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Review

Insect fungal symbionts: a promising source of detoxifying enzymes*

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SUMMARY

Many species of insects cultivate, inoculate, or contain symbiotic fungi. Insects feed on plant materials that contain plant-produced defensive toxins, or are exposed to insecticides or other pesticides when they become economically important pests. Therefore, it is likely that the symbiotic fungi are also exposed to these toxins and may actually contribute to detoxification of these compounds. Fungi associated with bark beetles, ambrosia beetles, termites, leaf-cutting ants, long-horned beetles, wood wasps, and drug store beetles can variously metabolize/detoxify tannins, lignins, terpenes, esters, chlorinated hydrocarbons, and other toxins. The fungi (*Attamyces*) cultivated by the ants and the yeast (*Symbiotaphrina*) contained in the cigarette beetle gut appear to have broad-spectrum detoxifying abilities. The present limiting factor for using many of these fungi for large scale detoxification of, for example, contaminated soils or agricultural commodities is their slow growth rate, but conventional strain selection techniques or biotechnological approaches should overcome this problem.

INTRODUCTION

At a time when environmental concerns have been highlighted, the search for detoxifying enzymes has greatly expanded. Microorganisms have shown promise in decontaminating some pesticide residues and agricultural commodities such as oil seed meals. However, in many cases these microorganisms can only detoxify a single, or closely related toxins. In addition, there are many toxins for which no effective detoxifying microorganism or other process is available. Thus, there is still a need for microorganisms or other sources of new detoxifying enzymes, preferably with good stability and broad-spectrum detoxifying ability. In this review, fungal insect symbionts will be considered as a source of detoxifying enzymes. The discussion will begin with a review of the types, distribution and species of insects and fungi involved in symbiotic relationships. The discussion will continue by considering why insect symbionts might produce detoxifying enzymes. Representative examples of different detoxifying reactions from the different types of symbioses will be described. Finally, the feasibility of using these symbionts for detoxification will be explored.

INSECT FUNGAL SYMBIONTS

In the less highly evolved relationships, it is often difficult to determine if a microorganism associated with an insect is a symbiont. The presence of saprophytes in materials colonized by symbionts has frequently confounded matters (see below). In addition, the fungi that act as symbiotic partners of insects are varied in regard to the intimacy of the association. The number of diagnostic tests that can be performed on the fungi, due to their oftentimes obligate relationship, is frequently limited. These limitations often result in a particular fungus being classified in a variety of ways. Two extremes appear to be morphological co-identity, where the fungi are identified as free-living species, and symbiosis identity, where all symbionts from the same insect species are classified as unique species, and those from related insects

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are put in the same genus. The intent of this paper is not to offer yet another view on this problem. Recent success in molecular taxonomy of fungi hopefully will soon be applied to insect fungal symbionts and thereby clarify taxonomic relationships. The majority of the following material has been compiled from reviews. The reader is advised to check recent taxonomic fungal compendia for synonymies.

One type of symbiotic association is where the relationship is extracorporeal and insects serve as inoculators (Table 1). For these insects, it is sometimes difficult to determine which fungi are symbionts, and which are casual associates. Bark beetles (Coleoptera: Scolytidae) bore into and feed on the vascular phloem (bark) tissue of woody plants [8], and sometimes may kill trees [7, 145]. Ambrosia beetles (Coleoptera: Scolytidae and Platypodidae) are so-called because of the growth patterns of the fungi they inoculate and subsequently feed upon in the xylem of dead tress or other woody plants [7, 50]. The larvae of horntails (Hymenoptera: Siricidae) and wood wasps (Hymenoptera: Xyphriydiidae) feed on dead or dying trees [88]. Ship timber worms (Coleoptera: Lyxmelidae) also feed on wood [50]. Other insects that may inoculate fungi include detritivorous sap beetles (Coleoptera: Nitidulidae) [39, 98], cactus-feeding fruit flies (Diptera: Drosophilidae) [130], adult lacewings (Neuroptera: Chrysopidae) [55], and some dung beetles (Coleoptera: Scarabaeidae) [89]. Some scale insects are shielded with fungi that invade the body cavity, but not individual cells [32]. Other associations that may evolve into symbiotic relationships include cases where insects are known consumers of mushrooms [19], Ascomycetes [51], or yeast [56].

Some insects appear to actively cultivate, and then consume, their symbiotic fungi. Some termites that inhabit Africa and Asia build nests in soil that extend mound-shaped from the earth [6, 149]. The fungi form spherules as they grow on masticated woody material and/or primary feces of the termites [6]. Fungus-growing ants are distributed in South, Central, and North America and also build nests in the ground that may also form mounds. The fungi form swellings (gongylidiae) as they grow on masticated plant debris, insect frass, or fresh plant material [142]. Adults primarily feed on plant sap [85], and use the fungal growths to feed the larvae [29].

Although intracellular bacterial symbionts appear more common, many species of insects also contain intracellular symbiotic fungi. Several families of scale insects (Coccoidea), plant hoppers (Fulgoroidea), and cicada-like insects (Cicadoidea) all of which are sap feeders, contain symbiotic yeasts which have not been identified. Intracellular yeasts are also found in certain genera [90] of the longhorned beetles (Coleoptera: Cerambycidae),

TABLE 1

Hosts and genera of fungal insect symbionts

Insect host	Symbiont	References
Extracorporeal Associations – Inoculators		
Bark beetles	Various[a]	[144]
(Scolytidae)	*Ceratocystis*	[50, 144]
	(*Ceratocystiopsis*	[57])
	Sporothrix	[144]
	Trichosporium	[50]
	Tubercullariella	[50]
	Hansenula	[7]
	Pichia	[7]
Ambrosia beetles	Various[a]	[50, 77]
(Scolytidae, Platypodidae)	*Ambrosiella*	[7, 50]
	Cephalosporium	[7, 32, 50]
	Ceratocystis	[7, 32, 50]
	Fusarium	[7, 32, 50]
	Graphium	[7, 32]
	Monacrosporium	[32]
	Monilia	[32, 50]
	Phialophorpsis	[32]
	Sporothrix	[32, 50]
	(*Raffaelea*)	
	Ascoidea	[32]
	Endomyces	[32, 37]
	Endomycopsis	[7, 50]
	(*Ambrosiozyma*	[78])
	(*Pichia*	[80])
	Hansenula	[7]
	Pichia	[7]
Lyxmelidae	*Ascoidea*	[32]
Horntails and wood wasps	*Amylostereum*	[52]
(Siricidae and Xyphrydiidae)	*Cerrena*	[52]
	Daedalea	[32]
Sap beetles (Nitidulidae)	*Ceratocystis*	[39]
	Yeasts	[98]
	Various[a]	[39]
Fruit flies (Drosophilidae)	Yeasts	[130]
Lacewings (Chrysopidae)	Yeasts	[55]
Scarabaeidae	Yeasts	[89]
Scale insects-*Aspidiotus*	*Septobasidium*	[32]
Extracorporeal Associations – Cultivators		
Termites (Termitidae)	*Termitomyces*	[6, 119, 149]
	Xylaria[a]	[6, 149]
Ants (Formicidae: Attinae)	*Attamyces*	[21]
	Lepiota	[142]
	Tyridiomyces	[142]
Intracorporeal Associations		
Scale insects (Coccoidea)	Unidentified yeasts	[20, 76, 135]
(some families)	(Ascomycetes	[76, 135])
	(*Dermatia pullulans*	[121])
Planthoppers (Fulgoroidea)	Unidentified yeasts	[20, 76, 135]
Cicadas and relatives	Unidentified yeasts	[20, 76, 135]
(Cicadinae)		
Longhorned beetles	*Candida*	[105]
(Cerambycidae – some genera)	*Rhodotorula*	[105]
	Torulopsis	[26]
Drug store beetles	*Candida*	[97]
(Anobiidae)	*Symbiotaphrina*	[73]
	Torulopsis	[105]
Bark beetles (*Ips*)	Unidentified yeasts	[50]

[a] Indicates fungi are associated with insects, but status as symbionts is unknown or uncertain.

which have wood-boring larvae. In the larvae, the gut is modified into swollen pockets (mycetomes) that contain cells with enlarged nuclei and that are filled with yeast. Similar structures are found in the drugstore beetles (Coleoptera: Anobiidae). Most of these insects are wood-boring (including in furniture), but few are pests of virtually all stored products [96].

The fungal symbionts of these insects are generally considered to be a food or nutrient source, but may also produce enzymes that aid in digestion. Fungus-invaded wood is consumed by bark beetles, horntails, and wood wasps; while primarily the fungus itself is consumed by ambrosia beetles, and fungus-growing termites and ants. The fungus thus may make up a significant portion of the diet and/or provide nutrients [88], specifically vitamins [26, 72, 144], lipids and sterols [77, 114, 144], and amino acids [7]. Similarly, intracellular symbiotic fungi appear to contribute vitamins, sterols, and amino acids to their hosts as well [23, 42, 105]. The symbiotic fungi of wood-inhabiting insects may also produce cellulolytic enzymes [1, 6, 79, 90–92], although this is not always the case [26, 104, 120]. Proteolytic enzymes may also be produced by the symbionts [15, 16, 90, 126].

WHY DETOXIFICATION?

As indicated above, insect fungal symbionts (as well as bacterial symbionts) are well recognized as providers of nutrients for their insect hosts. The rationale behind this is that the insects are feeding on nutrient-poor resources, and need the microbial symbionts to provide a balanced diet. The effects of the presence of toxins in these food materials has been relatively overlooked, yet in many cases there is just as much need for detoxification as there is for nutrient provision. A common factor in all of these instances is that the insects are either inoculating plant material with the fungi, and then eating the fungi, or consuming plant material itself. The ability of plants to produce secondary compounds toxic to insects and fungi is well accepted. Their role in protecting herbaceous parts has been demonstrated repeatedly. Defensive compounds include alkaloids, cardenolides, cyanogenic glycosides, flavonoids and terpenes (e.g. [10, 118]). These toxins are often concentrated in areas of greater 'importance' to the plant, such as growing tips (except for tannins and lignins), flowers, and seeds. Toxicity may be increased when insect chewing destroys compartmentalization of toxins and enzymes, such that more toxic aglycones are liberated from stored glycosides (e.g. flavonoids and phenolics [56]), or cyanic compounds are liberated (e.g. cyanogenic glycosides [31] or thiocyanates [137]).

These toxin interactions not only involve herbaceous, but also woody tissues. Tannins in wood and leaves have long been recognized for their digestibility-reducing and protein-binding properties [131]. Recently tannins have been reported to cause acutely toxic effects [116]. Lignins may present physical barriers [139], interfere with digestion [122], or be acutely toxic [129]. Intercalation of lignin with cellulose also interferes with the degradation of cellulose [90]. Other toxins also may be found in woody tissues. Outer bark may contain alkaloids and phenolics [24, 69], while heartwood may contain terpenes, polyphenol glycosides, alkaloids, stilbenes, and tropolones [59, 127].

Plant sap is generally thought to be relatively free of defensive toxins, so theoretically sap-sucking insects may have a lower risk of encountering toxins. However, the sap of milkweeds contains cardiac glycosides [12], that of broom plants contains quinollizidine alkaloids [147], and stachyose is a transport sugar in *Fraxinus* and *Curcubita* [46]. Sap-sucking insects also may encounter foliar toxins when probing [136].

The number and types of hosts fed upon can also influence the exposure of insect hosts and their symbionts to toxins. Certain classes of plants typically are considered to contain high levels of particular toxins that are only overcome by a few specialist insects. They include: Umbelliferae, which contain furanocoumarins, Asclepiadaceae which contain cardiac glycosides, and Euphorbiacae and Solanaceae, which contain alkaloids. On the other hand, insects that feed on a wide variety of hosts encounter a diversity of toxins, and would be expected to have broad-spectrum detoxifying activity. Studies have associated the specialization on particular toxin-containing plants with enzymes that are efficient in degrading these toxins (see [41] for review). On the other hand, insects with a wide host range often have higher levels of activity of more 'general' detoxifying enzymes (such as unspecific monooxygenases), although this has been disputed (see [41]).

When symbionts are inoculated or cultured on plant material, the exposure to toxins is obvious. In cases where plant material is consumed, fungal symbionts that inhabit the gut of the insect would presumably be exposed to toxins. Due to the lipophilic nature of these toxins, symbionts within gut cells are not likely to be particularly well protected from any antifungal agents. Thus, insects that consume undetoxified plant materials would be expected to be exposed to the toxins. Inoculation or cultivation of detoxifying fungi on toxic plant materials provides a solution to this problem, as will be seen later.

Use of insecticides further exposes the insects and their fungal symbionts to compounds toxic to insects. Because insects may inhabit commodities also treated for fungi, symbiont exposure to fungicides may be subsequently increased as well. Especially prone to exposure

are those insects found in agricultural situations or that are actually agricultural or stored product pests, due to associated pesticide use.

However, due to prior research orientations, concern has been primarily with what plant parts/secondary metabolites are toxic to the symbionts, versus which ones are not (and thus presumably are detoxified). Cherrett [27] theorized that the fungi cultivated by leaf-cutting ants were involved in detoxifying plant toxins. Jones [71] suggested that detoxification by insect symbionts was likely to be widespread. These concepts are especially attractive when considering that target sites of several toxins for insects (nervous system, hormone system, etc.) are not likely to be present in fungi. Thus, problem compounds for insects may not be problems for the fungi, but instead be degraded by the fungi. The exposure to toxins, implied detoxification, and actual detoxification or demonstration of detoxifying enzymes produced by insect fungal symbionts will be discussed in the following section.

EXAMPLES OF DETOXIFICATION

Detoxifying enzymes produced by insect symbionts have not been widely studied. This is not the case, though, for other enzymes (or chemical reactions). Detoxification may be inferred in other instances by the ability of the symbiont to grow on toxin-containing materials or utilize toxins in media. This type of information is relevant because in many cases insects that were first found to feed on toxin-containing plants were subsequently found to enzymatically detoxify the associated toxins [41]. In some cases, actual detoxifying enzyme activity has been detected, whether by use of actual toxins as substrates, or 'indicator substrates', i.e. those compounds often used to indicate the production of a particular type of detoxifying enzyme. Examples include 4-nitroanisole *O*-demethylation to indicate unspecific monooxygenase activity, 1-naphthyl acetate hydrolysis to indicate hydrolysis of carboxyl esters or phosphate esters (including organophosphorus insecticides), glutathione conjugation of 1-chloro-2,4-dinitrobenzene as an indicator of glutathione transferase activity, and syringaldazine oxidation as an indication of laccase activity. Enzymes that are considered to have primarily another physiological role may be involved in detoxification as well. For example, proteolytic enzymes are thought to be capable of hydrolyzing toxic esters [58], pyrethroid esters can be hydrolyzed by leucine aminopeptidase [44], and pepsin will hydrolyze the mycotoxin ochratoxin A (Dowd, unpublished data). Glycosidases, although often involved in activating plant glycosides to more toxic aglycones, may also hydrolyze toxic sugar-containing compounds into less toxic forms. One such example is the hydrolysis of tannic acid to gallic acid, which is thought to be performed by β-glucosidases [93]. In the following discussion, detoxification primarily will be viewed from the point of the insect host.

Bark beetles

In spite of the wide distribution of bark beetles, relatively little work has been done on the ability of associated fungi to detoxify, other than the conversions that involve terpenes and produce pheromones or repellents. As discussed previously, the associations between these insects and fungi can be rather loose, so it is questionable whether some can be considered symbionts in the truest sense.

Enzymes that hydrolyze cellulose or other sugar complexes (osidases), including β-glucosidases are produced by unspecified microorganisms of bark beetles such as *Ips typographus* [33]. The associated species *Candida pulcherina* was most efficient at degrading trehalose, but also degraded amidon and pectin [82]. Thus, degradation of hydrolyzable tannins or other toxic glycoside esters is a potential role for bark beetle symbionts.

Terpenes appear to be effectively degraded by bark beetle symbiotic fungi. These resin-derived terpenes are a recognized defense and host resistance mechanism against the bark beetles and some of their associates [35, 112, 113, 115]. The fungal associates are thought to be important in detoxifying these compounds for the insects [8]. Although increased levels of terpenes are associated with resistance, conifers always have low levels of terpenes present [36], which are apparently tolerated or detoxified by the fungi. The terpenes α- and β-pinene actually stimulate the growth of a *Sporothrix* species [18]. Raffa et al. [113] demonstrated that although β-pinene, camphene, D-3-carene, limonene, myrcene, and resin slowed the growth of the *Scolytus ventralis* fungus *Trichosporium symbioticum*, they were not lethal to it, suggesting some tolerance. A *Sporothrix* from *Dendroctonus frontalis* could convert *trans*-verbenol to verbenone and 3-methyl-2-cyclohexene-1-ol to the ketone [17]. The yeast *Candida molischiana* (*Hansenula capsulata*) and *Candida nitrophila* from *Ips typographus* can convert verbenols to verbenone. The *C. nitrophila* converted *cis*- to *trans*-verbenol as well [83]. In spite of the fact that these 'bark beetles' and their fungi would be expected to encounter tannins and lignins, breakdown of these compounds by symbionts has apparently not been investigated (but see following discussion on ambrosia beetles). Considering the variety of insects and fungi involved, and initial work with terpenes, these fungi should be further investigated for detoxifying abilities.

Ambrosia beetles

In spite of the more specific relationship between these insects and their associated fungi compared to bark

beetles, apparently no detoxification studies have been performed. However, known ambrosia fungi, such as *Aspergillus*, *Ceratocystis*, and *Fusarium* (although some may be contaminants), are recognized as being able to degrade different toxins. *Aspergillus* has long been recognized as a producer of enzymes that degrade tannin [75]. *Ceratocystis* spp. produce hydrolases [2] and phosphatases [4]. Interestingly, the types of phosphatases produced depends not only on the species of *Ceratocystis*, but also on the substrate on which they are grown [4]. The production of phosphatases of *Sporothrix* depends on whether the fungus is growing in a yeast or filamentous form [4]. These types of enzyme activity suggest an ability to degrade toxic esters. *Ceratocystis* has also been suggested as a good fungus for bioreactors, due to its cellulose and lignin-degrading abilities [30]. Different strains of *Fusarium solani* can *O*-demethylate the phytoalexin pisatin [138], can *N*-dealkylate alkaloids such as glaucine [38], and can degrade carbamates and derivatives such as EPTC [87], carbaryl and 1-naphthol [141]. It can also degrade anilides such as propanil and bromoxynil [114]. Thus, the symbionts of these insects also appear to be good candidates for detoxifying enzymes, and should be investigated further.

Wood wasps

The detoxifying enzymes of wood wasps also have been explored only to a limited extent. These insects apparently encounter polyphenols [34, 59], and terpene resins [74], so by analogy with bark beetles and ambrosia beetles, detoxification should occur, whether by insect and/or symbionts. For example, resin from *Pinus radiata* incorporated into media at 1% only inhibited fungal growth by 30%, while terpene volatiles α- and β-pinene only partly inhibited growth [74], suggesting some mechanism for tolerance, such as detoxification. Presumably the standard wood defensive compounds tannins and lignins would also be important. Kukor and Martin [79] demonstrated that cellulases and related enzymes used in digestion by *Syrex cyaneus* were produced by the fungus *Amylostereum chailletii*. Again, this suggests that hydrolyzable tannins may be degraded, and that the enzymes involved may have useful stability. Gilbertson [52] considers these fungi to be white-rotters, implying lignin degradation. Other white-rot fungi, such as *Phanerochaete chrysosporium* have a wide detoxification range that includes chlorinated hydrocarbons, phenolics, tannins and lignins [22]. Thus, the wood wasp fungi may also have some of these detoxifying abilities. This is yet another example of a symbiotic fungus that should be further investigated.

Termites

The fungal symbionts of termites have been studied to some degree. Since wood is the 'food' of the fungus, tannins, lignins, and other secondary metabolites found in wood are the relevant toxins to be considered. Hydrolysis of crystalline, non-crystalline, and cellulose degradation products (by β-glucosidases) is contributed to or due totally to enzymes acquired from the fungus *Termitomyces* cultured by *Macrotermes natalensis* [90–92]. Interestingly, the termite itself produces different forms of non-crystalline cellulolytic and β-glucosidase enzymes [90]. Sugar-degrading enzymes produced by the fungi have been reported by Ghosh and Sengupta [51], although activity may be much reduced in cultured mycelia [134]. The β-glucosidase and cellulase produced by *Termitomyces* conidiophores are highly active [108]. Conceivably, some of these enzymes may degrade hydrolyzable tannins, and it would be interesting to see the differences in substrate specificity for termite vs. fungus-produced enzymes, i.e., which is more effective in degrading hydrolyzable tannins.

More work has been done on lignin degradation by termite fungi compared to other toxins. Grasse and Noirot [53] reported that *Termitomyces* breaks down lignin, based on staining with safranin, and that the utility of this is to release cellulose for digestion. This lignin degradation is thought to be the major function of the fungus [119]. Ligninases are reported to be produced by *Termitomyces* [117, 134], but not *Xylaria* [117]. However, *Xylaria* can *N*-dealkylate 6,12-*endo*-ethenotetrahydrothebain alkaloids [101]. Polyphenol oxidase is produced by *X. nigripes* and *T. albuminosus* [6], and production is correlated with lignin degradation [134]. Differences in activity of this enzyme occur in *Termitomyces* associated with *Macrotermes* vs. *Microtermes* [134]. Studies by Osore [106, 107] using lignocelluloses and mono-dimeric lignin model compounds have indicated polyphenol oxidase, quinone oxidoreductase, and hydrogen peroxide dependent ligninase activity produced by termite-comb associated *Fusarium*, *Aspergillus*, and *Trichoderma*. These enzymes appear to interact in concert with cellulolytic enzymes produced by *Termitomyces* in wood degradation [107]. Laccase production by *T. albuminosus* has been detected by using syringaldazine [6]. Thus, the symbiotic fungi cultivated by termites are a demonstrated source of lignin-degrading enzymes, and may also produce enzymes that detoxify toxic esters. Since the white-rot fungi are also known as good lignin degraders, it would be interesting to see if the termite symbionts have as broad a detoxifying-spectrum as other white rot fungi.

Ants

Prior research orientations dealing with potential control strategies for ants and/or their fungi have primarily

been concerned with what plant, plant parts, and/or secondary metabolites are repellent to the ants, or which are toxic to the ants and their symbionts, and not with which are preferred (and thus presumably are detoxified). The ants do not cut leaves from plants that repel them and also presumably contain toxins that affect the symbiotic fungi [64, 123]. Biologically active compounds include terpenoids [67], and in some cases phenolics [64–66].

However, leaf-cutting ants are reported to cut materials (leaves, stems, flowers, fruits) from 50 to 75% of plant species present in the tropics [29]. The leaf cutting ants are thought to be the major foliage consumers in the tropics, and thus the fungus becomes the major foliage degrader [29]. The diversity of defensive compounds that the host plants are likely to produce suggests that the fungus is likely to be a potent detoxifier [29], and the ants are thereby spared the effects of plant toxins through the activity of the fungus [27, 29, 146]. The mastication that leaf-cutting ants perform on the plant tissues [111] also is likely to promote the liberation of activated compounds, through previously discussed decompartmentalization of enzymes. Unfortunately, very little work has been done to directly test the detoxifying ability of the ant symbionts.

There is much indirect evidence that this detoxifying ability is potent. Plants containing hydrolyzable tannins are preferred, while the presence of alkaloids has no effect on leaf-cutting by *Atta cephalotes* [62]. In contrast, *Atta columbica* prefers hosts that contain condensed tannins, but the presence of hydrolyzable tannins has no influence on host selection [63]. Thus, tannins and alkaloids are potentially degraded by the fungi cultivated by these ants, although relative rates are likely to vary depending on the species. The reported host range for *Acromyrmex octospinosus* [133], and the associated toxic compounds (indicated in parentheses) includes plants in the genera *Bidens* (chalcones [14, 94], acetylenes [14]), *Cassia* (anthraquinones [3, 5, 148], alkaloids [3, 9], coumarins [9]), *Euphorbia* (diterpenes [70]), and *Phyllanthus* (alkaloids [132], flavonoids [54, 60], terpenes [128]). The ant *Atta sexdens rubropilosa* is a problem in Brazil because it cuts leaves from *Eucalyptus* trees [28]. These plants typically contain isoprenoids, which are toxic to a variety of insects [143, 103]. The reported host range for *Atta texana* [140] includes (with associated toxins in parentheses) mature leaves of *Ligustrum japonica* (iridoid glycosides [68, 81]), *Lonicera japonica* (secoiridoids [95]), *Salix nigra*, *Quercus fusiformis*, *Smilax bona-nox*, and *Melia azedarach* (leaves and berry pulp). Tannins are well known components of *Quercus* leaves, which can adversely affect a variety of insects [49], while *Salix* generally contains toxic phenolic glycosides [109]. The leaves and berry pulp of the Chinaberry tree, *M. azedarach*, are a good source [102] of the potent and commercially sold insecticidal compounds, azadiractins, which are toxic to insects, and appear to act both as antifeedants and toxins. Assuming the ants are not immune to these compounds, apparently the ants utilize a food source that is not available to other insects, through their symbiotic fungi. An interesting question is whether the fungi detoxify these compounds or selectively screen them out.

Considering the wide number of potentially toxic compounds that may be encountered by the ant symbionts, relatively few detoxifying studies have been performed. Two proteases from *Atta texana* fungi (AP2 and AP3) are insensitive to diisopropylfluorophosphate (DFP) [15, 16]. Because enzymes that hydrolyze organophosphorus or carbamates are not inhibited by these compounds, this resistance suggests that the proteases may degrade organophosphorus or carbamate insecticides. Interestingly, these enzymes appear to be recycled through to the fungus by deposition in ant feces; they remain active during passage through the ants [15, 16]. The proteinase AP1 is relatively non-specific [90]. This information suggests that these enzymes may have useful stability and wide substrate ranges.

A variety of cellulose/sugar degrading enzymes are also reported to be produced by the ant fungi. The symbiont of *Atta octospinosus* produces α- and β-galactosidase and glucosidase, β-mannosidase, and α- and β-xylosidase [47, 48]. This sort of enzyme activity suggests that hydrolyzable tannins could be detoxified by this fungus. Arylamidase, esterase, and lipase activity is also reported from this fungus [47, 48], suggesting that it may be able to degrade naturally-produced toxic amides or esters, or organophosphorus or carbamate pesticides.

Some resistance of the ant fungi to tannic acid has been reported [110, 111] but this has been disputed [123]. Fungal resistance to naringen also has been reported [123]. Actual studies dealing directly with hydrolyzable tannins and lignins also have been performed. Tannase and polyphenol oxidase apparently were produced by the ant symbionts, as indicated by colorimetric reactions [13]. Fungi from *Atta*, *Acromyrmex* and *Trachymyrmex* could produce polyphenol oxidase [110]. Lignin was dephenolized and *Ligustrum* (privet) phenolics were degraded as well [110]. Cherrett et al. [29] have pointed out that these ant symbionts, because they degrade lignin, can be considered as white rot fungi. As was indicated with the wood wasps, the known range of compounds that are degraded by white rot fungi suggests the ant symbionts may also have a diverse ability to degrade toxins. For example, the insect juvenile hormone analog hydroprene may be degraded by the ant fungi, since it had no effect on the insects when added to the fungal diet [84].

The differences in host ranges may influence enzyme profiles for the associated fungi. An examination of fungi

TABLE 2

Relative activity of *Attamyces* detoxifying enzymes from four different species of *Atta* and *Acromyrmex* leaf cutting ants. Values are corrected for mg protein used in assays (Dowd, unpublished data).

Enzyme	% Relative activity of strains			
	1	2	3	4
1-Naphthyl acetate esterase	9.1	100.0	61.5	78.1
CDNB[a] glutathione transferase	100.0	22.2	11.6	0.0

[a] CDNB = 1-chloro-2,4-dinitrobenzene.

cultivated by four different leaf-cutting ants indicated variation in 1-naphthyl acetate and glutathione transferase activity, with one strain/species being particularly effective in dechlorination (Table 2). Thus, the host range for the fungus, suspected toxin profiles, and prior enzyme work suggest that these fungi have tremendous potential for detoxifying a variety of compounds.

Homoptera

The yeasts and fungi of the Homoptera, primarily of scales, cicadas, and Fulgoridae have not been examined for detoxifying enzymes. The host ranges involved include a wide variety of toxic plants. The potential for exposure to toxins in the sap, and encountered by probing, has already been discussed, so involvement of the symbionts in detoxification is certainly a possibility. Although cicadas (due to their underground larval habits) and scale insects (due to their often cryptic and permanent attachment to hosts) are more difficult to work with, many of the plant hoppers are readily collected and reared and should be interesting sources for investigation.

Cerambycids

Potential detoxifying enzymes produced by the symbiotic yeast from long horned beetles have been investigated to a limited extent. Because only some genera contain symbionts, only those with recognized or suspected symbionts will be discussed here. Again, for the most part we are dealing with a wood-boring insect. The *Eucalyptus* feeding cerambycids should be exposed to toxic terpenes, as discussed previously, but only sugar-degrading enzyme activity of symbionts has been studied. The yeast isolated from *Phoracantha semipunctata*, which feeds on *Eucalyptus*, could degrade saccharose (*Saccharomyces* sp.), maltose (*Saccharomyces* and *Candida* spp.), amidon (both genera), and pectin (both genera) [25]. Further studies were performed on different isolates from this insect: *Candida guilliermondii*, *C. diddensii*, *C. tenuis*, *C. intermedia*, and *Torulopsis molischiana* (although the authors were not sure all were symbionts) [26]. High levels of β-glucosidase were produced by all yeasts except *C. guilliermondii*, while all yeasts produced other sugar-hydrolyzing enzymes [26]. Again, these enzymes, especially the β-glucosidases, may be important in degrading hydrolyzable tannins. The symbionts from *Stromatium barbatum* produced polysaccharide-hydrolyzing enzymes (xylanase, mannanase, arabanase, galactanase, inulinase) but not cellulolytic enzymes (which were instead produced by the insects) [100]. Many of the same compounds were hydrolyzed by the symbionts of *Homochrambyx spinicornis*, with the addition of melibiase and raffinase activity [99]. Thus, the wood-feeding habits of these insects and presence of sugar-hydrolyzing enzymes suggest that toxic glycosides can be hydrolyzed by the symbionts.

Anobiids

As discussed previously, early workers with wood-boring anobiids suggested that the symbionts may be involved in degrading cellulose, but this was subsequently disproven [104]. The stored product pest anobiids, the drug store beetle (*Stegobium paniceum*) and cigarette beetle (*Lasioderma serricorne*), can feed on a diversity of dried seeds and plant tissue, including tobacco, spices, and straw [96]. Thus, their symbionts may be exposed to a variety of toxins. Jurzitza [73] reported that the symbiont could utilize salicin, a compound toxic to many insects, suggesting some role in detoxification. The wide host range, availability and easy rearing of the insect, and reports of the ability to culture the symbiont (*Symbiotaphrina kochii*) apart from the insect and to readily obtain symbiont-free insects (see Jurzitza [73] for review) prompted our investigation of the toxin-degrading ability of the cigarette beetle symbiont.

The insect tissues that contained the symbionts were more active than symbiont-free tissues in producing enzymes in situ that detoxify 1-naphthyl acetate, tannic acid, diazinon, and ochratoxin A (as indicated by histochemical analysis) [40, 42]. Production of 1-naphthyl acetate esterase is significantly reduced in the absence of the symbionts [40, 43]. Larvae rendered aposymbiotic, or treated with fungicides and then fed on diets that were nutritionally fortified had survival and development rates equivalent to those with the normal complement of symbionts [43]. However, when plant toxins such as tannic acid or flavone were incorporated into these diets, those insects that had the symbionts poisoned had higher rates of mortality and/or longer development rates, suggesting the compounds were more toxic [43]. Thus, the symbionts appeared to be involved in detoxifying plant toxins, and presumably produced detoxifying enzymes.

Cultures of the symbionts were examined for detoxify-

ing spectra and enzyme production. Certain plant secondary metabolites, insecticides, herbicides, and mycotoxins appeared to be used as carbon sources [126]. Enzymes that may be involved in detoxification that were detected included esterases, glucosidases, lipases, proteases, phosphatases, and glutathione transferases [126]. The insecticide parathion was also degraded [126]. The esterase activity could be slightly to greatly induced by representative plant allelochemicals, mycotoxins, and insecticides [124]. The 1-naphthyl acetate esterase was resistant to inhibition by the potent serine-hydroxyl esterase inhibitor paraoxon [125], suggesting this enzyme may be able to hydrolyze organophosphorus insecticides. This enzyme was also relatively stable in water : acetone mixtures [126]. Thus, this symbiont appears to have a broad-spectrum ability to detoxify.

FEASIBILITY

As indicated in the prior discussion, notwithstanding the great potential for insect symbionts to detoxify a variety of toxins, further testing is needed to determine actual rates of detoxification and to compare detoxifying ability and enzyme activity with other microorganisms known to be effective detoxifiers. It would also be useful to determine desirable properties of these detoxifying enzymes, e.g. stability and substrate range. However, because the relationships that have been described are symbiotic, the question of potential feasibility for various applications arises. If the symbionts are obligate, then the only place they may produce enzymes is in association with the insect. However, a number of examples have already been discussed indicating that cultures of the symbionts produce detoxifying enzymes. Other desirable properties of the fungal symbionts themselves are that they typically nutritionally enrich their environment (from the standpoint of the insect) and they typically do not produce toxins harmful to people or animals.

The ability to cultivate the organisms is an initial concern. Those fungi that also occur as free-living forms (many bark beetle and ambrosia beetle associates) can be cultivated readily. In addition, unlike many of the bacterial symbionts of insects, most of the other fungal symbionts of insects can be cultivated, even the intracellular ones. Unfortunately, many of these (fungi cultivated by termites and ants, intracellular ones from beetles) are relatively slow-growing. These fungi would not be appropriate to use in situations where low-inoculum levels of the symbionts would be used and where the symbionts then would be expected to compete with other, more aggressive microorganisms that may be casual contaminants.

There are some circumstances where this slow growth and lack of competitive ability may be an advantage. If the symbionts can be applied as previously grown cells (e.g. [150]), they would not be expected to perturb the environment, due to lack of persistence. The symbionts might find use in soil pesticide residue (especially herbicide) problems. In cases where the symbionts can be applied to sterilized materials and maintained under sterile conditions, their feasibility in detoxifying other materials through 'fermentation' (such as oil-processed meals still containing livestock toxins) may still be appropriate.

When rapid growth is warranted, it should be possible to develop strains that have a more desirable growth rate. There are many instances where a particular fungus has a desirable property (e.g. antibiotic production), coupled with undesirable properties (low level production, strict nutrient requirements for production) that have been overcome through selection of appropriate strains (e.g. [45]). As far as the insect symbionts are concerned, growth of *Termitomyces* frequently improves after several transfers in culture media [6]. The original cigarette beetle strain we isolated has been mutated to produce two rapidly growing strains (Alexander, unpublished data), which still retain activity of representative detoxifying enzymes (Table 3). Insect symbionts are also known to rapidly adapt to antibiotics [76] suggesting that potential competitors could be controlled using symbiont strains selected for resistance to antibiotics. Thus, the slow growth rate problem does not appear to be insurmountable.

Genetic engineering allows for the possibility of transferring genetic material that codes for useful detoxifying enzymes from symbionts into more desirable organisms, such as *E. coli*. Plasmids are known to code for detoxifying enzymes in various microorganisms [11, 21]. Possibly plasmids code for detoxifying enzymes in the symbionts, and thus could be transferred to desired organisms by appropriate means. Similarly, other genetic material cod-

TABLE 3

Relative detoxifying enzyme activity in parent and mutant strains of *Symbiotaphrina kochii*. Values are corrected for mg protein or whole cell volume (parathion) used in assays (Alexander et al., unpublished data).

Enzyme	% Relative activity of strains		
	Parent	Mutant 1	Mutant 2
1-Naphthyl acetate esterase	86.8	92.8	100.0
CDNB[a] glutathione transferase	99.5	85.8	100.0
Parathion esterase	100.0	39.5	80.2

[a] CDNB = 1-chloro-2,4-dinitrobenzene.

ing for detoxifying enzymes could be identified and transferred using available techniques in molecular biology. However, the wide-spectrum detoxifying ability that appears to be present in the symbionts from leaf-cutting ants and the cigarette beetles suggests that stimulating growth rates may be more desirable.

Thus, insect fungal symbionts represent a relatively untapped source for novel detoxifying, or other bioconversion enzymes. Their novel origin suggests that many enzymes with interesting and useful properties may abound but must be elucidated.

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