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Novel and co-evolved associations between insects and microorganisms as drivers of forest pestilence

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Abstract Some of the most devastating diseases of trees involve associations between forest insects and microorganisms. Although a small number of native insect-microorganism symbioses can cause tree mortality, the majority of associations with tree health implications involve one or more exotic organisms. Here, we divide damaging symbioses between forest insects and microorganisms into four categories based on the native/exotic status of the species involved: (1) insect and microorganism are native; (2) insect is native, microorganism is exotic; (3) insect is exotic, microorganism is native; and (4) insect and microorganism are both exotic. For each category, we describe several well-researched examples of forest insect symbioses and discuss some of the consequences of the types of interactions within each category. We then discuss priorities for research on forest insect symbioses that could help to further elucidate patterns in the complexity of such interactions in the context of invasion biology. We argue that a nuanced understanding of insect-pathogen relationships is lacking, even for the few well-studied examples. Because novel associations between insects, microorganisms, and trees are increasing with globalization, such symbioses and their potential to negatively impact forest ecosystems demand focused research in the future.

Keywords Insects · Microorganisms · Pathogens · Symbiosis · Tree disease

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Introduction

Associations between forest insects and symbiotic "microorganisms" (a term which we herein use to refer both to microbes and to metazoans such as mites and nematodes) have increasingly been recognized as major drivers of biotic damage to trees in forests throughout the world (Hulcr and Dunn 2011; Six et al. 2011; Wingfield et al. 2010). Most symbioses between forest insects and microorganisms (herein referred to as forest insect symbioses) are benign and rarely cause tree death in their native ranges. However, over the past century insects and microorganisms have been moved, together and separately, all over the world with increasing frequency (Aukema et al. 2010; Liebhold et al. 1995, 2012). The global movement of insects and microorganisms has resulted in numerous new associations between insects, microorganisms and tree hosts (Wingfield et al. 2010). Although these novel associations are likely incredibly common and the majority may be transitory and/or inconsequential from the perspective of forest health, some of these novel associations have resulted in virulent diseases that far exceed the amount of damage that would have been caused by either the insect or microorganism in the absence of the other (Ploetz et al. 2013; Wingfield et al. 2010). As the number of new forest insect symbioses causing tree health problems is expected to increase in the future, a better understanding of the driving factors behind these associations is a high priority.

The damage caused by forest insect symbioses is frequently unexpected and unpredictable (Ploetz et al. 2013), making management of these associations very difficult. While the importance of microorganisms to tree decline and mortality (Ayres and Lombardero 2000), digestion of recalcitrant materials (Engel and Moran 2013), and the exhaustion or detoxification of tree defences (Lieutier et al. 2009; Mason et al. 2014) has long been recognized, the degree to which forest insect symbioses drive forest pestilence remains to be fully explored. Thorough studies have been conducted for only a handful of symbioses, specifically those with dramatic economic and/or forest health consequences such as the southern pine beetle (and more recently the mountain pine beetle), Dutch elm disease, the Sirex woodwasp, and pine wilt caused by the pinewood nematode. Generalizing across these disparate interactions affecting forest ecosystem health is tremendously difficult, especially in the context of exotic invasion where origins and/or introduction histories are often unknown and where transient dynamics dominate. It is therefore difficult to develop frameworks to better predict, research and manage damaging forest insect symbioses.

In this review we explore the importance of native origin for both the insect and microorganismal associates involved in key forest insect symbioses as they influence patterns and processes of infestation, disease, levels of pathogenicity and spread. We broadly divide these associations into four categories as summarized in Fig. 1, namely associations where: (1) both the insect and microorganism are native; (2) the insect is native and has acquired exotic microorganisms; (3) the insect is exotic and has acquired native microorganisms; and (4) both the insect and microorganism of interest are exotic. We then investigate a number of known examples that demonstrate the general importance of such novel and/or coevolved symbionts on forest pestilence in a globalized world. We conclude by describing future research that should be conducted on forest insect symbioses in the context of invasion biology.

Insects and microorganisms are native

Despite the predominant focus in the scientific literature on emerging pests and diseases, including those characterized by important symbioses, the vast majority of forest insect symbioses are associations between native organisms. Depending on the tightness and frequency of the interactions, some degree of coevolution may have occurred between the partners (Janzen 1980). The majority of these associations are benign from the perspective of tree hosts. The low virulence is hypothesized to be a result of coevolution between the trees, insects and symbionts (Ploetz et al. 2013), as well as population suppression of nuisance insects and/or pathogens by coevolved natural enemies (Keane and Crawley 2002). Some mortality may be observed if trees are stressed, or if the trees undergoing mortality are non-native, such as trees planted in a plantation (Wingfield et al. 2010). Several of these insects and symbionts occur in small population densities in their native ranges, and many are not even known to science until they have formed an epidemic in an invaded range.

In a small number of native forest insect symbioses, the insect associate can undergo cyclical population eruptions whereby the insect is able to mass-aggregate to colonize and kill healthy trees. For example, some species of *Dendroctonus* bark beetles (e.g., *D. frontalis*—the southern pine beetle, *D. ponderosae*—the mountain pine beetle) are capable of forming periodic tree-killing epidemics in their native ranges under specific environmental and demographic circumstances (Raffa et al. 2008). These species are dependent on fungal mutualists, which are only mildly pathogenic to the trees, but are still critically important to the tree-killing behaviour of the insect by virtue of concentrating nutrients upon which the developing

		Insects	
		native	exotic
Microorganisms	native	• Insects, microorganisms and host trees are often coevolved • Forest insect symbioses can be obligate, facultative or casual; usually benign, except for outbreak-forming insect species • Numerous examples; many more that are currently unknown to science	• Exotic insects usually vector congeners of their native microorganism associates • Result in altered interactions between hosts and microorganisms • Increased access to hosts for microorganisms • Examples include the symbiosis that causes beech bark disease
	exotic	• Microorganisms usually vectored by congeners of their native insect vectors • Result in disruption of host tree resistance and increased access to hosts for insects • Potential for altered host range for insects • Examples include the Dutch elm disease and pinewood nematode symbioses	• Insects and microorganisms are frequently coevolved, but trees are naïve • Potential for high aggressiveness on hosts by insects or microorganisms • Numerous examples, with varied characteristics • Examples include the Sirex woodwasp and laurel wilt symbioses

Fig. 1 Categories of forest insect symbioses based on the native/exotic status of the organisms involved

beetle larvae depend (Ayres et al. 2000; Six 2012). As such, these mutualistic fungi reduce intra-specific competition among beetles and facilitate population growth (Ayres et al. 2000).

Native insect, exotic microorganism

Many of the most famous damaging forest insect symbioses involve exotic symbionts acquired by native insect species. The classic example is that of the fungal pathogens *Ophiostoma ulmi* and *O. novo-ulmi* that cause Dutch elm disease (DED; Gibbs 1978; Santini and Faccoli 2014; Fig. 2a). *Ophiostoma ulmi*, which is believed to have originated in Asia (Brasier 1990), was introduced to Europe in the early twentieth century where it was acquired by native elm bark beetles in the genus *Scolytus* (Coleoptera: Curculionidae). This association later spread to other parts of the globe, largely because of accidental introductions of the smaller European elm bark beetle, *Scolytus multistriatus*, which is one of the most important vectors of *O. ulmi* (Santini and Faccoli 2014). *Ophiostoma novo-ulmi* largely replaced *O. ulmi* around the world decades after the outbreaks caused by *O. ulmi*. After *O. novo-ulmi* was introduced into North America, it was acquired by the North American elm bark beetle, *Hylurgopinus rufipes*, which has contributed to the spread of the pathogen across the continent (Westwood 1991). The bark beetle species were minor pests before coming into association with the fungal pathogens, as the beetles primarily colonized stressed or damaged elm trees (*Ulmus* spp.). However, in environments where the beetles acquired one of the pathogens, the beetles became part of a devastating symbiosis that has killed millions of elms globally, and transformed numerous forests and urban/suburban landscapes.

Another famous example is that of the pinewood nematode *Bursaphelenchus xylophilus* (Fig. 2b). This nematode species is native to North America where it is vectored by various longhorned beetles (Coleoptera: Cerambycidae), especially in the genus *Monochamus* (Akbulut and Stamps 2012). While these insects have been known in North America and elsewhere in the Northern Hemisphere for a long period of time, their relationship with *B. xylophilus* was not recognized until relatively recently. This knowledge emerged from the discovery that the nematode is the causal agent of pine wilt in Japan where it has devastated forests of native pine trees (*Pinus* spp.). The nematode had established a relationship with the native *Monochamus alternatus* (Mamiya 1983; Mamiya and Enda 1972). It was only after this unique discovery in Japan

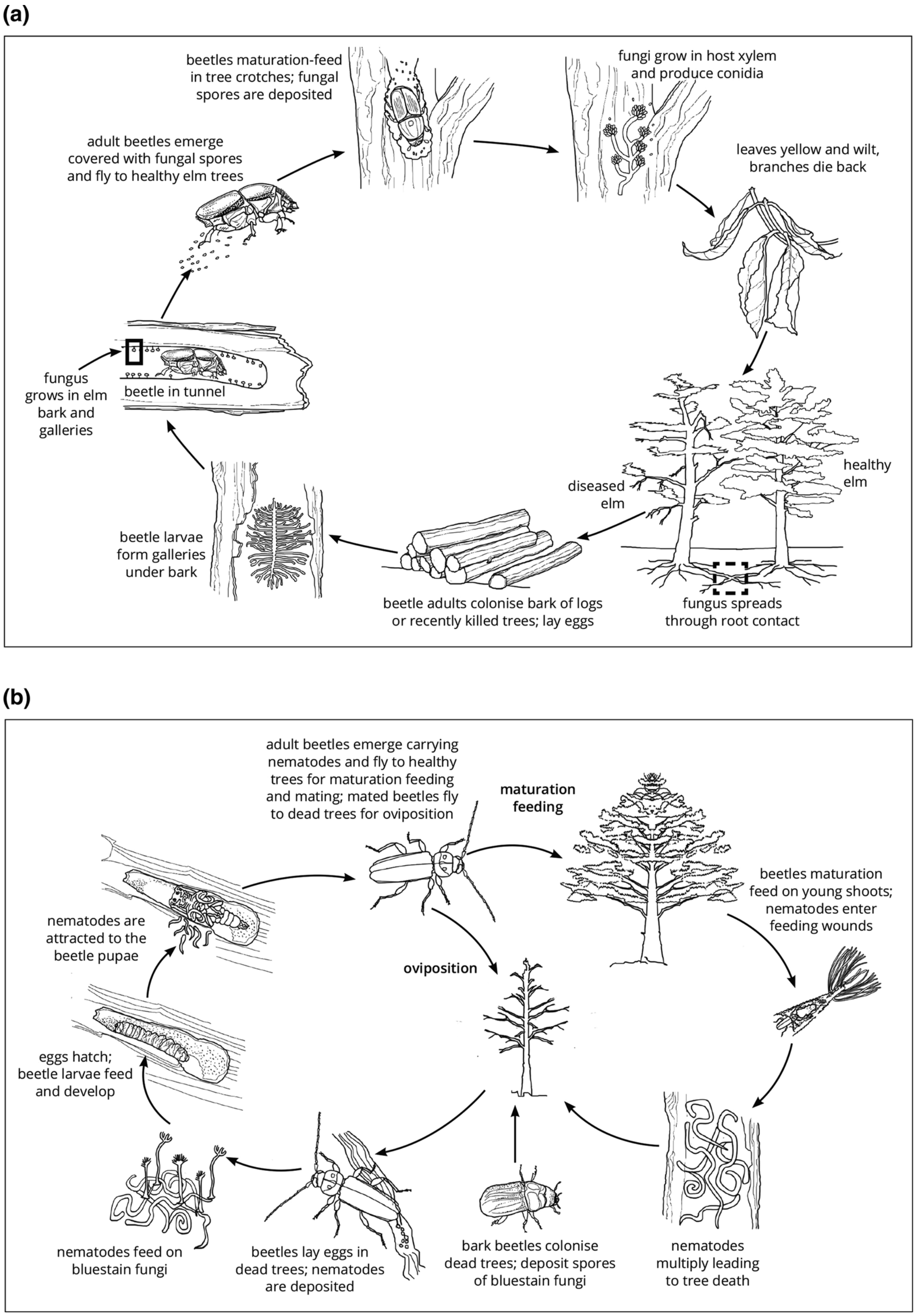

Fig. 2 Life cycles of three damaging forest insects which have well-studied associations with microorganisms. **a** The life cycle of *Scolytus multistriatus* associated with *Ophiostoma ulmi*/*Ophiostoma novo-ulmi*, the causative agents of Dutch elm disease (modified from D'Arcy 2000), **b** the life cycle of *Monochamus alternatus* associated with *Bursaphelenchus xylophilus*, the causative agent of pine wilt (modified from Wingfield 1987b), **c** the life cycle of *Sirex noctilio* associated with *Amylostereum areolatum* (modified from Ryan and Hurley 2012)

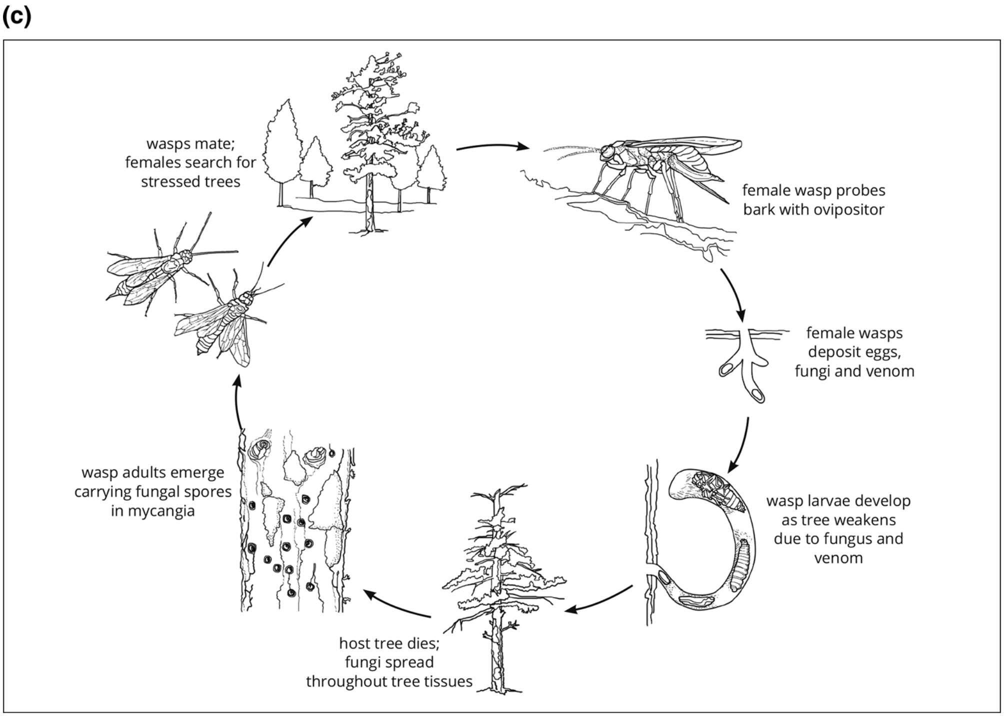

Fig. 2 continued

that the relationship between native North American *Monochamus* spp. and *B. xylophilus*, which is clearly also native to North America, was recognized (Dropkin et al. 1981; Wingfield et al. 1982; Wingfield 1983). After invading Japan the nematode has also been introduced into China, Korea and Portugal and in each case has become associated with a native species of *Monochamus* (Akbulut and Stamps 2012).

In both the DED and pinewood nematode examples, native beetles appear to have benefited from the associations with the exotic microorganisms as the beetles have gained access to a larger number of potential host trees weakened by the microorganisms (Mamiya 1983; Santini and Faccoli 2014). In turn, the beetles serve as a crucial vector shuttling the pathogens to tree hosts during maturation feeding. Maturation feeding is an essential component of both disease cycles as this process allows the pathogens to gain access to healthy trees which they subsequently kill. Without maturation feeding, it is likely that such symbioses would only affect stressed or dying hosts (preferred by beetles as oviposition sites) and would be far less damaging to forest environments.

In both examples the high degree of conservatism present in the novel interactions between native insects and introduced microorganisms is apparent. The new associations maintain fidelity at the generic, or at least the family level, and in both cases, the general ecological niche is preserved. The pinewood nematode, *B. xylophilus*, is vectored by native cerambycid beetles in Asia (*M. alternatus*; Mamiya and Enda 1972) and Portugal (*Monochamus galloprovincialis*; Sousa et al. 2001) that are closely related to the native vectors of the nematode in the United States (*Monochamus scutellatus* and *Monochamus carolinensis*; Akbulut and Stamps 2012). Likewise, the fungi that cause DED in North America were acquired by *H. rufipes* which, despite being distantly related to *Scolytus* spp. (Jordal and Cognato 2012; Jordal and Kambestad 2014), is ecologically comparable to the European elm bark beetles. In these examples, the requirement appears to be that the native potential vector insects would have comparable biological characteristics (e.g., morphology, behaviour, life cycle or host species) as those associated with the pathogens in their native ranges. Similarly, the fungi that cause DED are believed to have supplanted *Ophiostoma quercus*, a non-pathogenic fungus that is closely related to *O. ulmi* and *O. novo-ulmi*, and was commonly associated with elm bark beetles in Europe before the introduction of *O. ulmi* (Brasier 1990; Santini and Faccoli 2014). In addition, *Monochamus* spp. in Asia and Europe were most likely associated with nematodes that were closely related to *B. xylophilus* before the arrival of the pathogenic nematode (Kulinich and Orlinskii 1998). This suggests that the pathogens were able to form associations with native insects in the invaded environments largely because the insects were previously associated with close relatives of the pathogens. Overall, pre-adaptation to particular forms of association appears to be an important element of emerging/damaging symbioses, though the details and potential constraints on ecological novelty with respect to novel interactions is not known.

Exotic insect, native microorganism

There are comparatively few well-studied examples of damaging associations between exotic insects and native symbionts. The most iconic example is that of beech bark disease in North America. This disease is a decline disease complex arising from the interaction between the invasive scale insect *Cryptococcus fagisuga* (Hemiptera: Eriococcidae) and one of several species of fungi from the ascomycete genus *Neonectria*. The scale insect was introduced from Europe into Nova Scotia, Canada, in the 1890's, and has spread to its current distribution across much of eastern North America (Garnas et al. 2011a). Scale insects colonize the smooth bark of American beech (*Fagus grandifolia*), during which the insects facilitate infection by one of at least two species of *Neonectria*: *N. faginata* and *N. ditissima* (formerly *Neonectria galligena*). These fungi appear to be native. Infections can lead to the formation of cankers causing the tree to appear gnarled and twisted, or, in some cases, the infections can rapidly kill large amounts of vascular cambium without triggering the cankering response. The symbiosis causing beech bark disease has resulted in increased mortality of beech trees in North America (Garnas et al. 2011a; Houston 1994), as well as a shift toward smaller denser stands (Garnas et al. 2011a, b) with dramatic effects on biodiversity and ecosystem function (Cale et al. 2013).

Neonectria faginata is the most commonly isolated fungus in the core range of beech bark disease in North America, and is known only from beech trees (Castlebury et al. 2006). *Neonectria ditissima* is also clearly a native fungus and can be found causing disease, particularly along the leading front of the disease (Kasson and Livingston 2009). Unlike *N. faginata*, *N. ditissima* frequently occurs outside the relationship with insects, causing disease symptoms on at least eight genera of native and naturalized trees (Plante et al. 2002). Both species of fungi have benefited from the invasive insect as the pathogens were provided with millions of new hosts, and a rapid mode of transmission between these hosts. The benefits of the association (if any) to *C. fagisuga* are less clear, although it has been hypothesized that infection-induced cankering creates colonization sites and refugia for the insects (e.g., from stemflow, winter cold, and/or predation) on what is otherwise smooth bark (Houston 1994). However, despite this apparent mutualism, *C. fagisuga* and *Neonectria* do not exhibit coupled population dynamics inter-annually (Garnas et al. 2011b), nor is there evidence of cross-correlation in abundance across space (Garnas et al. 2013).

As with the examples given in the previous category, the associations observed in the beech bark disease example are not random. In Europe, *C. fagisuga* is associated with *Neonectria coccinea* (Castlebury et al. 2006), a fungal species that is closely related to *N. faginata* and *N. ditissima*. In fact, beech bark disease was long attributed to *N. coccinea* var. *faginata* assumed to have been introduced from Europe (Mahoney et al. 1999). In this example, the ability of *C. fagisuga* to vector *N. faginata* and *N. ditissima* appears to be largely due to the prior ability of the insect to vector phylogenetically similar fungal species.

Exotic insect and exotic microorganism

Many of the examples of insect-symbiont associations where both species are exotic involve associations between forest insects and nutritional mutualists. The relationships between various species of woodwasp (Hymenoptera: Siricidae) and basidiomycete fungi provide fitting examples. In this case, the Sirex woodwasp *Sirex noctilio* and its fungal symbiont *Amylostereum areolatum* have been most thoroughly studied (Fig. 2c; Slippers et al. 2003). *Sirex noctilio* is native to Europe, Asia, and North Africa, and has invaded pine forests throughout the world with devastating effects in many of the invaded environments (Hurley et al. 2007; Slippers et al. 2015). This woodwasp is fully dependent on the fungus for the development of its larvae (Talbot 1977) with the fungus contributing enzymes and functioning primarily as an "external rumen" (Thompson et al. 2014). However, although the fungus is clearly able to infect living tree tissues and may contribute to the tree-killing process, it is not capable of inducing mortality in the absence of its insect vector (Bordeaux and Dean 2012).

Another example of a nutritional mutualism involves the mountain pine beetle, *D. ponderosae*. This bark beetle has formed an unprecedented epidemic, most likely due to climate change and forest management practices, and has killed millions of pine trees in western North America (Aukema et al. 2006). The beetle has expanded its range and invaded forests dominated by naïve pines to the north and east of its native range (Janes et al. 2014). The beetle depends on nutrients concentrated by its fungal symbionts, *Grosmannia clavigera*, *Ophiostoma montium*, and *Leptographium longiclavatum* (Bentz and Six 2006; Lee et al. 2006). The fungi were introduced by the insect to the invasive range of the beetle (Roe et al. 2011), and are considered to be mild pathogens of infected pine trees (Rice and Langor 2008). As with the *S. noctilio*-*A. areolatum* association, the fungi are not capable of killing healthy trees in the absence of their insect vector, except at unrealistically high densities in experimental work (Yamaoka et al. 1995). However, as with *S. noctilio*, the co-invading fungi are essential to the invasiveness of *D. ponderosae* as the fungi are obligate mutualists of the beetles.

A recent example of a co-invading insect-symbiont association is that of laurel wilt that is devastating red bay trees (*Persea bobonia*) and threatening other Lauraceae in the southeastern United States (Harrington et al. 2008). The disease is caused by the fungus *Raffaelea lauricola*, which is vectored by the wood-boring ambrosia beetle *Xyleborus glabratus* (Harrington et al. 2008). Both species are native to Asia, where the association does not cause disease on native trees (Haack and Rabaglia 2013; Harrington et al. 2008, 2011). Similarly to *S. noctilio* and *D. ponderosae*, *X. glabratus* depends on its associated fungus for nutrition. However, unlike the other associations, the symbiont of *X. glabratus* is the primary tree-killer in this symbiosis (Harrington et al. 2008). Since the forest insect symbiosis was introduced to North America, the fungus has made lateral shifts onto native ambrosia beetles that are closely related to *X. glabratus*, potentially expanding the range of the pathogenic fungus (Carillo et al. 2014). Several other invasive ambrosia beetle-fungus symbioses are also causing serious damage throughout the world, although in most cases even less is known about these associations than with the symbiosis that causes laurel wilt (Hulcr and Dunn 2011; Ploetz et al. 2013).

There are also examples of co-invading forest insects and symbionts where the symbionts do not appear to be nutritional mutualists of the insect vectors. In the case of the red turpentine beetle (*Dendroctonus valens*) invasion in China (Sun et al. 2013; Yan et al. 2005) it has been suggested that the associated fungus *Leptographium procerum* could be an important driver of the insect invasion (Lu et al. 2010, 2011). *Dendroctonus valens* invaded China from North America in the 1980s. Although the beetle does not form tree-killing epidemics in North America, it has killed millions of native pine trees in China since its introduction. Lu et al. (2010, 2011) provided evidence that the dominant pine tree species in the area affected by *D. valens* in China produces greater amounts of 3-carene, a monoterpene that is very attractive to *D. valens*, when infected with certain strains of *L. procerum*. The authors hypothesized that the interactions between *D. valens*, *L. procerum*, and the naïve tree hosts in China resulted in a population feedback loop for *D. valens*, allowing the beetle to form a tree-killing epidemic. However, there is emerging evidence that *L. procerum* may in fact not have co-invaded with *D. valens* from North America, and may actually represent a novel association in China (S. J. Taerum, unpublished data). If the fungus was introduced from elsewhere in the world, this association would in fact be an example of a novel symbioses between an exotic insect and an exotic microorganism that were independently introduced.

The examples given above are all situations where the symbiont co-invaded (or is suspected to have co-invaded) with its native vector. In such examples, the importance of the symbiont to the insect vector depends on whether the symbiont is an obligate mutualist (as it is in the examples of the Sirex woodwasp, the mountain pine beetle, and the laurel wilt disease), or a facultative mutualist or commensalist (as it is in the case of the red turpentine beetle). Insects that depend on an obligate nutritional mutualist often evolve structures that ensure the transmission of the symbiont (e.g., mycangia; Six 2012; Slippers et al. 2003). Because of these structures, and the fact that the survival and reproduction of the insect depends on these fungi, the insect and symbiont are unlikely to become decoupled during or after an invasion event, unless a suitable replacement for the nutritional mutualist is present in the invaded environment (e.g., exchanges of *Amylostereum* spp. for *Sirex noctilio* invading North America; Wooding et al. 2013). In the case of facultative mutualists, the presence of the symbiont can enhance the fitness of the vector by, for example, providing the insect access to more host trees. However, species involved in facultative symbioses are more likely to lose their partners than species involved in obligate mutualisms, and as a result, successful co-invasions by the symbiont would depend on stochastic processes.

Interestingly, there appear to be few clear examples of damaging novel associations between exotic forest insects and exotic microorganisms. This is in stark contrast with co-invading forest insect symbioses, of which there are numerous examples. However, as insects and microorganisms are moved around the globe, it is likely that many new associations between independently introduced insects and microorganisms will form. This category of associations should be closely monitored as such novel forest insect symbioses could have negative implications for forest health in many unpredictable ways.

Synthesis and priorities for future research

Microorganisms that are symbiotic with insects are clearly important drivers of tree mortality in forest ecosystems. However, the "driver" effect clearly varies among different symbioses and depends on the biology of the organisms involved. The native/exotic status of the insect and the symbiotic microorganism may also influence the proximate and ultimate outcomes of novel associations, though more research is required to adequately test this hypothesis. Several natural experiments add credence to the importance of forest insect symbiosis to ecosystem health. For example, *S. multistriatus* was introduced to Australia without *O. ulmi* or *O. novo-ulmi* (Parbery and Rumba 1991). This is in contrast to the situation in New Zealand, where both *S. multistriatus* and *O. novo-ulmi* have been introduced (Brasier 2001; Brockerhoff et al. 2006), leading to dramatic death of elm trees and a long-term serious attempt to eradicate the insect as well as the pathogen. This and other examples clearly demonstrate the importance of the association between the insects and microorganisms to tree health.

In most known cases of invasions of tree-infesting insects where microorganisms are important drivers of pestilence, the microorganisms have been introduced along with their associated insects. There are however numerous examples where novel associations have been established between insects and microorganisms where one of those players is native and the other is exotic. A unifying characteristic of these associations is that of conservatism. In examples where native insects have acquired exotic microorganisms, or where invasive insects have acquired exotic microorganisms, both players were previously associated with organisms that were phylogenetically related and/or ecologically similar to the organisms involved in the novel associations. This suggests a certain degree of predictability with respect to novel associations that could form in the future, because exotic organisms should be more likely to form symbioses with congeners of existing associates (or at least organisms with a similar ecological niche). In turn, native organisms should be more likely to form symbioses with exotic species that are close relatives and/or ecologically similar to species to which the native organisms are naturally associated. These observations also support the hypothesis that coevolution within existing symbioses paves the way for novel interactions. Further research should be conducted on novel associations between forest insects and microorganisms to further test and elucidate these hypotheses.

A major complication in conducting research on damaging symbioses is that it is frequently difficult to identify the insects and (especially) the microorganisms involved. Accurate identifications are essential because misidentifications can result in ineffective management regimes that target the wrong species. Cryptic species are common with many forest insects (Mapondera et al. 2012; Rugman-Jones et al. 2013) and microorganisms that are symbionts of the insects (Alamouti et al. 2011; Blouin 2002). Molecular genetics have proven useful for differentiation between cryptic species. However, selection of appropriate molecular markers is essential because identifications can be complicated by issues such as the presence of NUMTs (nuclear mitochondrial DNA; Jordal and Kambestad 2014) and inherited symbionts (Hurst and Jiggins 2005). In addition, sufficient numbers of markers are needed to adequately differentiate among different cryptic species. Management regimes for forest insect symbioses must involve the appropriate utilization of molecular tools.

With many associations, it is unknown whether the insects or symbionts involved in a damaging symbiosis are native or exotic, or how the organisms came to be associated. Often, species involved in damaging symbioses are not known in their native ranges because they are not problematic there. For example, numerous serious tree diseases have arisen because of novel relationships between species of *Ceratocystis* fungi, nitidulid or wood-boring beetles, and tree hosts (Al Adawi et al. 2013; Tarigan et al. 2011; van Wyk et al. 2007). In most of these cases, all of the associates involved are poorly studied, and it is unclear where either the insects or microorganisms originated. Future research should address these questions with this example as well as other damaging forest insect symbioses.

Another difficulty with elucidating insect-microorganism associations is the inability to separate the roles of each in tree or forest decline. Although the ways in which some microorganisms damage their hosts have been well-studied (e.g., fungi associated with bark beetles; Krokene and Solheim 1998; Lieutier et al. 2009; Schmidt 2006), little is known about the mechanisms by which many other microorganisms damage trees. For example, in the case of the pinewood nematode, the mechanisms leading to tree death remain to be described (Wingfield 1987a). The nematodes enter the trees during maturation feeding of their cerambycid beetle vectors (Dropkin et al. 1981; Mamiya 1983). Where trees are highly susceptible, as is the case with native *Pinus* spp. in Asia (Mamiya 1983), the trees die very rapidly. Because the nematodes are unable to move rapidly through the vascular tissue of the infected trees, mechanisms other than nematode feeding must be involved in tree death. Various hypotheses have been raised including the possible involvement of bacteria associated with the nematodes (Mamiya 1983). However, more research is required to pinpoint the ways in which microorganisms such as *B. xylophilus* contribute to tree death.

An additional complication is that many forest insect symbioses are not limited to only two associates, but are instead complex multipartite associations. The pinewood nematode provides an interesting example because the nematodes are dependent on fungi that are not vectored by the cerambycid vectors of the nematodes, but rather ophiostomatoid fungi that are carried by bark beetles (Mamiya 1983; Wingfield et al. 1984; Wingfield 1987a). The bark beetles colonize the trees that are dying as a result of *B. xylophilus* infestation. These fungi provide nutrition for the nematodes in what has been termed the "mycophagous phase" (Wingfield et al. 1984) of the pinewood nematode life cycle. In the native range of the nematode where native *Pinus* spp. are not susceptible to the pinewood nematode, this mycophagous phase sustains populations in the absence of any tree disease (Wingfield et al. 1982, Wingfield 1983, Wingfield et al. 1984). In addition, in the DED symbiosis, mites associated with elm bark beetles have been demonstrated to carry spores of *O. novo-ulmi*, and have been hypothesized to contribute to the transmission of the pathogen (Moser et al. 2005, 2010). Here, the mites may facilitate the vector shifts by the pathogens. Further research on these and other associations will contribute to the overall understandings of these associations, and may contribute to the management of damaging forest insect symbioses.

It is important to recognize that available data on the complexity of insect-pathogen symbioses and the role that the pathogens play in insect invasions are lacking, and that most data have been collected from a small number of studies. This is unfortunate given that there are many available tools such as those linked to molecular genetics that would substantially improve our understanding of this topic. Where such new invasions are emerging, the identity of the pathogens is commonly based on small collections, little is known regarding the interactions of the pathogens with their various associates, and the population structures of the insects and the pathogen associates have seldom been investigated. For example, the invasion of the Eurasian bark beetle *Tomicus piniperda* in North America occurred together with *Leptographium wingfieldii*, known to be pathogenic in Europe (Haack and Poland 2001; Lieutier et al. 2004). It has been shown that the fungus has established associations with various native bark beetle species in North America (Jacobs et al. 2004) but the consequences of this association, if any exist, have not been considered. There is clearly much work to be done in this field and this should be strongly encouraged.

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