

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

Tod D. Ramsfield

Natural Resources Canada, Canadian Forest Service, Northern Forestry Centre, 5320-122 Street, Edmonton, AB, T6H 3S5, Canada

E-mail: Tod.Ramsfield@NRCan-RNCan.gc.ca

Telephone: 780-435-7394

Fax: 780-435-7359

1 Abstract

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

15

16 Introduction

17 Conifers and hardwood trees are independently attacked by both insects and
18 fungi; however, the focus of this review is tree mortality caused by insects and
19 filamentous fungi that have developed symbiotic relationships to help overwhelm tree
20 defenses and facilitate colonization by both organisms. Several thorough reviews
21 have been published that outline the mutualistic symbiotic relationships that exist
22 between insects and fungi (i.e., Six and Klepzig 2004; Klepzig *et al.* 2009; Six and

23 Wingfield 2011). The nature of the symbiotic relationship can vary; some insects,
24 such as some bark beetles, all ambrosia beetles and *Sirex* woodwasps require fungi
25 for larval development (Klepzig *et al.* 2009). In other cases, such as Dutch elm
26 disease, pathogens rely upon insects for long distance dispersal to host trees (Brasier
27 2001). Within the native ranges of host trees, co-evolved symbiotic relationships
28 between insects and fungi have independently arisen multiple times (Six and Klepzig
29 2004; Klepzig *et al.* 2009). Typically damage is restricted to unhealthy trees, where
30 the fungal symbiont has a role in overcoming defensive compounds that benefit the
31 insect by concentrating nutrients that are made available to their larvae (Hulcr and
32 Dunn 2011). Mycangia are structures of adult insects that carry the inoculum of
33 symbiotic fungi for transmission to the tree host during insect attack and oviposition;
34 these structures demonstrate the close evolutionary relationship between insects and
35 fungi (Six and Klepzig 2004). Other mechanisms by which fungi are vectored by
36 insects are within exoskeleton cavities or even on the surface of phoretic mites that
37 are associated with bark beetles (Levieux *et al.* 1989; Hofstetter and Moser 2014).
38 Upon inoculation, trees become colonized by these fungi that either provide nutrition
39 to developing larvae, or are pathogenic to the host tree, or both.

40 Over the past century, through the increased movement of pests that has
41 occurred via international trade, multiple urban and forest tree populations have been
42 devastated by invasive pests, many of which are not problematic within their native
43 range. Some of these invasive pests consist of a symbiotic complex that requires the
44 introduction of both partners for effective establishment. Emerging threats to trees

45 are new symbioses that have developed through the introduction of an insect or
46 pathogen and the opportunistic development of a new symbiotic relationship with a
47 native partner (Wooding *et al.* 2013). This can be considered as a threat because
48 these new relationships may result in the initiation of a new evolutionary trajectory
49 that could potentially provide an adaptive advantage to an insect or pathogen to cause
50 elevated levels of damage or mortality to attacked trees.

51 In Canada, both established and recently developed symbiotic relationships
52 are present, and due to range expansion, naïve hosts (i.e., tree species that are not
53 recorded to have had contact with the insects or symbionts) are being exposed to
54 long-established symbiotic partners. With climate change and the inadvertent
55 introduction of new species through expansion of global trade, there are new risks
56 posed to trees from insects and pathogens separately, and also through the emergence
57 of new symbiotic partnerships due to host range expansion (Langor *et al.* 2009). In
58 this review, the mountain pine beetle (*Dendroctonus ponderosae* Hopkins
59 (Coleoptera: Curculionidae)), the invasive brown spruce longhorn beetle (*Tetropium*
60 *fuscum* (Fabricius) (Coleoptera: Cerambycidae)), *Sirex* woodwasps, and Dutch elm
61 disease are reviewed as examples of emerging symbiotic relationships that are either
62 already causing damage, or are new combinations that may pose threats, to forests or
63 trees within the urban and forested landscapes of Canada. These examples also all
64 illustrate evolutionary mechanisms, such as vectoring of multiple fungal symbionts to
65 facilitate adaptation during range expansion, fungal symbiont exchange between
66 native and exotic vectors, the ability of pathogens to be vectored by closely related

67 bark beetles, and the potential for interspecific hybridization between native and
68 exotic species. These outcomes may initiate new evolutionary trajectories and
69 although they are less obvious than sudden tree mortality that can occur as a result of
70 the establishment of non-native species, they provide further impetus for the actions
71 of regulatory agencies that are responsible for the exclusion of exotic organisms.

72

73 Native insect-fungus symbioses

74 In Canada, the native mountain pine beetle has caused extensive damage to
75 millions of hectares of lodgepole pine (*Pinus contorta* Douglas var. *latifolia*
76 Engelmann (Pinaceae)) forest within its historical range in British Columbia
77 (Safranyik *et al.* 2010). In approximately 2002 the range of the mountain pine beetle
78 expanded across the Rocky Mountains into north-eastern British Columbia, likely via
79 mass airborne dispersal (de la Giroday *et al.* 2012), and by 2006 the beetle had
80 advanced eastward into the lodgepole pine × jack pine (*Pinus banksiana* Lamb.
81 (Pinaceae)) hybrid zone of boreal Alberta (Rice and Langor 2009). The insect has
82 since been confirmed to attack pure jack pine outside of the hybrid zone in Alberta,
83 and it is considered a serious threat to jack pine across the boreal forest (Cullingham
84 *et al.* 2011). The government of the Province of Alberta has aggressively responded
85 to the threat posed by the mountain pine beetle through management actions directed
86 at slowing the spread of the insect as outlined in their action plans and management
87 strategy (Alberta Environment and Parks 2007). The Government of Saskatchewan
88 has also recognized the potential impact of this pest and since 2011 it has provided

89 funds directly to the Province of Alberta to manage mountain pine beetle in eastern
90 Alberta (Government of Saskatchewan 2015).

91 As demonstrated *in-vitro* by Myrholm and Langor (2015), ophiostomatalean
92 fungi are critical for larval development of *D. ponderosae*, and optimal larval
93 development may be achieved with a specific fungal associate. Although a specific
94 fungal associate may provide optimal larval nutrition and growth, the probability of
95 insect survival is increased through the ability of the insect to vector multiple fungi.
96 Three ophiostomatalean fungi are vectored by *D. ponderosae*: *Grosmannia clavigera*
97 (Robinson-Jeffry and Davidson) Zipfel, de Beer and Wingfield, *Ophiostoma montium*
98 (Rumbold) von Arx (Bleiker and Six 2009), and *Leptographium longiclavatum* Lee,
99 Kim and Breuil (Lee *et al.* 2005). As range expansion occurs, the ability to shift
100 fungal associates to an optimal symbiont mixture is a mechanism that can facilitate
101 survival in new environments on a new host. This was illustrated by Roe *et al.*
102 (2011), who observed that the relative abundance of *L. longiclavatum* increased with
103 latitude within *D. ponderosae* populations in Alberta and British Columbia. This is
104 likely due to the lower optimal growth temperature of *L. longiclavatum* relative to
105 other symbionts hosted by *D. ponderosae*, leading to competitive exclusion of the
106 other symbionts (Roe *et al.* 2011). Another hypothesis is that interspecific
107 competition between the fungal associates based on fungal virulence to pine is a
108 mechanism that may explain the differences in the abundance of the fungal
109 associates; *G. clavigera* was found to be more virulent on lodgepole pine and
110 lodgepole pine × jack pine hybrids than *O. montium* but there was no difference

111 between the two fungi on jack pine (Rice *et al.* 2007a). A second inoculation study
112 that included all three ophiostomatalean fungi found comparable virulence between
113 all fungi and that lodgepole pine was less susceptible to the fungi than the hybrids
114 with jack pine and pure jack pine, as assessed by lesion length (Rice *et al.* 2007b).

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

129

130 Invasive insects with symbiotic partners

131 A. Brown spruce longhorn beetle

132 Brown spruce longhorn beetle, *Tetropium fuscum* (Fabricius) (Coleoptera:
133 Cerambycidae), an insect native to Europe where it infests declining Norway spruce
134 (*Picea abies* (L.) Karst. (Pinaceae)), was confirmed to be present on red spruce
135 (*Picea rubens* Sarg.) in Point Pleasant Park, Halifax, Nova Scotia in 1999, in close
136 proximity to a shipping container port. Re-examination of samples collected in 1990
137 indicated that the beetle has been present there since at least 1990 (Smith and Hurley
138 2000; Harrison and Smith 2013). When fungi were isolated from trees that were
139 infested by *T. fuscum*, three species of ophiostomatalean fungi were commonly
140 isolated: *Ophiostoma piceae* (Münch) Syd. & P. Sydow (Jacobs *et al.* 2003),
141 *Pesotum fragrans* Mathiesen-Käärik (Jacobs *et al.* 2003; Jacobs and Seifert 2004a),
142 and *Ophiostoma tetropii* Mathiesen (Jacobs *et al.* 2003; Jacobs and Seifert 2004b),
143 but only *O. tetropii* was strictly associated with *P. rubens* infested by *T. fuscum*
144 (Jacobs *et al.* 2003). Following the identification of *T. fuscum* in 1999, the Canadian
145 Food Inspection agency (CFIA) initiated an eradication campaign that was in place
146 until 2006; in 2007 the campaign was shifted to a containment programme to slow the
147 spread of the insect (CFIA 2014). The exotic *T. fuscum* is morphologically similar to
148 the native *Tetropium cinnamopterum* Kirby and both insects utilise the same mating
149 pheromone, allowing trapping surveys to be conducted. In surveys conducted
150 between 2008 and 2010, Rhains *et al.* (2011) determined that *T. fuscum* was
151 relatively rare compared with *T. cinnamopterum* beyond 80 km from the site of
152 introduction. The beetle is able to disperse via flight and by human-assisted dispersal
153 through the movement of infested spruce products; therefore, the movement of high

154 risk forest products is restricted by Ministerial Order (CFIA 2014). Further research
155 on host preference by *T. fuscum* has shown that the beetle prefers stressed trees over
156 healthy trees (Flaherty *et al.* 2011, 2013), thus lowering the risk profile associated
157 with healthy red spruce, and this has had a bearing on the decision of CFIA to shift to
158 a containment strategy (CFIA 2014).

159 The role of *O. tetropii* in the symbiosis in Canada has not been studied with
160 the same level of detail as *T. fuscum*, although Jacobs *et al.* (2003) suggest that *O.*
161 *tetropii* is not pathogenic to *P. rubens*. Inoculation studies of *P. rubens* seedlings and
162 larger trees within the regulated area, using *O. piceae* and *O. tetropii* indicated that
163 the native *O. piceae* is more aggressive than *O. tetropii* (Harrison and Smith 2013).
164 In Europe, artificial inoculation of juvenile (14±1.4 cm DBH) Norway spruce,
165 growing under natural conditions, with *O. tetropii* indicated that the fungus can be
166 pathogenic to Norway spruce (Sallé *et al.* 2005).

167 There is some uncertainty with respect to the historical presence of *O. tetropii*
168 in Canada. The fungus was reported as present in the Muskoka region of Ontario in
169 1968 (Griffin 1968), although this could not be confirmed since the herbarium
170 material was overgrown by an *Aspergillus* sp. (Jacobs and Seifert 2004b). In their
171 initial study, Jacobs *et al.* (2003) tentatively suggested that *O. tetropii* was introduced
172 into Nova Scotia with *T. fuscum* and in 2004 they confirmed the presence of *O.*
173 *tetropii* in New York State associated with the spruce beetle, *Dendroctonus*
174 *rufipennis* (Kby.) (Coleoptera: Curculionidae) (Jacobs and Seifert 2004b). It is
175 possible that a population of *O. tetropii* was introduced into Nova Scotia with the

176 introduction of *T. fuscum* from Europe and that another population of *O. tetropii* was
177 also already present in North America and associated with *D. rufipennis*. A
178 population genetics study of *O. tetropii* in North America would shed light on the
179 relationship between the potentially introduced and native populations of this fungus
180 and potential gene flow between the introduced and native populations. In their 2003
181 study, Jacobs *et al.* (2003) did not find *O. tetropii* in trees that were infested by the
182 native *T. cinnamopterum*. It would be interesting to re-examine the fungal associates
183 of the introduced *T. fuscum* and the native species of *Tetropium* in Canada, including
184 *T. cinnamopterum*, throughout the range of *T. cinnamopterum* to assess the plasticity
185 of the fungal symbionts and to answer the question “Can *O. tetropii* be vectored by *T.*
186 *cinnamopterum*, and which ophiostomatalean fungi are now vectored by *T. fuscum* in
187 Canada?”.

188 This insect has not dispersed with the same speed or impact as have other
189 invasive insects, such as the emerald ash borer, *Agrilus planipennis* Fairmaire
190 (Coleoptera: Buprestidae). Within the city of Halifax, the initial eradication
191 campaign severely impacted local stands of *P. rubens*; however, on a national scale
192 the impact of *T. fuscum* and *O. tetropii* have not been as great as that of other
193 invasive insects or fungi. The baseline studies that have been conducted on this
194 system provide a solid foundation for future studies within this system that may
195 generate knowledge that can be applied to other symbiotic invasive insects and fungi.
196 This insect-fungus system should therefore be retained as a research focus and
197 potential model system. Understanding invasion biology and the characteristics of

198 these systems provides opportunities to develop strategies that can be put into place
199 to prevent the establishment of other exotic species in the future.

200

201 B. *Sirex* woodwasp

202 The European woodwasp, *Sirex noctilio* (Fabricius) (Hymenoptera:
203 Siricidae), which attacks species of *Pinus*, was first discovered in southeastern
204 Ontario in 2005 during a trapping survey that was conducted in response to the
205 discovery of the woodwasp in New York state in 2004 (de Groot *et al.* 2006). In
206 Europe, where *S. noctilio* is native, it does not attack healthy trees (Ayres *et al.*
207 2014); however, in exotic locations *S. noctilio* is capable of attacking vigorous trees
208 following establishment (Francke-Grosmann 1963). Thus, *S. noctilio* is a species
209 with a demonstrated ability to be invasive as it has become established in New
210 Zealand, Australia, South Africa, Uruguay, Argentina, Brazil, and most recently in
211 North America (Ayres *et al.* 2014).

212 The fungus most often associated with *S. noctilio* is the basidiomycete
213 *Amylostereum areolatum* (Fr.) Boid., which is carried by females in internal
214 mycangia near the base of the ovipositor. This ensures inoculation of the fungus into
215 the wood during the oviposition process, which creates a small wound in the bark
216 (Slippers *et al.* 2003; Bergeron *et al.* 2011). The fungus plays an important role in
217 the lifecycle of the woodwasp through decomposition of wood, which provides a
218 nutritional resource and habitat to the larval stage (Gilmour 1965; Boissin *et al.*
219 2012). The discovery of *A. areolatum* in Canada presented an opportunity to utilise

220 population genetic tools to investigate the potential origin of the invasive *S. noctilio* -
221 *A. areolatum* complex (Bergeron *et al.* 2011), which can inform decision makers
222 about potential invasion pathways. In their assessment of the genetic diversity of *A.*
223 *areolatum*, Bergeron *et al.* (2011) discovered that the genetic variation in Canada is
224 very low, with only two multi-locus genotypes present here. One of these genotypes
225 was shared with the southern hemisphere, while the other genotype, which composed
226 74% of the population, was unique to North America and of unknown origin.

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

236 The nature of the associations between *S. noctilio* and *A. areolatum* and the
237 native *S. nigricornis* and *A. chailletii* has recently been studied, and it was discovered
238 that symbiont switching can occur (Wooding *et al.* 2013) with *A. areolatum*
239 sometimes in association with *S. nigricornis* and *A. chailletii* with *S. noctilio*. In a
240 separate study, Nielsen *et al.* (2009) found *A. areolatum* and *A. chailletii* within the
241 mycangia of different *Sirex* spp. emerging from the same section of a tree. Symbiont

242 sharing has important implications for the biology of invasive organisms. Through
243 switching symbiotic partners, an adaptive advantage may occur, resulting in increased
244 virulence and subsequent damage to infected trees (Wooding *et al.* 2013). Another
245 potential consequence of closely related fungal species growing within close
246 proximity is the development of interspecific hybrids that can result in the generation
247 of new species with altered virulence (Brasier 2001). The obvious threat posed by
248 the establishment of the *S. noctilio-A. areolatum* complex in Canada is that it will
249 behave as it has in other regions of the world; the more insidious threat is that
250 symbiont switching or genetic recombination will create new genotypes that could
251 facilitate greater impacts than anticipated.

252

253 Fungi that cause tree disease

254 Another type of symbiotic relationship is that in which insects serve as the
255 long distance dispersal vectors for pathogenic fungi. This is particularly important
256 because some pathogens, such as the Dutch elm disease pathogens, *Ophiostoma ulmi*
257 (Buisman) Nannf., and the more aggressive *Ophiostoma novo-ulmi* Brasier, that
258 cause a lethal vascular wilt disease of elm, can be vectored by multiple species of
259 scolytine bark beetles.

260 Dutch elm disease was first discovered in Canada in the Province of Quebec
261 in 1944. The importation of elm nursery material and lumber from Europe was
262 prohibited in 1928 and from the United States in 1934, and it is hypothesized that the
263 pathogen was introduced into Sorel, Quebec, as early as 1940, possibly on infected

264 elm wood crate material that was utilised during the importation of machinery from
265 Europe at the outbreak of World War II (Pomerleau 1961). The pathogen has also
266 likely expanded its range into Canada from the United States through spread into
267 southern Ontario from New York, Ohio or Michigan, into New Brunswick from the
268 Houlton area of Maine, and into Manitoba from the Red River area of Minnesota
269 (Campana and Stipes 1981). In Canada, the native North American elm bark beetle,
270 *Hylurgopinus rufipes* Eichhoff (Coleoptera: Scolytidae), can vector the disease, as
271 can the introduced European elm bark beetle, *Scolytus multistriatus* Marsham
272 (Coleoptera: Scolytidae) (Campana and Stipes 1981; Allen and Humble 2002). The
273 fact that this exotic pathogen can be vectored by a native insect was likely an
274 important factor in the establishment and spread of Dutch elm disease in Canada
275 (Allen and Humble 2002; Humble and Allen 2006). An emerging risk for this
276 disease is the potential new vector, *Scolytus schevyrewi* Semenov (Coleoptera:
277 Scolytidae), which was introduced from Asia and is established in Colorado, USA
278 (Loo 2009). It was first detected in southern Alberta in 2006 (Langor *et al.* 2009),
279 and it is now considered to be established in the Prairie Provinces (Veilleux 2012).

280 The impact of Dutch elm disease in Canada has been extensive as all elm trees
281 (*Ulmus* spp.) in North America are susceptible to the disease (Campana and Stipes
282 1981). The American elm, *Ulmus americana* L. (Ulmaceae), which is widely planted
283 in urban environments, has been particularly affected by the disease, and major
284 Canadian cities such as Toronto have lost a significant percentage of their elms
285 (Hubbes 1999). Management of Dutch elm disease has been a priority for cities such

286 as Winnipeg, which lost more than 21,000 trees between 2009 and 2013 (Hintz *et al.*
287 2013); the Province of Saskatchewan has invoked regulations to prevent spread of the
288 disease (Anonymous 2005); however, the disease is present in Saskatchewan and it
289 threatens Alberta (Hubbes 1999).

290 Although it is very unfortunate that Dutch elm disease was introduced into
291 Canada, there have been two distinct benefits associated with its introduction:
292 Firstly, as a high priority pathogen, several important research programmes were
293 launched in Canada to investigate topics such as population genetics of invasive
294 pathogens (i.e. Hintz *et al.* 1993; Temple *et al.* 2006), pathogen gene expression and
295 infection processes (i.e., Temple *et al.* 1997; Et-Touil *et al.* 2005), and alternative
296 pathogen management strategies (i.e. Hubbes 1999; Temple *et al.* 2006; Hintz *et al.*
297 2013). Secondly, public awareness of the impacts of invasive alien species has been
298 raised through the outreach activities of non-governmental agencies such as the
299 Society to Prevent Dutch Elm Disease in Alberta, which promotes healthy urban
300 forests. These organizations have served to raise the profile of invasive alien species
301 in the urban environment through programmes to slow the spread of Dutch elm
302 disease. Also, the networks and resources that these programmes have developed
303 could be utilised to raise the awareness of other devastating invasive species, such as
304 the emerald ash borer, which is currently present only in Ontario and Quebec. If we
305 can learn from this invasive organism, using techniques such as retrospective
306 analysis, as conducted in the United Kingdom where researchers studied the Dutch
307 elm disease outbreak of the 1970s to inform contemporary biosecurity policy (Potter

308 *et al.* 2011), and if we utilise existing outreach networks to prevent the spread of
309 other invasive pests, perhaps other species of trees in Canada will have a better
310 chance of survival.

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

325

326 International Considerations

327 Canadian forests are not unique in experiencing damage from invasive
328 organisms. Within many countries, forests and the ecosystem services that they
329 provide have been negatively impacted by non-native organisms that have become

330 established and spread following their arrival and the body of literature related to
331 invasive organisms and their impacts is extensive (e.g. Liebhold *et al.* 1995; Boyd *et*
332 *al.* 2013). Globalization and increased trade have facilitated the international
333 movement of insects and pathogens. Even 63 years after the formation of the
334 International Plant Protection Convention (IPPC) in 1952 and the development of a
335 set of International Standards for Phytosanitary Measures (ISPM), the number of
336 incursions of exotic organisms continues to rise (Evans 2010). National quarantine
337 policies and systems have been enacted by many countries to prevent the introduction
338 of exotic insects and pathogens, yet the volume of trade and the impracticality of
339 100% inspection of imported goods has permitted the introduction of exotic
340 organisms. The trade in live plants (Liebhold *et al.* 2012) and solid wood packaging
341 material (Humble 2010) have been recognized as two important invasion pathways
342 for exotic insects and pathogens. The management of pathways, rather than
343 individual high risk organisms, leading to the strategy of “manage once, remove
344 many”, can theoretically manage a larger number of species, including unknown and
345 potentially damaging species, in an effective manner (Evans 2010). The economic
346 costs of eradication, if feasible, or ongoing management to mitigate losses from
347 invasive insects or pathogens, are high and often borne by taxpayers (Hantula *et al.*
348 2014). The evolutionary events triggered by the establishment of invasive organisms,
349 such as new host associations and hybridization events (Wingfield *et al.* 2010) and
350 illustrated by the examples in this paper, demonstrate the adaptive capacity of exotic
351 pests to respond to new environments. The economic costs and biological

352 consequences of the establishment of exotic pests are high and mitigation of these
353 impacts requires commitment from regulatory agencies that is supported by a solid
354 foundation based in science and effective international cooperation.

355

356 Conclusion

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

367 In the context of Dutch elm disease control, Campana and Stipes (1981) stated
368 that control of the disease is “At best limited, always expensive, always difficult,
369 often frustrating, at times hopeless and never well understood”. This is because of
370 the complex interactions that are involved between the tree host, the environment, the
371 disease and the insect (Campana and Stipes 1981). Indeed, this sentiment can be
372 extended to all invasive insect and pathogen combinations that have developed a new
373 symbiotic relationship in a new environment where, as stated by Brasier (2008) in the

374 context of invasive plant pathogens, “Each imported pathogen is therefore an
375 uncontrolled, potentially dangerous, open-ended experiment in evolution”.

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

387 Range expansion by native and exotic organisms has resulted in the death of a
388 large number of trees in Canada over an evolutionarily short time period. The long
389 term consequences of these human-mediated introductions, and the new associations
390 and evolutionary trajectories that have been initiated through symbiont exchange and
391 new vector relationships are even more of a concern. The evolutionary consequences
392 facilitated by these processes are complex and the impacts on tree hosts are difficult
393 to predict, but continued study of these systems has the potential to generate
394 significant knowledge as we witness evolutionary processes unfolding before us.
395 Efforts to prevent the introduction of additional exotic organisms into Canada

396 through international trade are expensive in human terms, but these expenses need to
397 be balanced by the impacts on ecosystem services and societal well-being that are
398 afforded by healthy trees.

399

400 Acknowledgements

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

402 This manuscript was strengthened through reviews provided by David Langor, Rene
403 Alfaro and two anonymous reviewers.

404

405 References

406

407 Alberta Environment and Parks. 2007. Alberta's Strategy [online]. Available from
408 <http://aep.alberta.ca/lands-forests/mountain-pine-beetle/albertas-strategy/default.aspx>
409 [accessed 16 July 2015].

410

411 Allen, E.A., and Humble, L.M. 2002. Nonindigenous species introductions: a threat
412 to Canada's forests and forest economy. *Canadian Journal of Plant Pathology*, **24**:
413 103–110.

414

415 Anonymous. 2005. The Dutch Elm Disease Regulations. Chapter F-19.1 Reg 5. The
416 Forest Resources Management Act, Government of Saskatchewan [online].
417 Available from <https://www.saskatoon.ca/sites/default/files/documents/community->

- 418 [services/parks/The%20Dutch%20Elm%20Disease%20Regulations%202005.pdf](https://www.services/parks/The%20Dutch%20Elm%20Disease%20Regulations%202005.pdf)
419 [accessed 7 February 2015].
420
- 421 Ayres, M.P., Pena, R., Lombardo, J.A., and Lombardero, M.J. 2014. Host use
422 patterns by the European woodwasp, *Sirex noctilio*, in its native and invaded range.
423 PLOS ONE: e90321. Doi:10.1371/journal.pone.0090321.
424
- 425 Bergeron, M., Leal, I., Foord, B., Ross, G., Davis, C., Slippers, B., de Groot, P., and
426 Hamelin, R.C. 2011. Putative origin of clonal lineages of *Amylostereum areolatum*,
427 the fungal symbiont associated with *Sirex noctilio*, retrieved from *Pinus sylvestris*, in
428 eastern Canada. *Fungal Biology*, **115**: 750–758.
429
- 430 Bleiker, K.P. and Six, D.L. 2009. Competition and coexistence in a multi-partner
431 mutualism: Interaction between two fungal symbionts of the mountain pine beetle in
432 beetle-attacked trees. *Microbial Ecology*, **57**: 191–202.
433
- 434 Boissin, E., Hurley, B., Wingfield, M.J., Vasaitis, R., Stenlid, J., Davis, C., de Groot,
435 P., Ahumada, R., Carnegie, A., Goldarazena, A., Klasmer, P., Wermelinger, B., and
436 Slippers, B. 2012. Retracing the routes of introduction of invasive species: the case
437 of the *Sirex noctilio* woodwasp. *Molecular Ecology*, **21**: 5728–5744. doi:
438 10.1111/mec.12065.
439

- 440 Boyd, I.L., Freer-Smith, P.H., Gilligan, C.A., and Godfray, H.C.J. 2013. The
441 consequence of tree pests and diseases for ecosystem services. *Science*,
442 **342**:1235773-1–1235773-8. doi: 10.1126/science.1235773.
443
- 444 Brasier, C.M. 2001. Rapid evolution of introduced plant pathogens via interspecific
445 hybridization. *BioScience*, **51**: 123–133.
446
- 447 Brasier, C.M. 2008. The biosecurity threat to the UK and global environment from
448 international trade in plants. *Plant Pathology*, **57**: 792–808.
449
- 450 Campana, R.J., and Stipes, R.J. 1981. Dutch elm disease in North America with
451 particular reference to Canada: Success or failure of conventional control methods.
452 *Canadian Journal of Plant Pathology*, **3**: 252–259.
453
- 454 CFIA. 2014. Canadian Food Inspection Agency, Pest Risk Management Document
455 – Brown Spruce Longhorn Beetle (*Tetropium fuscum*) [online]. Available from
456 [http://www.inspection.gc.ca/plants/plant-protection/directives/risk-
457 management/brown-spruce-longhorn-beetle-tetropium-fuscum-/eng/1410452600838](http://www.inspection.gc.ca/plants/plant-protection/directives/risk-management/brown-spruce-longhorn-beetle-tetropium-fuscum-/eng/1410452600838)
458 [accessed 31 January 2015].
459

- 460 Cullingham, C.I., Cooke, J.E., Dang, S., Davis, C.S., Cooke, B.J., and Coltman, D.W.
461 2011. Mountain pine beetle host-range expansion threatens the boreal forest.
462 *Molecular Ecology*, **20**: 2157–2171.
- 463
- 464 de Groot, P., Nystrom, K., and Scarr, T. 2006. Discovery of *Sirex noctilio*
465 (Hymenoptera: Siricidae) in Ontario, Canada. *The Great Lakes Entomologist*, **39**:
466 49–53.
- 467
- 468 de la Giroday, H.C., Carroll, A.L., and Aukema, B.H. 2012. Breach of the northern
469 Rocky Mountain geoclimatic barrier: initiation of range expansion by the mountain
470 pine beetle. *Journal of Biogeography*, **39**: 1112–1123.
- 471
- 472 Et-Touil, A., Rioux, D., Mathieu, F.M., and Bernier, L. 2005. External symptoms
473 and histopathological changes following inoculation of elms putatively resistant to
474 Dutch elm disease with genetically close strains of *Ophiostoma*. *Canadian Journal of*
475 *Botany*, **83**: 656–667.
- 476
- 477 Evans, H.F. 2010. Pest risk analysis – organisms or pathways? *New Zealand Journal*
478 *of Forestry Science*, **40** suppl.: S35–S44.
- 479

- 480 Flaherty, L., Sweeney, J.D., Pureswaran, D., and Quiring, D.T. 2011. Influence of
481 host tree condition on the performance of *Tetropium fuscum* (Coleoptera:
482 Cerambycidae). *Environmental Entomology*, **40**: 1200–1209.
- 483
- 484 Flaherty, L., Quiring, D., Pureswaran, D., and Sweeny, J. 2013. Preference of an
485 exotic wood borer for stressed trees is more attributable to pre-alighting than post-
486 alighting behaviour. *Ecological Entomology*, **38**: 546–552.
- 487
- 488 Francke-Grosmann, H. 1963. Some new aspects in forest entomology. *Annual*
489 *Review of Entomology*, **8**: 415–438.
- 490
- 491 Gadgil, P.D., Bulman, L.S., Dick, M.A., Bain, J., and Dunn, C.P. 2000. Dutch elm
492 disease in New Zealand. *In* *The Elms: Breeding, Conservation and Disease*
493 *Management. Edited by C.P. Dunn. Kluwer Academic Publishers, Boston, USA. Pp.*
494 *189–199.*
- 495
- 496 Gilmour, J.W. 1965. The life cycle of the fungal symbiont of *Sirex noctilio*. *New*
497 *Zealand Journal of Forestry*, **10**: 80–89.
- 498
- 499 Government of Saskatchewan. 2015. Saskatchewan and Alberta renew agreement to
500 combat mountain pine beetle [online]. Available from

- 501 [http://www.saskatchewan.ca/government/news-and-
502 media/2015/january/15/mountain-pine-beetle](http://www.saskatchewan.ca/government/news-and-
502 media/2015/january/15/mountain-pine-beetle)
503 [accessed 16 July 2015].
504
- 505 Griffin, H.D. 1968. The genus *Ceratocystis* in Ontario. Canadian Journal of Botany,
506 **46**: 689–718.
507
- 508 Hantula, J., Müller, M.M., and Uusivuori, J. 2014. International plant trade
509 associated risks: laissez-faire or novel solutions. Environmental Science & Policy,
510 **37**: 158-160. doi: 10.1016/j.envsci.2013.09.11.
511
- 512 Harrison, K.J., and Smith, G.A. 2013. The discovery of *Ophiostoma tetropii* with
513 the brown spruce longhorn beetle (*Tetropium fuscum*) in Halifax, Canada. In The
514 Ophiostomatoid fungi: expanding frontiers. Edited by K.A. Seifert, Z.W. De Beer,
515 and M.J. Wingfield. CBS Biodiversity Series 12, CBS-KNAW Fungal Biodiversity
516 Centre, Utrecht, The Netherlands. Pp. 213–217.
517
- 518 Hintz, W.E., Carneiro, J.S., Kassatenko, I., Varga, A., and James, D. 2013. Two
519 novel mitoviruses from a Canadian isolate of the Dutch elm pathogen *Ophiostoma*
520 *novo-ulmi* (93-1224). Virology Journal, **10**: 252.
521

- 522 Hintz, W.E., Jeng, R.S., Yang, D.Q., Hubbes, M.M., Horgen, P.A. 1993. A genetic
523 survey of the pathogenic fungus *Ophiostoma ulmi* across a Dutch elm disease front in
524 Western Canada. *Genome*, **36**: 418–426.
- 525
- 526 Hofstetter, R.W., and Moser, J.C. 2014. The role of mites in insect-fungus
527 associations. *Annual Review of Entomology*, **59**: 537–557.
- 528
- 529 Hubbes, M. 1999. The American elm and Dutch elm disease. *Forestry Chronicle*,
530 **75**: 265–273.
- 531
- 532 Hulcr, J., and Dunn, R.R. 2011. The sudden emergence of pathogenicity in insect-
533 fungus symbioses threatens naïve forest ecosystems [online]. *Proceedings of the*
534 *Royal Society B*, **278**: 2866–2873. doi:10.1098/rspb.2011.1130.
- 535
- 536 Humble, L.M., and Allen, E.A. 2006. Forest biosecurity: alien invasive species and
537 vectored organisms. *Canadian Journal of Plant Pathology*, **28**: S256–S269.
- 538
- 539 Humble, L.M. 2010. Pest risk analysis and invasion pathways – insects and wood
540 packing revisited: What have we learned? *New Zealand Journal of Forestry Science*,
541 **40** suppl.: S57–S72.
- 542

- 543 Jacobs, K., Seifert, K.A., Harrison, K.J., and Hirstits, T. 2003. Identity and
544 phylogenetic relationships of ophiostomatoid fungi associated with invasive and
545 native *Tetropium* species (Coleoptera: Cerambycidae) in Atlantic Canada. Canadian
546 Journal of Botany, **81**: 316–329.
- 547
- 548 Jacobs, K., and Seifert, K.A. 2004a. Fungi Canadenses No. 347. *Pesotum fragrans*.
549 Canadian Journal of Plant Pathology, **26**: 79–80.
- 550
- 551 Jacobs, K., and Seifert, K.A. 2004b. Fungi Canadenses No. 346. *Ophiostoma*
552 *tetropii*. Canadian Journal of Plant Pathology, **26**: 76–78.
- 553
- 554 Klepzig, K.D., Adams, A.S., Handelsman, J., and Raffa, K.F. 2009. Symbioses: A
555 key driver of insect physiological processes, ecological interactions, evolutionary
556 diversification, and impacts on humans. Economic Entomology, **38**: 67–77.
- 557
- 558 Langor, D.W., DeHaas, L.J., and Footitt, R.G. 2009. Diversity of non-native
559 terrestrial arthropods on woody plants in Canada. Biological Invasions, **11**: 5–19.
- 560
- 561 Lee, S., Kim, J.J., and Breuil, C. 2005. *Leptographium longiclavatum* sp. nov., a
562 new species associated with the mountain pine beetle, *Dendroctonus ponderosae*.
563 Mycological Research, **109**: 116–170.
- 564

- 565 Leveux, J., Lieutier, F., Moser, J.C. and Perry, T.J. 1989. Transportation of
566 phytopathogenic fungi by the bark beetle *Ips sexdentatus* Boerner and associated
567 mites. *Journal of Applied Entomology*, **108**: 1–11.
- 568
- 569 Liebhold, A.M., MacDonald, W.L., Bergdahl, D., and Mastro, V.C. 1995. Invasion
570 by exotic forest pests: A threat to forest ecosystems. *Forest Science Monograph*, **30**.
571 49 p.
- 572
- 573 Liebhold, A.M., Brockerhoff, E.G., Garrett, L.J., Parke, J.L., and Britton, K.O. 2012.
574 Live plant imports: the major pathway for forest insect and pathogen invasions of the
575 US. *Frontiers in Ecology and the Environment*, **10**: 135–143. doi: 10.1890/110198.
- 576
- 577 Loo, J.A. 2009. Ecological impacts of non-indigenous invasive fungi as forest
578 pathogens. *Biological Invasions*, **11**: 81–96.
- 579
- 580 Myrholm, C.L., and Langor, D.W. 2015. Assessment of the impact of symbiont
581 Ophiostomatales (Fungi) on mountain pine beetle (Coleoptera: Curculionidae)
582 performance on a jack pine (Pinaceae) diet using a novel *in vitro* rearing method.
583 *The Canadian Entomologist*. *In Press*.
- 584
- 585 Nielsen, C., Williams, D.W., and Hajek, A.E. 2009. Putative source of the invasive
586 *Sirex noctilio* fungal symbiont, *Amylostereum areolatum*, in the eastern United States

- 587 and its association with native siricid woodwasps. *Mycological Research*, **113**:
588 1242–1253.
- 589
- 590 Pomerleau, R. 1961. History of the Dutch elm disease in the Province of Quebec,
591 Canada. *Forestry Chronicle*, **37**: 356–367.
- 592
- 593 Potter, C., Harwood, T., Knight, J., and Tomlinson, I. 2011. Learning from history,
594 predicting the future: the UK Dutch elm disease outbreak in relation to contemporary
595 tree disease threats. *Philosophical Transactions of the Royal Society B*, **366**: 1966–
596 1974.
- 597
- 598 Rhains, M., MacKinnon, W.E., Porter, K.B., Sweeney, J.D., and Silk, P.J. 2011.
599 Evidence for limited spatial spread in an exotic longhorn beetle, *Tetropium fuscum*
600 (Coleoptera: Cerambycidae). *Forest Entomology*, **104**: 1928–1933.
- 601
- 602 Rice, A.V., and Langor, D.W. 2009. Mountain pine beetle-associated blue-stain
603 fungi in lodgepole × jack pine hybrids near Grande Prairie, Alberta (Canada). *Forest*
604 *Pathology*, **39**: 323–334.
- 605
- 606 Rice, A.V., Thormann, M.N., and Langor, D.W. 2007a. Mountain pine beetle
607 associated blue-stain fungi cause lesions on jack pine, lodgepole pine, and lodgepole
608 × jack pine hybrids in Alberta. *Canadian Journal of Botany*, **85**: 307–315.

609

610 Rice, A.V., Thormann, M.N., and Langor, D.W. 2007b. Virulence of, and
611 interactions among, mountain pine beetle associated blue-stain fungi on two pine
612 species and their hybrids in Alberta. *Canadian Journal of Botany*, **85**: 316–323.

613

614 Roe, A.D., James, P.M.A., Rice, A.V., Cooke, J.E.K., and Sperling, F.A.H. 2011.
615 Spatial community structure of mountain pine beetle fungal symbionts across a
616 latitudinal gradient. *Microbial Ecology*, **62**: 347–360.

617

618 Safranyik, L., Carroll, A.L., Régnière, J., Langor, D.W., Riel, W.G., Shore, T.L.,
619 Peter, B., Cooke, B.J., Nealis, V.G. and Taylor, S.W. 2010. Potential for range
620 expansion of mountain pine beetle into the boreal forest of North America. *The*
621 *Canadian Entomologist*, **142**: 415–442.

622

623 Sallé, A., Monclus, R., Yart, A., Garcia, J., Romary, P., and Lieutier, F. 2005.
624 Fungal flora associated with *Ips typographus*: frequency, virulence, and ability to
625 stimulate the host defense reaction in relation to insect population levels. *Canadian*
626 *Journal of Forest Research*, **35**: 365–373.

627

628 Silk, P.J., Sweeney, J., Wu, J., Price, J., Gutowski, J.M., and Kettela, E.G. 2007.
629 Evidence for a male-produced pheromone in *Tetropium fuscum* (F.) and *Tetropium*

- 630 *cinnamopterum* (Kirby) (Coleoptera: Cerambycidae). *Naturwissenschaften*, **94**:
631 697–701.
632
- 633 Six, D.L., and Klepzig, K.D. 2004. *Dendroctonus* bark beetles as model systems for
634 studies on symbiosis. *Symbiosis*, **37**: 207–232.
635
- 636 Six, D.L., and Wingfield, M.J. 2011. The role of phytopathogenicity in bark beetle-
637 fungus symbioses: A challenge to the classic paradigm. *Annual Review of*
638 *Entomology*, **56**: 255–272.
639
- 640 Slippers, B., Coutinho, T.A., Wingfield, B.D., and Wingfield, M.J. 2003. A review
641 of the genus *Amylostereum* and its association with woodwasps. *South African*
642 *Journal of Science*, **99**: 70–74.
643
- 644 Smith, G., and Hurley, J.E. 2000. First North American record of the Palearctic
645 species *Tetropium fuscum* (Fabricus) (Coleoptera: Cerambycidae). *The Coleopterists*
646 *Bulletin*, **54**: 540.
647
- 648 Stillwell, M.A. 1966. Woodwasps (Siricidae) in conifers and the associated fungus,
649 *Stereum chailletii*, in eastern Canada. *Forest Science*, **12**: 121–128.
650

- 651 Temple, B., Pines, P.A., and Hintz, W.E. 2006. A nine-year genetic survey of the
652 causal agent of Dutch elm disease, *Ophiostoma novo-ulmi* in Winnipeg, Canada.
653 Mycological Research, **110**: 594–600.
654
- 655 Temple, B., Horgen, P.A., Bernier, L., and Hintz, W.E. 1997. Cerato-ulmin, a
656 hydrophobin secreted by the causal agents of Dutch elm disease, is a parasitic fitness
657 factor. Fungal Genetics and Biology, **22**: 39–53.
658
- 659 Veilleux, J. 2012. Establishment of *Scolytus schevyrewi* Semenov (Coleoptera:
660 Curculionidae: Scolytinae) in the prairies: life cycle, hosts and impact. M.Sc.
661 Thesis, University of Manitoba, 112 p. Available from
662 <http://hdl.handle.net/1993/8859> [accessed 26 February 2015].
663
- 664 Wingfield, M.J., Slippers, B., and Wingfield, B.D. 2010. Novel associations
665 between pathogens, insects and tree species threaten world forests. New Zealand
666 Journal of Forestry Science, **40** suppl.: S95–S103.
667
- 668 Wooding, A.L., Wingfield, M.J., Hurley, B.P., Garnas, J.R., de Groot, P., and
669 Slippers, B. 2013. Lack of fidelity revealed in an insect – fungal mutualism after
670 invasion. Biology Letters, **9**: 20130342. Available from
671 <http://dx.doi.org/10.1098/rsbl.2013.0342> [accessed 23 October 2014].
672

673

For Peer Review