Project title:	**The use of highly attractive yeast strains for controlling *Drosophila suzukii* (spotted wing drosophila).**
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Project leaders:	Rory Jones (author) Michelle Fountain (NIAB EMR) Matthew Goddard (University of Lincoln)
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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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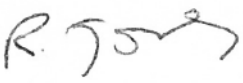
Signature  Date: 16.11.20

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GROWER SUMMARY

Headline

Four combinations of yeast have been found to be attractive to *D. suzukii* in laboratory choice tests; three of which are also attractive in the field. Additionally, three single yeasts previously shown to be attractive in the laboratory, in this project, also proved to be attractive in the field. Fruit type and fruit ripening stage have an impact on fungal community diversity on the surface of fruit.

Background

Since being identified in the UK, in 2012, *Drosophila suzukii* has caused considerable damage to commercial fruit, resulting in yield loss, and increasing expenditure on control methods. Currently *D. suzukii* is controlled with plant protection products, crop hygiene measures, and insect exclusion mesh. With more stringent measures being increasingly implemented on plant protection products (PPP) use, often resulting in the withdrawal of particular products; combined with the threat of insecticide resistance from a limited number of active ingredients, new control methods need to be developed and optimised.

There are complex interactions between fruit, microbes, and *Drosophila* species, and understanding these is important for the control of *D. suzukii*. Yeasts are an essential source of nutrients for *Drosophila*; they are important for oviposition and larval development. Some yeast species, most notably *Hanseniaspora uvarum,* are attractive to *D. suzukii* and have the potential to produce highly attractive and selective baits. There are two potential approaches for *D. suzukii* control using yeast: Firstly, precision monitoring, where numerous traps capture adult *D. suzukii.* This is widely available and easily to implement although it is labour-intensive. To date, this method has not yet been demonstrated to reduce crop damage. Trapping is recommended for the monitoring and detection of *D. suzukii* and lure-and-kill strategies could be used in integrated pest management of *D. suzukii*. However, more attractive, and selective baits are needed to reduce the capture rates of non-target species. This would also make detecting *D. suzukii* females easier as they can be mistakenly identified for other *Drosophila* species without the aid of a microscope. Secondly, another attract-and-kill strategy, which combines a phagostimulant with a PPP can be employed. The pest is attracted to the feeding bait upon which it feeds, thus killing the pest on ingestion. This method can enable a reduction in the amount of PPP applied whilst simultaneously increasing the targeted exposure of *D. suzukii.* This could also reduce the number of non-target species

which come into contact to PPPs and reduce residues on fruit and in the environment. A study of the literature and AHDB project SF 145 have both shown that combining plant protection products with the yeast species *H. uvarum* increases mortality and reduces egg laying.

Yeast has been used to trap *Drosophila* for many years; typically, dried baker's yeast has been used in fermentation-based baits. More recently *H. uvarum* has been investigated as it is associated with *D. suzukii*. Although, *H. uvarum* is known to be attractive to *D. suzukii* few other yeast species have been tested for attractiveness. This project will not only test the attractiveness of yeasts from an existing culture collection but also characterise fungal communities on ripening fruit (blueberries, cherries, raspberries, and strawberries), and will additionally provide a resources of microbes from relevant host fruit species that could be exploited in future projects in the control of this pest. Unlike most *Drosophila* species, *D. suzukii* oviposit in ripening fruit, therefore yeast from ripening fruit may be selectively attractive to *D. suzukii.* In nature, microbes on the surface of fruit are in complex communities and, currently, only single yeasts have been tested for attractiveness. This project will also test the attractiveness of combinations of yeasts.

The main aim of this project is to identify highly attractive yeast species alone and in combination, and then utilise these in the control of *D. suzukii.* Additionally, this project will aim to characterise microbial communities on ripening fruit and investigate identified yeast for attraction to *D. suzukii* and use in control strategies.

Summary

Yeasts vary in their attraction to *Drosophila* species and previous work in 2017/18 identified four candidate yeast species attractive to *D. suzukii*: *H. uvarum*, yeast coded 218, 164, and 190. *D. simulans* was indifferent to all four but *D. melanogaster* was attracted to all four. Both are common non-target species often captured in *D. suzukii* monitoring traps; highlighting the potential for yeast to produce attractive and selective baits for *D. suzukii.* Additionally, multiple strains of *H. uvarum* were attractive to *D. suzukii*. This yeast, in the context of *D. suzukii,* has received the most attention in the literature and is known to be attractive to *D. suzukii*, both in the literature and project SF 145. Building on this work, we tested the attractiveness of ferments of these yeasts in the field using standard commercially available traps. Three yeasts were attractive in the field; yeast coded 190, and two strains of *H. uvarum*. In addition, combinations of yeasts were screened, both in the laboratory and the field. Of the combinations tested, four proved to be significantly attractive in the laboratory; 201+164, 190+201, 190+218+201, and 190+218, but in the field only the latter three combinations attracted *D. suzukii*. Currently, none of the yeast-based attractants tested in the field proved

significantly more attractive than a commercial wine/vinegar liquid bait, Gasser. It is worth noting that these field-based trials were conducted between late October and early December when winter morph *D. suzukii* were more abundant than the summer form.

Microbial communities on ripening fruit are also currently being investigated. Fruit samples were collected in 2018 from four ripening stages of blueberries, cherries, strawberries, and raspberries. Fruits were surface-washed to collect microbes; DNA was extracted and ITS regions (conserved across fungal species) were amplified and sequenced. It was apparent that both fruit type and ripening stage have a significant impact on fungal community diversity; in terms absolute species richness (numbers of fungal phylotypes (taxon designation of 97% similarity)), relative species richness (types of fungal phylotypes) and community composition (abundances of fungal phylotypes). Additionally, fungal abundance (both relative and absolute) increases with ripening stage. Although, for absolute abundance we only present data for cherry, as this was the only fruit species that the quantitative analysis of fugal communities was successful for, andadditionally, when adjusted to cells per mm^2 this trend did not hold true. Cherry harboured significantly more fungal phylotypes than blueberry, raspberry, and strawberry, and with blueberry, raspberry and strawberry not differing significantly from one another. Ripening stage 1 had significantly fewer fungal phylotypes than 2 and 3 but not 4. Additionally, stage 2 and 4 were also not significantly different. For yeast phylotypes from the order *Saccharomycetales* fruit type but not ripening stage had a significant effect on species richness with raspberry harbouring significantly more yeast phylotypes than strawberry, cherry, and blueberry. Strawberry harboured significantly more than cherry and blueberry, and cherry harboured significantly more than blueberry.

Attractive yeasts, both single species and combinations (both single fermented then combined and co-fermented yeasts), are being assessed by exposing *D. suzukii* to a choice of baits in Drosophila Activity Monitoring (DAM) equipment. The baits have been presented in in SSJ (sterile strawberry juice) and YPD (yeast extract 1%, peptone 2%, dextrose 2%). YPD is a standard culture media and is a more realistic growth media for phagostimulant bait commercially (SF 145a). This data is currently being statistically analysed. Laboratory jar-bioassays experiments testing the efficacy of yeast /PPP combinations as control for *D. suzukii* have just finished and are also currently being statistically analysed (data will be included in final report). In addition, I have received training on the odour collection from yeast ferments at the Natural Resources Institute, University of Greenwich. This data is also being analysed and will be reported next year.

Financial Benefits

D. suzukii is an economically damaging pest that causes loss to soft and stone fruit yields. This project has the potential to improve *D. suzukii* control and reduce residues in fruits. The attractive yeast species and strains identified by this project could potentially be exploited in monitoring and control of *D. suzukii* in IPM strategies to combat this pest more effectively.

Action Points

Currently, a commercial product Combi-protec is available for growers to use as a feeding bait. This product should be used according to manufacturer recommendations and following the label specifications.

SCIENCE SECTION

Introduction

Drosophila suzukii (Matsumra) is a highly polyphagous pest that is endemic to Southeast Asia and has recently spread around the globe. In 2008 *D. suzukii* was first detected on the American mainland in California (Bolda *et al.,* 2010). *Drosophila suzukii* then spread quickly to many other countries and is now also present in most northern temperate regions (Rota-Stabelli *et al.,* 2013; Asplen *et al.,* 2015). The ability of *D. suzukii* to adapt to a wide range of environmental conditions has been an important factor in the success of this pest species around the globe (Little *at al.,* 2020), with *D. suzukii* being able to oviposit over a larger range of temperatures (18-30 °C) (Winkler *et al.,* 2020). It was first detected in the UK in 2012 (Harris and Shaw, 2014). It is likely that *D. suzukii's* global spread is due to human-aided transportation (Hauser, 2011; Calabria *et al.,* 2012). Since its recent spread around the globe, *D. suzukii* has caused massive economic losses. Total losses attributed to *D. suzukii* in 2008 in three states in the USA in strawberries, blueberries, raspberries, blackberries and cherries totalled $511.3 million (Bolda *et al.,* 2010). In Europe, *D. suzukii* causes substantial losses, with 80% losses to strawberry crops in a region in the south of France and between 60-80% losses of strawberry crops in Italy in 2010 and 90% losses of late-harvested cherries and 90-100% of blueberry crops being affected in some regions (Grassi *et al.,* 2011). Unlike most *Drosophila* species, *D. suzukii* oviposits in ripening fruit; this ability derives from female *D. suzukii* having a morphologically modified ovipositor (Atallah *et al.,* 2014). Oviposition can lead to mechanical damage in the form of puncture wounds in the surface of fruit. Once the

larvae hatch they cause additional mechanical damage by feeding, resulting in unmarketable fruit (Goodhue *et al.,* 2011). The open wounds inflicted on the fruit allow secondary infection by bacteria and yeast species (Loriatti *et al.,* 2017). In addition to this, the damage inflicted on fruit by *D. suzukii* allows oviposition entry by other insects that lack the ability to oviposit in undamaged ripe fruit (Walsh *et al.,* 2011).

Microbes associated with fruit

Microbes are essential components of agricultural and natural ecosystems. Fruits provide conditions that support microbial growth with good water availability and access to sugars and other nutrients (Berg *et al.,* 2016). Microbes inhabiting the surface of plants form complex communities whilst fulfilling a variety of roles, some beneficial, some neutral and some pathogenic to plants. Several factors have an effect on fruit microbial communities; geographic location (Gayevskiy and Goddard, 2012; Taylor *et al.,* 2014; Bokulich *et al.,* 2014), plant organ, fruit ripening stage (Barata *et al.,* 2012), harvest date (Shi *et al.,* 2020), farming practices (Martins *et al.,* 2014; Perazzolli *et al.,* 2020), fruit species (Leff and Fierer, 2013) and even fruit variety (Cordero-Bueso *et al.,* 2011; Gayevskiy and Goddard 2012). Some studies reported little variation between fungal communities and geographic region in some fruit species but variation in others (Vepštaitė-Monstavičė *et al.,* 2018). Niche generally explains more variance in fungal communities than geographic location (Morrison-Whittle and Goddard, 2015).

Yeasts, which are single-celled fungi, colonise various surfaces of plants and are more abundant and have greater community diversity on fruits compared to blossoms (Vadkertiová *et al.,* 2012). A small number of abundant taxa dominate yeast communities on fruit (Hamby *et al.,* 2012). *Hanseniaspora uvarum*, *Hanseniaspora guilliermondii*, *Metschnikowia pulcherrima* and *Pichia kluyveri* were regularly isolated from various fruit surfaces; including apples, plums and pears using culture-based methods (Vadkertiová *et al.,* 2012). Apples are dominated by the fungal phylum Ascomycota and Basidiomycota (Vepštaitė-Monstavičė *et al.,* 2018). Analogous to bacterial populations on apples, little difference was detected between geographic location and fungal population on apples (Vepštaitė-Monstavičė *et al.,* 2018). Yeast communities change over time on nectarines (Janisiewicz *et al.,* 2010), plums (Janisiewicz *et al.,* 2014) and grapes (Martins *et al.,* 2014). Strawberries harbour a wide variety of fungal taxa (Abdelfattah *et al.,* 2016). Like apples, the diversity of fungal communities on strawberries differs significantly with fruit maturity and between different parts of the plant including, flowers and leaves (Abdelfattah *et al.,* 2016).

Yeasts associated with Drosophila.

Drosophilae are saprotrophic and thus dependent on microbes for nutrition. Microbes are required for the development of both *D. suzukii* and *D. melanogaster* (Meigen) (Bing *et al.,* 2018). *Drosophila* are attracted to yeasts (Dobzhansky *et al.,* 1956) and yeast is used in fermentation baits to catch *Drosophila*. Female flies prefer to oviposit on yeast-colonised substrates (Oakeshott *et al.,* 1989), resulting in an increased development rate of larvae (Meshrif *et al.,* 2016; Bellutti *et al.,* 2018). The same yeast lineages are often present in *Drosophila* irrespective of species, geographical location, or diet (Chandler *et al.,* 2012). In *D. melanogaster* survival rate and development time vary with yeast species availability (Meshrif *et al.,* 2016). Giving *D. melanogaster* larvae access to either *Pichia toletana* or *Metschnikowia pulcherrima* decreased development time whilst simultaneously increasing survival rates. *Pichia toletana* was more beneficial than *M. pulcherrima*; in addition, choice tests revealed that *P. toletana* was more attractive to *D. melanogaster* larvae (Meshrif *et al.,* 2016). Both larvae and adult *D. melanogaster* engage in niche construction, manipulating yeast communities to differing degrees (Stamps *et al.,* 2012). Yeasts are immobile and therefore rely, in part, on insects to transport them to new habitats. *Drosophila* adults may vector yeast, potentially inoculating yeast onto new fruit surfaces (Gilbert, 1980; Starmer *et al.,* 1987; Stamps *et al.,* 2012; Buser *et al.,* 2014) with a significant effect on communities (Stamps *et al.,* 2012) and *Drosophila* have been shown to vector yeast in the laboratory (Christiaens *et al.,* 2014; Buser *et al.,* 2014). There are however limited studies demonstrating this in nature, but a strain of *Saccharomyces cerevisiae* that is attractive to *D. simulans* has been shown to be dispersed more frequently than an unattractive strain of *S. cerevisiae* in a vineyard setting (Buser *et al.,* 2014). Additionally, flies had higher fecundity when associated with more attractive yeasts (Buser *et al.,* 2014) and thus these studies provide some evidence to support the existence of a mutualism between yeast and *Drosophila* as both gain fitness advantages by interacting (Christiaens *et al.,* 2014; Buser *et al.,* 2014), but there is not enough evidence to assume that this mutualism extends to hold generally between yeast and *Drosophila* (Günther and Goddard, 2018). In addition, larvae have considerable effects on yeast species composition, reducing yeast diversity on fruits (Stamps *et al.,* 2012). Larvae and adults from four different *Drosophila* species preferred certain yeast species when given the choice; this preference differed between larvae and adults of the same species (Cooper, 1960). Stamps *et al.* (2012) also demonstrated that *D. melanogaster* larvae consistently encouraged the development of yeast communities comprised of three yeast species; *Candia cailfornica*, *Candidia zemplina* and *P. kluyveri* (Stamps *et al.,* 2012).

Yeast species vary in attractiveness to *D. melanogaster* with *Saccharomyces* species generally being more attractive than non-*Saccharomyces* species (Palanca *et al.,* 2013). Additionally, yeasts isolated from fruit were more attractive than non-fruit isolated yeast (Palanca *et al.,* 2013). Culture media of yeast also affect attraction of yeasts (Günther *et al.,* 2019; Lasa *et al.,* 2019; Koerte *et al.,* 2020). *Saccharomyces cerevisiae* strains also vary in attractiveness to both *D. melanogaster* (Palanca *et al.,* 2013; Schiabor *et al.,* 2014) and *D. simulans* (Sturtevant) (Buser *et al.,* 2014). Only single yeasts have been tested for attractiveness to *Drosophila* species (e.g. Palanca *et al.,* 2013; Buser *et al.,* 2014; Günther *et al.,* 2015; *Scheidler et al.,* 2015). It is not known whether there is a synergistic or additive effect of combinations of different yeasts, as occurs in nature, on attractiveness. Microbial communities on fruit are much more complex (e.g. Taylor *et al.,* 2014). Yeast combinations warrant further investigation and may have the potential to produce both attractive and selective baits (Fischer *et al.,* 2017).

D. suzukii and yeast.

There are complex interactions between fruit, microbes and *Drosophila* species and understanding these interactions is important in the control of *D. suzukii* (Hamby and Becher, 2016). Yeasts have been utilised for trapping *Drosophila* for a long time (e.g. Da Cunha *et al.,* 1951). Yeast can be exploited in baits to attract *D. suzukii,* with baits incorporating yeast capturing more *D. suzukii* than baits like apple cider vinegar (Iglesias *et al.,* 2014). *D. suzukii* is associated with the yeast species *H. uvarum*; one study found, from the total 28 yeast species identified, *H. uvarum* was the dominant yeast species isolated from both *D. suzukii* larvae and adults, followed by *Issatchenkia terricola* and *P. kluyveri* (Hamby *et al.,* 2012). Non-Saccharomyces yeasts from the genera *Hanseniaspora*, *Pichia* and *Candida* have also been identified from the guts of adult winter morph *D. suzukii* (Fountain *et al.,* 2018). Hamby *et al*. (2012) affirmed that as the abundance of *H. uvarum* was higher in *D. suzukii* compared to other *Drosophila* species (Heed *et al.,* 1976) this could mean that there was a specific association between *D. suzukii* and *H. uvarum* making it an ideal candidate for a yeast-based bait (Hamby *et al.,* 2012). If this is the case, it could be fundamentally important in the creation of selective and attractive bait for *D. suzukii.* In addition, *H. uvarum* was attractive to *D. suzukii* when tested against multiple yeast species (Scheidler *et al.,* 2015; Noble *et al.,* 2019; Lasa *et al.,* 2019; Bueno *et al.,* 2020). As well as being attractive to *D. suzukii*, *H. uvarum* also increases the fecundity of female flies, when provided as a food source, increasing the number of eggs being laid (Noble *et al.,* 2019, Spitaler *et al.,* 2020). The media that yeasts are cultured in also affects attraction, with *H. uvarum* strains isolated from fruit being more attractive to *D. suzukii* than a commercial *S. cerevisiae* strain grown in corn syrup media but

not when grown in sucrose-based media (Lasa *et al.,* 2019). *H. uvarum* is common in the environment and on fruits having been isolated from cherries, raspberries (Hamby *et al.,* 2012), apples, plum, pears (Vadkertiová *et al.,* 2012) and grapes (Barata *et al.,* 2012; Gayevisky and Goddard*,* 2012). *H. uvarum* is also attractive to other *Drosophila* species, including *D. melanogaster* (Scheidler *et al.,* 2015; Palanca *et al.,* 2013; Hoang *et al.,* 2015)*,* making it unlikely to be a species-specific relationship. However, as *H. uvarum* is attractive to *D. suzukii* (Scheidler *et al.,* 2015; Noble *et al.,* 2019) it is still an important candidate yeast which warrants future investigation into its attractiveness to *D. suzukii*.

D. suzukii control utilising yeast

Currently *D. suzukii* is controlled using plant protection products (PPP), crop hygiene measures, and insect exclusion mesh. With more stringent measures being increasingly implemented on the use of plant protection products, often resulting in the withdrawal of particular products combined with the threat of insecticide resistance from a limited number of active ingredients; new control methods need to be developed and improved.

There are two potential approaches for yeast in *D. suzukii* control. Firstly, precision monitoring is a relatively cheap method of control which is widely available and easily implementable, but this method can be labour-intensive (Lee *et al.,* 2011). Trapping is essential for the monitoring and detection of *D. suzukii*, and greater incidence of *D. suzukii* has been recorded in spring when fruit infestation levels were low and vice versa in early July. This suggests lures may be less attractive than ripening fruit (Beers *et al.,* 2011) highlighting the need to develop more attractive and selective baits. Lure-and-kill strategies using traps are very important candidates in the IPM management of *D. suzukii*. However, for precision monitoring more attractive and selective baits are needed to reduce the capture rates of non-target species; this would also make detecting *D. suzukii* females easier as they can be mistakenly identified for other *Drosophila* species without the aid of a microscope. Therefore, reducing the numbers of other *Drosophila* species in traps with a bait that is selective to *D. suzukii* would make it easier to process trap captures (Hamby *et al.,* 2012). Secondly, one promising avenue of research are phagostimulant attract-and-kill strategies which combine yeast with PPP thus attracting flies to feed and be exposed to the PPP (Hamby and Becher, 2016; Mori *et al.,* 2016; Noble *et al.,* 2019; SF145). This may enable a reduction of the amount of synthetic pesticide input generally whilst simultaneously increasing the targeted exposure of *D. suzukii.* Importantly, this means it may reduce the exposure to non-target species to pesticides and reduce residues on fruit. *Drosophila suzukii* females lay fewer eggs and mortality increases when they are exposed to *H. uvarum* combined with the Spinosad

pesticide (Mori *et al.,* 2017) demonstrating the potential for utilising yeast combined with pesticides for control.

Aims

In this project the following null hypotheses will be tested:

H1-There is no significant difference between the attractiveness of different yeast species to different *Drosophila* species *(D. melanogaster*, *D. simulans* and *D. suzukii)*.

H2-There is no significant difference between the attractiveness of different *H. uvarum* strains to *D. suzukii*.

H3-The effect of combining yeast on the attraction of *D. suzukii* will follow an additive (linear) model i.e. there will be no interaction effect or synergistic effect of combining yeast on attraction.

H4-Neither fruit type nor ripening stage has a significant effect on fungal communities on fruit by any ecological measure.

H5-There is no significant difference between highly attractive yeasts or yeast combinations to *D. suzukii* in comparison to currently available commercial baits.

H6-There is no significant difference in fly mortality and egg laying between the different yeast treatments, with combinations being no more effective in increasing mortality and deceasing egg laying compared to single yeasts.

The aims addressed by this report were;

1. Test attractiveness and repulsiveness of individual yeast species to *D. suzukii* in the field and combinations of yeast both in the laboratory and field.
2. Characterise yeast communities on ripening fruit, strawberries, raspberries, blueberries, and cherries.

Materials and methods

Drosophila maintenance

D. suzukii cultures were derived from an Italian strain, which was established at NIAB EMR in 2013. Summer morph *D. suzukii* populations were housed in BugDorm cages (32.5 x 32.5 x 32.5 cm) (MegaView Science Co., Ltd.) situated in a temperature-controlled room, which provided a constant temperature of 25°C and a 16:8 h light: dark photoperiod (Shaw *et al.,* 2018). Damp blue absorbent paper was placed on the base and roof of the cages to provide humidity (96%) inside the cages. *D. suzukii* were fed on Drosophila Quick Mix Medium blue (Blades Biological Ltd) sprinkled with dried baker's yeast and were also cultured on media

comprising of 100% distilled H_20, 1% agar, 9% sugar, 9% pre-cooked ground maize, 2% baker's yeast, 5% malt 1% soy flour, 0.3% propionic acid and 0.3% Methylparaben (methyl 4-hydroxybenzoate) pre-dissolved in 70% ethanol (as per Shaw *et al.,* 2018). Additionally, *D. suzukii* were also regularly provided with frozen raspberries (Shaw *et al.,* 2018). For winter morph cultures, summer morph flies were transferred from culture to square or circle-based Drosophila Bottles (177 ml, Fisherbrand) filled with 50 ml cornmeal media. Flies were left to oviposit and lava to develop for 7 days whereupon adult flies were removed, and the bottles were place at 10°C, 0:24 h light: dark. Before use in experiments winter morph *D. suzukii* were transferred to Drosophila Bottles containing 50ml of the Drosophila Quick Mix Medium sprinkled with yeast and were then acclimatised to 22°C and 16:8 h light: dark photoperiod over a three day period.

Yeast cultures

Yeast species were cultured from the Goddard culture collection, University of Lincoln, stored on glycerol at -80°C, originating from different locations including New Zealand, UK, and USA (Table 1).

Table1: Origin, source and strain of yeast species used in choice tests.

Yeast Strain	Origin	Source	Reference
EC-1118	France	Commercial wine yeast	Lallemand Inc.
162	New Zealand	Chardonnay juice	Anfang *et al.,* 2009.
218	New Zealand	Pinot noir ferment	Goddard culture collection
150	New Zealand	Beehive	Anfang *et al.,* 2009.
164	New Zealand	Chardonnay ferment	
212	New Zealand	Syrah fruit	Gayevskiy *et al.,* 2012.
198	New Zealand	Sauvignon Blanc ferment	Goddard culture collection
190	New Zealand	Sauvignon Blanc ferment	Goddard culture collection
166	New Zealand	Sauvignon Blanc ferment	Goddard culture collection
98-3	United Kingdom	*D. subobscura*	Goddard culture collection
44-1	United Kingdom	*D. subobscura*	
201	New Zealand	Chardonnay fruit	Gayevskiy *et al.,* 2012.
206	New Zealand	Chardonnay fruit	Goddard culture collection
209	New Zealand	Chardonnay fruit	
11-382	United States	*D. suzukii*	Phaff Yeast culture collection, UC-Davis
44-9	United Kingdom	*D. subobscura*	Goddard culture collection
28-1	United Kingdom	*Drosophila* sp.	
28-5	United Kingdom	*Drosophila* sp.	
28-9	United Kingdom	*Drosophila* sp.	

Ferment Preparation

A preculture was prepared using YPD (yeast extract 1%, peptone 2%, dextrose 2%) media. Individual yeast species were transferred into 15 ml falcon tube containing 3 ml of YPD media. This was incubated at 30°C and 180rpm for 24 hours; the optical density was ascertained

after inoculation and at 24 hours using a spectrophotometer (Jenway 6705). The ferments were then created by inoculating SSJ or YPD with approximately $1x10^6$ yeast cells per ml yeast cells. Strawberry juice was cold-sterilised by filtration, where juice was passaged through a 0.2 µL filter system (Corning 1L Filter system 0.2 µL) laboratory assays or sterilised using up to a maximum of 1000 µl dimethyl dicarbonate (DMDC) dissolved in ethanol at a ratio of 1:2 DMDC to ethanol per litre of fruit juice for field assays. Prior to being subjected to filtration, strawberry juice was pre-filtered through two layers of muslin to help reduce clogging of the filter which could potentially reduce efficiency of the filtration process. The brix and optical density (OD) of the yeast ferments were determined at 48 hours. The brix was ascertained using a refractometer (HI 96801, Hanna instruments) and the OD was again measured using a spectrophotometer. One ferment was used per yeast, to reduce variation.

Two-way *choice tests*

Two-way choice tests using multiple T-maze apparatus were employed to ascertain the attractiveness of yeasts (Palanca *et al.,* 2013). Situated at each side of the T-Maze apparatus was 10 ml of 1:1000 dilution of either a yeast ferment or sterile fruit juice control. Experiments were run for 30 minutes in the absence of light to ensure that any choice made was done solely on olfactory cues (Palanca *et al.,* 2013; Buser *et al.,* 2014; Günther *et al.,* 2015; Günther *et al.,* 2019). Where combinations of yeasts were tested, yeasts were fermented separately and then combined in in equal proportions. T-maze position was randomised regarding their orientation, in addition to treatments being reversed, to account for any directional bias in the room.

Flies were anaesthetised using CO_2 for a maximum of 6 minutes to separate out the number of females required and then starved for 24 ± 1 hours prior to the experiment start. For *D. suzukii* flies were starved for 17 ± 1 hours then anaesthetised and sexed. Flies were again anaesthetised briefly using CO_2 before being inserted in the centre of the T-Maze apparatus (Fig. 1). Between 60-80 mated adult *D. suzukii* females between 3-12 days old were added to each T-maze. Each replicate run consisted of one replicate per yeast for all treatments; this was repeated six times. Thus, ensuring that each cohort of flies, bred from the original populations, was subjected to all treatments. Ensuring that the physiological state of each cohort of *Drosophila* was similar, to reduce variation between yeasts tested.

After each replicate run, T-mazes were placed in a freezer to euthanise the flies prior to counting. To determine attractiveness of each yeast species an Attraction Index (AI) was calculated (AI = the total number of flies in the arm corresponding to yeast ferments subtracted from the number of flies in the arm corresponding to the sterile strawberry juice,

divided by the total number of flies making a choice) (Palanca *et al.,* 2013; Buser *et al.,* 2014; Günther *et al.,* 2015). After completion of the experiment the sliding doors of the T-maze were closed (Fig. 1). Any flies that left the central compartment, passed either sliding door thus occupying either arm, were deemed to have made a choice (Palanca *et al.,* 2013; Buser *et al.,* 2014; Günther *et al.,* 2015). The data was analysed using the binomial distribution to determine whether the choices made by the flies differed significantly from a random distribution (Palanca *et al.,* 2013; Buser *et al.,* 2014; Günther *et al.,* 2015).

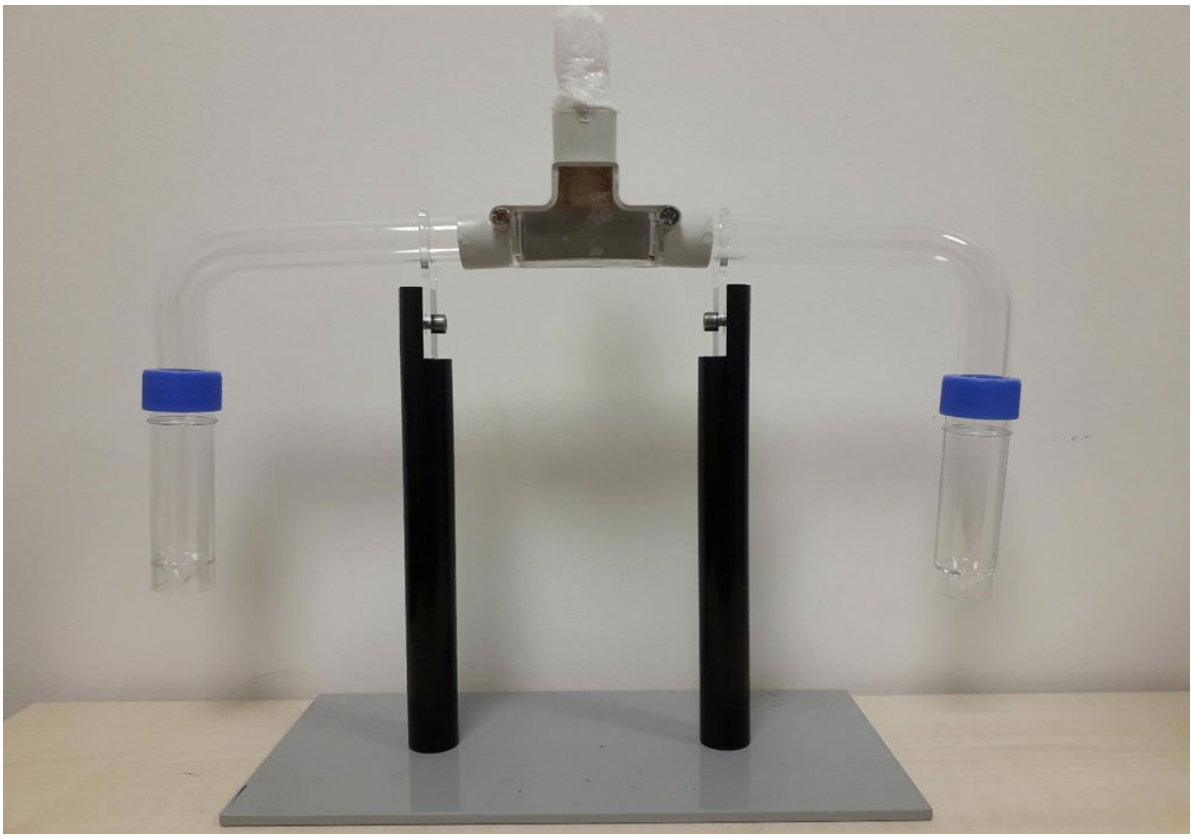

Fig. 1. Set up of T-maze apparatus. 10 ml of 1:1000 dilution of yeast ferment or sterile fruit juice for a control was placed in the vials attached to the T-maze arms, a piece of circular mesh was placed in between the top of the vial and the end of the T-maze arms. This prevented the *Drosophila* individuals interacting with the ferments/fruit juice, whilst simultaneously allowing diffusion of odours from the different treatments throughout the T-maze. For all experiments using *D. suzukii,* damp blue absorbent paper was included in the centre of the T-maze to increase humidity. *Drosophila* individuals were placed in the T-maze after being anaesthetised. A cotton wool plug prevented any flies escaping. The doors of the T-maze were opened in the absence of light, to prevent the flies making a choice based on visual cues.

Field trials

Field experiments were conducted on a commercial fruit farm in Kent, UK. Drososan field traps (Koppert Biological Systems) were deployed with a 200 ml drowning solution of either single, or combinations of yeast fermented in strawberry juice. Where combinations of yeasts were required, equal volumes of singly fermented yeast were mixed to produce the 200 ml. In each trial, three control treatments were included: sterile strawberry juice, distilled water (negative control) and commercially available Gasser lure (RIGA) (positive control). Triton x 100 (Sigma-Aldrich, concentration of 0.005%) was added to all drowning solutions, regardless of treatment, to reduce surface tension. Traps were arranged in a randomised block design where one trap from each treatment was present in a random order per block. For tests 1 and 2, traps were situated in a native hedgerow approximately 3 m apart and 1 m from the ground adjacent to raspberries canes. For test three, traps were sited in a deciduous, bramble woodland, adjacent to the hedgerow close to the same raspberry canes. Traps were placed 7-8 m apart and 1 m from the ground. After 72 hours trap contents were filtered through muslin and numbers of male, female and other *Drosophila* were counted in the laboratory.

Microbes on fruit

Methods

Fruit sampling and processing

Fruit were sampled at four ripening stages, ranging from green to harvest fruit, from blueberries, cherries, raspberries, and strawberries (supplementary material, Fig S1). All fruit samples were collected from four separate locations, a different one for each fruit, from three commercial fruit growers in Kent all within a 55 km^2 area and maximum distance of 19 km apart. All fruit were subject to growers' spray programmes to control pest and diseases. Samples were collected throughout the 2018 growing season between June and September. Six replicates of 10 fruit (except blueberries N=20) for each fruit species from each of the ripening stage were collected. Fruit were randomly selected from within their respect fields or orchards and were aseptically removed from plants with as much of the stalks or calyx removed as possible without damaging the fruit's epicarp. After collection, the fruit was transported straight to the lab, in sterile bags, where it was processed immediately. Upon arrival in the lab fruit was surface washed with sterile water to remove microbes and collected in 50 ml falcon tubes and centrifuged for 30 mins at 4,500 rpm, whereupon the supernatant

was discarded down to approximately 2 ml. The pellet was re-suspended in the remaining water and 1 ml of the suspension was then transferred to eppendorf tubes and centrifuged at 13,000 rpm for 10 mins, whereupon the supernatant was discarded and the pellet stored at -80°C in preparation for DNA extraction.

DNA extraction

Pellets derived from samples were thawed and resuspended in sterile water then split into two equal parts (resulting in N=12) with one half of each sample being spiked with 265 cells (determined using a haemocytometer) of *Plectosphaerella cucumerina*. Samples were again centrifuged 13,000 rpm for 10 mins and the supernatant removed in preparation for DNA extraction. *P. cucumerina* was grown in potato dextrose broth at 25°C with 180 rpm shaking for 7 days. Half the samples were spiked with *P. cucumerina* so that later in the analysis absolute abundance of phylotypes (number of cells) could be quantified. 265 cells were used as this was estimated to be approximately 0.5% of cells likely to be present in the samples with the lowest cell count (estimated from spare samples). The *P. cucumerina* strain used was isolated from pumpkins and was chosen as it was deemed to be unlikely to be present in the fruit samples.

DNA was extracted using the DNeasy Blood and Tissue kit (QIAGEN) following the manufacturer's instructions but with an additional bead beating step before incubation. Pellets were resuspended in 750 µL ATL buffer and moved to a bead tube containing 1g of glass beads at a 1:1 ratio of <106 µm and 0.5mm size beads (Sigma-Aldridge), then placed in a bead beater (Bead Ruptor 12, Omni international INC) and set on maximum speed for 30s, this was repeated 5 times.

Barcode amplification

PCR reactions comprised of 15 µl Kapa 2x master mix (Kapa Biosystems), 6 µl of 1x ITS primer mix (ITS2 forward TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCATCGATGAAGAACGCAGC and ITS2 reverse GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTCCGCTTATTGATATGC), 7 µl sterile water and 2 µl template DNA. Each batch of PCR reactions included a negative control (2 µl sterile water) and a positive control (*S. cerevisiae* DNA). The PCR cycle parameters were 95°C for 3mins, 29 cycles of 98°C for 20 seconds 64°C for 20 seconds 72°C for 40 seconds, followed by a final extension time at 72°C for 5mins. The PCR product was electrophoresed through a 2% agarose gel containing 10 µl SYBR safe dye™ (Invitrogen) per 100 ml TAG

buffer for 25mins at 120V to ascertain the size of products. PCR amplicons were sequenced on Illumina MiSeq instruments with a 300PE metric by Eurofins genomics.

Bioinformatics pipeline

The processing of sequences was carried out using QIIME 2 (2019.4) (Bolyen *et al*., 2018). FastQC was utilised to assess sequence quality (Andrews, 2010) and Dada 2 was used to denoise the paired end sequences (Callahan *et al*., 2016). ASVs (Amplicon Sequence Variants) were clustered together at an identity of 97% using vsearch (Rognes *et al*., 2016). At this point fungi were identified using q2-feature-classifier plugin and unite_ver7dynamic database (Bokulich *et al*., 2018). Additionally, any unassigned ASVs were subjected to a manual online Blast search and after both these steps all non-fungi identified ASVs (320 in total), and the benchmark ASV, when not needed for quantification of cell numbers, were removed. Raw sequence counts were then subjected to variance-stabilizing normalization using CSS normalisation (metagenomSeq and phyloseq, R packages; McMurdie and Holmes, 2013; Paulson *et al*., 2013; Weiss *et al*., 2017; Muletz Wolz *et al*., 2018). The ASVs were then annotated using q2-feature-classifier plugin and unite_ver7dynamic database (Bokulich *et al*., 2018). For quantitative analysis of fungal communities, samples containing detectable amounts of the spiked fungal benchmark (*P. cucumerina*) were run through the bioinformatics pipeline again, this time without the benchmark ASV being removed. Normalised counts for each of the samples were then adjusted using the known number of *P. cucumerina* cell in the samples to estimate actual cell numbers.

Jar-bioassays

Laboratory choice tests

Three separate multiple-choice tests were carried out to test: 1) attractiveness of single yeast volatiles; 2) attractiveness of combinations after post fermentation blending of singly cultured yeasts; and 3) attractiveness of combinations, co-fermented yeasts. The attractiveness of all yeast treatments was tested separately when grown in YPD and sterile strawberry juice, to both summer and winter morphs. Attractiveness of yeasts were tested in YPD as this is a more realistic growth media for yeast combined with plant protection products as used in phagostimulantory baits in (SF 145a). Multiple choice tests, using a 32-channel modified LAM10H Locomotor Activity Monitor rig with open ended tubes (Fig. 3), was set up in a BugDorm cage (475 × 475 × 475 mm) (Noble *et al.,* 2019; SF 145). Each tube in the activity monitor had a planar array of three infra-red beams 23 mm from the base of the tubes and were set up at an angle of 20° from horizontal. Flies, 200 summer morphs females or 130-

140 winter morphs females, were added to the cage and left for 24 hours. Females were considered mated as they had access to males until they were sexed and mating generally occurs one day post eclosion (Cini *et al.,* 2012). Flies were allowed to acclimatise before the commencement of the experiment (five minutes for summer morph and two hours for winter morph). Flies were free to enter/exit tubes at will, when they did they broke the infra-red beams in the corresponding tubes and this registered as a count. Total number of counts after 24 hours was recorded. Yeasts were grown in either sterile strawberry juice or YPD media and tested alongside water, Combi-protec (5%) and growth media (either sterile strawberry juice or YPD) controls.

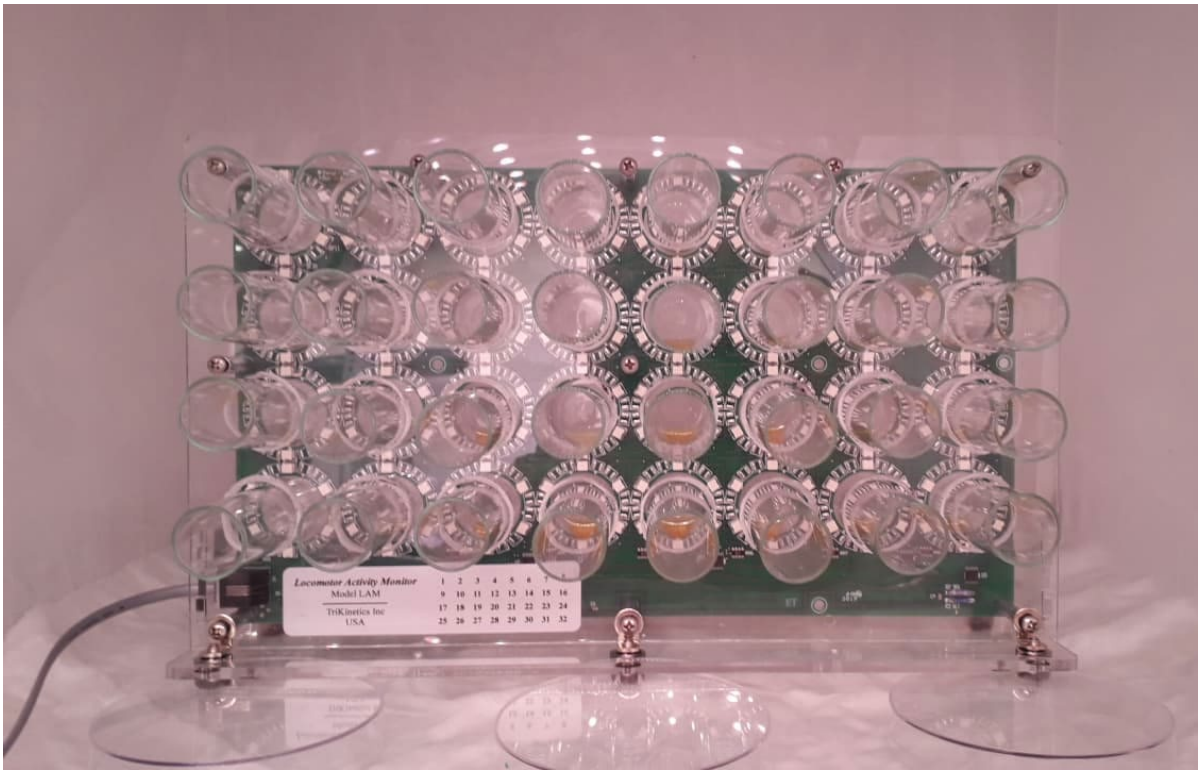

Fig. 3. Activity monitor apparatus, 32-channel modified LAM10H Locomotor Activity Monitor rig with open ended tubes. Modifications were resiticted to enlarging the holes in the front sheet of perspex so that they could accommodate 25 mm diameter tubes. The activity monitor was set up at an angle of 20° to ensure that the baits in the tubes did not pass the 23 mm mark on the tube which was where the infra-red beams were positioned. Each tube contained 200 µm of yeast ferments or controls (either YPD or SSJ for media control, Combi-protec and water negative controls). Each treatment was present on each row in a random order.

Jar-bioassays

Following Noble *et al.* 2019, jar-bioassays were set up to determine the effect of combined different yeast treatments with PPPs. Jars (750 ml clear plastic jars; 103 mm diameter, 95 mm height, Involvement Packaging Ltd.) modified with a fine mesh covered ventilation hole (10 mm diameter), with damp filter paper (90 mm, Fisherbrand) on the base were used (Noble *et al.,* 2019). Filter paper was re-wetted with 500 µl distilled water at 24 hours if required. Each jar contained three similar sized (approximately 30 × 20 mm) blackberry leaves, picked the day before the experiment and stored at 2°C. Each leaf had 6 x 10 µl droplets of the test bait (or control) per leaf (three on each side); two leaves had insecticide or control and bait or control, the other leaf had sugar solution (160 g l^{-1}, 16%) (Noble *et al.,* 2019). Leaves were left to dry for 1-2 hours prior to use and were arranged with the two pesticide or control leaves on one side of the jar and the sugar leaf on the opposite side (Fig. 4). A Petri dish (35 mm, Corning) containing grape juice agar (34.7 g Agar, 333 ml red grape juice, 33.3 g dextrose and 2.0 g Nipagin per litre distilled water) for egg laying was also placed in each jar (Noble *et al*., 2019; SF145).

Flies were anesthetised using CO_2 and sexed, then starved for 7 hours prior to the experiment starting, whereupon they were again anesthetised briefly using CO_2 and inserted into the jars in the space between leaves (Fig. 4). Twelve flies (eight females and four males) were added to each replicate (N=5 replicates per treatment). Five yeast treatments (three single yeast species and two co-culture combination of species) were tested alongside water positive and negative controls, YPD positive and negative media controls and Combi-protec (commercially available phagostimulatory bait). Mortality was recorded at 1, 2, 4, 8, 24, 32 and 48 hours post set-up. Flies that were heavily moribund (defined as individuals clearly close to death, on their back or side with one or more legs twitching) were classified as dead. Numbers of eggs laid in the grape juice agar were also recoded at 48h.

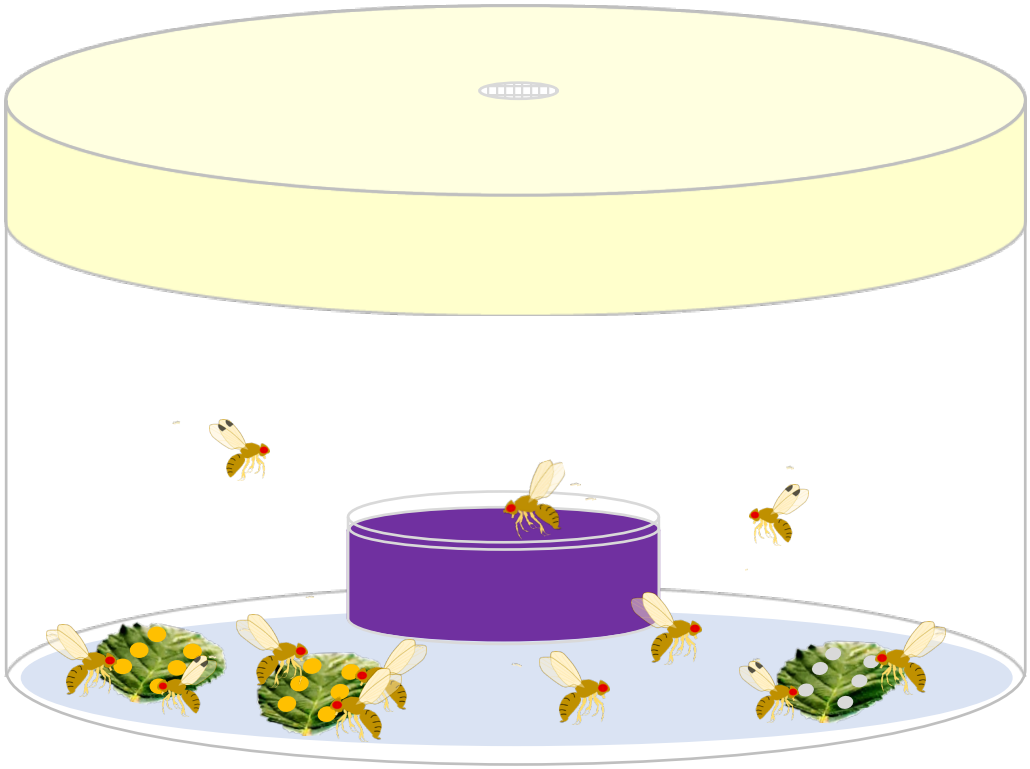

Fig. 4. Jar-bioassay set up used in these experments. 12 flies (eight females and four males) were added to each pot (10 treatments, N=5 replicates). Jars were 750 ml with 103 mm diameter and 95 mm height (Involvement Packaging Ltd.) and were modified with a fine mesh covered ventilation hole (10 mm diameter) in the lid. Damp filter paper (90 mm, Fisherbrand) was place on the base of the jars. The jars also contained three leaves each with 6 x 10 µl droplets per leaf (three on each side of the central leaf vein); two leaves had insecticide or control plus bait or control (orange dots), the other leaf had sugar solution only (grey dots) (160 g l^{-1}, 16%) (Noble *et al.,* 2019). The jar also contained grape juice agar for egg laying. Flies were anesthetised briefly using CO_2 and inserted into the jars in the space between leaves.

Statistical analysis

Choice tests

Yeast attractiveness was analysed by ANOVA and binomial logistic regression, where treatment and *Drosophila* species were the main factors in the logistic regression model, and significance was assayed using ANOVA following model simplification by removal of the interaction between the two factors from the null model (Crawley, 2005). The attraction of individual yeast was analysed using the binomial distribution to determine whether the choices made by the flies differed significantly from a random choice distribution (Palanca *et*

al., 2013; Buser *et al.,* 2014; Günther *et al.,* 2015; Günther *et al.,* 2019). Permutation analysis was used to test whether yeasts interacted linearly or non-linearly in terms of attraction when combined into communities. The predicted AI based on an additive (linear) model was compared to the observed AI of yeast combinations. Predicted AI values for yeast combinations under an additive model were created by randomly selecting individual AI values from the replicates of each of the corresponding single yeast experiments for each of the constituent yeasts in the combinations and calculating the mean AI from these. This procedure was then repeated 1,000 times for each combination to generate a null/additive distribution of community AI values for each of the yeast combinations employed. The experimentally observed AI values from yeast combinations was then compared to this null distribution and used to calculate the probability the observed yeast combination AI was due to an additive interaction between constituents' yeasts in the communities. Additionally, a linear model was also used to test whether the preferences for the communities of yeast differed from that predicted based on a linear mixture of the preferences of the individual yeasts ($y = X\beta + \varepsilon$).

Field trials

Field trap capture data was analysed using a linear regression with Poisson distribution. For total *D. suzukii*, interaction terms between the fixed effects of treatment and sex were included, and block was included as a random effect. Significance was tested using ANOVA following model simplification by removal of interaction between treatment and sex from the null model. If there was a significant interaction with sex, *D. suzukii* males and females were analysed separately.

Microbes on fruit

The effect of both fruit species and ripening stage on total species richness was assessed using two-way ANOVA with Tukey HSD for post hoc pairwise comparisons. Where the data did not conform to the assumption of normality as determined by Shapiro-Wilks test for normality, a square root transformation was applied. Shannon's and Simpson's diversity indices were analysed for significance using Kruskal-Wallis tests as even when transformed the data did not conform to the assumptions of normality. Differences in presences or absences of fungal phylotypes and relative abundances of phylotypes were analysed with two-way full factorial permutational multivariate ANOVA (permanova, with 10000 permutations) on binary and abundance based Jaccard dissimilarities (Anderson, 2001). Pairwise PermANOVAs were conducted to analyse differences within fruit species and

ripening stages where required. For quantitative analysis of fungal communities, the effect of ripening stage on cell numbers was analysed using Kruskal-Wallis. Indicator analysis was used to determine fungal phylotypes that were over-represented in the different fruit species (indicspecies package in R) (Dufrêne and Legendre, 1997). R version 3.6.1 was used for all statistical analyses (R Core Team, 2019).

Results

H1-There is no significant difference between the attractiveness of different yeast species to *D. suzukii.*
H2-There is no significant difference between the attractiveness of different *H. uvarum* strains to *D. suzukii*.

There was a significant interaction between yeast type and sex for numbers of *D. suzukii* captured, and this explained a significant amount of deviance in the trap data (Δdev=44, df=7, p<0.001). We therefore analysed male and female data separately. There was a significant difference between yeast type for numbers of male and female *D. suzukii* and other *Drosophila* caught (Δdev=4568, df=7, p<0.001, Δdev=1874, df=7, p<0.001 and Δdev=1118, df=7, p<0.001 respectively). Confidence intervals revealed all traps had significantly greater numbers of male and female *D. suzukii* (and other *Drosophila*) than the sterile water negative control. Overall, the commercial Gasser-lure bait was the most attractive treatment, attracting significantly more individuals than other treatments for both male and female *D. suzukii*. For other *Drosophila,* however, Gasser-lure was significantly more attractive than all treatments apart from *H. uvarum* (201). Three of the yeast treatments were more attractive than sterile strawberry juice: *H. uvarum* (201), *H. uvarum* (11-382) and 190 for male and female *D. suzukii* and other *Drosophila*. *H. uvarum* (201) was significantly more attractive than both *H. uvarum* (11-382) and 190 (Fig. 5).d *H. uvarum* (11-382) and 190 were equally as attractive as each other and both significantly more attractive than 218 and *S. cerevisiae* which were equally as attractive as each other and not different in attraction from the sterile fruit control (Fig. 5).

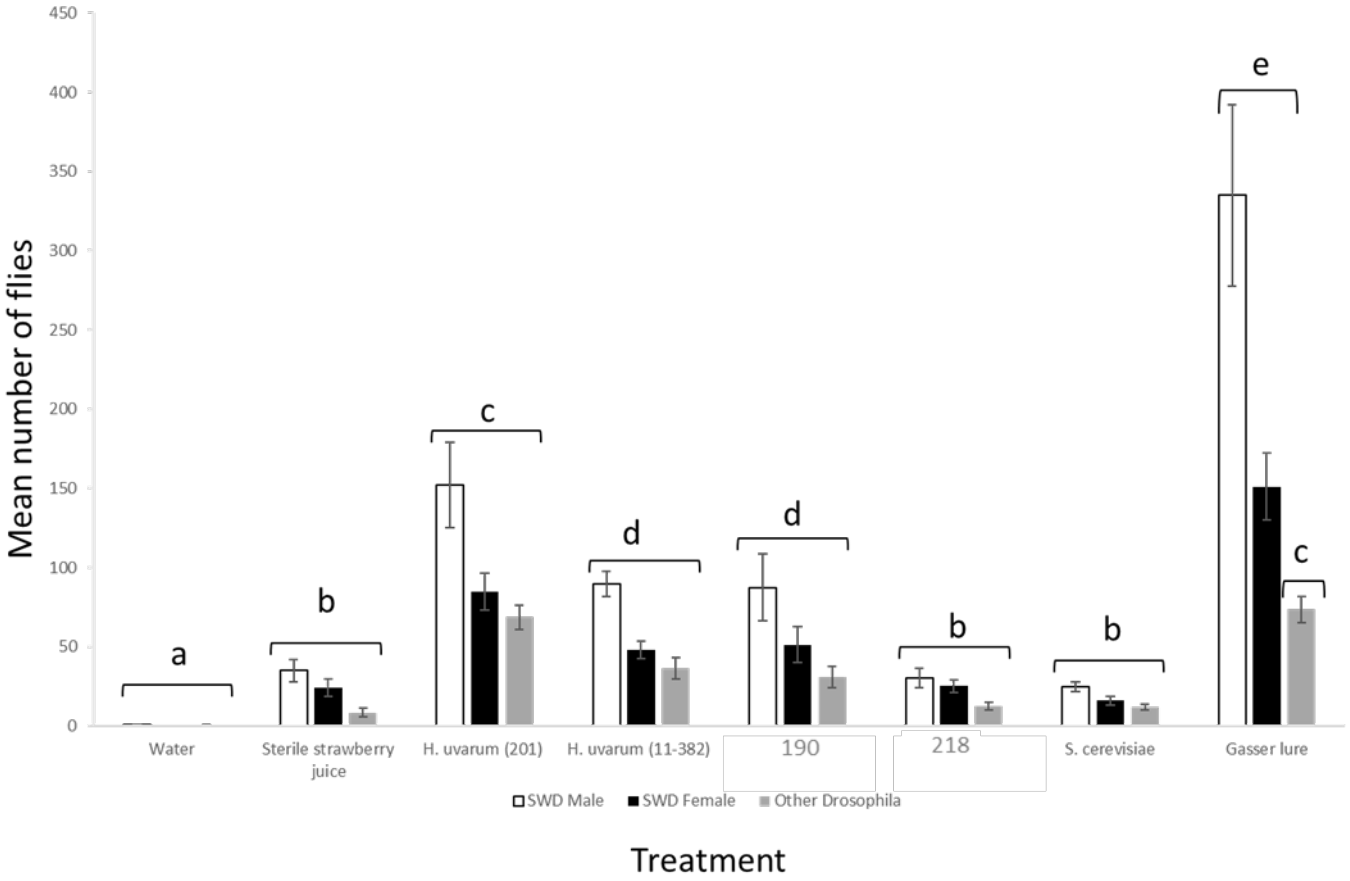

Fig. 5: Mean numbers (± SE) of *D. suzukii* and other *Drosophila* attracted to five individual yeasts, four different species (*H. uvarum,* 190, 218 and *S. cerevisiae*) and two strains of *Hanseniaspora uvarum* (201 and 11-382) fermented in strawberry juice in comparison to sterile strawberry juice, water and commercial Gasser lure (N=6). Attraction is measured as the total number of flies caught over a 72-hour period. Lower case letters above bars show statistical differences for non-overlapping confidence intervals, calculated from Estimated Marginal Means (95%).

H3- The effect of combining yeast on the attraction of *D. suzukii* will follow an additive (linear) model i.e. there will be no interaction effect or synergistic effect of combining yeast on attraction.

Two-way choice tests

There was a significant difference in attractiveness between yeast combinations (following model simplification, by removing treatment, deviance = -39.16, df=-12, p>0.001). Individual binomial analyses reveal four yeast combinations to be significantly more attractive than sterile strawberry juice: 190+201 (AI=0.33, P<0.001), 201+164 (AI=0.23, P=0.0026), 190+218+201 (AI=0.19, P=0.010) and 190+218 (AI=0.18, P=0.0096). *H. uvarum* (201) alone was significantly attractive (AI=0.15, P=0.027), and the positive control Gasser lure was also attractive (AI=0.28, P<0.001). *D. suzukii* was indifferent to seven yeast combinations compared to sterile strawberry juice (Fig. 6). We next tested whether there was any evidence that combinations of yeast volatiles interacted in a non-linear manner to affect the degree to which *D. suzukii* are attracted. Permutation analyses reveal an interaction between yeasts in two of the combinations: 190+218+201+164+98-3 and all yeasts (P=0.012 and P=0.006 respectively; Fig. 6). For these combinations, the observed AI values were significantly lower compared to predicted values assuming an additive interaction of the AIs from the constituent component yeasts in the combinations. This indicates there may be some types of yeast combinations that produce a combined volatile profile that is repulsive to *D. suzukii*. Analysis with a linear model also revealed that 190+218+201+164+98-3 (P=0.0057) differed significantly from predicted attractiveness assuming a linear model of attraction, but the all-yeast combination did not (P=0.072).

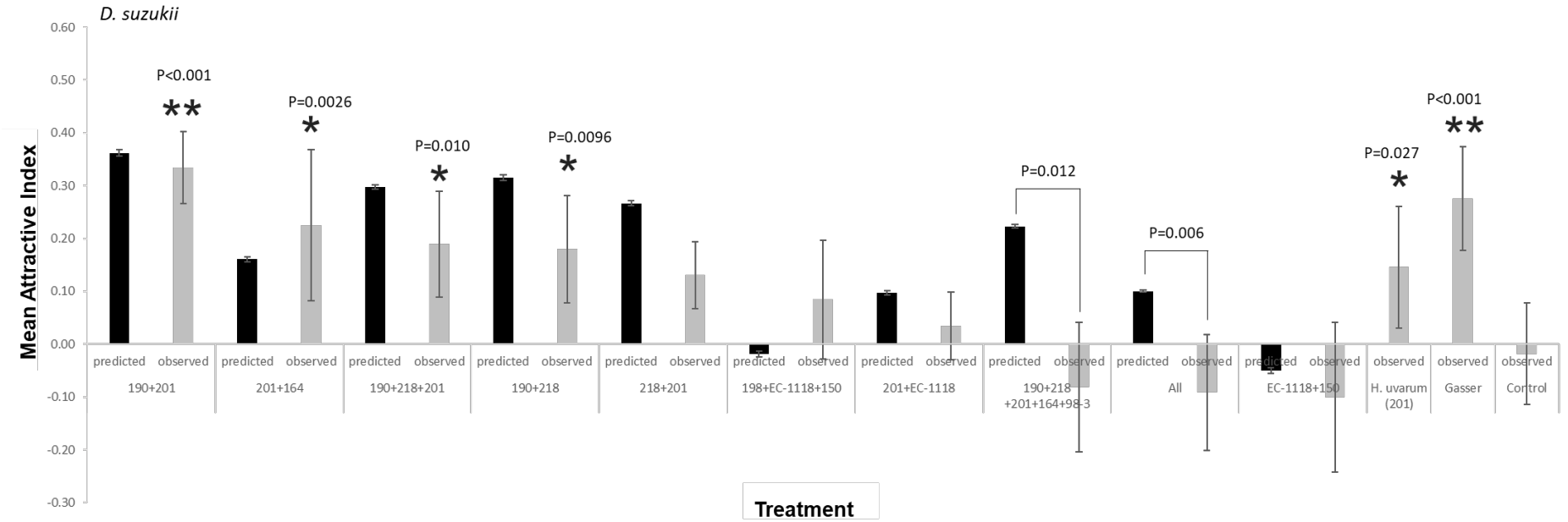

Fig. 6: Mean ± standard error of the mean Attraction Index (AI) of *D. suzukii* to ten yeast combinations, fermented individually in strawberry juice then mixed in equal proportions. *H. uvarum* (201), sterile fruit and commercial Gasser lure act as controls. N=6 for all treatments except Gasser, Hu (201), All, 190+218+201+164+ 98-3 and EC-1118+198+150 and control (N=7). *D. suzukii* had no significant preference for either side in the sterile fruit juice control (AI=-0.02, P=0.36). Grey bars represent observed AI and black bars the mean predicted AI, calculated from 10,000 permuted individual AI values. Asterisks above bars represent any significant attraction.

Field trials

Overall, there was a significant interaction between treatment and sex and this explained a significant amount of the deviance (Δdev=65, df=8, p<0.001 and Δdev=247, df=8, p<0.001, first and second sites respectively), and so male and female *D. suzukii* were analysed separately. There was significant variance in attraction to different yeast treatments for male and female *D. suzukii* and other *Drosophila* (male: Δdev=1323, df=8, p<0.001, Δdev=1299, df=8, p<0.001 and female: Δdev=1437, df=8, p<0.001 first site, and male: Δdev=9366, df=8, p<0.001, Δdev=9661, df=8, p<0.001 and female: Δdev=2613, df=8, p<0.001 second site). The confidence intervals for male and female *D. suzukii* and other *Drosophila* revealed all treatments were more attractive that the negative water control for both sites and that Gasser-lure was the most attractive. *H. uvarum* (201) was significantly more attractive than sterile strawberry juice for male *D. suzukii* at the first site only. Individual analysis of male *D. suzukii* in traps from the first site revealed two yeast combinations were more attractive than sterile fruit: 190+218+201 and 190+201, and only one yeast combination was more attractive than sterile fruit for female *D. suzukii:* 190+201. Four of the yeast treatments proved to be more attractive than sterile strawberry juice for other *Drosophila*; *H. uvarum* (201), 190+201, 218+201 and 201+EC-1118 (Fig. 7a; first site). For the second site only, one yeast community was more attractive than sterile fruit for male *D. suzukii:* 190+201. Sterile strawberry juice, *H. uvarum* (201), 190+218+201, 190+218 and 190+201 were more attractive than 218+201. 190+218 and 190+201 were also more attractive than 201+EC-1118. For female *D. suzukii,* two combinations were more attractive than sterile strawberry juice; 190+218 and 190+201, and these combinations were significantly more attractive than 201+EC-1118 and 218+201. For other *Drosophila* the ‘all yeast’ treatments were more attractive than sterile strawberry juice, with *H. uvarum* (201) and 190+201 also being more attractive than 190+218 and 218+201 (Fig. 7b).

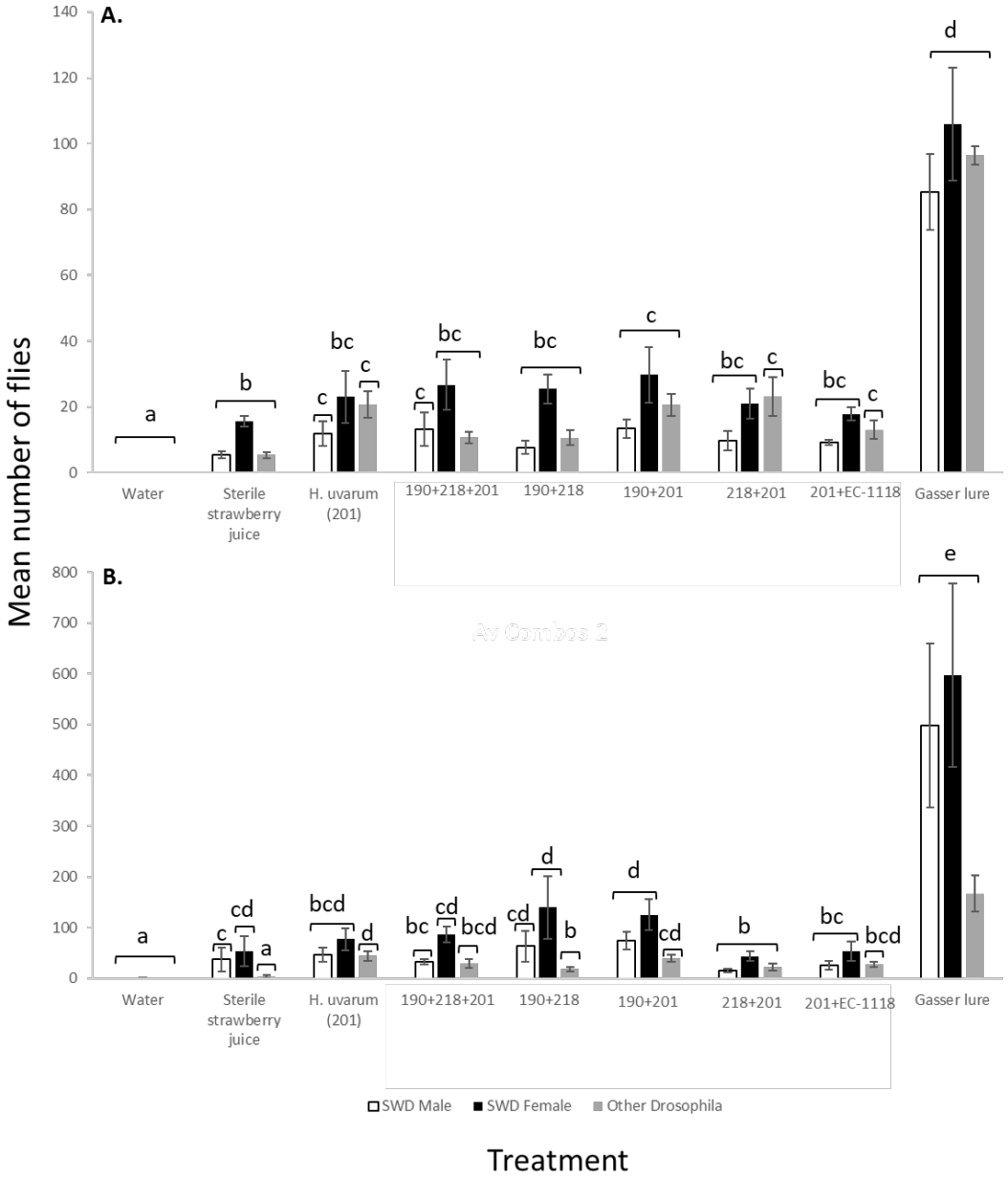

Fig. 7. Mean numbers (± SE) of *D. suzukii* and other *Drosophila* attracted to five combinations of yeast species (190+218+201, 190+218, 190+201, 218+201, 201+EC-1118) fermented separately in strawberry juice and then combined in equal proportions. *H. uvarum* (201), sterile strawberry juice, water and commercial Gasser lure serve as controls. N=7 except 190+218 N=6 at the second site. A. first site carried out in November 2018 at the same native hedgerow at the commercial fruit producer in Kent, UK as the single yeast field test; B. carried out December 2018 in a deciduous bramble woodland, adjacent to the site used in A. Attraction was measured as the total number of flies caught over a 72-hour period. Lower case letters above bars shows statistical differences (with in group) for none overlapping confidence intervals, calculated from Estimated Marginal Means (95%).

H4- Neither fruit type nor ripening stage has a significant effect on fugal communities on fruit by any ecological measure.

When quantifying the similarities and differences in the fungal communities from the different fruit species and different ripening stages, we analysed three biodiversity metrics for fungal communities: 1, absolute species richness (differences in the number of fungal phylotypes); 2, relative species richness (differences in types of fungal phylotypes present) and 3, community composition (differential abundances of fungal phylotypes) (Morrison-Whittle *et al*., 2017; Morrison-Whittle and Goddard, 2018).

Overview of fungal diversity on ripening fruit

A total 1,712 fungal phylotypes with >97% ASV identity were revealed in the sequence data overall comprising seven phyla, 26 classes, 96 orders, 197 families, 282 genera. The most abundant and diverse phylum was Ascomycota which made up 92.2% of the total reads and 57.3% of phylotypes, followed by Basidiomycota (7.7% reads and 33.6% phylotypes), Zygomycota (0.1% and 1.1%), Chytridiomycota (>0.1% and 0.7%), Mucoromycota (>0.1% and 0.3%), Glomeromycota and Rozellomycota (both >0.1% and 0.1%)(supplementary material, Fig. S2a). A phylotype from the *Cladosporium* genus was the most common fungal phylotype across all samples, making up 60.8% of the total reads. A total of 87 distinct yeast phylotypes with >97% ASV identity from the order *Saccharomycetales* were detected across all samples comprising 9 families and 25 genera. With the most abundant genus being *Metschnikowia* (40.0% of the reads), followed by *Hanseniaspora* (38.2%) then Pichia (5.2%) with the rest of the genera contributing less than 3% of the reads each. *Candida* was the most diverse genus within the order *Saccharomycetales* with 21.8% of the phylotypes, despite only having 2.5% of the reads, followed by *Metschnikowia* (11.5%), *Hanseniaspora* (8.0%) and *Pichia* (6.9%), with each of the remaining genera contributing less than 3.4% of the phylotypes each. (supplementary material, Fig. S2b). The most common yeast across all samples was a phylotype from the genus *Hanseniaspora*, which comprised of 38.2% of the total reads for *Saccharomycetales* yeasts.

Quantitative analysis of fugal communities

Using amplicon sequencing to characterise fungal diversity is generally not quantitative, giving only relative abundance and not actual abundance. The benchmark fungi, *P. cucumerina*, was recovered from 25% of all samples. The only fruit species where the benchmark was recovered consistently across spiked samples was cherry, with the

benchmark being recovered from 50% of cherry samples, from all but one benchmark spiked cherry samples and only one of the non-spiked samples. Cell numbers generally, but not significantly, increased during ripening, with stage four having an average of 465190 cells, followed by stage 3 (425713), then stage 1 (263139) and stage 2 (234205) (N=6 replicates fruit N=60, except stage 3 N=5 and N=50). However, fruit surface area also increased significantly (across the ripening stages Kruskal-Wallis, chi-squared=20.64, $P = 0.00013$) and when cell numbers were calculated per mm^2 of surface area the pattern of cells increase was no longer apparent, with stage 1 having the largest number of cells per mm^2 (28), then 3 (22), 4 (21) and 2 (19) (Fig. 8).

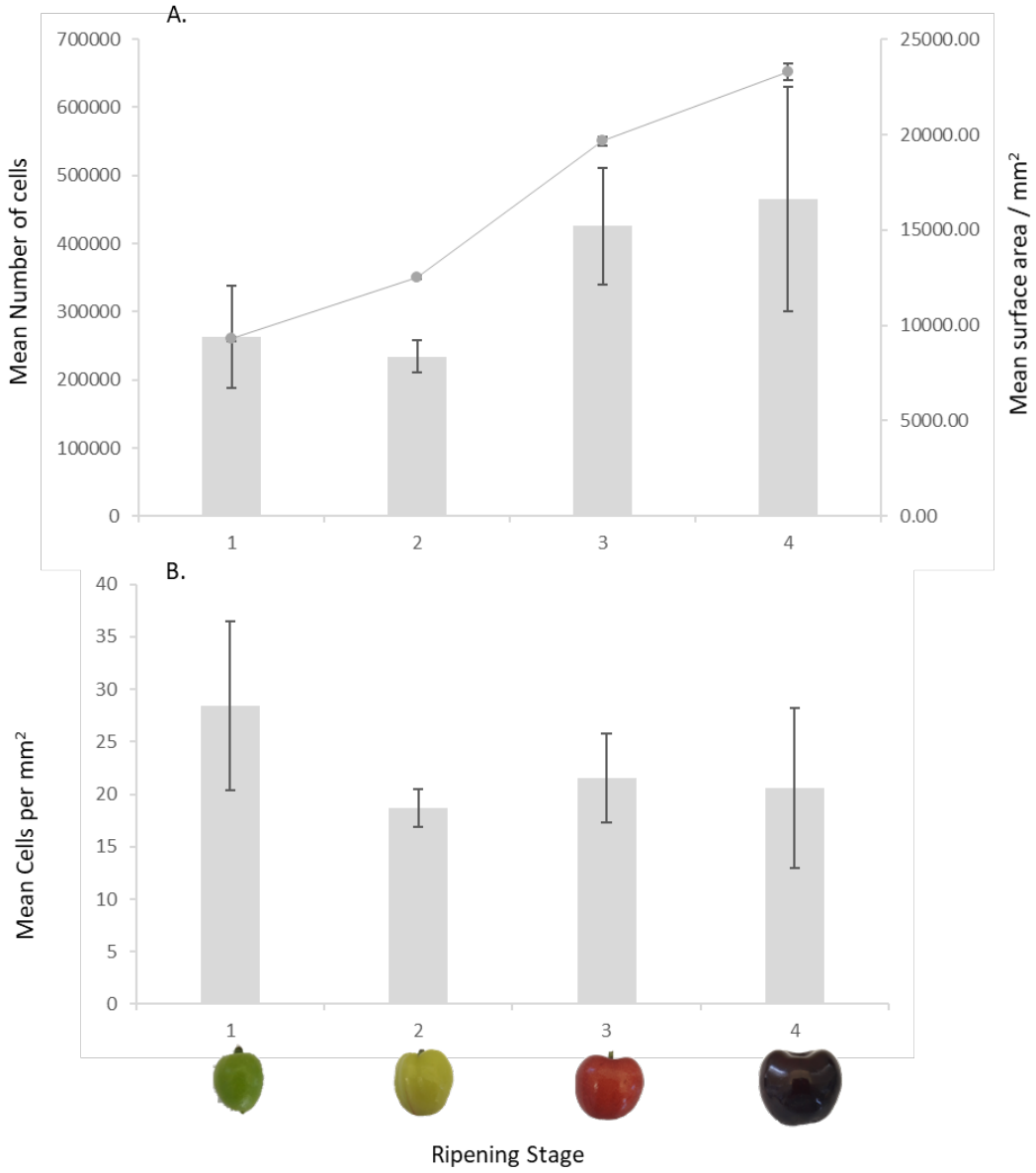

Fig. 8. A. Mean (±SE) number of fungal cells across the four ripening stages of cherry (N=6, except stage 3 N=5) (grey bars, primary y axis) and mean (±SE) surface are of cherries at the four ripening stages (grey line, secondary y axis) B. Mean number of fungal cells per mm^2 (N=6 except, stage 3 N=5).

The effect of fruit and ripening stage on fungal phylotype richness.

To start, we analysed the effect of fruit type and ripening stage on the total number (richness) of fungal phylotypes using a two-way ANOVA. The data was not normally distributed (Shapiro-Wilks, $P = 0.0079$) so a square root transformation was applied. There was a significant effect of fruit type and ripening stage on the number of fungal phylotypes, as well as an interaction between the two ($F_{3,175} = 18.58$, $P = 1.65 \times 10^{-10}$; $F_{3,175} = 5.00$, $P = 0.0024$ and $F_{9,175} = 6.69$, $P = 3.25 \times 10^{-8}$ respectively). With cherry (average number of phylotypes = 98) harbouring significantly more fungal phylotypes than blueberry (68), raspberry (72) and strawberry (76) (Tukey's HSD, $P < 1.0 \times 10^{-7}$, $P = 2.0 \times 10^{-7}$ and $P = 2.56 \times 10^{-5}$ respectively), and with blueberry, raspberry and strawberry not differing significantly from one another (supplementary material Fig. S3). Additionally, ripening stage 1 (average of 69) had significantly fewer fungal phylotypes than 2 (85) and 3 (82) ($P = 0.0015$ and $P = 0.033$, respectively) but not 4 (79), additionally stage 2 and 4 were also not significantly different (supplementary material Fig S2). Comparisons of E^2 values showed fruit type ($E^2 = 0.23$) has 3.7 times greater influence than ripening stage ($E^2 = 0.063$) on fungal phylotype richness.

As there was a significant effect of fruit type on absolute species richness, we also investigated the effect of ripening stage for our four fruit species individually. When each fruit species was analysed separately, data was normally distributed (Shapiro-Wilks, $P > 0.05$ for all) and one way ANOVAs revealed that there was a significant effect of ripening stage on the number of fungal phylotypes present on cherry, raspberry and strawberry ($F_{3,44} = 4.33$, $P = 0.0093$; $F_{3,44} = 13.56$, $P = 2.11 \times 10^{-6}$ and $F_{3,44} = 13.86$, $P = 1.84 \times 10^{-6}$, respectively), but not blueberry ($F_{3,44} = 2.27$, $P = 0.055$). For cherry, the number of fungal phylotypes increased during ripening peaking at ripening stage 4, with stage 4 harbouring significantly more fungal phylotypes than both stage 1 and 2 ($P = 0.012$ and $P = 0.027$, respectively). For raspberry, ripening stage 2 harboured the most fungal phylotypes having significantly more than stage 1 and 4 ($P = 0.0068$ and $P = 3.4 \times 10^{-6}$, respectively). Additionally, stage 3 also harboured significantly more than 4 ($P = 1.13 \times 10^{-4}$. Again, for strawberry ripening stage 2 had significantly more fungal phylotypes than stage 1 and 4 (both $P = 2.27 \times 10^{-4}$ and $P = 2.23 \times 10^{-5}$, respectively). Additionally, stage 3 also harboured significantly more phylotypes than

stages 1 and 4 (P = 0.0035 and P = 4.25 x10^{-4}, respectively). For blueberry there were no significant differences between any of the stages (Fig. 9).

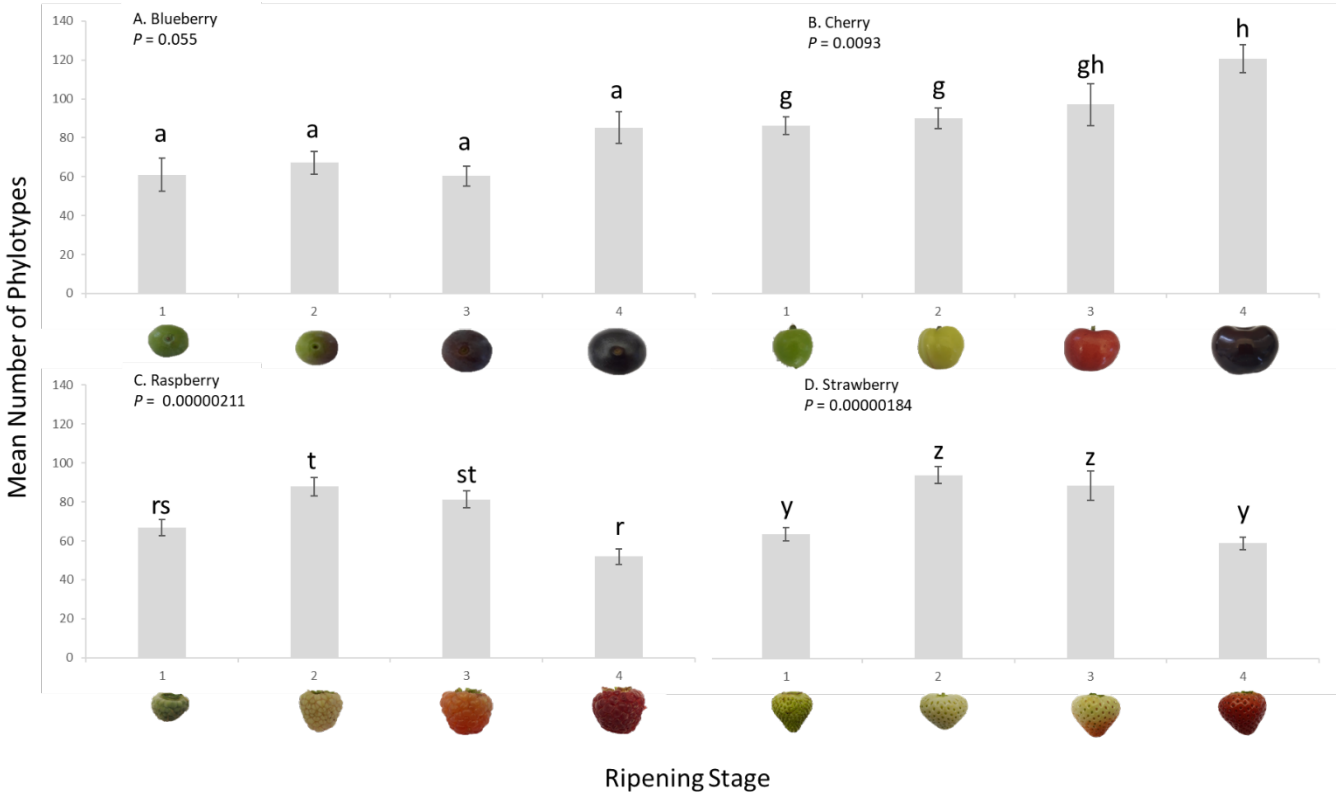

Fig. 9. Average number of fungal phylotypes present across the four ripening stages for the four fruit (N=12 except strawberry stage 3 N=11). A. Blueberry, B. Cherry, C. Raspberry and D. Strawberry. Different lowercase letters represent any significant difference in phylotypes number between ripening stages within fruit species and not between fruit species.

The effect of fruit type and ripening stage on species richness of yeast phylotypes from the order *Saccharomycetales* was analysed using Kruskal-Wallis as square transformed data did not conform to the assumption of normality. There was a significant effect of fruit, but not ripening stage on the number of yeast phylotypes (Kruskal-Wallis, chi-squared=75.66, df = 3, P = 2.61 x10^{-16} and chi-squared = 5.50, df = 3, P = 0.14 respectively). With raspberry harbouring significantly more yeast phylotypes than strawberry (mean 10), cherry (7) and blueberry (4) (Tukey's HSD, P = 0.044, P = 2.9 x10^{-6} and P = 1.5 x10^{-16} respectively). Strawberry harboured significantly more than cherry and blueberry (Tukey's HSD, P = 0.0066 and P = 2.6 x10^{-9}), and cherry harboured significantly more than blueberry (Tukey's HSD, P = 0.0011) (Fig S3).

Both Shannon's and Simpson's diversity indexes demonstrated difference between phylotypes distribution for both fruit phylotypes (average Shannon's diversity indexes = 1.04, 1.41, 1.13 and 1.36; average Simpson's indices = 0.41, 0.58, 0.41 and 0.48; for blueberry, cherry, raspberry, and strawberry respectively). Difference between species distribution were significant for fruit species stage (Kruskal-Wallis, chi-squared=46.98, $P = 3.52 \times 10^{-10}$ and chi-squared = 50.19 $P = 7.27 \times 10^{-11}$, for Shannon and Simpson indices respectively). Ripening stage also demonstrated difference between phylotypes distribution (average Shannon's diversity indexes 1.26, 1.29, 1.08 and 1.31; average Simpson's indices 0.034, 0.044, 0.051 and 0.041; for ripening sage 1, 2, 3 and 4 respectively). These differences were also significant (Kruskal-Wallis, chi-squared=12.18, $P = 0.0068$ and chi-squared = 16.55 $P = 0.00088$ for Shannon and Simpson indices respectively).

The effect of fruit and ripening stage on types of fungal phylotypes

Next, we analysed the impact of fruit species and ripening stages on types of fungal phylotypes present with a 2-way PermANOVA on binary Jaccard distance matrices**.** This revealed that both fruit type and ripening stage significantly influenced the types of phylotypes present ($R^2 = 0.094$, $P = 9.999 \times 10^{-5}$ and $R^2 = 0.017$, $P = 9.999 \times 10^{-5}$, respectively, Fig. 10) with a significant interaction between fruit type and ripening stage ($R^2 = 0.013$, $P = 9.999 \times 10^{-5}$). Comparisons of R^2 values showed fruit type has approximately five and a half times greater influence than ripening stage on the types of fungal phylotypes present. Pairwise PermANOVAs showed that there were significance differences in presence of different types of fungal phylotypes between all fruits and ripening stages ($P = 9.999 \times 10^{-5}$, R^2 range 0.09 – 0.20; supplemental Tables S1 and S2).

As there was a significant effect of fruit type on relative species richness, we again also investigated the effect of ripening stage on fungal communities for our four fruit species individually. When the four different fruit species were analysed individually, ripening stage significantly influenced the types of fungal phylotypes present for blueberry, cherry, raspberry and strawberry ($R^2 = 0.043$, $P = 2.00 \times 10^{-4}$; $R^2 = 0.060$, $P = 9.999 \times 10^{-5}$; $R^2 = 0.13$, $P = 9.999 \times 10^{-5}$ and $R^2 = 0.055$, $P = 9.999 \times 10^{-5}$, respectively). The relationships between the structure and composition of fungal communities from the four fruit species and four ripening stages are shown in Nonmetric Multidimensional Scaling (NMDS) of non-binary Jaccard measures of community dissimilarity (Fig. 10; supplementary material Fig. S4).

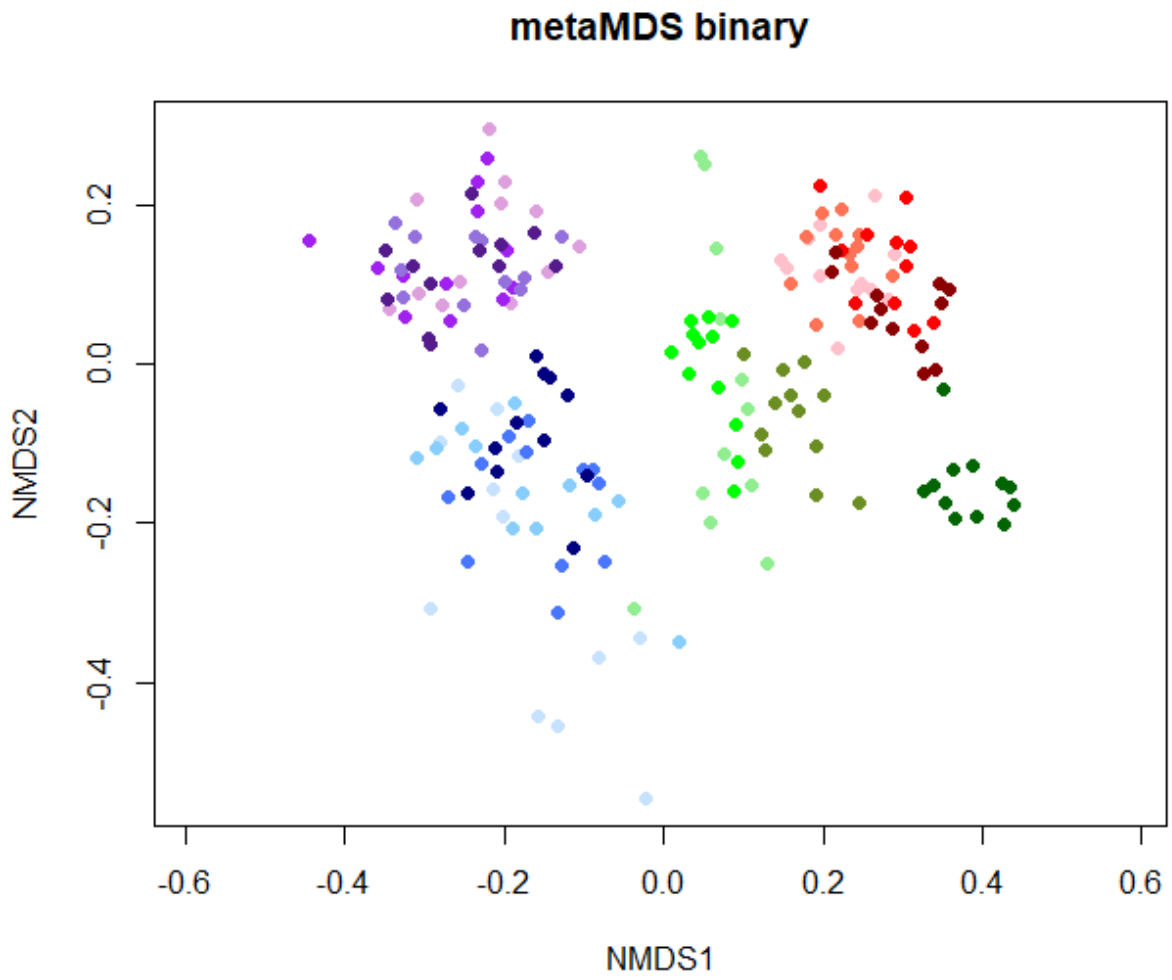

Fig. 10. Nonmetric Multidimensional Scaling analysis of binary Jaccard measures of community dissimilarity of fungal communities on four fruit species, blueberry (blue dots), cherry (purple), raspberry (green) and strawberry (red) and four ripening stages for each fruit, from green fruit through to harvest fruit (ripening stages denoted by shade of fruit colour, lightest shade for green fruit and moving through to darkest shade for harvest).

For yeast phylotypes from the order *Saccharomycetales* 2-way PermANOVA on binary Jaccard distance matrices showed that both fruit type and ripening stage significantly influenced the types of phylotypes present (R^2 = 0.082, P = 9.999 x10^{-5} and R^2 = 0.026, P = 9.999 x10^{-5}, respectively, supplementary material, Fig. S5) with there also being a significant interaction between fruit type and ripening stage (R^2 = 0.024, P = 9.999 x10^{-5}). Comparisons of R^2 values showed fruit type has approximately 3.15x greater influence than ripening stage on the types of phylotypes present. Pairwise PermANOVAs showed that there were

significance differences in presences of different types of yeast phylotypes between all fruits and ripening stages (P= 9.999 x10^{-5}, R^2 range 0.051 – 0.15; supplemental Tables S5 and S6). For *Saccharomycetales* yeasts there was also a significant effect of fruit type on relative species richness. When the four different fruit species were analysed individually, ripening stage significantly influenced the types of fungal phylotypes present for blueberry, cherry, raspberry and strawberry (R^2 = 0.065, P = 0.00080; R^2 = 0.080, P = 0.00080; R^2 = 0.27, P = 9.999 x10^{-5} and R^2 = 0.084, P = 9.999 x10^{-5}, respectively) (NMDS binary, supplementary material Fig. S5).

The effect of fruit and ripening stage on the abundances of fungal phylotypes

We then analysed the impact of fruit species and ripening stage on fungal community composition with a 2-way PermANOVA on abundance Jaccard distance matrix. This showed that both fruit type and ripening stage significantly influenced the relative abundances of phylotypes in fungal communities (R^2 = 0.15, P = 9.999 x10^{-5} and R^2 = 0.027, P = 0.00020, respectively, Fig 3). There was also a significant interaction between fruit type and ripening stage (R^2 = 0.018, P = 0.0031). Fruit species has approximately 5.6 times greater influence than ripening stage on the relative abundances of phylotypes. Pairwise PermANOVAs showed that there were significant differences in fungal community composition between all the different fruit species and ripening stages (P= 9.999 x10^{-5}, R^2 range 0.11 – 0.43; supplemental Tables S3 and S4).

As there was a significant effect of fruit type on fungal community composition, the effect of ripening stage on fungal communities for the four fruit species was investigated individually. As with types of species, when blueberry, cherry, raspberry and strawberry were all analysed individually ripening stage also had a significant effect on relative abundances of species in fungal communities (R^2 = 0.16, P = 9.999 x10^{-5}; R^2 = 0.061, P = 0.0074; R^2 = 0.24, P = 9.999 x10^{-5} and R^2 = 0.15, P = 9.999 x10^{-5}, respectively). The relationships between the structure and composition of fungal communities from the four ripening stages for each fruit are shown in NMDS of non-binary Jaccard measures of community dissimilarity (Fig. 11; supplementary material Fig. S6).

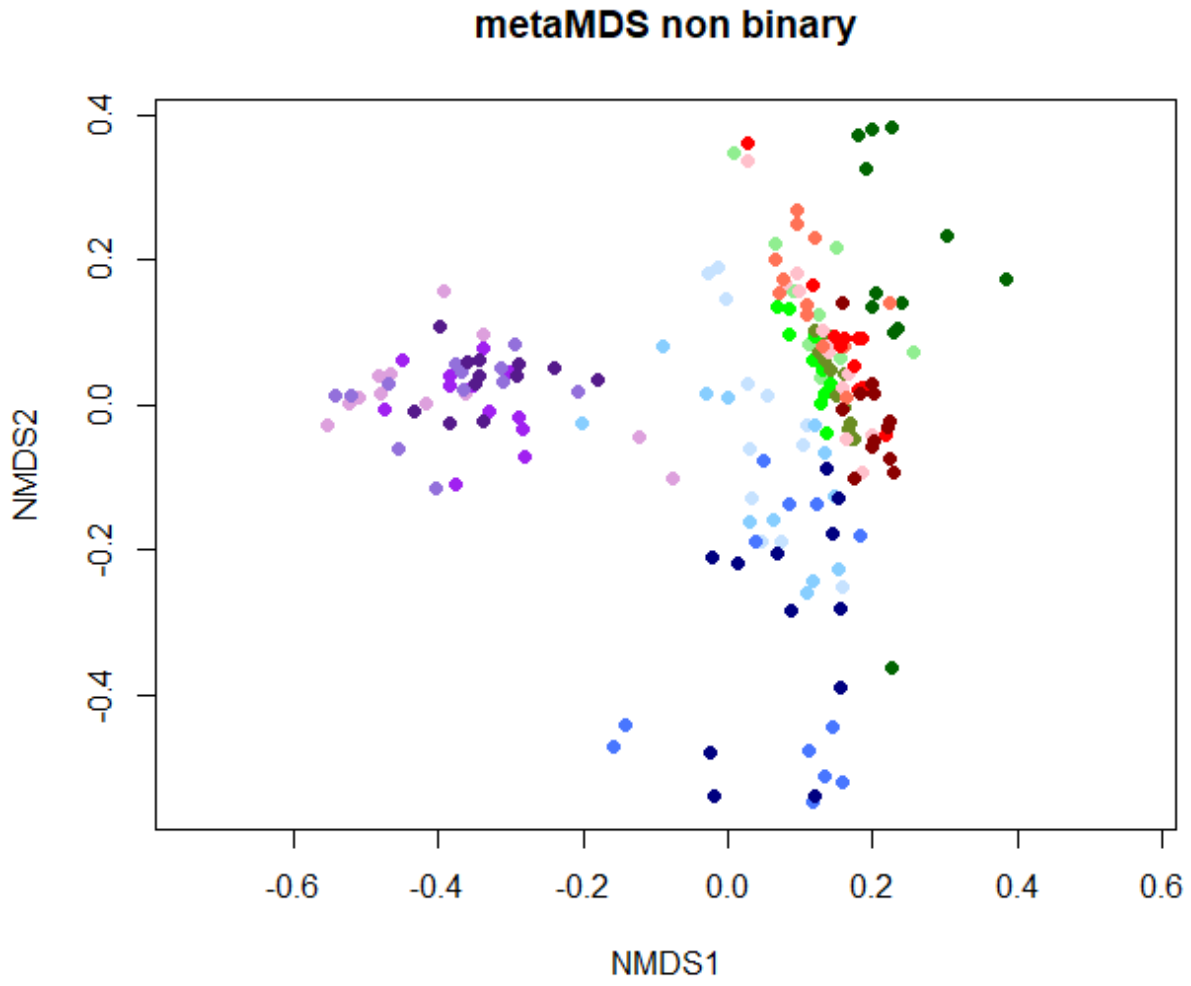

Fig. 11. Nonmetric Multidimensional Scaling analysis of non-binary Jaccard measures of community dissimilarity of fungal communities on four fruit species, blueberry (blue dots), cherry (purple), raspberry (green) and strawberry (red) and four ripening stages for each fruit, from green fruit through to harvest fruit (ripening stages denoted by shade of fruit colour, lightest shade for green fruit and moving through to darkest shade for harvest).

For yeast phylotypes from the order *Saccharomycetales* 2-way PermANOVA on abundance Jaccard distance matrix again revealed fruit type and ripening stage significantly influenced the relative abundances of phylotypes in fungal communities (R^2 = 0.038, P = 9.999 x10^{-5} and R^2 = 0.024, P = 9.999 x10^{-5}, respectively, Fig 3), with there also being a significant interaction between fruit type and ripening stage (R^2 = 0.016, P = 9.999 x10^{-5}). Fruit species has approximately 1.6 times greater influence than ripening stage on the relative abundances of phylotypes. Pairwise PermANOVAs showed that there were significance differences in fungal community composition between all the different fruit species and ripening stages (P = 9.999

x10^{-5}, R^2 range 0.038 – 0.13; supplemental Tables S3 and S4). As with types of species, when blueberry, cherry, raspberry and strawberry were all analysed individually, ripening stage also had a significant effect on relative abundances of phylotypes for *Saccharomycetales* yeasts (R^2 = 0.043, P = 0.0038; R^2 = 0.64, P = 0.0031; R^2 = 0.19, P = 9.999 x10^{-5} and R^2 = 0.070, P = 0.00080, respectively) (NMDS non-binary, supplementary material Fig. S5).

Indicator species.

Of the four fruit species, all had indicator species that were significantly over-represented when compared to the other fruits. Blueberry had 33 indicator species, cherry 70, raspberry 39 and strawberry 53 (FDR corrected P values ranging from P = 0.011 to P = 0.044 for all fruit except strawberry, P = 0.011 to P = 0.036).

For blueberry, the two indicator species with the highest indicator species value (indval) were *Polyphialoseptoria tabebuiae-serratifoliae* (indval=0.83) and *Ramularia endophylla* (indval=0.78). For cherry, a fungi from the genus *Exobasidium* (indval= 0.99) a genus of fungi containing species that are patristic on angiosperms (Reddy and Saravanan, 2013) and *Collophorina germanica* (indval= 0.98), a recently identified fungal species that was found in necrotic wood from *Prunus avium* (sweet cherry) in Germany (Bien *et al.,* 2019). For raspberry, *Metschnikowia kunwiensis* (indval= 0.77) the teleomorph of *Candida kunwiensis* (Brysch-Herzberg, 2004) which is associated with flowers and bumblebees (Hong *et al*., 2003) and a yeast of the genus *Hanseniaspora,* mostly likely *H. uavrum* (indval= 0.75) a genus of yeast often associated with fruit. For strawberry, *Kalmanozyma fusiformata* (indval= 0.9996) a yeast species that has been isolated from cabbages and cauliflowers (Buhagiar, 1979), and *Podosphaera aphanis* (indval=0.99) causal agent of strawberry powdery mildew (Carisse and Bouchard, 2010).

When analysed individually for indicator species for the different ripening stages, only strawberry and raspberry had any significant indicator species, 16 for strawberry with 3 from stage 1, 2 from both stage two and three and 9 from stage 4 (FDR corrected P = 0.043 for all), 36 for raspberry with 8 from stage 1, 12 stage two, three stage three and 13 from stage four (FDR corrected P values ranging from P = 0.023 to P = 0.038).

H6-There is no significant difference in fly mortality and egg laying between the different yeast treatments, with combinations being no more effective in increasing mortality and deceasing egg laying compared to single yeasts.

Attractiveness data has been collected for all three experiments (single yeasts, combination singly fermented then combined and combinations co-fermented) for yeast grown both in SSJ and YPD for summer morph and winter morph flies. Multiple choice tests were carried out to determine yeasts to combine with PPPs for phagostimulatory baits. For jar bioassays, data form all three experiments using Tracer (spinosad), Exirel (cyantraniliprole) and Hallmark (lambda-cyhalothrin) have been collected for both summer morph and winter morph flies. Data from both the multiple-choice tests and jar-bioassays has yet to be fully analysed so is not included in this report.

Discussion

The attractiveness of different yeast species to different *Drosophila* was shown to vary (Fig. 5); this is in line with the findings of previous studies (Palanca *et al.,* 2013; Buser *et al.,* 2014; Günther *et al.,* 2015; Scheidler *et al.*, 2015). In the field, *Drosophila suzukii* was significantly attracted to three yeasts; the two *H. uvarum* strains and 190, and *S. cerevisiae* was no more attractive than sterile strawberry juice (Fig. 5). However, unlike the laboratory findings, 218 was not significantly attractive in the field. Overall, this suggests that 2-way choice test with T-mazes are a good proxy for attraction in the field but highlight the need for field testing to confirm attraction.

Scheidler *et al.* (2015) found that *H. uvarum* and *S. cerevisiae* were both significantly attractive to *D. suzukii.* In contrast, we have again reported that *D. suzukii* is indifferent to *S. cerevisiae* (EC-1118). We assayed the attractiveness of yeast species fermented in strawberry juice whereas Scheidler *et al.* (2015) used culture media, Potato Dextrose Broth, which could account for the differences. Also, different strains of *S. cerevisiae* were used which could also account for the difference in attractiveness (Scheidler *et al.*, 2015) as it has been shown that different *S. cerevisiae* genotypes vary in their attractiveness to *D. simulans* (Buser *et al.,* 2015) and *D. melanogaster* (Palancar *et al.,* 2013). The attractiveness of *H. uvarum* strains seems to vary, with there being a significant effect of attraction between the two strains tested in the field (Fig. 5).

To date, only single yeasts have been tested for attraction to *Drosophila* (e.g. Palanca *et al*., 2013; Buser *et al*., 2014; Scheidler *et al*., 2015). We combined singly fermented yeast to create combinations and found that 4 of the 10 combinations tested were significantly attractive to *D. suzukii* Including 190+218+201, 190+218, 190+201, 201+164 (Fig. 6). In the field, three of the combinations tested caught significantly more flies than sterile strawberry juice for both female and male *D. suzukii* 190+201 (across both field assays), 190+218 for female (only second) and 190+218+201 for males (only first) (Fig. 7). Additionally, there seemed to be some evidence that, at least for some combinations, attraction does not follow a linear model as two of the combinations 190+218+201+164+98-3 and 'all yeast' differed significantly from expected additive values of attractiveness (Fig. 6). The combinations tested here are post ferment blended combinations of single fermented then combined yeasts. This is not ecologically realistic as the yeast have not had the chance to interact when growing. Co-fermentation of *S. cerevisiae* and *P. kluyveri* produce increased amounts of certain volatiles, including varietal thiols like 3-mercaptohexyl acetate, compared to single cultures (Anfang *et al.,* 2009). Furthermore, when the yeast *S. cerevisiae* was co-cultured with bacteria it was more attractive to *D. melanogaster* than post-growth blending (Fischer *et al.,* 2017). The testing of post ferment blended combinations represents an important step in testing more ecologically realistic yeast combinations, but one that can potentially be optimised to improve attractiveness. Experiments have been carried out looking at the attraction of co-fermented yeasts alongside single yeast and their use as phagostimulatory baits (data not presented, currently being analysed).

Fruit surfaces are home to complex microbial communities which are often dynamic. Microbial communities are known to be affected by a range of factors including fruit species, ripening stage, geographic location and damage sustained by fruit (Leff and Fierer, 2013; Barata *et al.,* 2012; Taylor *et al.,* 2014 Vepštaitė-Monstavičė *et al.,* 2018). Findings from this study are in line with other studies that have shown fungal and yeast communities to vary with fruit type and ripening stage (Janisiewicz *et al.,* 2014; Kecskeméti *et al.,* 2016; Abdelfattah *et al.,* 2016; Vepštaitė-Monstavičė *et al*., 2018). It is apparent from this study that both fruit type and ripening stage have a significant impact on fungal community diversity, in terms of absolute species richness, relative species richness and community composition. Additionally, it seems that fungal abundance (both relative and absolute) increases with ripening stage. Although, for absolute abundance we only present data for cherry and the increase was not significant. Additionally, when adjusted to cells per mm^2 this trend did not hold true. In addition to fungal communities varying in diversity, this was also true for *Saccharomycetales* yeasts.

Fruit type but not ripening stage had a significant effect on absolute species richness for *Saccharomycetales* yeasts, with raspberry harbouring the most yeast phylotypes. Additionally, community composition and relative fungal abundance were both significantly impacted by fruit type and ripening stage for *Saccharomycetales* yeast communities, with fruit type having the bigger impact in both cases.

The genera of yeast with the highest relative abundance identified by this study were *Metschnikowia, Hanseniaspora* and *Pichia,* with the most diverse genera being *Candida Metschnikowia, Hanseniaspora* and *Pichia.* This is not surprising as these are well known yeasts genera that are associated with fruit (e.g. Hamby *et al.,* 2012 Vadkertiová *et al.,* 2012; Vepštaitė-Monstavičė *et al.,* 2018). Various yeast species from these genera are attractive to *D. suzukii*, including *H. uvarum, C. zemphilina, C. californica* and *P. terricola* (Scheidler *et al.,* 2015; Lasa *et al.,* 2019; Bueno *et al.,* 2020). Additionally, for raspberry the two most prominent indicator species that were overrepresented in raspberry samples compared to other fruits were both yeasts, *Metschnikowia kunwiensis* and yeast of the genus *Hanseniaspora* mostly likely *H. uavrum.* This further suggests that there are individual yeast species that differ significantly in relative abundance between the different fruits and these overrepresented species would make for interesting candidates for attraction testing.

The fact that yeast communities vary between fruit type and ripening stages has potentially interesting implications. Various yeast species from the order *Saccharomycetales* are attractive to *D. suzukii* (Scheidler *et al.,* 2015; Lasa *et al.,* 2019), as well as to other *Drosophila* species (Palanca *et al.,* 2013; Günther *et al.,* 2019), and there may be differences in yeast preferences between different *Drosophila* species (Scheidler *et al.,* 2015; Günther *et al.,* 2019). Coupled with the fact that *D. suzukii* can lay eggs in ripening fruit and is one of only two *Drosophila* species with this ability (Atallah *et al.,* 2014). Additionally, there is a microbial component to fruit attraction with *D. suzukii* females preferring to oviposit on yeast-colonised fruit as opposed to sterile fruit (Bellutti *et al*. 2018). Taken together this leads to interesting possibilities, namely can we exploit yeast communities on ripening fruit to develop and optimise more attractive and specific yeast baits that can be utilised as phagostimulatory baits in control of this pest.

Conclusions

Yeasts vary in their attraction to *Drosophila* species and previous work in 2017/18 identified four candidate yeast species attractive to *D. suzukii*: *H. uvarum*, yeast coded 218, 164, and 190. Of these yeasts three were also attractive in the field, yeast coded 190, and two stains of *H. uvarum*. Four combinations proved to be significantly attractive in the laboratory 201+164, 190+201, 190+218+201 and 190+218, but in the field only the latter three combinations attracted *D. suzukii*. There seems to be a good correlation between attraction in the laboratory and field, suggesting that 2-way choice test with T-mazes are a good proxy for *D. suzukii* attraction in the field, but also highlighting the need for field testing to confirm attraction. Currently, none of the yeast-based attractants tested in the field proved significantly more attractive than the commercial Gasser lure. It is worth noting that these field-based trials were conducted between late October and early December. This study has demonstrated for that both fungal and yeast communities fruit type and ripening stage have a significant impact on diversity. A greater knowledge of how yeast communities change during ripening and how they differ between fruit species could be invaluable in the pursuit of attractive yeasts that can be exploited in control of this pest. The ecological reality is that yeast communities on fruit are complex and potentially could be exploited to produce attractive baits. Yeast combinations are attractive to *D. suzukii*, though not more so than single yeast at this stage. A greater knowledge of relevant yeast communities could therefore be potentially important in the design and optimization of ecologically realistic communities of yeast that may be exploited to control *D. suzukii* and in the identification of new candidate single yeast species. Additionally, this project has samples stored from four ripening stages of four ecologically relevant fruit species that represent an important microbial resource that could be mined for attractive yeasts candidates in the future.

Knowledge and Technology Transfer

ABB Crop Protection in Southern Britain November 2018.

PGR symposium at University of Lincoln on in October 2018 (Poster).

AHDB Studentship Conference 2017(flash talk), 2018 (Poster) and talk (2019).

ABB Advances in IPM 2018: Making it Work for the Farmer. 12-13 December 2018 (Poster).

AHDB/NIAB EMR Association Soft Fruit Day November 2018 (talk and poster, winning poster in poster competition) and November 2019 (Poster).

ABB Advances in Biocontrol & IPM 2019: Addressing the innovation crisis. November 2019 (talk).

SF145a Field meeting October 2019 (talk)
DTP University of Nottingham October 2019 (talk).
CTP University of Reading November 2019 (talk).

AHDB/NIAB EMR Tree Fruit Day February 2020 (talk).

Royal Entomological Society Behaviour Special Interest Group. September 2020 (poster).

Publication

Günther, C.S., Knight, S.J., Jones, R. and Goddard, M.R., 2019. Are Drosophila preferences for yeasts stable or contextual? *Ecology and evolution*, *9*(14), pp.8075-8086.

One paper submitted to Scientific Reports (currently under review): 'Separate and combined volatile profiles produced by *Hanseniaspora uvarum* and *Metschnikowia pulcherrima* yeasts are attractive to *Drosophila suzukii* In the laboratory and field.'

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Upcoming

Accepted to present a talk at the International Congress of Entomology (ICE) conference in Helsinki 2020 (postponed until 2021).

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Supplementary material

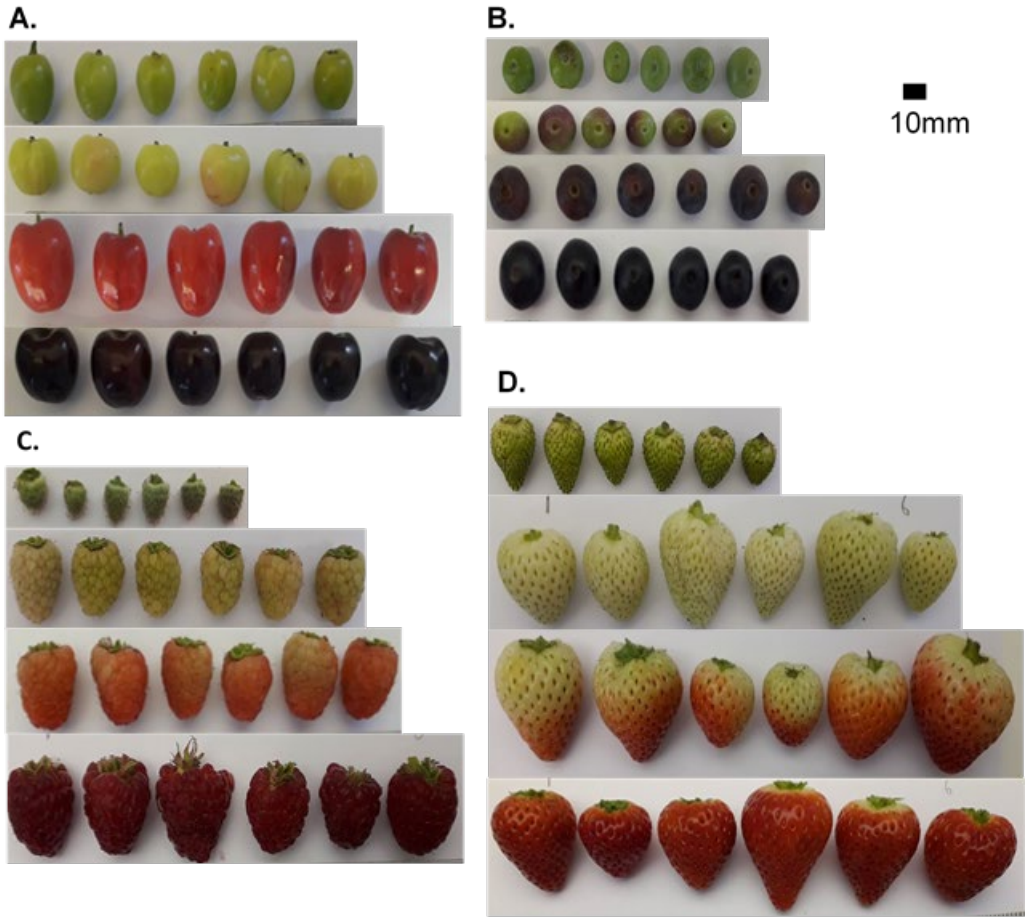

Fig S1: Four stages of fruit sampled and processed. A. green, white/pink, red and harvest (top to bottom) of cherry. B. green, green/purple, purple and harvest (top to bottom) of blueberry. C. green, white, pink and harvest (top to bottom) of raspberry, D. green, white and red/white (pink) and harvest (top to bottom) of strawberry.

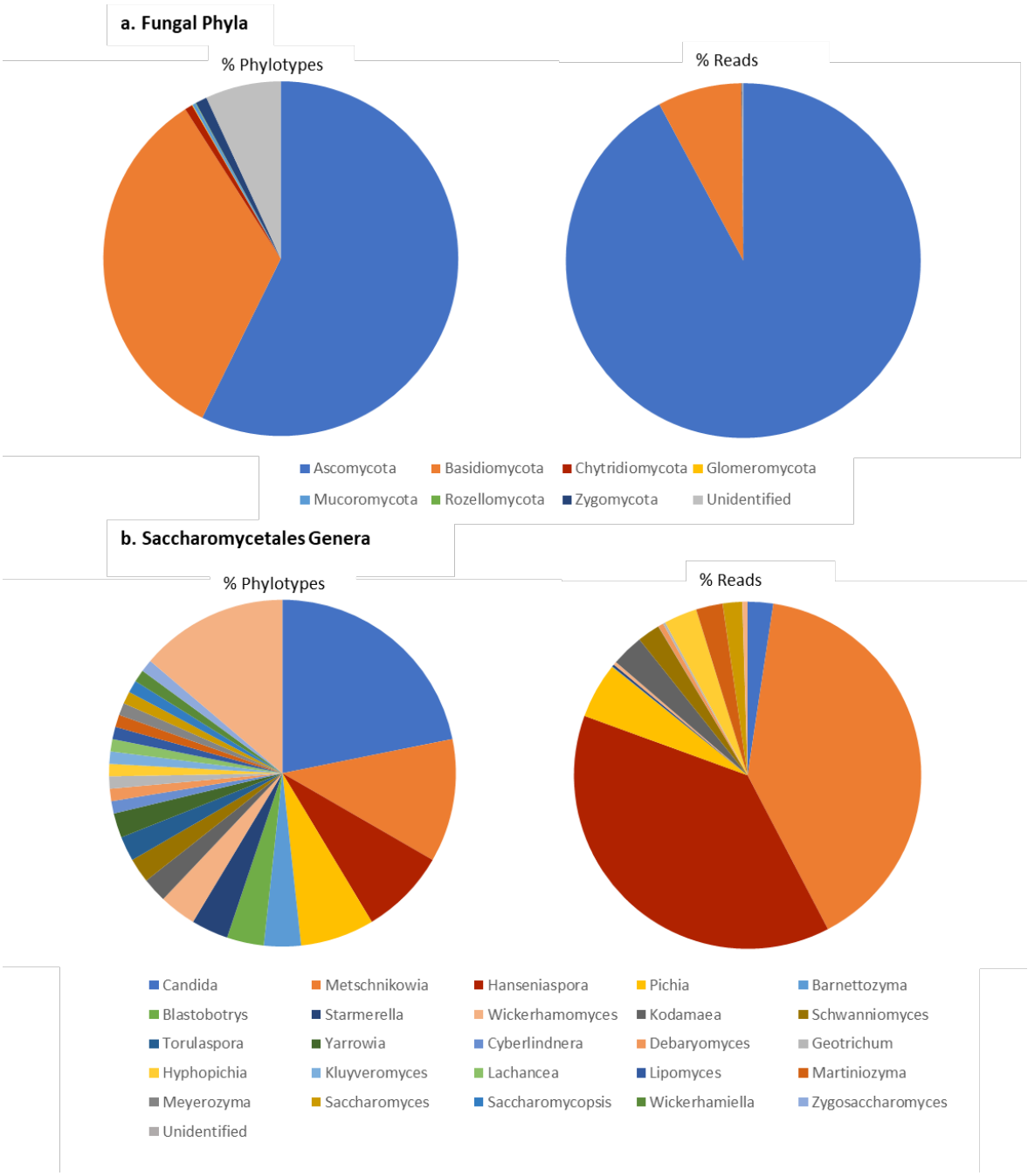

Fig.S2 a. pie charts showing the proportion of both phylotypes (Left) and reads (right) for the different fungal Phyla detected across all samples and b. pie charts showing the proportion of both phylotypes (Left) and reads (right) for the different *Saccharomycetales* yeast genera detected.

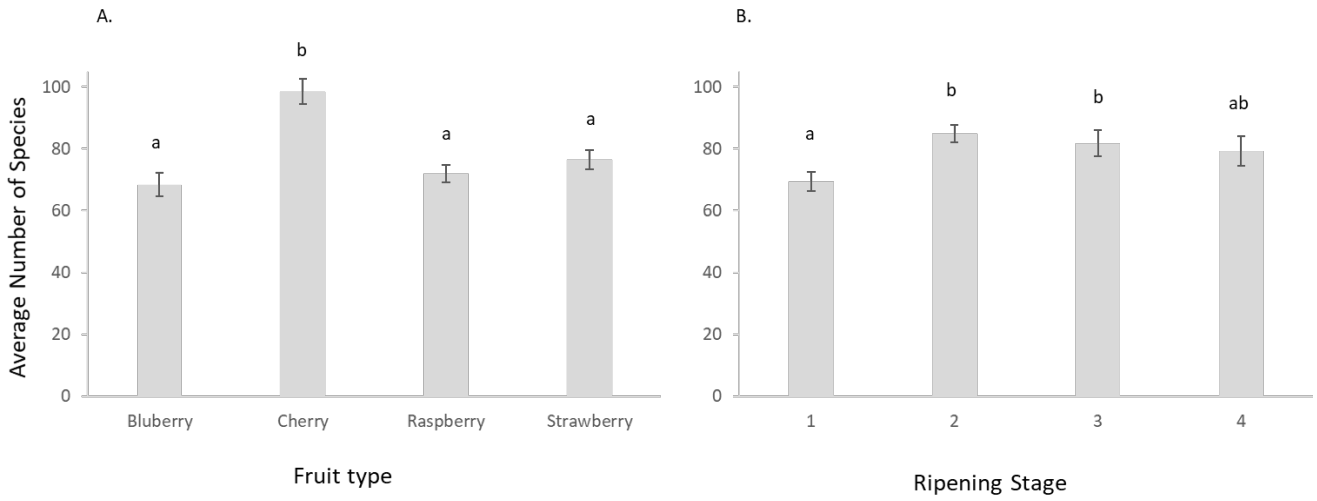

Fig. S3. A. Average number of fungal phylotypes present across the four fruit species. B. Average number of fungal phylotypes present across the four ripening stages (N=12 except strawberry stage 3 N=11). Different lowercase letters represent any significant difference in phylotype numbers between fruit type or ripening stages.

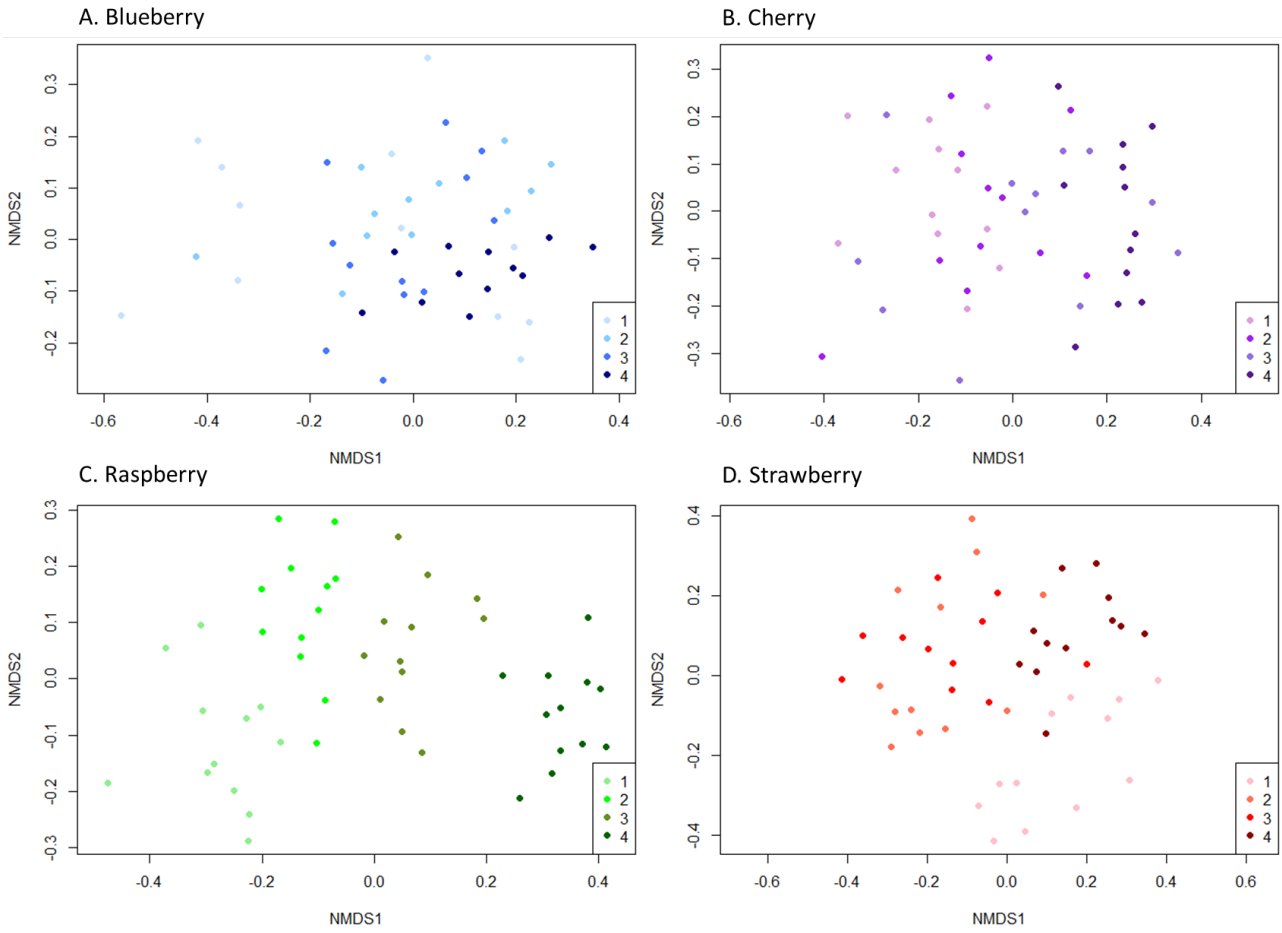

Fig. S4. Four separate Nonmetric Multidimensional Scaling analyses of binary Jaccard measures of community dissimilarity of fungal communities on four ripening stage for four separate fruit species. Panel A. blueberry (blue dots), B. cherry (purple), c. raspberry (green) and strawberry (red). Ripening stages for each fruit, are from green fruit through to harvest fruit and are denoted by shade of fruit colour, lightest shade for green fruit and moving thought to darkest shade for harvest). Exact colours for fruit and stage are denoted in the keys (boxes bottom righthand side of each plot).

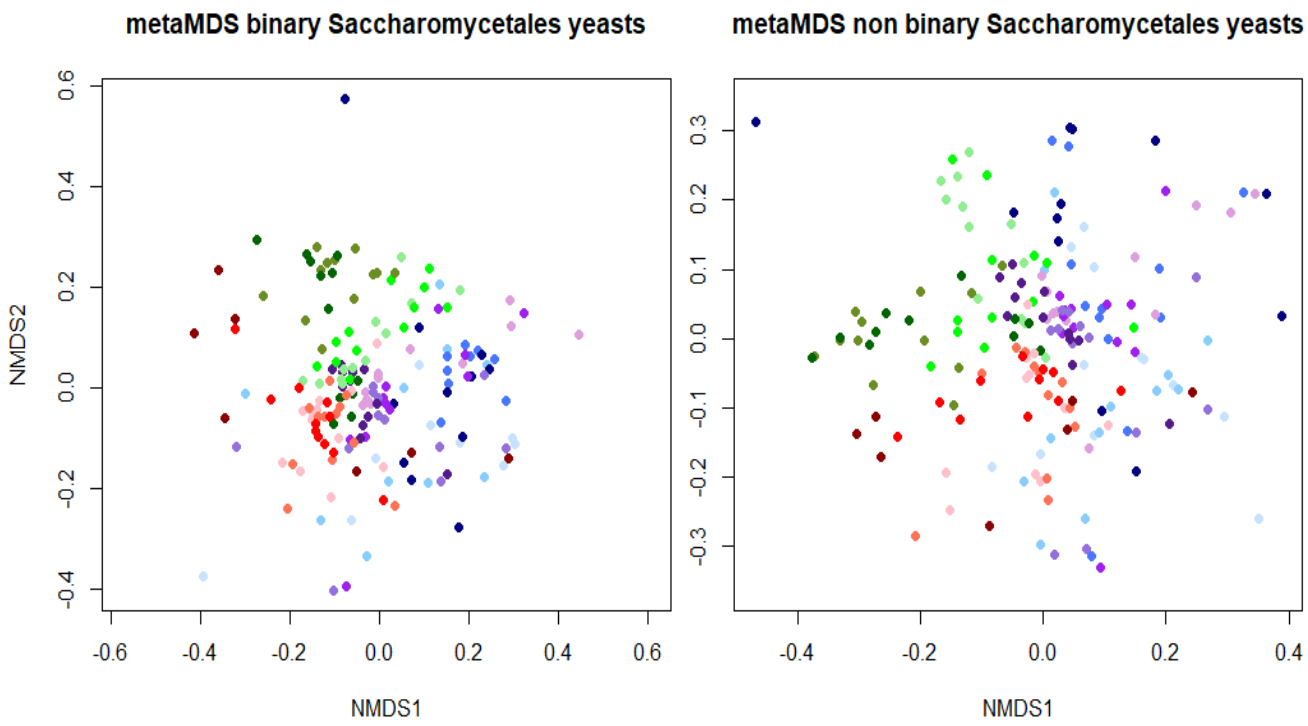

Fig. S5. Nonmetric Multidimensional Scaling analysis of binary Jaccard measures of community dissimilarity (left) and non-binary (right) of *Saccharomycetales* yeast communities on four fruit species, blueberry (blue dots), cherry (purple), raspberry (green) and strawberry (red). As well as four different ripening stages for each fruit, from green fruit through to harvest fruit (ripening stages denoted by shade of fruit colour, lightest shade for green fruit and moving through to darkest shade for harvest).

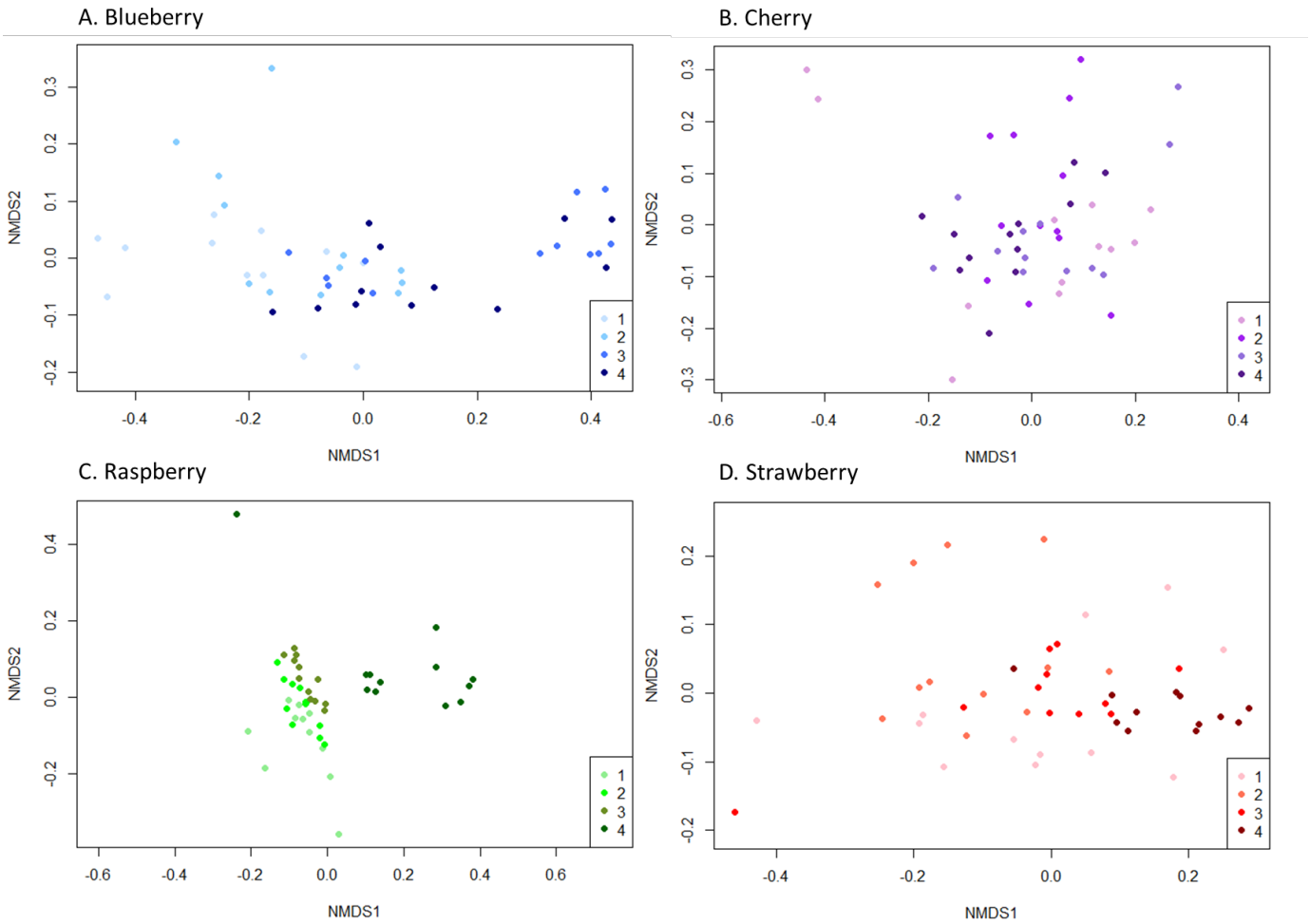

Fig. S6. Four separate Nonmetric Multidimensional Scaling analyses of non-binary Jaccard measures of community dissimilarity of fungal communities on four ripening stage for four separate fruit species. Panel A. blueberry (blue dots), B. cherry (purple), c. raspberry (green) and strawberry (red). Ripening stages for each fruit, are from green fruit through to harvest fruit and are denoted by shade of fruit colour, lightest shade for green fruit and moving thought to darkest shade for harvest). Exact colours for fruit and stage are denoted in the keys (boxes bottom righthand side of each plot).

Table S1: Results from pairwise PERMANOVAs on binary Jaccard distance matrices for fruit species. Benjamini-Hochberg correction was applied to all *P* values

Fruit species		R^2	*P*
blueberry	cherry	0.09	9.999×10^{-5}
blueberry	raspberry	0.10	9.999×10^{-5}
blueberry	strawberry	0.16	9.999×10^{-5}
cherry	raspberry	0.14	9.999×10^{-5}
cherry	strawberry	0.20	9.999×10^{-5}
raspberry	strawberry	0.09	9.999×10^{-5}

Table S2: results from pairwise PERMANOVAs on binary Jaccard distance matrices for ripening stages. Benjamini-Hochberg correction was applied to all P values

Ripening stage		R^2	*P*
1	2	0.09	9.999×10^{-5}
1	3	0.10	9.999×10^{-5}
1	4	0.10	9.999×10^{-5}
2	3	0.11	9.999×10^{-5}
2	4	0.11	9.999×10^{-5}
3	4	0.12	9.999×10^{-5}

Table S3: results from pairwise PERMANOVAs on abundance Jaccard distance matri x for fruit species. Benjamini-Hochberg corection was applied to all P values

Fruit species		R^2	*P*
blueberry	cherry	0.42743	9.999×10^{-5}
blueberry	raspberry	0.23158	9.999×10^{-5}
blueberry	strawberry	0.25026	9.999×10^{-5}
cherry	raspberry	0.54396	9.999×10^{-5}
cherry	strawberry	0.57456	9.999×10^{-5}
raspberry	strawberry	0.10963	9.999×10^{-5}

Table S4: results from pairwise PERMANOVAs on abundance Jaccard distance matri x for ripening stages. Benjamini-Hochberg correction was applied to all P values

Ripening stage		R^2	*P*
1	2	0.1511	9.999×10^{-5}
1	3	0.16129	9.999×10^{-5}
1	4	0.12251	9.999×10^{-5}
2	3	0.20548	9.999×10^{-5}
2	4	0.15594	9.999×10^{-5}
3	4	0.20305	9.999×10^{-5}

Table S5: Results from pairwise PERMANOVAs of Saccharomycetales yeasts on binary Jaccard distance matrices for fruit species.

Fruit species		R^2	*P*
blueberry	cherry	0.05997	9.999×10^{-5}
blueberry	raspberry	0.15077	9.999×10^{-5}
blueberry	strawberry	0.13973	9.999×10^{-5}
cherry	raspberry	0.13687	9.999×10^{-5}
cherry	strawberry	0.10161	9.999×10^{-5}
raspberry	strawberry	0.13193	9.999×10^{-5}

Table S6: results from pairwise PERMANOVAs of Saccharomycetales yeasts on binary Jaccard distance matrices for ripening stages.

Ripening stage		R^2	*P* adjusted
1	2	0.09203	9.999×10^{-5}
1	3	0.09593	9.999×10^{-5}
1	4	0.07454	9.999×10^{-5}
2	3	0.09833	9.999×10^{-5}
2	4	0.08003	9.999×10^{-5}
3	4	0.12478	9.999×10^{-5}

Table S7: Results from pairwise PERMANOVAs of Saccharomycetales yeasts on abundance Jaccard distance matrix for fruit species.

Fruit species		R^2	*P*
blueberry	cherry	0.05088	9.999×10^{-5}
blueberry	raspberry	0.07072	9.999×10^{-5}
blueberry	strawberry	0.06644	9.999×10^{-5}
cherry	raspberry	0.09652	9.999×10^{-5}
cherry	strawberry	0.09091	9.999×10^{-5}
raspberry	strawberry	0.08518	9.999×10^{-5}

Table S4: results from pairwise PERMANOVAs of Saccharomycetales yeasts on abundance Jaccard distance matrix for ripening stages.

Ripening stage		R^2	*P*
1	2	0.04518	9.999×10^{-5}
1	3	0.04724	9.999×10^{-5}
1	4	0.03801	9.999×10^{-5}
2	3	0.04694	9.999×10^{-5}
2	4	0.04016	9.999×10^{-5}
3	4	0.06843	9.999×10^{-5}