

Evolving symbioses between insects and fungi that kill trees in Canada: new threats associated with invasive organisms

Tod D. Ramsfield¹

Abstract—Symbiotic relationships between insects and fungi are known to cause tree mortality either through direct damage by larval feeding that can be facilitated by symbiotic fungi, or through insects vectoring pathogens directly to healthy trees. Within their native ranges, the impacts of many insect-fungus symbioses are restricted to weakened and declining trees; however, within the last century tree mortality caused by globally invasive insect–fungus associations has had a devastating impact on trees in both urban and natural forest ecosystems. Unfortunately, Canadian forests have been seriously affected by invasive organisms and an emerging threat is the expansion of a native bark beetle into the boreal forest of Alberta. This paper reviews the symbiotic relationships between selected invasive insects and pathogens that cause tree mortality within the urban and forested landscapes of Canada; it uses these case studies to illustrate potentially damaging new evolutionary trajectories.

Introduction

Conifers and hardwood trees are independently attacked by both insects and fungi; however, the focus of this review is tree mortality caused by insects and filamentous fungi that have developed symbiotic relationships to help overwhelm tree defenses and facilitate colonisation by both organisms. Several thorough reviews have been published that outline the mutualistic symbiotic relationships that exist between insects and fungi (*i.e.*, Six and Klepzig 2004; Klepzig *et al.* 2009; Six and Wingfield 2011). The nature of the symbiotic relationship can vary; some insects, such as some bark beetles, all ambrosia beetles and *Sirex* Linnaeus (Hymenoptera: Siricidae) woodwasps require fungi for larval development (Klepzig *et al.* 2009). In other cases, such as Dutch elm disease, pathogens rely upon insects for long distance dispersal to host trees (Brasier 2001). Within the native ranges of host trees, co-evolved symbiotic relationships between insects and fungi have independently arisen multiple times (Six and Klepzig 2004; Klepzig

et al. 2009). Typically damage is restricted to unhealthy trees, where the fungal symbiont has a role in overcoming defensive compounds that benefit the insect by concentrating nutrients that are made available to their larvae (Hulcr and Dunn 2011). Mycangia are structures of adult insects that carry the inoculum of symbiotic fungi for transmission to the tree host during insect attack and oviposition; these structures demonstrate the close evolutionary relationship between insects and fungi (Six and Klepzig 2004). Other mechanisms by which fungi are vectored by insects are within exoskeleton cavities or even on the surface of phoretic mites (Acari) that are associated with bark beetles (Coleoptera: Curculionidae) (Levieux *et al.* 1989; Hofstetter and Moser 2014). Upon inoculation, trees become colonised by these fungi that either provide nutrition to developing larvae, or are pathogenic to the host tree, or both.

Over the past century, through the increased movement of pests that has occurred via international trade, multiple urban and forest tree populations have been devastated by invasive

Received 1 April 2015. Accepted 31 August 2015. First published online 18 January 2016.

T.D. Ramsfield,¹ Natural Resources Canada, Canadian Forest Service, Northern Forestry Centre, 5320-122 Street, Edmonton, Alberta, T6H 3S5, Canada

¹Corresponding author (e-mail: Tod.Ramsfield@Canada.ca).

Langor, D.W. and Alfaro, R.I. (eds.) Forest Entomology in Canada: Celebrating a Century of Science Excellence doi:10.4039/tce.2015.65.

pests, many of which are not problematic within their native range. Some of these invasive pests consist of a symbiotic complex that requires the introduction of both partners for effective establishment. Emerging threats to trees are new symbioses that have developed through the introduction of an insect or pathogen and the opportunistic development of a new symbiotic relationship with a native partner (Wooding *et al.* 2013). This can be considered as a threat because these new relationships may result in the initiation of a new evolutionary trajectory that could potentially provide an adaptive advantage to an insect or pathogen to cause elevated levels of damage or mortality to attacked trees.

In Canada, both established and recently developed symbiotic relationships are present, and due to range expansion, naïve hosts (*i.e.*, tree species that are not recorded to have had contact with the insects or symbionts) are being exposed to long-established symbiotic partners. With climate change and the inadvertent introduction of new species through expansion of global trade, there are new risks posed to trees from insects and pathogens separately, and also through the emergence of new symbiotic partnerships due to host range expansion (Langor *et al.* 2009). In this review, the mountain pine beetle (*Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae)), the invasive brown spruce longhorn beetle (*Tetropium fuscum* (Fabricius) (Coleoptera: Cerambycidae)), *Sirex* woodwasps, and Dutch elm disease are reviewed as examples of emerging symbiotic relationships that are either already causing damage, or are new combinations that may pose threats, to forests or trees within the urban and forested landscapes of Canada. These examples also all illustrate evolutionary mechanisms, such as vectoring of multiple fungal symbionts to facilitate adaptation during range expansion, fungal symbiont exchange between native and exotic vectors, the ability of pathogens to be vectored by closely related bark beetles, and the potential for interspecific hybridisation between native and exotic species. These outcomes may initiate new evolutionary trajectories and although they are less obvious than sudden tree mortality that can occur as a result of the establishment of non-native species, they provide further impetus for the actions of regulatory agencies that are responsible for the exclusion of exotic organisms.

Native insect-fungus symbioses

In Canada, the native mountain pine beetle has caused extensive damage to millions of ha of lodgepole pine (*Pinus contorta* var. *latifolia* Engelmann; Pinaceae) forest within its historical range in British Columbia (Safranyik *et al.* 2010). In approximately 2002 the range of the mountain pine beetle expanded across the Rocky Mountains into northeastern British Columbia, likely via mass airborne dispersal (de la Giroday *et al.* 2012), and by 2006 the beetle had advanced eastward into the lodgepole pine × jack pine (*Pinus banksiana* Lambert (Pinaceae)) hybrid zone of boreal Alberta (Rice and Langor 2009). The insect has since been confirmed to attack pure jack pine outside of the hybrid zone in Alberta, and it is considered a serious threat to jack pine across the boreal forest (Cullingham *et al.* 2011). The government of the Province of Alberta has aggressively responded to the threat posed by the mountain pine beetle through management actions directed at slowing the spread of the insect as outlined in their action plans and management strategy (Alberta Environment and Parks 2007). The government of Saskatchewan has also recognised the potential impact of this pest and since 2011 it has provided funds directly to the Province of Alberta to manage mountain pine beetle in eastern Alberta (Government of Saskatchewan 2015).

As demonstrated *in vitro* by Myrholm and Langor (2016), ophiostomatalean fungi are critical for larval development of *D. ponderosae*, and optimal larval development may be achieved with a specific fungal associate. Although a specific fungal associate may provide optimal larval nutrition and growth, the probability of insect survival is increased through the ability of the insect to vector multiple fungi. Three Ophiostomatales (Fungi) are vectored by *D. ponderosae*: *Grossmannia clavigera* (Robinson-Jeffrey and Davidson) Zipfel, de Beer, and Wingfield; *Ophiostoma montium* (Rumbold) von Arx (Bleiker and Six 2009); and *Leptographium longiclavatum* Lee, Kim, and Breuil (Lee *et al.* 2005). As range expansion occurs, the ability to shift fungal associates to an optimal symbiont mixture is a mechanism that can facilitate survival in new environments on a new host. This was illustrated by Roe *et al.* (2011), who observed that

the relative abundance of *L. longiclavatum* increased with latitude within *D. ponderosae* populations in Alberta and British Columbia, Canada. This is likely due to the lower optimal growth temperature of *L. longiclavatum* relative to other symbionts hosted by *D. ponderosae*, leading to competitive exclusion of the other symbionts (Roe *et al.* 2011). Another hypothesis is that interspecific competition between the fungal associates based on fungal virulence to pine is a mechanism that may explain the differences in the abundance of the fungal associates; *G. clavigera* was found to be more virulent on lodgepole pine and lodgepole pine × jack pine hybrids than *O. montium* but there was no difference between the two fungi on jack pine (Rice *et al.* 2007a). A second inoculation study that included all three ophiostomatalean fungi found comparable virulence between all fungi and that lodgepole pine was less susceptible to the fungi than the hybrids with jack pine and pure jack pine, as assessed by lesion length (Rice *et al.* 2007b).

The eastward range expansion of *D. ponderosae* into the boreal forest of Canada represents a risk to forestry and the socioeconomic benefits associated with the forest industry (Safranyik *et al.* 2010), and the ecosystem services provided by healthy jack pine forests. Range expansion into the boreal forest is a complex process that is mediated by abiotic conditions (climate), host availability, and the symbiotic relationship between *D. ponderosae* and the fungal symbionts that are necessary for larval development. The factors that drive mountain pine beetle outbreaks are well understood within its native range, but with the arrival of the insect and its fungal symbionts in the boreal forest, there is a level of uncertainty that exists making it difficult to predict exactly how devastating the beetle will be in this naïve system. Investigations in Alberta have shown that the beetle arrived in the boreal forest with its fungal symbionts; therefore, jack pine will be exposed to the insect in combination with its fungal symbionts for the first time in recorded history, providing an opportunity for researchers to study evolution in action.

Invasive insects with symbiotic partners: brown spruce longhorn beetle

Brown spruce longhorn beetle, *Tetropium fuscum* (Fabricius) (Coleoptera: Cerambycidae),

an insect native to Europe where it infests declining Norway spruce (*Picea abies* (Linnaeus) Karst. (Pinaceae)), was confirmed to be present on red spruce (*Picea rubens* Sargent) in Point Pleasant Park, Halifax, Nova Scotia, Canada in 1999, in close proximity to a shipping container port. Re-examination of samples collected in 1990 indicated that the beetle has been present there since at least 1990 (Smith and Hurley 2000; Harrison and Smith 2013). When fungi were isolated from trees that were infested by *T. fuscum*, three species of ophiostomatalean fungi were commonly isolated: *Ophiostoma piceae* (Münch) Sydow and Sydow (Jacobs *et al.* 2003), *Pesotum fragrans* Mathiesen-Käärik (Jacobs *et al.* 2003; Jacobs and Seifert 2004a), and *Ophiostoma tetropii* Mathiesen (Jacobs *et al.* 2003; Jacobs and Seifert 2004b), but only *O. tetropii* was strictly associated with *P. rubens* infested by *T. fuscum* (Jacobs *et al.* 2003). Following the identification of *T. fuscum* in 1999, the Canadian Food Inspection Agency initiated an eradication campaign that was in place until 2006; in 2007 the campaign was shifted to a containment programme to slow the spread of the insect (Canadian Food Inspection Agency 2014). The exotic *T. fuscum* is morphologically similar to the native *Tetropium cinnamopterum* Kirby and both insects use the same mating pheromone, allowing trapping surveys to be conducted. In surveys conducted between 2008 and 2010, Rhoads *et al.* (2011) determined that *T. fuscum* was relatively rare compared with *T. cinnamopterum* beyond 80 km from the site of introduction. The beetle is able to disperse via flight and by human-assisted dispersal through the movement of infested spruce products; therefore, the movement of high-risk forest products is restricted by ministerial order (Canadian Food Inspection Agency 2014). Further research on host preference by *T. fuscum* has shown that the beetle prefers stressed trees over healthy trees (Flaherty *et al.* 2011, 2013), thus lowering the risk profile associated with healthy red spruce, and this has had a bearing on the decision of Canadian Food Inspection Agency to shift to a containment strategy (Canadian Food Inspection Agency 2014).

The role of *O. tetropii* in the symbiosis in Canada has not been studied with the same level of detail as *T. fuscum*, although Jacobs *et al.* (2003) suggest that *O. tetropii* is not pathogenic to

P. rubens. Inoculation studies of *P. rubens* seedlings and larger trees within the regulated area, using *O. piceae* and *O. tetropii* indicated that the native *O. piceae* is more aggressive than *O. tetropii* (Harrison and Smith 2013). In Europe, artificial inoculation of juvenile (14 ± 1.4 cm diameter at breast height) Norway spruce, growing under natural conditions, with *O. tetropii* indicated that the fungus is pathogenic to Norway spruce (Sallé *et al.* 2005).

There is some uncertainty with respect to the historical presence of *O. tetropii* in Canada. The fungus was reported as present in the Muskoka region of Ontario in 1968 (Griffin 1968), although this could not be confirmed since the herbarium material was overgrown by an *Aspergillus* Micheli (Trichocomaceae) species (Jacobs and Seifert 2004b). In their initial study, Jacobs *et al.* (2003) tentatively suggested that *O. tetropii* was introduced into Nova Scotia with *T. fuscum* and in 2004 they confirmed the presence of *O. tetropii* in New York State, United States of America, associated with the spruce beetle, *Dendroctonus rufipennis* (Kirby) (Coleoptera: Curculionidae) (Jacobs and Seifert 2004b). It is possible that a population of *O. tetropii* was introduced into Nova Scotia with the introduction of *T. fuscum* from Europe and that another population of *O. tetropii* was also already present in North America and associated with *D. rufipennis*. A population genetics study of *O. tetropii* in North America would shed light on the relationship between the potentially introduced and native populations of this fungus and potential gene flow between the introduced and native populations. In their 2003 study, Jacobs *et al.* (2003) did not find *O. tetropii* in trees that were infested by the native *T. cinnamopterum*. It would be interesting to re-examine the fungal associates of the introduced *T. fuscum* and the native species of *Tetropium* Kirby in Canada, including *T. cinnamopterum*, throughout the range of *T. cinnamopterum* to assess the plasticity of the fungal symbionts and to answer the question “Can *O. tetropii* be vectored by *T. cinnamopterum*, and which ophiostomatalean fungi are now vectored by *T. fuscum* in Canada?”.

Tetropium fuscum has not dispersed with the same speed or impact as other invasive insects, such as the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). Within the city of Halifax, the initial eradication campaign

severely impacted local stands of *P. rubens*; however, on a national scale the impact of *T. fuscum* and *O. tetropii* have not been as great as that of other invasive insects or fungi. The baseline studies that have been conducted on this system provide a solid foundation for future studies within this system that may generate knowledge that can be applied to other symbiotic invasive insects and fungi. This insect-fungus system should therefore be retained as a research focus and potential model system. Understanding invasion biology and the characteristics of these systems provides opportunities to develop strategies that can be put into place to prevent the establishment of other exotic species in the future.

Invasive insects with symbiotic partners: *Sirex* woodwasp

The European woodwasp, *Sirex noctilio* (Fabricius) (Hymenoptera: Siricidae), which attacks species of *Pinus*, was first discovered in southeastern Ontario in 2005 during a trapping survey that was conducted in response to the discovery of the woodwasp in New York State, United States of America, in 2004 (de Groot *et al.* 2006). In Europe, where *S. noctilio* is native, it does not attack healthy trees (Ayres *et al.* 2014); however, in exotic locations *S. noctilio* is capable of attacking vigorous trees following establishment (Francke-Grosman 1963). Thus, *S. noctilio* is a species with a demonstrated ability to be invasive as it was established in New Zealand, Australia, South Africa, Uruguay, Argentina, Brazil, and most recently in North America (Ayres *et al.* 2014).

The fungus most often associated with *S. noctilio* is the basidiomycete *Amylostereum areolatum* (Chaillet ex Fries) Boidin (Amylostereaceae), which is carried by females in internal mycangia near the base of the ovipositor. This ensures inoculation of the fungus into the wood during the oviposition process, which creates a small wound in the bark (Slippers *et al.* 2003; Bergeron *et al.* 2011). The fungus plays an important role in the lifecycle of the woodwasp through decomposition of wood, which provides a nutritional resource and habitat to the larval stage (Gilmour 1965; Boissin *et al.* 2012). The discovery of *A. areolatum* in Canada presented an opportunity to use population genetic tools to investigate the potential origin of

the invasive *S. noctilio*-*A. areolatum* complex (Bergeron *et al.* 2011), which can inform decision makers about potential invasion pathways. In their assessment of the genetic diversity of *A. areolatum*, Bergeron *et al.* (2011) discovered that the genetic variation in Canada is very low, with only two multilocus genotypes present here. One of these genotypes was shared with the southern hemisphere, while the other genotype, which composed 74% of the population, was unique to North America and of unknown origin.

Several species of *Sirex* are present in North America, including *S. edwardsii* Brullé (Bergeron *et al.* 2011) and *S. nigricornis* Fabricius (Wooding *et al.* 2013), which are associated with the basidiomycete *Amylostereum chailletii* (Persoon) Boidin (Bergeron *et al.* 2011). The natural history of the North American native species has not been studied as thoroughly as that of *S. noctilio*, although they are considered to be similar in many respects (Nielsen *et al.* 2009), including their preference for weakened trees (Stillwell 1966). The establishment of an exotic insect and its fungal symbiont within the range of closely related native species has facilitated interactions between native and exotic species and their respective symbiotic partners.

The nature of the associations between *S. noctilio* and *A. areolatum* and the native *S. nigricornis* and *A. chailletii* has recently been studied, and it was discovered that symbiont switching can occur (Wooding *et al.* 2013) with *A. areolatum* sometimes in association with *S. nigricornis* and *A. chailletii* with *S. noctilio*. In a separate study, Nielsen *et al.* (2009) found *A. areolatum* and *A. chailletii* within the mycangia of different *Sirex* species emerging from the same section of a tree. Symbiont sharing has important implications for the natural history of invasive organisms. Through switching symbiotic partners, an adaptive advantage may occur, resulting in increased virulence and subsequent damage to infected trees (Wooding *et al.* 2013). Another potential consequence of closely related fungal species growing within close proximity is the development of interspecific hybrids that can result in the generation of new species with altered virulence (Brasier 2001). The obvious threat posed by the establishment of the *S. noctilio*-*A. areolatum* complex in Canada is that it will behave as it has in other regions of the world; the more insidious threat is that symbiont

switching or genetic recombination will create new genotypes that could facilitate greater impacts than anticipated.

Fungi that cause tree disease

Another type of symbiotic relationship occurs when insects serve as the long distance dispersal vectors for pathogenic fungi. This is particularly important because some pathogens, such as the Dutch elm disease pathogens, *Ophiostoma ulmi* (Buisman) Nannfeldt (Ophiostomataceae), and the more aggressive *Ophiostoma novo-ulmi* Brasier, that cause a lethal vascular wilt disease of elm, can be vectored by multiple species of bark beetles (Coleoptera: Curculionidae: Scolytinae).

Dutch elm disease was first discovered in Canada in the province of Québec in 1944. The importation of elm nursery material and lumber from Europe was prohibited in 1928 and from the United States of America in 1934, and it is hypothesised that the pathogen was introduced into Sorel, Québec, as early as 1940, possibly on infected elm wood crate material that was used during the importation of machinery from Europe at the outbreak of World War II (Pomerleau 1961). The pathogen has also likely expanded its range into Canada from the United States of America through spread into southern Ontario from New York, Ohio, or Michigan, into New Brunswick from the Houlton area of Maine, and into Manitoba from the Red River area of Minnesota (Campana and Stipes 1981). In Canada, the native North American elm bark beetle, *Hylurgopinus rufipes* Eichhoff (Coleoptera: Curculionidae), can vector the disease, as can the introduced European elm bark beetle, *Scolytus multistriatus* Marsham (Coleoptera: Curculionidae) (Campana and Stipes 1981; Allen and Humble 2002). The fact that a native insect vectors this exotic pathogen was likely an important factor in the establishment and spread of Dutch elm disease in Canada (Allen and Humble 2002; Humble and Allen 2006). An emerging risk for this disease is the potential new vector, *Scolytus schevyrewi* Semenov (Coleoptera: Curculionidae), which was introduced from Asia and is established in Colorado, United States of America (Loo 2009). It was first detected in southern Alberta in 2006 (Langor *et al.* 2009), and it is now considered to be established in the prairie provinces (Veilleux 2012).

The impact of Dutch elm disease in Canada has been extensive as all elm trees (*Ulmus* Linnaeus; Ulmaceae) in North America are susceptible to the disease (Campana and Stipes 1981). The American elm, *Ulmus americana* Linnaeus, which is widely planted in urban environments, has been particularly affected by the disease, and major Canadian cities such as Toronto, Ontario have lost a significant percentage of their elms (Hubbes 1999). Management of Dutch elm disease has been a priority for cities such as Winnipeg, Manitoba, which lost more than 21 000 trees between 2009 and 2013 (Hintz *et al.* 2013); the province of Saskatchewan has invoked regulations to prevent spread of the disease (Anonymous 2005); however, the disease is present in Saskatchewan and it threatens Alberta (Hubbes 1999).

Although it is very unfortunate that Dutch elm disease was introduced into Canada, there have been two distinct benefits associated with its introduction: First, as a high priority pathogen, several important research programmes were launched in Canada to investigate topics such as population genetics of invasive pathogens (*i.e.*, Hintz *et al.* 1993; Temple *et al.* 2006), pathogen gene expression and infection processes (*i.e.*, Temple *et al.* 1997; Et-Touil *et al.* 2005), and alternative pathogen management strategies (*i.e.*, Hubbes 1999; Temple *et al.* 2006; Hintz *et al.* 2013). Second, public awareness of the impacts of invasive alien species has been raised through the outreach activities of non-governmental agencies such as the Society to Prevent Dutch Elm Disease in Alberta, which promotes healthy urban forests. These organisations have served to raise the profile of invasive alien species in the urban environment through programmes to slow the spread of Dutch elm disease. Also, the networks and resources that these programmes have developed could be used to raise the awareness of other devastating invasive species, such as the emerald ash borer, which is currently present only in Ontario and Québec. If we can learn from this invasive organism, using techniques such as retrospective analysis, as conducted in the United Kingdom where researchers studied the Dutch elm disease outbreak of the 1970s to inform contemporary biosecurity policy (Potter *et al.* 2011), and if we use existing outreach networks to prevent the spread of other invasive pests, perhaps other species of trees in Canada will have a better chance of survival.

Another instance of the introduction of Dutch elm disease occurred in New Zealand where exotic *Ulmus procera* Salisbury were discovered to be infected by *O. novo-ulmi* in 1990. Inspection of the diseased material revealed the presence of the insect vector *Scolytus multistriatus*. Surveys in Auckland discovered *S. multistriatus* without *O. novo-ulmi* outside of the area affected by *O. novo-ulmi*, indicating that the bark beetle had been introduced before the pathogen and that two separate introductions occurred (Gadgil *et al.* 2000). The ability of *O. novo-ulmi* to be vectored by multiple bark beetles, the fact that it likely spread widely in Canada on a bark beetle that is native to North America, and its ability to re-associate with its European vector after independent establishment of the vector and the pathogen in New Zealand, illustrate the risks associated with novel vector-pathogen relationships. These facts clearly indicate that there are multiple mechanisms that can facilitate the establishment and long distance dispersal of exotic pathogens, which must be considered for effective regulatory policy.

International considerations

Canadian forests are not unique in experiencing damage from invasive organisms. Within many countries, forests and the ecosystem services that they provide have been negatively impacted by non-native organisms that have become established and spread following their arrival and the body of literature related to invasive organisms and their impacts is extensive (*e.g.*, Liebhold *et al.* 1995; Boyd *et al.* 2013). Globalisation and increased trade have facilitated the international movement of insects and pathogens. Even 63 years after the formation of the International Plant Protection Convention in 1952 and the development of a set of International Standards for Phytosanitary Measures, the number of incursions of exotic organisms continues to rise (Evans 2010). National quarantine policies and systems have been enacted by many countries to prevent the introduction of exotic insects and pathogens, yet the volume of trade and the impracticality of 100% inspection of imported goods has permitted the introduction of exotic organisms. The trade in live plants (Liebhold *et al.* 2012) and solid wood packaging material (Humble 2010) have been recognised as two important invasion pathways for

exotic insects and pathogens. The management of pathways, rather than individual high-risk organisms, leading to the strategy of “manage once, remove many”, can theoretically manage a larger number of species, including unknown and potentially damaging species, in an effective manner (Evans 2010). The economic costs of eradication, if feasible, or ongoing management to mitigate losses from invasive insects or pathogens, are high and often borne by taxpayers (Hantula *et al.* 2014). The evolutionary events triggered by the establishment of invasive organisms, such as new host associations and hybridisation events (Wingfield *et al.* 2010) and illustrated by the examples in this paper, demonstrate the adaptive capacity of exotic pests to respond to new environments. The economic costs and biological consequences of the establishment of exotic pests are high and mitigation of these impacts requires commitment from regulatory agencies that is supported by a solid foundation based in science and effective international cooperation.

Conclusion

These examples of both native and invasive insects and their symbionts in Canada illustrate the potential damage that can occur when an organism becomes established in a new region. They also demonstrate the flexibility and adaptive capacity that is inherent within symbiotic relationships that have allowed the survival of insects, such as the mountain pine beetle or the brown spruce longhorn beetle, in new environments. The example of symbiont exchange following invasion, as illustrated by *Sirex* woodwasps, clearly demonstrates how human actions can have potentially long-term evolutionary implications. The Dutch elm disease example illustrates the fact that exotic forest pathogens can be vectored by another species of insect that share the niche with its traditional symbiotic partner.

In the context of Dutch elm disease control, Campana and Stipes (1981) stated that control of the disease is “At best limited, always expensive, always difficult, often frustrating, at times hopeless and never well understood”. This is because of the complex interactions that are involved between the tree host, the environment, the disease and the insect (Campana and Stipes 1981). Indeed, this sentiment can be extended to all

invasive insect and pathogen combinations that have developed a new symbiotic relationship in a new environment where, as stated by Brasier (2008) in the context of invasive plant pathogens, “Each imported pathogen is therefore an uncontrolled, potentially dangerous, open-ended experiment in evolution”.

Another direct consequence of the introduction of plant pathogenic fungi is the potential for interspecific hybridisation, which may result in hybrids with increased virulence (Brasier 2001). This type of hybridisation can occur when two closely related species are brought together through anthropogenic actions (*e.g.*, inadvertent long distance dispersal facilitated by international trade) that place these species geographically close enough together. The introduction of exotic insects may also lead to interspecific hybridisation; as determined by Silk *et al.* (2007) and used by Rhainds *et al.* (2011), the exotic *T. fuscum* and the native *T. cinnamopterum* share a common sex/aggregation pheromone. This leads to the question “What is the potential for intra-specific hybridisation between these species that respond to the same pheromone?”.

Range expansion by native and exotic organisms has resulted in the death of a large number of trees in Canada over an evolutionarily short time period. The long-term consequences of these human-mediated introductions, and the new associations and evolutionary trajectories that have been initiated through symbiont exchange and new vector relationships are even more of a concern. The evolutionary consequences facilitated by these processes are complex and the impacts on tree hosts are difficult to predict, but continued study of these systems has the potential to generate significant knowledge as we witness evolutionary processes unfolding before us. Efforts to prevent the introduction of additional exotic organisms into Canada through international trade are expensive in human terms, but these expenses need to be balanced by the impacts on ecosystem services and societal well-being that are afforded by healthy trees.

Acknowledgements

Funding for this manuscript was provided by the Canadian Forest Service. This manuscript was

strengthened through reviews provided by David Langor, René Alfaro, and two anonymous reviewers.

References

- Alberta Environment and Parks. 2007. Alberta's strategy [online]. Available from <http://aep.alberta.ca/landsforests/mountain-pine-beetle/albertas-strategy/default.aspx> [accessed 16 July 2015].
- Allen, E.A. and Humble, L.M. 2002. Nonindigenous species introductions: a threat to Canada's forests and forest economy. *Canadian Journal of Plant Pathology*, **24**: 103–110.
- Anonymous. 2005. The Dutch elm disease regulations. Chapter F-19.1 Reg 5. The Forest Resources Management Act, Government of Saskatchewan [online]. Available from <https://www.saskatoon.ca/sites/default/files/documents/community-services/parks/The%20Dutch%20Elm%20Disease%20Regulations%202005.pdf> [accessed 7 February 2015].
- Ayres, M.P., Pena, R., Lombardo, J.A., and Lombardero, M.J. 2014. Host use patterns by the European woodwasp, *Sirex noctilio*, in its native and invaded range. *Public Library of Science One*, **9**: e90321. doi:10.1371/journal.pone.0090321.
- Bergeron, M., Leal, I., Foord, B., Ross, G., Davis, C., Slippers, B., *et al.* 2011. Putative origin of clonal lineages of *Amylostereum areolatum*, the fungal symbiont associated with *Sirex noctilio*, retrieved from *Pinus sylvestris*, in eastern Canada. *Fungal Biology*, **115**: 750–758.
- Bleiker, K.P. and Six, D.L. 2009. Competition and coexistence in a multi-partner mutualism: interaction between two fungal symbionts of the mountain pine beetle in beetle-attacked trees. *Microbial Ecology*, **57**: 191–202.
- Boissin, E., Hurley, B., Wingfield, M.J., Vasaitis, R., Stenlid, J., Davis, C., *et al.* 2012. Retracing the routes of introduction of invasive species: the case of the *Sirex noctilio* woodwasp. *Molecular Ecology*, **21**: 5728–5744. doi:10.1111/mec.12065.
- Boyd, I.L., Freer-Smith, P.H., Gilligan, C.A., and Godfray, H.C.J. 2013. The consequence of tree pests and diseases for ecosystem services. *Science*, **342**: 1235773-1–1235773-8. doi:10.1126/science.1235773.
- Brasier, C.M. 2001. Rapid evolution of introduced plant pathogens via interspecific hybridization. *BioScience*, **51**: 123–133.
- Brasier, C.M. 2008. The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology*, **57**: 792–808.
- Campana, R.J. and Stipes, R.J. 1981. Dutch elm disease in North America with particular reference to Canada: success or failure of conventional control methods. *Canadian Journal of Plant Pathology*, **3**: 252–259.
- Canadian Food Inspection Agency. 2014. Pest risk management decision document – brown spruce longhorn beetle (*Tetropium fuscum*) [online]. Available from <http://www.inspection.gc.ca/plants/plant-protection/directives/risk-management/brown-spruce-longhorn-beetle-tetropium-fuscum-eng/1410452600838> [accessed 31 January 2015].
- Cullingham, C.I., Cooke, J.E., Dang, S., Davis, C.S., Cooke, B.J., and Coltmán, D.W. 2011. Mountain pine beetle host-range expansion threatens the boreal forest. *Molecular Ecology*, **20**: 2157–2171.
- de Groot, P., Nystrom, K., and Scarr, T. 2006. Discovery of *Sirex noctilio* (Hymenoptera: Siricidae) in Ontario, Canada. *The Great Lakes Entomologist*, **39**: 49–53.
- de la Giroday, H.C., Carroll, A.L., and Aukema, B.H. 2012. Breach of the northern Rocky Mountain geoclimatic barrier: initiation of range expansion by the mountain pine beetle. *Journal of Biogeography*, **39**: 1112–1123.
- Et-Touil, A., Rioux, D., Mathieu, F.M., and Bernier, L. 2005. External symptoms and histopathological changes following inoculation of elms putatively resistant to Dutch elm disease with genetically close strains of *Ophiostoma*. *Canadian Journal of Botany*, **83**: 656–667.
- Evans, H.F. 2010. Pest risk analysis – organisms or pathways? *New Zealand Journal of Forestry Science* (Supplement), **40**: S35–S44.
- Flaherty, L., Quiring, D., Pureswaran, D., and Sweeny, J. 2013. Preference of an exotic wood borer for stressed trees is more attributable to pre-alighting than post-alighting behaviour. *Ecological Entomology*, **38**: 546–552.
- Flaherty, L., Sweeny, J.D., Pureswaran, D., and Quiring, D.T. 2011. Influence of host tree condition on the performance of *Tetropium fuscum* (Coleoptera: Cerambycidae). *Environmental Entomology*, **40**: 1200–1209.
- Francke-Grosmann, H. 1963. Some new aspects in forest entomology. *Annual Review of Entomology*, **8**: 415–438.
- Gadgil, P.D., Bulman, L.S., Dick, M.A., Bain, J., and Dunn, C.P. 2000. Dutch elm disease in New Zealand. *In* The elms: breeding, conservation and disease management. *Edited by* C.P. Dunn. Kluwer Academic Publishers, Boston, Massachusetts, United States of America. Pp. 189–199.
- Gilmour, J.W. 1965. The life cycle of the fungal symbiont of *Sirex noctilio*. *New Zealand Journal of Forestry*, **10**: 80–89.
- Government of Saskatchewan. 2015. Saskatchewan and Alberta renew agreement to combat mountain pine beetle [online]. Available from <http://www.saskatchewan.ca/government/news-and-media/2015/january/15/mountain-pine-beetle> [accessed 16 July 2015].
- Griffin, H.D. 1968. The genus *Ceratocystis* in Ontario. *Canadian Journal of Botany*, **46**: 689–718.

- Hantula, J., Müller, M.M., and Uusivuori, J. 2014. International plant trade associated risks: laissez-faire or novel solutions. *Environmental Science & Policy*, **37**: 158–160. doi:10.1016/j.envsci.2013.09.011.
- Harrison, K.J. and Smith, G.A. 2013. The discovery of *Ophiostoma tetropii* with the brown spruce longhorn beetle (*Tetropium fuscum*) in Halifax, Canada. In *The ophiostomatoid fungi: expanding frontiers*. CBS Biodiversity Series 12. Edited by K.A. Seifert, Z.W. De Beer, and M.J. Wingfield. Fungal Biodiversity Centre, Centraalbureau voor Schimmelcultures, Royal Netherlands Academy of Arts and Sciences, Utrecht, The Netherlands. Pp. 213–217.
- Hintz, W.E., Carneiro, J.S., Kassatenko, I., Varga, A., and James, D. 2013. Two novel mitoviruses from a Canadian isolate of the Dutch elm pathogen *Ophiostoma novo-ulmi* (93-1224). *Virology Journal*, **10**: 252.
- Hintz, W.E., Jeng, R.S., Yang, D.Q., Hubbes, M.M., and Horgen, P.A. 1993. A genetic survey of the pathogenic fungus *Ophiostoma ulmi* across a Dutch elm disease front in western Canada. *Genome*, **36**: 418–426.
- Hofstetter, R.W. and Moser, J.C. 2014. The role of mites in insect-fungus associations. *Annual Review of Entomology*, **59**: 537–557.
- Hubbes, M. 1999. The American elm and Dutch elm disease. *Forestry Chronicle*, **75**: 265–273.
- Hulcr, J. and Dunn, R.R. 2011. The sudden emergence of pathogenicity in insect-fungus symbioses threatens naïve forest ecosystems. *Proceedings of the Royal Society B*, **278**: 2866–2873. doi:10.1098/rspb.2011.1130.
- Humble, L.M. 2010. Pest risk analysis and invasion pathways – insects and wood packing revisited: what have we learned? *New Zealand Journal of Forestry Science (Supplement)*, **40**: S57–S72.
- Humble, L.M. and Allen, E.A. 2006. Forest biosecurity: alien invasive species and vectored organisms. *Canadian Journal of Plant Pathology*, **28**: S256–S269.
- Jacobs, K. and Seifert, K.A. 2004a. Fungi Canadenses no. 347. *Pesotum fragrans*. *Canadian Journal of Plant Pathology*, **26**: 79–80.
- Jacobs, K. and Seifert, K.A. 2004b. Fungi Canadenses no. 346. *Ophiostoma tetropii*. *Canadian Journal of Plant Pathology*, **26**: 76–78.
- Jacobs, K., Seifert, K.A., Harrison, K.J., and Hiristits, T. 2003. Identity and phylogenetic relationships of ophiostomatoid fungi associated with invasive and native *Tetropium* species (Coleoptera: Cerambycidae) in Atlantic Canada. *Canadian Journal of Botany*, **81**: 316–329.
- Klepzig, K.D., Adams, A.S., Handelsman, J., and Raffa, K.F. 2009. Symbioses: a key driver of insect physiological processes, ecological interactions, evolutionary diversification, and impacts on humans. *Economic Entomology*, **38**: 67–77.
- Langor, D.W., DeHaas, L.J., and Footitt, R.G. 2009. Diversity of non-native terrestrial arthropods on woody plants in Canada. *Biological Invasions*, **11**: 5–19.
- Lee, S., Kim, J.J., and Breuil, C. 2005. *Leptographium longiclavatum* sp. nov., a new species associated with the mountain pine beetle, *Dendroctonus ponderosae*. *Mycological Research*, **109**: 116–170.
- Levieux, J., Lieutier, F., Moser, J.C., and Perry, T.J. 1989. Transportation of phytopathogenic fungi by the bark beetle *Ips sexdentatus* Boerner and associated mites. *Journal of Applied Entomology*, **108**: 1–11.
- Liebholt, A.M., Brockerhoff, E.G., Garrett, L.J., Parke, J.L., and Britton, K.O. 2012. Live plant imports: the major pathway for forest insect and pathogen invasions of the US. *Frontiers in Ecology and the Environment*, **10**: 135–143. doi:10.1890/110198.
- Liebholt, A.M., MacDonald, W.L., Bergdahl, D., and Mastro, V.C. 1995. Invasion by exotic forest pests: a threat to forest ecosystems. *Forest Science Monograph*, **30**: 1–49.
- Loo, J.A. 2009. Ecological impacts of non-indigenous invasive fungi as forest pathogens. *Biological Invasions*, **11**: 81–96.
- Myrholm, C.L. and Langor, D.W. 2016. Assessment of the impact of symbiont Ophiostomatales (Fungi) on mountain pine beetle (Coleoptera: Curculionidae) performance on a jack pine (Pinaceae) diet using a novel *in vitro* rearing method. *The Canadian Entomologist*, **148**: 68–82.
- Nielsen, C., Williams, D.W., and Hajek, A.E. 2009. Putative source of the invasive *Sirex noctilio* fungal symbiont, *Amylostereum areolatum*, in the eastern United States and its association with native siricid woodwasps. *Mycological Research*, **113**: 1242–1253.
- Pomerleau, R. 1961. History of the Dutch elm disease in the province of Quebec, Canada. *Forestry Chronicle*, **37**: 356–367.
- Potter, C., Harwood, T., Knight, J., and Tomlinson, I. 2011. Learning from history, predicting the future: the UK Dutch elm disease outbreak in relation to contemporary tree disease threats. *Philosophical Transactions of the Royal Society B*, **366**: 1966–1974.
- Rhainds, M., MacKinnon, W.E., Porter, K.B., Sweeney, J.D., and Silk, P.J. 2011. Evidence for limited spatial spread in an exotic longhorn beetle, *Tetropium fuscum* (Coleoptera: Cerambycidae). *Forest Entomology*, **104**: 1928–1933.
- Rice, A.V. and Langor, D.W. 2009. Mountain pine beetle-associated blue-stain fungi in lodgepole × jack pine hybrids near Grande Prairie, Alberta (Canada). *Forest Pathology*, **39**: 323–334.
- Rice, A.V., Thormann, M.N., and Langor, D.W. 2007a. Mountain pine beetle associated blue-stain fungi cause lesions on jack pine, lodgepole pine, and lodgepole × jack pine hybrids in Alberta. *Canadian Journal of Botany*, **85**: 307–315.

- Rice, A.V., Thormann, M.N., and Langor, D.W. 2007b. Virulence of, and interactions among, mountain pine beetle associated blue-stain fungi on two pine species and their hybrids in Alberta. *Canadian Journal of Botany*, **85**: 316–323.
- Roe, A.D., James, P.M.A., Rice, A.V., Cooke, J.E.K., and Sperling, F.A.H. 2011. Spatial community structure of mountain pine beetle fungal symbionts across a latitudinal gradient. *Microbial Ecology*, **62**: 347–360.
- Safranyik, L., Carroll, A.L., Régnière, J., Langor, D.W., Riel, W.G., Shore, T.L., *et al.* 2010. Potential for range expansion of mountain pine beetle into the boreal forest of North America. *The Canadian Entomologist*, **142**: 415–442.
- Sallé, A., Monclus, R., Yart, A., Garcia, J., Romary, P., and Lieutier, F. 2005. Fungal flora associated with *Ips typographus*: frequency, virulence, and ability to stimulate the host defense reaction in relation to insect population levels. *Canadian Journal of Forest Research*, **35**: 365–373.
- Silk, P.J., Sweeney, J., Wu, J., Price, J., Gutowski, J.M., and Kettela, E.G. 2007. Evidence for a male-produced pheromone in *Tetropium fuscum* (F.) and *Tetropium cinnamopterum* (Kirby) (Coleoptera: Cerambycidae). *Naturwissenschaften*, **94**: 697–701.
- Six, D.L. and Klepzig, K.D. 2004. *Dendroctonus* bark beetles as model systems for studies on symbiosis. *Symbiosis*, **37**: 207–232.
- Six, D.L. and Wingfield, M.J. 2011. The role of phytopathogenicity in bark beetle-fungus symbioses: a challenge to the classic paradigm. *Annual Review of Entomology*, **56**: 255–272.
- Slippers, B., Coutinho, T.A., Wingfield, B.D., and Wingfield, M.J. 2003. A review of the genus *Amylostereum* and its association with woodwasps. *South African Journal of Science*, **99**: 70–74.
- Smith, G. and Hurley, J.E. 2000. First North American record of the Palearctic species *Tetropium fuscum* (Fabricius) (Coleoptera: Cerambycidae). *The Coleopterists Bulletin*, **54**: 540.
- Stillwell, M.A. 1966. Woodwasps (Siricidae) in conifers and the associated fungus, *Stereum chailletii*, in eastern Canada. *Forest Science*, **12**: 121–128.
- Temple, B., Horgen, P.A., Bernier, L., and Hintz, W.E. 1997. Cerato-ulmin, a hydrophobin secreted by the causal agents of Dutch elm disease, is a parasitic fitness factor. *Fungal Genetics and Biology*, **22**: 39–53.
- Temple, B., Pines, P.A., and Hintz, W.E. 2006. A nine-year genetic survey of the causal agent of Dutch elm disease, *Ophiostoma novo-ulmi* in Winnipeg, Canada. *Mycological Research*, **110**: 594–600.
- Veilleux, J. 2012. Establishment of *Scolytus schevyrewi* Semenov (Coleoptera: Curculionidae: Scolytinae) in the prairies: life cycle, hosts and impact. M.Sc. thesis. University of Manitoba, Winnipeg, Manitoba, Canada. Available from <http://hdl.handle.net/1993/8859> [accessed 26 February 2015].
- Wingfield, M.J., Slippers, B., and Wingfield, B.D. 2010. Novel associations between pathogens, insects and tree species threaten world forests. *New Zealand Journal of Forestry Science (Supplement)*, **40**: S95–S103.
- Wooding, A.L., Wingfield, M.J., Hurley, B.P., Garnas, J.R., de Groot, P., and Slippers, B. 2013. Lack of fidelity revealed in an insect–fungal mutualism after invasion. *Biology Letters*, **9**: 20130342. Available from <http://dx.doi.org/10.1098/rsbl.2013.0342> [accessed 23 October 2014].