Symbiosis between yeasts and insects

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Summary

Mutualistic relationships between insects and microorganisms have been widely described for bacterial symbionts associated with sap feeding insects and fungi associated with bark beetles. Recently, the importance and widespread distribution of mutualistic yeasts in plant-insect interactions has been demonstrated. Several examples with *Drosophila melanogaster* among other insects have shown the ability of the insect to survive in a diet based on yeast consumption only. Moreover, yeasts have shown the ability of suppressing pathogens that might hamper the development of the insects. From the point of view of the yeasts, the main benefit of the mutualism is the facilitation of processes such as outbreeding and spreading offered by contact with insects. Understanding the functions and key elements in yeast-insect interactions could lead to the development of better pest management strategies, for example by exploiting the attraction of insects to yeasts to lure them into entomopathogenic viruses. In this review, I present an overview of the current knowledge in yeast-insect interactions, highlighting what has been studied to date and what research gaps remain to be addressed.

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1. Introduction

Traditionally in ecology, the relationships or interactions between organisms of different species have been studied and considered from a bilateral point of view, in which the relationship between two organisms of different species is defined by their neutral, beneficial or detrimental effects with each other (Henderson *et al.*, 1949).

When two species interact with no positive or negative effects between them their relationship is considered neutral. When the relationship is negative for one species and positive for the other, the relationship is called either predation (when one species kills the other) or parasitism (when one species feeds on the other without killing it). Commensalism describes the relationship in which one species maintains a condition that is beneficial for the survival of the other but without affecting its own fitness. Finally, when both species benefit the interaction is considered mutualistic (Smith & Smith, 2003; Molles, 2002).

The term symbiosis is used to describe the relationship between two organisms living together, which includes parasitism (Klepzig *et al.*, 2009; Smith & Smith, 2003). However, symbosis is mainly used to describe positive relationships in which one or both species benefit (Gibson & Hunter, 2010).

The advancement of our knowledge regarding interlinked interactions between the components of the communities has extended our view of interactions between insects and plants. However, the importance of other organisms in such bilateral relationships, e.g. plants and phytophagous insects, has become apparent, demonstrating that interactions often involve multiple trophic levels including predators, parasites and mutualistic organisms (Janson *et al.*, 2008; Schoonhoven *et al.*, 2005).

Most studies dealing with multitrophic interactions in entomology have focused mainly on systems with plants acting as mediators between herbivores and their natural enemies: predators and parasitoids (Singer & Stireman, 2005; Van der

Putten *et al.*, 2001). Nevertheless, ongoing research has shown the importance of incorporating different trophic levels in ecology and evolutionary studies. For instance, tritrophic interactions that consider organisms from three different levels: primary producers (plants) and primary and secondary consumers (herbivores and their predators) have shown that the development of one organism depends on the development of the other components of the interaction (Dicke & Baldwin, 2010; Van der Putten *et al.*, 2001; Vet & Dicke, 1992).

There is expanding evidence demonstrating that microorganisms also play an important role in the existence, abundance and interactions between the three trophic levels mentioned previously (Pineda *et al.*, 2013; Davis *et al.*, 2012; Ohgushi, 2005; Van der Putten *et al.*, 2001). The range of possible relationships between plants, insects and microorganisms covers different scenarios, depending on which organism mediates the interaction between the other two (Biere & Bennett, 2013).

Although microorganisms are widespread globally and are present in almost every environment, their study in entomology has focused mainly on their role as insect parasites and their potential use as biological control agents (Davis *et al.*, 2013). However, in recent decades the role of microbes (fungi and bacteria) as insect symbionts has drawn attention. One of the most promising fields of study is the interaction between yeasts and insects due to the diversity of yeasts and their presence in almost every environment, including the gastrointestinal system of important pests (Urubschurov & Janczyk, 2011; Backman & Sikora, 2008; Ganter, 2006).

Important questions about mutualistic interactions between insects and microorganisms still have to be answered as positive interactions between microbes and insects are less studied than negative interactions such as competition and predation (Hofstetter *et al.*, 2006, (Ganter, 2006). Here, I highlight the main characteristics and contributions to mutualistic relationships between insects and microbes. I also specifically discuss the presence of insect-

yeast interactions within the main insect orders and their potential use for pest monitoring and biological control. Finally, I indicate areas where further research is needed.

2. Plant-Microbe-Insect Interactions

Plants, insects and microbes are involved in complex tripartite interactions, which form integral components of the ecosystem. The study of the interlinked effects of these three groups of organisms has joined multiple disciplines including entomology, and plant and insect pathology (Biere & Bennett, 2013). Each member of this tripartite interaction could act as mediator of the relationship between the other two members, hence the effects will depend on which organism is mediating the interaction. Mediation means that the presence and biological activities of one organism affect the relationships between the other two. For instance (as further explained below), microbial plant pathogens that affect the suitability of host plants for insects because of the changes they induce in plant phenology, physiology and biochemistry (Biere & Bennett, 2013).

2.1 Plants as mediators

Plant suitability, physiological state and nutritional quality can increase or decrease the susceptibility of insects to entomopathogens; however, results vary depending on the organisms involved (Cory & Ericsson, 2010; Cory & Hoover, 2006). Volatiles produced by *Nicotiana tabacum* inhibit the development of the fungal entomopathogen *Pandora neoaphidis*, increasing the fitness of aphids (Brown *et al.*, 1995). Whereas, volatiles released by *Vicia faba* infested with pea aphids, *Acyrthosiphon pisum*, did not affect the development of *P. neoaphidis* (Baverstock *et al.*, 2005). Plant chemistry can also affect the availability of symbionts of insects. Davis and Hofstetter (2011) demonstrated that differences in monoterpene compositions of pine phenotypes affected the availability of nutritional fungal symbionts of the tree-killing beetle (*Dendroctonus brevicomis*).

2.2 Insects as mediators

Microbe dissemination is positively affected by disease-vectoring insects (Caesar, 2000). For instance, transmission of tomato viruses such as the spotted wilt virus and the tomato yellow curl virus are vectored by thrips and whiteflies, respectively (Pan *et al.*, 2012; Wijkamp *et al.*, 1993). Transmission of bacteria

and fungi by insects also occurs. Transmission of "Huanglongbing" is mediated by two psyllid species that feed on citrus and transmit the disease (Hansen *et al.*, 2008). On the other hand, negative effects of insects on microbes have also been demonstrated; for example, insects can affect the abundance, accessibility or suitability of plant tissues, which prevents the development of microorganisms (Thaler *et al.*, 2012). Changes in plant chemical defenses due to herbivory also affect the development of microbes. For instance, tomato plants damaged by *Helicoverpa zea* accumulate more proteinase inhibitor mRNA and polyphenol oxidases, which increases resistance to the pathogen *Pseudomonas syringae* (Stout *et al.*, 1999).

2.3 Microbes as mediators

The effect of microbes as mediators of insect-plant interactions seems to be highly variable. Microbes can establish parasitic or mutualistic relationships with plants, increasing or decreasing the susceptibility to herbivore attacks (Pineda *et al.*, 2013). The effects on insects seem to vary among the associated microorganisms, plant genotypes and herbivores tested. For example, association with the fungus *Acremonium* spp. has detrimental effects on the performance of *Helicoverpa armigera* in tomato and *Plutella xylostella* in cabbage, while plant bacterial symbionts increase insect performance and plant susceptibility to herbivory in tomato (Megali *et al.*, 2013; Jallow *et al.*, 2004; Raps & Vidal, 1998).

In the past few decades, the role of microbes as mediators of plant-insect interactions has received increasing attention because of their potential use for pest control. For example, some plant endophytes increase secondary plant defenses decreasing plant susceptibility to herbivory (Backman & Sikora, 2008). Moreover, fungal endophytes associated with grasses increase the production and accumulation of alkaloids, which has negative effects on the development of aphids and some leaf chewers such as *Spodoptera frugiperda* (Clay, 1996; Siegel *et al.*, 1990; Hardy *et al.*, 1985). Similarly, endophytical entomopathogenic fungi are able to accumulate their mycotoxins inside plant tissues decreasing the herbivore's performance (Gurulingappa *et al.*, 2010; Vega *et al.*, 2008).

In the case of microbes positively affecting insects, most research is biased towards bacteria-insect mutualisms, meanwhile, research on fungal-insect symbiosis is lacking.

2.3.1 Mutualism between insects and bacteria

Due to the diversity and presence of bacteria on almost every multicellular organism, bacterial symbionts have been widely studied and characterized (Gibson & Hunter, 2010; Janson *et al.*, 2008). Bacteria that nutritionally benefit their insect hosts have received particular attention. For instance, it is currently considered that most aphid species are associated with bacterial mutualists that supply their host insect with essential amino acids complementing their diets (Moran *et al.*, 2005).

Extensive research has shown that associations between bacteria and xylem and phloem feeding insects are vital, allowing the insects to diversify into a highly species-rich and geographically widespread order, Hemiptera (Bennett & Moran, 2013). These microorganisms are mainly members of the *Buchnera* genus, and are characterized by being obligate, vertically transmitted endosymbionts, with strict co-speciation with their hosts (Clark *et al.*, 2000). These bacteria are located in special cells called "mycetocytes" present in the hemocoel. In this symbiosis, both the insect and the bacteria are obligate partners; the microbe cannot grow outside of the insect tissue, and the insect performs poorly in absence of the microbe (Douglas, 1998).

Among hemipterans, mutualism between aphids and gut bacteria has been well characterized. Aphids are highly dependent on dietary amino acids, the ability of gut-associated bacteria to produce most essential amino acids makes them an exceptional symbiotic partner (Prosser & Douglas, 1992; Prosser *et al.*, 1992). One example is the association between pea aphid (*Acyrthosiphom pisum*) and *Buchnera aphidicola.* Similarly, bacteria living in the gut of the green peach aphid, *Myzus persicae,* are able to produce all but four amino acids required by the insect (Mittler, 1970). In exchange for nutritional supply, insect association

assures the transmission of bacteria to further generations, especially for obligate endosymbionts such as *B. aphidicola* (Engel & Moran, 2013).

Bacteria associated with insects can have even more complex functions apart from supplying amino acids. There are a vast number of reports in which microbes act as detoxifiers of allelochemicals and as suppressors of plant defenses (Lauzon *et al.*, 2003; Suh *et al.*, 2003; Shen & Dowd, 1991a). For instance, the Colorado potato beetle, *Leptinotarsa decemlineata*, use the associated bacteria present in their oral secretions to suppress jasmonic acid (JA) associated plant defenses in tomato (Chung *et al.*, 2013). Similarly, the mountain pine beetle, *Dendroctonus ponderosae*, is able to overcome plant defenses through mutualistic relationships with bacteria communities dominated by *Pseudomonas*, *Rahnella*, *Serratia* and *Burkholderia* spp. that are able to degrade the high concentrations of terpenes produced by the trees (Adams *et al.*, 2013).

Another proposed function for bacteria in mutualistic relationships with insects is their ability to prevent the colonization of the gut by other microorganisms such as insect pathogens. Gut microbes in silkworm larvae decrease the proliferation of pathogens such as streptococci and *Serratia piscatorum* (Kodama & Nakasuji, 1971). Further research has shown that endosymbiotic bacteria play important roles in stress tolerance. In aphids, heat tolerance is supported by the expression of heat-shock proteins from *Buchnera* and protection from parasitoid wasps is granted by association with *Hamiltonella defensa* (Degnan *et al.*, 2009; Fares *et al.*, 2004).

Finally, bacteria are able to produce volatiles that can be detected by insects, and have important ecological consequences (Davis *et al.*, 2013). Volatiles produced by *Klebsiella pneumonea* and *Enterobacter agglomerans* are highly attractive to the Mexican fruit fly, *Anastrepha ludens*, and to the Mediterranean fruit fly, *Ceratitis capitata*, due to the production of nitrogen containing compounds such as amines and pyrazines (Lauzon *et al.*, 2009; Robacker *et al.*, 2004; Robacker & Bartelt, 1997). The compound guaiacol produced by some bacteria found in the

locust gut (e.g. *Pantoea agglomerans*) promotes mating aggregations (Dillon *et al.*, 2000). Bacterial volatiles can also act as oviposition stimulants for insects such as *Rhagoletis pomonella* and *Lucillia cuprina* that result in oviposition preference and higher rates of egg laying when sites contain bacterial colonies (Lauzon *et al.*, 1998; Emmens & Murray, 1983). The role of volatiles produced by bacteria as infochemicals of host and food resources has been demonstrated mainly with necrophagous dipteran species (Stensmyr *et al.*, 2002). Nevertheless, other insect orders seem to be attracted not to specific bacteria, but to the microbial communities (fungal and bacterial) associated with their hosts (Davis *et al.*, 2013).

2.3.2 Mutualism between insects and fungi

Research on mutualisms between insects and microbes is biased towards understanding the associations with bacterial communities, especially vertically transmitted obligate endosymbionts. Nevertheless, fungal mutualisms are also relatively common. Contrary to bacterial mutualisms, fungal symbioses with insects are mainly facultative and horizontally transmitted, with microbial cells living extracellularly in the hemolymph, fat bodies or in specialized structures (Gibson & Hunter, 2010; Klepzig *et al.*, 2009).

Similar to bacterial symbionts, fungal mutualists provide different services to the insects. One well-known case is the nutritional role of fungi for leaf cutting ants. The ants cannot acquire all their energy requirements from plant sap or from the fungus only, forcing them to grow, select and harvest highly specialized strains of fungi (Seal *et al.*, 2012; Quinlan & Cherrett, 1979).

Other well-characterized examples of fungus-insect mutualisms are the association between bark beetles and ambrosia beetles with fungi (Klepzig *et al.*, 2009). In this system, the fungus provides nutritional supplements for most beetle life stages, while insects carry the spores of the fungus infecting new trees (Harrington, 2005; Paine *et al.*, 1997). Fungi associated with these beetles also help them to colonize the host because of their ability to produce extracellular

enzymes that breakdown indigestible wood into nutritious compounds for the insects (Valiev *et al.*, 2009). Similarly, wood wasps, such as *Sirex noctilio*, also use a symbiotic relationship with a wood decay fungus, *Amylostereum areolatum*, to colonize trees in non-native habitats. This fungus supports the wasps through cellulose degrading enzymes, which allows them to weaken and colonize healthy trees (Nielsen *et al.*, 2009; Kukor & Martin, 1983; Talbot, 1977).

Another example of mutualism between fungi and insects is the association between the plant endophyte, *Epichloe* spp., and *Botanophila* flies. The flies visit, feed and oviposit where the sclerotium or stroma emerges from the leaf. After consuming fungal spores, the flies transfer them through fecal deposition in unfertilized stroma, allowing the cross fertilization of the fungi (Bultman & Leuchtmann, 2008; Bultman *et al.*, 1998).

The role of fungal mutualists as producers of chemical indicators for insect communication has also been demonstrated. For instance, the fungi *Pichia pinus* and *Hansenula capsulate* are able to convert *cis* and *trans*-verbenol into verbenone, which act as an anti-aggregation pheromone for bark beetles (Hunt & Borden, 1990; Leufvén & Nehls, 1986).

Recent research has determined that yeast associations mainly dominate mutualisms between insects and fungi. The presence of yeast has been found in at least 143 insect species from many different orders, demonstrating that yeasts have produced highly diverse associations with insects (Suh *et al.*, 2005; Vega & Dowd, 2005).

3. Mutualism between yeasts and insects

3.1 Yeast biodiversity

The word "yeast" is broadly used to describe a fungal growth form represented by single cell organisms that reproduce by budding at some stage of their life cycle (Vega & Dowd, 2005). This fungal group is ubiquitous, occupying a variety of different ecological niches present in terrestrial and aquatic ecosystems. Still despite their widespread presence, it is thought that only 1% of the diversity of yeast species has been described so far, demonstrating that there is a huge number of undescribed species (Kurtzman & Fell, 2006).

Yeasts lack specialized sex organs and thereby reproduce via sexual spores from somatic cells that are not enclosed in fruiting bodies (Kurtzman *et al.*, 2011). There are at least 1500 known yeast species, belonging mainly to the phyla *Ascomycota* and *Basidiomycota* (Urubschurov & Janczyk, 2011). Almost 700 species described in 93 genera belong to the class Saccharomycetes of Ascomycota, and are generally called the "true yeasts" (Vega & Dowd, 2005). Genera of true yeasts include *Candida, Kluveromyces, Metschnikowia, Pichia,* and *Saccharomyces* among others (Nguyen *et al.*, 2007; Lachance *et al.*, 2001; O'shea & Walsh, 2000; Sipiczki, 2000). *Cryptococcus, Fellomyces*, *Tremella, Ustilago* and *Cystofilobasidium* are examples of yeasts belonging to Basidiomycota (Urubschurov & Janczyk, 2011; Landell *et al.*, 2009; Suh *et al.*, 2005; Fell *et al.*, 1999).

Most of the known yeasts associated with insects belong to a few members of true yeasts genera, especially *Candida*, *Metschnikowia* and *Pichia,* but associations with other yeasts such as *Cryptococcus* and *Pseudozyma* are also reported (Urubschurov & Janczyk, 2011). Most of these yeasts are housed and frequently found in the gastrointestinal tract of insects. Their relationships with the host seem to be facultative, as they can be recovered from feces, ovipositors and specialized organs of the insect (Gibson & Hunter, 2010). In most cases these symbionts are compact, unicellular organisms that grow intercellularly in the hosts (Vega & Dowd, 2005). The diversity among hosts and habitats has

produced an unknown number of yeast species. For example, the number of yeast species harbored in the guts of *Erotylidae* and *Tenebrionidae* beetle families is suggested to match the total number of beetle species, i.e. each beetle species might carry their own unique yeast species (Boekhout, 2005; Suh *et al.*, 2005).

Although true yeasts represent most of the symbiosis between yeasts and insects, "yeast-like symbionts" (YLS) are a group of fungal endosymbionts that differs from true yeasts. This group is characterized by evolutionary reduced forms of yeasts from ascocarpic ascomycetes (filamentous), especially from the subphylum Pezizomycotina (Vega & Dowd, 2005).

Phylogenetic analyses have shown that despite their ability to form similar mutualisms with insects, YLS are more closely related to the Hypocreales than to the Saccharomycetales (true yeasts). This raises particular interest in mutualism development because the family Clavipitaceae, which contains entomopathogenic fungi, also forms part of the Hypocreales, thus YLS are more related to entomopathogenic fungi than to yeasts (Suh *et al.*, 2001). This points to two different origins of symbiosis, with mutualistic YLS originating from insect pathogenic microbes, while other associated yeasts probably evolving from commensal interactions (Gibson & Hunter, 2010). However, the diversity and distribution of the association between YLS and insects seem to be more limited than true yeasts and insects. To date, mutualistic YLS-insect interactions have been observed mainly in anobiid beetles and in some planthopper and aphid species (Fukatsu & Ishikawa, 1996; Noda & Kodama, 1996; Sasaki *et al.*, 1996). Overall, it appears mutualism between yeasts and YLS with insects can vary with different fungal phyla, subphyla and orders (Figure 1).
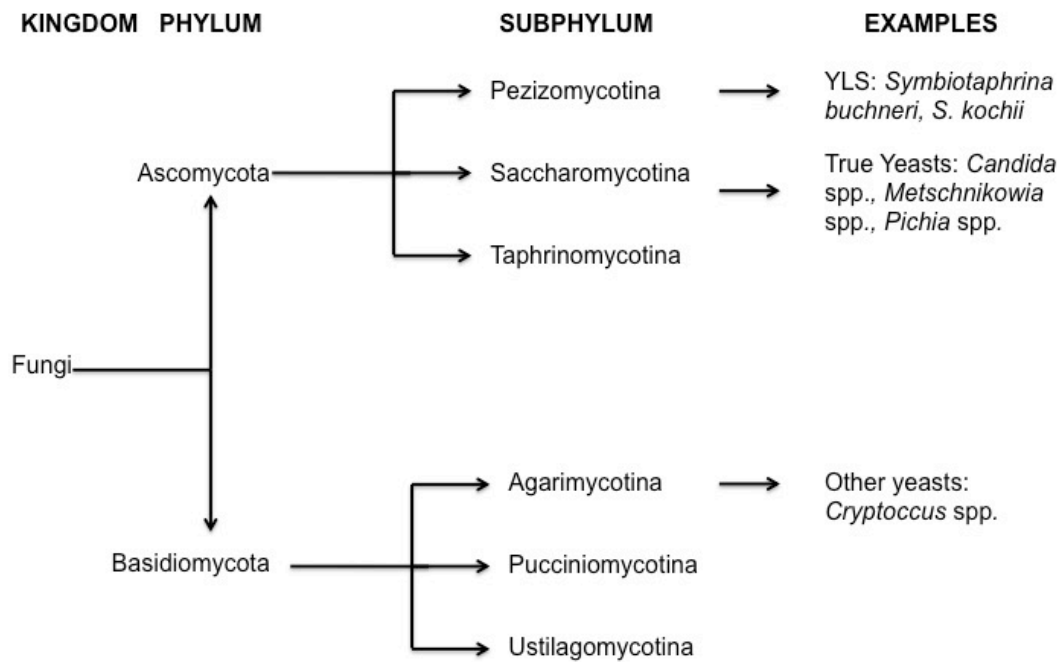
Figure 1. Representation of the taxonomic classification of yeast and yeast-like symbionts of insects (Adapted from Suh et al, 2001 and Suh et al., 2005).

Clearly, there is specificity in the association of yeasts with insects; moreover, some yeasts and yeast-like symbionts are often found within specific groups of insects (Gibson & Hunter, 2010). For instance, as mentioned previously YLS are mainly found in sap feeders, but others like *Metschnikowia,* are linked to several groups of nectivorous insects, suggesting this specificity could be correlated to the services provided by the yeast to the insect or vice versa (Pozo *et al.*, 2012; Suh *et al.*, 2005).

3.2 Roles in Symbiosis

3.2.1 Benefits for the yeasts

The benefits of the association of yeasts with insects have been subject to continuous debate (Douglas & Smith, 1989). Currently, it is thought that the main services offered to the fungus by the insects are related to the protection and

dispersal of their spores, along with outbreeding facilitation (Vega & Dowd, 2005).

Insect guts are considered unstable habitats for microbial colonization; however, associated and evolved microorganisms are able to endure such inhospitable conditions and be passed on to host conspecifics. The insect digestive tract consists of three main regions: foregut, midgut and hindgut, each one with specialized functions in the digestion process. The anatomy of all three segments can vary among insect orders and this leads to varying abilities to form symbiosis with microorganisms. Generally, it is considered that symbionts are, first, acquired by ingestion or interaction with conspecifics and environment. Second, they colonize the guts, and finally are released from insect molts and feces for dispersion of microbial cells. Depending on the insect behavior, yeasts can be re-ingested (through coprophagy, trophalaxis or by colonization of food sources) or inoculated in eggs by females. The survival and transfer of microbe mutualists is widely demonstrated for bacterial symbionts and is expected to be similar for yeasts (Engel & Moran, 2013; Coluccio *et al.*, 2008).

Contrary to filamentous fungi that are able to produce complex reproductive structures to assure the dispersal of conidiospores and ascospores, yeasts elaborate cell types are able to survive numerous extreme environmental conditions (Carlile *et al.*, 2001). The survival of spores in a laboratory experiment in which different stress conditions (ether vapor, heat shocks, incubation at 42 °C, high salt concentrations, and high and low extremes of pH) were applied to vegetative cells and spores of baker's yeast, Sac*charomyces cerevisiae*, demonstrated that spores are more adapted than vegetative cells to endure environmental stresses (Coluccio *et al.*, 2008). The same study also showed that the cell wall of spores allows them to survive passage through the gut of *Drosophila melanogaster* (Coluccio *et al.*, 2008). Recovery of significantly more intact spores than vegetative cells in the frass of flies implies that the spore wall confers resistance to stresses associated with digestion allowing the yeast to be transported and dispersed by insect feeding and oviposition.

Besides dispersion by ingestion and subsequent excretion through feces, some insects such as bark and ambrosia beetles have developed specialized structures to collect, carry, protect and disperse spores (Figure 2). These structures are called mycangia, and are glandular cavities or cuticular invaginations that allow the entry and exit of fungal spores (Stone *et al.*, 2005; Beaver & Wilding, 1989). Mycangia have evolved in two ways, as pocket-like expansions of the cuticle or as newly developed hollow glands, presumably from previous glands that produced defensive compounds or oils used to avoid excessive sap flow. Some fungi were able to survive and use the cavities for their benefit (Beaver & Wilding, 1989). In these pockets, spores are able to reproduce, while being transported to new hosts by the insect. Once in contact with the tree, beetles produce galleries in which the yeasts develop, spreading to the phloem and wood, assuring food for the insect and new inoculum for the dispersion of the fungi (Beaver & Wilding, 1989).

Another benefit to yeasts after their consumption by insects is the putative role of digestion in the promotion of outbreeding (Coluccio *et al.*, 2008). Outbreeding is fundamental to maintain genetic variation among the progeny of an organism and to increase the probability of adaptation and evolution. Sexual reproduction of yeasts is activated by adverse environmental conditions, in which the diploid vegetative cells enter into meiosis and produce a tetrad of haploid spores that can germinate, mate and restore the diploid state. Frequently, selfing (inbreeding) between the four spores housed in the same capsule (ascus) is observed. To release these spores and avoid selfing, enzymes are required to break up this capsule. It has been proposed that the enzymes and overall conditions of insect guts accomplish this function, breaking down the tetrads and promoting outbreeding (Reuter *et al.*, 2007; Zambonelli & Giudici, 2002). An increase up to 10 times in outbreeding was observed by Reuter et al. (2007), when genetically marked strains of *S. cerevisiae* were divided into 2 groups, one that had contact with fruit flies and another group with no contact with insects. The results indicate that digestion by insects significantly increased the number of heterozygotes in comparison to the yeasts that were kept for non-insect mediated mating.
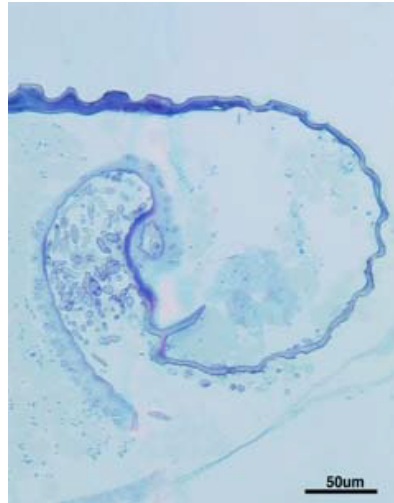
Figure 2. Fungal spores inside mycangium of *Xylosandrus mutylatus* (from Stone *et al.* 2005).

One example of yeast benefitting from insects is the protection, outbreeding and especially dispersal of spores of *Metschnikowia* spp. by insects involved in pollination. This fungal genus encompasses 47 species distributed globally, with some species having a broad geographic range while others are confined to specific areas (Guzmán *et al.*, 2013). Members of this genus are mainly found in flowers, and particularly in nectar, being mainly vectored by insect pollinators (Lachance & Bowles, 2002). At least three orders of insects (Diptera, Coleoptera and Hymenoptera) have shown associations to different *Metschnikowia* species (Lachance *et al.*, 2003; Lachance *et al.*, 2001).

The ingestion of yeasts through nectar consumption by bumblebees and honeybees is proposed as the mechanism used by yeasts to survive in temperate zones in which flowers and active insects are absent during winter. In a study with different *Bombus* spp., Brysch-Herzberg (2004) demonstrated that *Metschnikowia* and *Candida* yeasts are present in the digestive tracts of workers and queens during the whole year and are inoculated in new flowers during early spring.

As a consequence of association with pollinators, nectar-dwelling yeasts have the advantage of being specialized to overcome and use the high concentrations of

sugars in the nectar. This ability coupled with their resistance to plant defensive compounds and high growth rates allows them to dominate the flower microhabitat and thereby assuring their dispersal (Pozo *et al.*, 2012).

Evidence supporting the benefits for the yeasts in mutualistic relationships with insects has increased with experimental work on insect attraction to yeast species with different odor profiles. Christiaens et al. (2014) demonstrated that by turning off the expression of the ATF1 gene in *S. cerevisiae* (dedicated to the production of acetate esters which are important signals of ripening fruits) the attraction of fruit flies was hampered and, consequently, reduced the dispersion of yeasts. Moreover, studies with different baker's yeast strains have shown that more odor-attractive yeasts are correlated with greater dispersal of the yeast and higher fecundity in *Drosophila simulans* (Buser et al., 2014). Although it is still difficult to demonstrate that specific genes in yeasts evolved solely with the purpose of attracting insects for their dispersal, evidence indicates a strong mutualism with not only clear benefits for both partners, but also with sophisticated strategies used to obtain these benefits (Christiaens *et al.*, 2014).

3.2.2 Benefits for the insects

Yeasts can provide insects with many benefits, which include acting as a nutrition source, detoxifying harmful substances, protection from biotic stresses and can aid in chemical communication (Gibson & Hunter, 2010; Janson *et al.*, 2008).

Nutrition

The role of yeasts in insect nutrition has been inferred from the fact that insect performance decreases in the absence of yeast associates (Vega & Dowd, 2005). Yeast cells are sources of B vitamins (e.g. B3 and B5), proteins, trace metals and amino acids that could be easily assimilated through simple digestion. Yeasts contain 7.5-8.5% nitrogen by dry weight, thus in many cases feeding on yeasts represents a better source of nitrogen and other dietary requirements than the plant tissue itself (Gibson & Hunter, 2010; Vega & Dowd, 2005).

Early research demonstrated that digestion of yeasts provided nitrogen and vitamins required by insects such as scales, *Pseudococcus citri*, and wood boring cerambycids, *Leptura* and *Rhagium* spp. (Jurzitza, 1970; Koch, 1954). Further investigation has shown the nutritional importance of yeasts for insect development. *Drosophila* flies are especially dependent on yeast feeding for nutrition during egg maturation and larval development due to the rich source of nitrogen, vitamins and lipids that these fungi provide (Starmer & Aberdeen, 1990).

Becher et al. (2012) reported the importance of yeasts as food for *D. melanogaster* larvae. They showed that the percentage of survival of larvae feeding on minimal medium or grape berries inoculated with baker's yeast was significantly higher than those feeding on sterilized grape berries or non-inoculated minimal media. Additionally, the difference between the survival of larvae feeding on grape berries with yeast and on minimal media with yeast was not significant, reinforcing the hypothesis that the yeast could be sufficient to support the larval development of fruit flies.

The nutritional role of yeasts has also been shown with the association of insects with YLS. Studies with *Symbiotaphrina* and anobiid beetles have shown that nitrogen, sterols, vitamins and essential amino acids (e.g. tryptophan) are provided by the YLS that otherwise wouldn't be supplied by the plant (Bismanis, 1976; Jurzitza, 1970). Similar to the case of anobiid beetles, YLS are essential to produce intermediate precursors for ergosterol biosynthesis in rice planthoppers (Noda & Koizumi, 2003).

The role of yeasts as facilitators of insect host nutrition through the production of digestive enzymes that degrade plant compounds into molecules with nutritional value for herbivores has been demonstrated (Gibson & Hunter, 2010; Vega & Dowd, 2005). Furthermore, bark beetles associated with plant pathogenic fungi such as *Ceratocytis* and *Ophiostoma* rely on these organisms to colonize trees (Six & Bentz, 2003; Krokene & Solheim, 1996). Similarly, *Sirex* and

Xyphidria woodwasps associated with fungi (*Amylostereum areolatum* and *Daldinia decipiens*, respectively) depend on the extracellular enzymes produced by the fungal mutualists to degrade cellulose (Pažoutová *et al.*, 2013; Slippers *et al.*, 2000).

The types of enzymes produced by insect-associated yeasts range from exoproteases and peptidases (for protein degradation), lipases (for fatty acids) and hydrolytic enzymes normally involved in sugar degradation. Yeast endosymbionts in the deathwatch beetle, *Lasioderma serricorne*, produce and release lipases, glucosidases, phosphatases and trypsin in order to degrade cellobiose. A similar ability has been found in yeast species from wood boring beetles *Ernobius mollis, E. abietis* and *Xestobium plumbeum* (Shen & Dowd, 1991a; Shen & Dowd, 1991b).

Research on the role of enzymes produced by yeasts has focused on degradation of polysaccharides and complex polymers into glucose or sugars that the insect can absorb directly. However, this role has been mainly demonstrated for YLS, since these fungi actively release digestive enzymes into their environment to colonize them and to spread towards new areas, while true yeasts are more sessile and do not produce enzymes for active degradation unless they are trapped in their own erosion zone (Gibson & Hunter, 2010). However, true yeasts species of the genus *Candida* have the ability to degrade wood components such as cellulose, pectin and glucosides. This suggests that cerambycid beetles might rely on their associated species to assimilate different sugars or to degrade cellulose, especially considering that their larval stages are woodborers and their symbionts produce pectinases and glucosidases (Chararas *et al.*, 1983).

Detoxification

Closely interlinked with the nutritional role of yeasts for insects is the ability of yeasts to break down plant allelochemicals and other types of toxins. Detoxification by yeasts has demonstrated that mutualism between yeasts and insects is an important driver in herbivory. Detoxification is related to digestion

because certain nutrients become available when toxins are neutralized or decomposed allowing them to be used as sources of nutrients or in some cases converted into more polar forms that are excreted through the digestive system (Engel & Moran, 2013; Vega & Dowd, 2005).

Detoxification by yeast and yeast-like symbionts has been demonstrated extensively with the YLS, *S. kochii,* associated with the beetle *L. serricorne* (Vega & Dowd, 2005). Shen and Dowd (1991a) confirmed that *S. kochii* can detoxify a wide range of toxins for *L. serricorne* including plant allelochemicals, metal toxins, insecticides and herbicides. Furthermore, *S. kochii* can produce many detoxifying enzymes, including aromatic ester hydrolase, glucosidase, phosphatase, and glutathione transferase, allowing the use of toxic substances as carbon sources.

The role of yeasts as detoxifiers of plant defenses has been proposed because of similar evidence observed with other fungi. For instance, the fungus, *Ophiostoma piliferum*, has the ability to degrade diterpene acids. These acids are one of the most important phytochemical defenses present in the resins and phloem tissue of conifers. By degrading these acids the tree immune system is lowered and allows the colonization of the tree by both *O. piliferum* and bark beetles (Kopper *et al.*, 2005).

Although the ability of certain yeast species to break down plant secondary metabolites has been shown experimentally, there is not enough evidence to support that this role takes place in the insect's digestive system and that it is common among yeasts associated with insects (Hansen & Moran, 2013). So far, results are mixed and not consistent among studies. For example, at least 2 of 5 yeasts species (C*andida guilliermondii* and *Debaromyces hansenii*) found in the gut of the red fire ant (*Solenopsis invicta)* were able to degrade the toxin salicin (Ba & Phillips Jr, 1996). On the other hand, the yeasts *Pichia burtonii* and *Candida fermentati* recovered from the coffee berry borer, *Hypothenemus hampei*, are both associated with the insect, but neither are able to degrade caffeine. It is

possible that in this case the yeasts have a nutritional effect or perhaps are able to degrade other compounds apart from caffeine (Vega *et al.*, 2003).

Protection from biotic stresses

Competitors, parasites and predators are examples of biotic hazards that herbivores face in addition to plant chemical defenses. In the case of insect-yeast associations, evidence demonstrating protective roles of yeasts against natural enemies of herbivores is scarce. Yeasts providing protection of plant tissues against pathogens and the consequent benefits for their herbivores is a more plausible scenario and has been discussed since the 1980's (Listemann, 1988). Plant diseases could have negative effects on the development of insects by damaging plant tissues or producing toxins. Therefore, the mutualism of insects with yeasts promotes the availability of safe food sources.

Yeasts could limit the development of other fungi inside plant tissues, favoring the development of their associated insects. The presence of *Metschnikowia* spp. reduces the incidence of molds inside apples, which also coincides with lower mortality and lower larval developmental time for *Cydia pomonella* (Witzgall et al. 2012)*.* These results indicate that yeasts not only supply insects with nutritional factors but might also suppress the development of opportunistic microbes that might hamper the development of both participants in the symbiosis.

Members of the *Metschnikowia* genus are effective biological control agents against post-harvest rots such as *Botrytis cinerea*, *Penicillium expansum, Monilia* sp. and *Alternaria* sp. (Manso & Nunes, 2011; Spadaro *et al.*, 2002; Kurtzman & Droby, 2001; Piano *et al.*, 1997). *Metschnikowia* is able to suppress the development of pathogens mainly due to their ability to outcompete other microorganisms for nutrients and space, and is probably not related to the production of antibiotics or toxins (Pozo *et al.*, 2012). Furthermore, some strains of *Metschnikowia* are able to inhibit the fungal and bacterial growth of pathogens by depleting the available iron that it is necessary for the production of their characteristic reddish pigment (Sipiczki, 2006). The presence and development

of living cells of *M. pulcherrima* is necessary to achieve control, as filtrates are not antagonistic against pathogens (Spadaro *et al.*, 2002).

The colonization of plant tissues by specific yeasts could have effects on community composition and niche availability (Biere & Bennett, 2013; Davis *et al.*, 2011). For example, the colonization of fruits with *M. fructicola* antagonizes *B. cinerea*, a pathogenic fungus associated with the grapevine moth, *Lobesia botrana,* which not only controls the pathogen but also decreases the attractiveness of the fruits for these moths (Tasin *et al.*, 2009). Thus, this effect could decrease the pressure of competition for the insects associated with *M. pulcherrima* such as *C. pomonella*. The effects of microbes as shapers of community structures have also been explored for belowground microbes (such nitrogen fixing bacteria and mycorrhiza) and endophytes (Rodriguez *et al.*, 2009; Van der Putten *et al.*, 2001).

Finally, insect associations with yeasts could also benefit insects by promoting the development of other mutualistic and beneficial organisms in the community while decreasing the presence of insect pathogens. Davis et al. (2011) reported that volatiles produced by the yeast *Ogatea pini*, found in bark beetle mycangia, inhibited the development of the entomopathogenic fungus *Beauveria bassiana* and increased the development of the mutualistic fungus *Entomocorticium* sp., demonstrating that yeasts could selectively shape microbial communities.

Chemical communication

Yeasts produce complex aroma profiles with a range of volatiles within multiple functional groups. Depending on the case, insects use some of these volatiles as information sources for intraspecific and interspecific communication (Christensen, 2010).

As mentioned previously, mountain pine beetles (*Dendroctonus ponderosae)* and spruce bark beetles (*Ips* spp.) establish symbiotic associations with the fungi *Hansenula capsulata* and *Candida nitrophila*, respectively. The fungi are able to produce the anti-aggregation pheromone verbenone from *cis* and *trans*-verbenol,

allowing the insects to stop aggregating and populations to mass attack more trees (Hunt & Borden, 1990; Leufvén & Nehls, 1986).

Yeasts volatiles aide in host location and attraction to food sources in several insect species, especially dipterans and coleopterans (Becher *et al.*, 2012; Nout & Bartelt, 1998). Compounds produced by yeasts on fermenting fruits such as ethyl acetate, ethyl hexanoate, and phenylethanol are attractive to *Ceratitis capitata*, *Blaberus discoidalis* and *Agrochola helvola* (Jang *et al.*, 1994; Bestmann *et al.*, 1977; Brossut *et al.*, 1974). Some yeasts volatiles could act as oviposition cues for lepidopterans, signaling suitable food sources for larval development as shown for *C. pomonella* in apples with *Metschnikowia* yeasts (Witzgall *et al.*, 2012). Nevertheless, the specific compounds that mediate the attractiveness are still undetermined. It is possible that the most important compounds are esters, because they are signals of rotting fruits and sugars emitted by fermenting yeasts and attractive to lepidopterans (El-Sayed *et al.*, 2005).

4. Associations of yeasts with main insect orders

4.1 Coleoptera

Urubschurov and Janczyck (2011) comprised a list of yeasts associated with insects and found almost 100 cases corresponding to mutualisms between beetles and different yeasts species (For further details see Urubschurov and Janczyck, 2011). This exemplifies how extensive this mutualism is in the Coleoptera.

The most studied cases of association between yeasts and members of the Coleoptera correspond with mutualisms between wood-feeding beetles and yeasts, mentioned previously. The yeasts are necessary to degrade wood sugars such as xylose and cellobiose and at the same time produce volatile organic compounds used as semiochemicals by the beetles (Long *et al.*, 2012; Tanahashi *et al.*, 2010; Ganter, 2006).

Scolytinae beetles use pheromones that are wood compounds modified by fungi. The interaction is based on the attraction of adults by their mutualistic fungi to trees in the early stages of the infestations, but deterred by the same compounds during the later stages. Yeasts and other fungi benefit through dispersal, while beetles feed on the fungi and develop in a tree with reduced defensive abilities since the physical and chemical defenses of the plant are blocked by fungal growth (Ganter, 2006). Although bark beetles are primarily associated with ophiostomatoid Ascomycetes, the presence of yeasts in their mycangia has been demonstrated. For example, Six and Bentz (2003) found that all *Dendroctonus rufipennis* beetles sampled from 6 populations across the U.S.A. had associations with yeasts, and some were associated exclusively with yeasts.

Beetles feeding on dead wood, such as Passalid beetles, also rely on gut inhabiting yeasts for degradation of xylose and cellobiose. As in the Scolytinae

subfamily, the Passalidae have a large number of yeasts in their gut, with many unknown species, ranging from Ascomycetes like *Scheffersomyces shehatae* and *Scheffersomyces stipites,* to Basidiomycetes like *Cryptococcus* and *Trichosporon* (Urbina *et al.*, 2013).

Other xylophagous beetles including Anobiid and Cerambycid beetles, establish mutualisms with yeasts (Ganter, 2006). Cerambycid beetles are mainly associated with endocytobionts of the *Candida* genus (Grunwald *et al.*, 2010). The roles of these yeasts in nutrition and detoxification for both (Anobiid and Cerambycid beetles) are clear, even when their phylogenetic origin seem to differ. This supports the idea that the endosymbiotic association of *Symbiotaphrina* and *Candida* species with Anobiid and Cerambycid beetles are examples of convergent evolution (Jones *et al.*, 1999). These findings indicate that symbiosis between intracellular yeasts and beetles has polyphyletic origins, but have been selected due to the benefits for the microbes and the insects.

Other yeasts, such as *Pichia* and *Candida*, associated with coleopterans are found in internal organs of the coffee bean borer and likely provide nutritional factors to the beetle (Vega *et al.*, 2003).

The importance of yeasts as indicators of fermenting substances for beetles has been also demonstrated. Nout and Bartelt (1998) demonstrated attraction of *Carpophilus humeralis* towards volatiles produced by baker's yeast in fermenting corn. Volatiles produced from bacterial cultures did not attract the beetles whereas volatiles produced by yeasts did. They also demonstrated that compounds produced by fermentation and even independent from fermentation, are part of the microbial stimuli that these beetles use to locate suitable hosts.

Floriculous beetles are also associated with yeasts. There is a wide diversity of yeasts species present and dispersed by visiting beetles (Lachance *et al.*, 2003). Associations between beetles and flower-inhabiting yeasts are dominated by ascomycetous yeasts of the *Metschnikowia* clade and Nitidulids beetles (especially from the *Conotelus* genus) (Lachance *et al.*, 2003). Although, there is

no clear evidence of the role of yeasts as nutritional suppliers for floriculous beetles, beetles carry a community of yeasts to the flowers in which they feed, and this role has been fundamental for yeasts speciation (Lachance *et al.*, 2003; Lachance *et al.*, 2001; Lachance *et al.*, 1998).

4.2 Diptera

Many yeast species associated with beetles are also associated with dipterans (e.g. scarab beetles and flies associated to *Metschnikowia proteae* in flowers of *Protea caffra*) (de Vega *et al.*, 2012).

Among dipterans, Drosophillid-yeast interactions are the most researched. These flies are able to survive feeding only on yeasts (Becher *et al.*, 2012), and it is thought that any substrate where *Drosophila* flies feed or oviposit are sources of yeast inoculum (Ganter, 2006). Different Drosophilids show preference for different yeasts species, of which *Pichia, Candida* and *Cryptococcus* seem to be the most abundant (Starmer & Fogleman, 1986). However, different Drosophilid species could prefer specific yeasts depending on their host plants. For instance, isolations from frass, midguts and fruit hosts of *D. suzukii* have shown that even when multiple yeasts could be re-isolated, the yeast *Hanseniaspora uvarum* predominates above the others representing a strong association between both organisms (Hamby *et al.*, 2012).

The role of flies as vectors of yeasts is clear and the nutritional factors offered by yeasts are conspicuous as well. The nutritional quality of plant resources can be improved or substituted by yeasts, although the degree in which yeasts benefit the flies depends on the yeasts species and the environment in which they grow (Ganter, 2006).

Yeasts speciation and co-evolution with Drosophilids might go beyond the simple role of nutrient providers. Some fruit flies probably form specific symbiotic combinations with yeasts that allow them to metabolize host plant toxins. For instance, the volatile 2-propanol is a compound found on decaying plant material of cacti with toxic effects on insects; however, larvae of *Drosophila*

are able to feed and develop in these tissues most likely due to the ability of associated yeasts (mainly *Candida sonoriensis* and *Cryptoccus cereanus*) to use 2-propanol and acetone as sole sources of carbon (Starmer *et al.*, 1986). A similar situation is observed with the Organ Pipe Cactus, in which the yeast community (dominated by *Dipodascus starmeri* and *Pichia mexicana*) secretes extracellular lipases that degrade triterpene glycosides, allowing their use as sources of carbon for the yeasts and thereby increasing the performance of the larvae of *D. mojavensis* (Fugleman & Armstrong, 1989; Starmer & Fogleman, 1986).

Yeasts also have an important role in reproduction and oviposition of Drosophilids. Microbes are transferred during courtship, and in some cases males present females with drops regurgitated from the crop that are rich in yeasts. Furthermore, evidence indicates that both females and males base their mate choice on their previous yeast diets, demonstrating the importance of yeasts as nutritional factors and the consequences on mating behavior (Starmer *et al.*, 1988; Steele, 1986). Similarly, in most *Drosophila* species, females prefer to oviposit on substrates dominated by yeasts, and differences in preferences among different yeasts species is also observed and are probably related to genetic and environmental effects (Barker, 1992).

The symbiosis between yeasts and fruit flies is so well established that some plants have used this association to their advantage. The Solomon Lilly (*Arum palaestinum*) is not a rewarding species and bases its pollination on the deception of flies (Benton, 2010). They mimic the association of plants with yeasts through the production of yeast and fruit fermentation volatile compounds. This deceptive strategy targets highly conserved odor receptors of *Drosophila* specially tuned to yeasts derived compounds. This allows the plant to use the strong association between yeasts and fruit flies for their pollination even without yeasts as mediators (Stökl *et al.*, 2010).

Recent work has shown that the importance of volatiles produced by yeasts for insects might be more widespread than expected, even for Diptera (Davis & Landolt, 2013). The yeast *Pichia guilliermondii* has been isolated from the gut of

Suila sp. larvae feeding on truffles (Zacchi & Vaughan-Martini, 2002), and some scattered reports point to the presence of yeasts (mainly *Pichia*) in mosquito guts (Ricci *et al.*, 2012). Davis and Landolt (2013) captured insects from 7 different orders in a field experiment with baits of fungal cultures of *Aureobasidium pullulans*. Diptera was the most represented order and interestingly 80% of the caught dipterans corresponded to members of the Syrphidae family (hoverflies) and not to Drosophilidae as one might expect.

4.3 Hymenoptera

The association between yeasts and members of this order has been reported in many species including bees, bumblebees, ants and wasps (Davis *et al.*, 2012; de Vega & Herrera, 2012; Engel *et al.*, 2012; Mendes *et al.*, 2012; Brysch‑Herzberg, 2004).

Bees and bee-pollinated plants have attracted most research related to the association between Hymenopterans and yeasts (de Vega & Herrera, 2012). The study of yeasts communities of bees have shown that many different species establish associations with these insects and their occurrence depends on the species, life stage, and niche of the bees. For example, some yeasts are found in the flowers, pollen and nectar and therefore recovered from the gut and frass of adults, while some others are re-isolated from larval provisions, larvae and pupae only (Teixeira *et al.*, 2003; Rosa *et al.*, 1999; Inglis *et al.*, 1993).

In most cases, yeast species associated with honeybees, stingless bees and solitary bees correspond to species of *Candida, Cryptococcus, Metschnikowia* and *Starmerella* (Rosa *et al.*, 2003). Yeasts can produce and release enzymes that enhance, protect and preserve pollen and also have a putative role as producers of antimycotic substances that protect the hives from diseases (Rosa *et al.*, 2003; Gilliam, 1997). However, it is likely that yeasts only play a complementary role in this sense because the gut of bees contains bacterial communities with analogous functions (Engel *et al.*, 2012).

Similar to the bees, the diversity of yeasts associated to bumblebees range from broadly distributed yeasts (yeasts present on soil, phylloplane and other insects) to highly specialized associations (only present in bumblebees bodies). For instance, Basidiomycetes yeasts, such as *Cryptococcus*, seem to be more widely distributed among multiple insect orders, while some others such as *M. kunwiensis* need to establish close symbiosis with bumblebees in order to overwinter (Brysch‑Herzberg, 2004). In the case of bumblebees, it has been proposed that bumblebees benefit from the yeasts present in flowers through host recognition and improvement on nutritional factors of the nectar, the yeasts benefit, not only because of their dispersal but also because of the possibility of overwintering in the bodies of the bumblebees (Brysch‑Herzberg, 2004).

The presence of yeasts in nectar seems to have deeper effects on bumblebee behavior and consequently affect pollination success, along with quantity and quality of seeds (Herrera *et al.*, 2013). In laboratory and field experiments, workers of *Bombus terrestris* preferred flowers of *Helleborus foetidus* containing nectar with yeast (*M. reukafii*) and that this preference negatively affected the general pollination of this plant (Herrera et al. 2013). They hypothesized that the increase in attraction and quality of flowers by yeasts might be costly for the plant and in cases of crossed pollination might represent a risk that bumblebees would not need to visit multiple flowers to fulfill their nutritional requirements, causing an increase in selfing.

Fungus-gardening ants cultivate diverse Basidiomycetes fungi, such as *Attamyces bromatificus,* that are not considered yeasts (Seal *et al.*, 2012). However, the importance of yeasts for ants has been the subject of controversy, although some studies have started to show examples of mutualisms. For instance, Mendes et al. (2012) demonstrated that yeasts growing in fungus gardens, along with the main fungus, produce a set of extracellular enzymes, like cellulytic, pectinolytic, proteases and amylases, which potentially detoxify the garden and help the assimilation of nutrients. In the case of ant-pollinated plants, the importance of yeasts is similar to the case of bees and bumblebees. Furthermore, evidence suggests that yeasts transported by ants are able to change the sugar content of

nectar (decreasing content of sucrose and increasing content of glucose and fructose), changing the pollination dynamics of ants and other pollinators (de Vega & Herrera, 2013; de Vega & Herrera, 2012).

Social wasps are also associated with yeasts (Stefanini *et al.*, 2012). The most studied case corresponds to *S. cerevisiae.* Although this yeast has been widely used and linked to human-related activities, their natural survival and spread is dependent on wasps. Queens of *Vespa crabro* and *Polistes* spp. can harbor *S. cerevisiae* from autumn to spring and transfer it to their offspring, demonstrating their role in dispersal and likely effect on yeast diversity.

The importance of yeasts as chemical indicators for eusocial wasps has also been demonstrated. Volatiles produced by *Aureobasidium pullulans* attracted significantly more individuals of *Vespula* spp. than unbaited traps, implying that wasps might use these volatiles as indicators of nutritional resources (Davis *et al.*, 2012).

4.4 Hemiptera

As mentioned before, the association between YLS and hemipterans has been subject of deep analysis, especially with aphids and planthoppers. In these systems, the endosymbionts form obligatory relationships with the insects and are transmitted between generations, and provide the homopterans with amino acids and sterols that they cannot get from their host plants (Ganter, 2006).

Apart from plant feeding homopterans, volatile production by yeasts may be used as olfactory cues for blood-feeding insects (e.g., Chagas disease vector *Triatoma infestans*) (Davis *et al.*, 2013). These insects use CO_2 as an indicator of their prey; therefore, the use of baker's yeasts for trapping has been proposed (Guerenstein *et al.*, 1995). Furthermore, electroantennograms of the hematophagous bug, *Rhodnius prolixus*, have shown that several volatiles produced by yeasts induce important responses in the insect indicating that

yeasts might play a role in host finding and opening a potential use for the control of disease vectors (Lorenzo *et al.*, 1999).

4.5 Neuroptera

The study of lacewing guts has shown the presence of yeasts cells in high concentrations along their alimentary track, especially in the midgut (Urubschurov & Janczyk, 2011). Comparisons between larvae and adults of *Chrysoperla carnea* have shown that bacteria predominate in larvae while yeasts are found mainly in adults (Chen *et al.*, 2006). These observations coincide with the feeding behavior of *C. carnea* adults, which contrary to other species are not predacious as adults, and therefore need to feed on other nutritional sources such as honeydew, pollen and nectar. Since honeydew cannot supply all the amino acids and lipids requirements, the role of yeasts as nutritional suppliers for lacewings seems conspicuous (Gibson & Hunter, 2005).

Although the majority of isolations find that *M. pulcherrima* is the most abundant yeast found in lacewings digestive tracks, evidence supports a large diversity of yeasts associated with lacewings (Nguyen *et al.*, 2006; Suh *et al.*, 2004; Woolfolk & Douglas Inglis, 2004). Adults from *Chrysoperla* spp. yielded 14 different yeast isolates ranging from three main taxa, and representing new species such as *Metschnikowia chrysoperlae*, *Candida picachoensis* and *C. pimensis* (Suh et al. 2004). As mentioned by several authors different insect species could contain completely different gut microbiota, pointing out the immense diversity of yeasts.

4.6 Lepidoptera

Although some lepidopterans represent serious pests for agriculture and have been subject of intensive research, their symbiosis with yeasts has been less well studied than in other insect orders. Cases of yeasts associated with moths have been reported to some extent. Yeasts such as *Clavispora opuntiae, Candida sonoriensis, Pichia cactophila, P. barkeri* and *Geotrichum* spp. were isolated from individuals and feeding sites of the moth *Sigelgaita* sp. indicating a likely association between organisms (Rosa *et al.*, 1992).

Potential mutualisms between lepidopterans and fungus have been mentioned for decades with an increasing interest in the past years. For instance, Listemann (1988) mentioned an apparent protection from other microbes and also a likely nutritional association between *C. pomonella* larvae and the yeast *M. pulcherrima* when he recovered the yeast from larval feces. Years later, Witzgall et al. (2012) demonstrated that this moth uses the yeast for host finding, feeding, protection from detrimental microbes and probably for more unrevealed reasons. These findings made way for the possibility of using yeasts as attractants and combined with a pathogenic virus for control of this pest (Knight & Witzgall, 2013).

In a survey conducted by Davis and Landolt (2013), a few specimens of lepidopterans were captured in traps with volatiles of *A. pullulans.* Many insects were attracted to yeasts volatiles, including moths such as *Caradrina morpheus* and *Xestia* spp. By comparison with other insect orders, the presence of lepidopterans was one of the lowest (3.0%), but this finding does not neglect the fact that yeasts are important for them, especially considering that the yeast strain used to attract insects to the traps was originally isolated from fecal material of codling moth.

In an experiment analyzing the presence of yeasts in the guts of different pests of maize, the yeast *M. pulcherrima* could survive after ingestion and digestion by *Ostrinia nubilalis* (Molnár & Prillinger, 2005). Similarly, a posterior analysis showed that multiple yeasts such as *Cryptococcus lutelus, C. flavenses, Pichia guilliermondii, Pseudozyma* and others are present in guts and feces of *O. nubilalis* and also in *Helicoverpa armigera*, another lepidopteran pest of maize (Molnár *et al.*, 2008). Such results demonstrate that ingestion of yeasts is probably more widespread among lepidopterans than expected.

5. Applying the know-how of yeast-insect interactions

5.1 Yeast-derived semiochemicals

As demonstrated for multiple pests, there are common volatiles that elicit powerful responses in the insect's sensory system (Davis *et al.*, 2012; Lorenzo *et al.*, 1999; Nout & Bartelt, 1998). Some of these compounds come from yeasts and signal the presence of fermented fruits and tissues and thereby suitable host plants, substrates and food for the insect. These signals represent a source of new putative attractants for pest monitoring and control, with the advantage that attraction to yeasts volatiles follows a different communication channel than plant volatiles. This decreases the interference of background odors that are normally observed when using attractants based on plant volatiles (Witzgall *et al.*, 2012; Knudsen *et al.*, 2008)

Some studies have used yeasts directly applied to traps for the attraction of herbivores (Davis & Landolt, 2013; Leblanc *et al.*, 2010). As mentioned previously, Davis and Landolt (2013) captured a plethora of different insect species by baiting traps with *A. pullulans.* From a more applied point of view, an experiment carried out under field conditions, demonstrated that traps baited with *Candida utilis* were more attractive and specific for fruit flies (*Bactrocera dorsalis*) than commercial chemical traps (Leblanc *et al.*, 2010).

Most of the yeast volatiles that elicit insect responses are produced during yeast fermentation. Witzgall et al. (2012) showed that compounds such as isoamyl acetate, phenyl acetaldehyde, 2-phenylethyl acetate, 2-phenylethanol and others, elicited antennal responses in codling moth. As observed by Stökl et al. (2010), the attraction of insects to yeast volatiles is possible even without the yeasts presence. Therefore, the commercial availability of these chemical compounds would allow their use for attraction and monitoring of insects in field traps.

Nevertheless, specific ratios, synergisms and relevance of yeast's volatiles must still be elucidated.

Beyond agricultural pest control the possibility of using yeasts volatiles for deception and trapping of animal disease vectors has been proposed (Ricci *et al.*, 2012; Lorenzo *et al.*, 1999; Guerenstein *et al.*, 1995). However, trap efficacy and design needs be tested in field experiments since most of the cases have been tested in laboratory conditions only.

5.2 Biological control

The use of yeasts in biological control has been neglected, probably because in general yeasts seem to have positive effects on insects considered pests. However, the putative role of yeasts as enhancers of the efficacy of other control agents has been proposed (Knight & Witzgall, 2013).

One example is the control of codling moth with microbial agents. Since *C. pomonella* larvae cause damage in apples and are particularly difficult to reach once they emerge, the use of entomopathogenic virus is preferred, especially considering the inefficacy and environmental consequences of available pesticides. Unfortunately, to be effective the virus must cover the apples and be ingested by the larvae. Taking into account the wandering behavior of the first larval stages and the detrimental exposure of the virus to UV light, the need to attract the larvae and protect the virus has led to research on the possibilities of combining the virus with attractants and feeding stimulants.

Attempts to increase the effect of the virus with larval attractive plant volatiles such as pear ester and (E,E)-α-farnesene have yielded inconsistent results (Arthurs *et al.*, 2007; Hughes *et al.*, 2003). However, Knight and Witzgall (2013), demonstrated that combining yeasts with the pathogenic virus CpGV increases the mortality of *C. pomonella* larvae and decreases the damage to fruits. The discovery of how codling moth is attracted to yeasts volatiles (both adults and larvae) has opened the possibility of developing more accurate control methods (Witzgall *et al.*, 2012). Since odor cues produced by the yeasts could attract and

stimulate larval feeding and the virus (due to its specificity) would kill only the target organism, this system represents a novel effective use of yeast for biological control.

6. Conclusions and future perspectives

Currently, it is thought that the main role offered by microbes associated with herbivores is for nutritional contribution to their diets (Hansen & Moran, 2013). Consequently, the study of how insects detect microbes has been focused on insects that depend on microorganisms for dietary requirements. For this reason in the case of yeast-insect mutualism, most of the available knowledge derives from the study of bark beetles, anobiid and cerambycid beetles, and Drosophilids, while other insects have been greatly ignored. Hence, the study of lepidopterans and their association with yeasts emerges as a promising field to understand the ecological roles of yeasts and their potential use to develop management strategies of important agricultural pests.

Beyond the nutritional functions of yeasts for insect development, there are several roles that have been proposed but with limited or inexistent experimental evidence. For instance, the putative effect of yeasts on decreasing plant defenses. Recent developments have shown that herbivores could use bacteria and fungi (including yeasts) to interfere with the JA-regulated defenses (Megali *et al.*, 2013). While Chung et al. (2013) found that bacteria in the Colorado potato beetle produced effectors that decreased JA-regulated defenses, Megali et al. (2013) found that a mixture of microorganisms (including *S. cerevisiae*) impaired the synthesis of alkaloids and JA related defenses, promoting herbivory by *S. littoralis.* However, the specific identity of these effectors and whether the effects are observed on direct defenses such as alkaloids or indirect defenses such as plant volatiles must still be elucidated for yeasts.

Another important knowledge gap that must be investigated is the molecular mechanism through which yeasts volatiles are perceived. Functional characterization of odor receptors is ongoing for many insect species (Zhang *et al.*, 2013; Bengtsson *et al.*, 2012; Montagné *et al.*, 2012; Stocker, 2001). However, those attempts have focused mainly on pheromones and plant odors, while odors produced by microorganisms have been overlooked. Understanding what

compounds and what receptors are expressed during different insect life stages and physiological states will be helpful to determine the key elements for insect behavior leading to the development of more accurate insect management strategies.

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