1007/s10886-013-0278-z.
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. https://doi.org/10.1603/029.102.0301.
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. https://doi.org/10.1146/annurev-ento-031616-035040.
59. Bailes EJ, Ollerton J, Pattrick JG, Glover BJ. 2015. How can an understanding of plant–pollinator interactions contribute to global food security? Curr Opin Plant Biol 26:72–79. https://doi.org/10.1016/j.pbi.2015.06.002.
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 Phylogenet Evol 65:855–870. https://doi.org/10.1016/j.ympev.2012.08.006.
61. Kurtzman CP, Droby S. 2001. *Metschnikowia fructicola*, a new ascosporic yeast with potential for biocontrol of postharvest fruit rots. Syst Appl Microbiol 24:395–399. https://doi.org/10.1078/0723-2020-00045.
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. https://doi.org/10.1016/j.syapm.2005.05.009.
63. Lachance MA. 2011. *Metschnikowia kamienski* (1899), p 575–620. *In* Kurtzman CP, Fell JW, Boekhout T (ed), The yeasts, 5th ed. Elsevier, Amsterdam, Netherlands.
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 Syst Evol Microbiol 40:415–420. https://doi.org/10.1099/00207713-40-4-415.
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. https://doi.org/10.1007/s10482-012-9774-3.
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. https://doi.org/10.1111/j.1365-2621.1964.tb00439.x.
67. Miller RL, Bills DD, Buttery RG. 1989. Volatile components from Bartlett and Bradford pear leaves. J Agric Food Chem 37:1476–1479. https://doi.org/10.1021/jf00090a005.
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.). Naturwissenschaften 88:333–338. https://doi.org/10.1007/s001140100243.
69. Light DM, Knight A. 2005. Specificity of codling moth (Lepidoptera: Tortricidae) for the host plant kairomone, ethyl (2*E*,4*Z*)-2,4-decadienoate: field bioassays with pome fruit volatiles, analogue, and isomeric compounds. J Agric Food Chem 53:4046–4053. https://doi.org/10.1021/jf040431r.
70. 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.
71. Takeoka GR, Buttery RG, Teranishi R, Flath RA, Guentert M. 1991. Identification of additional pineapple volatiles. J Agric Food Chem 39:1848–1851. https://doi.org/10.1021/jf00010a032.
72. 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. https://doi.org/10.1016/j.scienta.2015.12.053.
73. 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 Syst Evol Microbiol 50:1351–1371. https://doi.org/10.1099/00207713-50-3-1351.
74. 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 Syst Evol Microbiol 53:905–911. https://doi.org/10.1099/ijs.0.02374-0.
75. 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 Res 1:1–8. https://doi.org/10.1111/j.1567-1364.2001.tb00007.x.
76. Lachance MA. 2016. *Metschnikowia*: half tetrads, a regicide and the fountain of youth. Yeast 33:563–574. https://doi.org/10.1002/yea.3208.

77. 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. https://doi.org/10.7717/peerj.3063.
78. 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. https://doi.org/10.1007/s10886-019-01060-w.
79. Pogue MG. 2002. A world revision of the genus *Spodoptera* Guenee (Lepidoptera: Noctuidae.). Mem Am Entomol Soc 43:1–202.
80. 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. https://doi.org/10.1890/12-0907.1.
81. Saveer AM, Kromann SH, Birgersson 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 Biol Sci 279:2314–2322. https://doi.org/10.1098/rspb.2011.2710.
82. 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. https://doi.org/10.1242/jeb.083188.
83. Wolfin MS, Volo SL, Chilson RR, Liu Y, Cha DH, Cox KD, Loeb GM, Linn CE. 2019. Plants, microbes, and odorants involved in host plant location by a specialist moth: who's making the message? Entomol Exp Appl 167:313–322. https://doi.org/10.1111/eea.12778.
84. Tasin M, Bäckman A-C, Bengtsson M, Ioriatti C, Witzgall P. 2006. Essential host plant cues in the grapevine moth. Naturwissenschaften 93:141–144. https://doi.org/10.1007/s00114-005-0077-7.
85. Borrero-Echeverry F, Becher PG, Birgersson GO, 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.
86. Hatano E, Saveer AM, Borrero-Echeverry F, Strauch M, Zakir A, Bengtsson M, Ignell 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 signaling pathways. BMC Biol 13:75. https://doi.org/10.1186/s12915-015-0188-3.
87. 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. https://doi.org/10.1016/j.phytochem.2011.01.019.
88. 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. https://doi.org/10.1007/s10886-008-9517-0.
89. 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. https://doi.org/10.1007/s10886-012-0087-9.
90. 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 codling moth (Lepidoptera: Tortricidae). Environ Entomol 40:420–430. https://doi.org/10.1603/EN10283.
91. Becher PG, Hagman A, Verschut V, Chakraborty A, Rozpędowska E, Lebreton S, Bengtsson M, Flick G, Witzgall P, Piškur J. 2018. Chemical signaling and insect attraction is a conserved trait in yeasts. Ecol Evol 8:2962–2974. https://doi.org/10.1002/ece3.3905.
92. 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. https://doi.org/10.1093/femsre/fuy041.
93. 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. https://doi.org/10.1104/pp.19.00098.
94. Hedges SB, Blair JE, Venturi ML, Shoe JL. 2004. A molecular timescale of eukaryote evolution and the rise of complex multicellular life. BMC Evol Biol 4:2. https://doi.org/10.1186/1471-2148-4-2.
95. Taylor JW, Berbee ML. 2006. Dating divergence in the fungal tree of life: review and new analyses. Mycologia 98:838–849. https://doi.org/10.1080/15572536.2006.11832614.
96. Labandeira CC. 2013. A paleobiologic perspective on plant-insect interactions. Curr Opin Plant Biol 16:414–421. https://doi.org/10.1016/j.pbi.2013.06.003.
97. Labandeira CC, Currano ED. 2013. The fossil record of plant-insect dynamics. Annu Rev Earth Planet Sci 41:287–311. https://doi.org/10.1146/annurev-earth-050212-124139.
98. 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. https://doi.org/10.4161/psb.4.11.9874.
99. Aleklett K, Hart M, Shade A. 2014. The microbial ecology of flowers: an emerging frontier in phyllosphere research. Botany 92:253–266. https://doi.org/10.1139/cjb-2013-0166.
100. 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. https://doi.org/10.1007/s10886-010-9794-2.
101. Gonzalez F, Witzgall P, Walker WB. 2016. Protocol for heterologous expression of insect odourant receptors in *Drosophila*. Front Ecol Evol 4:24.
102. 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. Sci Rep 7:41105. https://doi.org/10.1038/srep41105.
103. Steuer R, Kurths J, Fiehn O, Weckwerth W. 2003. Observing and interpreting correlations in metabolomic networks. Bioinformatics 19:1019–1026. https://doi.org/10.1093/bioinformatics/btg120.
104. Weckwerth W. 2003. Metabolomics in systems biology. Annu Rev Plant Biol 54:669–689. https://doi.org/10.1146/annurev.arplant.54.031902.135014.
105. 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. https://doi.org/10.1016/j.mimet.2010.12.001.
106. 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. https://doi.org/10.1016/j.fgb.2013.02.005.
107. 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. https://doi.org/10.1016/j.jchromb.2012.05.038.
108. Sipiczki M, Horvath E, Pfliegler WP. 2018. Birth-and-death evolution and reticulation of ITS segments of *Metschnikowia andauensis* and *Metschnikowia fructicola* rDNA repeats. Front Microbiol 9:1193. https://doi.org/10.3389/fmicb.2018.01193.
109. 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. https://doi.org/10.1007/s10886-005-3552-x.
110. 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 Neurosc 12:264. https://doi.org/10.3389/fnbeh.2018.00264.
111. 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.
112. 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). Environ Sci Pollut Res 25:10383–10391. https://doi.org/10.1007/s11356-017-9490-7.
113. Binyameen M, Anderson P, Ignell R, 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. https://doi.org/10.1093/chemse/bju046.
114. 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. https://doi.org/10.1371/journal.pone.0165632.
115. Battisti DS, Naylor RL. 2009. Historical warnings of future food insecu-

rity with unprecedented seasonal heat. Science 323:240–244. https://doi.org/10.1126/science.1164363.
116. Wheeler T, von Braun J. 2013. Climate change impacts on global food security. Science 341:508–513. https://doi.org/10.1126/science.1239402.
117. Ehrlich PR, Harte J. 2015. Opinion: to feed the world in 2050 will require a global revolution. Proc Natl Acad Sci U S A 112:14743–14744. https://doi.org/10.1073/pnas.1519841112.
118. Witzgall P, Kirsch P, Cork A. 2010. Sex pheromones and their impact on pest management. J Chem Ecol 36:80–100. https://doi.org/10.1007/s10886-009-9737-y.
119. Lacey LA. 2017. Microbial control of insect and mite pests: from theory to practice. Academic Press, London, United Kingdom.
120. Knight AL, Witzgall P. 2013. Combining mutualistic yeast and pathogenic virus - a novel method for codling moth control. J Chem Ecol 39:1019–1026. https://doi.org/10.1007/s10886-013-0322-z.
121. Beck JJ, Vannette RL. 2017. Harnessing insect-microbe chemical communications to control insect pests of agricultural systems. J Agric Food Chem 65:23–28. https://doi.org/10.1021/acs.jafc.6b04298.
122. 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. https://doi.org/10.1016/j.postharvbio.2008.11.009.
123. 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. https://doi.org/10.1016/j.biocontrol.2009.05.001.
124. Manso T, Nunes C. 2011. *Metschnikowia andauensis* as a new biocontrol agent of fruit postharvest diseases. Postharv Biol Technol 61:64–71. https://doi.org/10.1016/j.postharvbio.2011.02.004.
125. 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. https://doi.org/10.1093/ee/nvv008.
126. 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. https://doi.org/10.1111/1365-2664.12688.
127. Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. 2015. The importance of the microbiome of the plant holobiont. New Phytol 206:1196–1206. https://doi.org/10.1111/nph.13312.
128. Müller DB, Vogel C, Bai Y, Vorholt JA. 2016. The plant microbiota: systems-level insights and perspectives. Annu Rev Genet 50:211–234. https://doi.org/10.1146/annurev-genet-120215-034952.
129. Junker RR, Tholl D. 2013. Volatile organic compound mediated interactions at the plant-microbe interface. J Chem Ecol 39:810–825. https://doi.org/10.1007/s10886-013-0325-9.
130. Leeder AC, Palma-Guerrero J, Glass NL. 2011. The social network: deciphering fungal language. Nat Rev Microbiol 9:440. https://doi.org/10.1038/nrmicro2580.
131. 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. https://doi.org/10.1371/journal.pbio.2001793.
132. White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p 315–322. *In* Innis MA, Gelfand DH, Sninsky JJ, White TJ (ed), PCR protocols: a guide to methods and applications. Academic Press, New York, NY.
133. Kurtzman CP, Robnett CJ. 2003. Phylogenetic relationships among yeasts of the "*Saccharomyces* complex" determined from multigene sequence analyses. FEMS Yeast Res 3:417–432. https://doi.org/10.1016/S1567-1356(03)00012-6.
134. Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/N. Nucleic Acids Symp Ser 41:95–98.
135. Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599. https://doi.org/10.1093/molbev/msm092.
136. Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454.
137. 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, NY.
138. Merico A, Sulo P, Piskur J, Compagno C. 2007. Fermentative lifestyle in yeasts belonging to the *Saccharomyces* complex. FEBS J 274:976–989. https://doi.org/10.1111/j.1742-4658.2007.05645.x.
139. 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. https://doi.org/10.1021/jf0100548.
140. Amigo JM, Skov T, Bro R, Coello J, Maspoch S. 2008. Solving GC-MS problems with PARAFAC2. Trends Anal Chem 27:714–725. https://doi.org/10.1016/j.trac.2008.05.011.
141. Jonsson P, Johansson AI, Gullberg J, Trygg AJ, 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. https://doi.org/10.1021/ac050601e.
142. 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. https://doi.org/10.4039/Ent1081345-12.