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RAPID COMMUNICATION

Monochamus species from different continents can be effectively detected with the same trapping protocol

Celia K. Boone[1] · Jon Sweeney[2] · Peter Silk[2] · Cory Hughes[2] · Reginald P. Webster[3] · Fred Stephen[4] · Lorraine Maclauchlan[5] · Barbara Bentz[6] · Alain Drumont[7] · Boguang Zhao[8] · Nick Berkvens[9] · Hans Casteels[9] · Jean-Claude Grégoire[10]

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Abstract

Pine wilt disease is one of the most serious introduced threats to coniferous forests worldwide. Its causal agent, the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is vectored primarily by cerambycids of the genus *Monochamus* Dejean throughout its native (North America) and introduced (Japan, China, Korea, Taiwan, Portugal) ranges. Despite strict import regulations and phytosanitary measures, interception records indicate that PWN and *Monochamus* species continue to be moved worldwide. Following its introduction in Portugal in the late 1990s, extensive monitoring programs for PWN and its vectors have been conducted throughout the European Union, using locally developed and tested lures and traps. The trapping system developed in Europe and used in this study is composed of a Crosstrap® and Galloprotect Pack® lures. These trapping systems were deployed in two locations in the USA, two locations in Canada, and one location in China in order to test their capacity to detect *Monochamus* species exotic to Europe. Large numbers of *M. carolinensis, M. mutator, M. notatus, M. s. scutellatus, M. clamator*, and *M. titillator* were trapped in North America, while large numbers of *M. alternatus* were trapped in China. The trapping systems developed in Europe for monitoring the European *Monochamus* species are also effective for the detection of many exotic *Monochamus* species and could thus be used as an early detection tool in ports and other high-risk sites.

Keywords Longhorn beetle · Vector · Pine wilt disease · Pinewood nematode · Traps · Lures

Key message

1. This study was conducted to confirm that traps and lures developed in Europe are also effective in detecting non-European *Monochamus* species, vectors of the pinewood nematode.
2. Several North American and Chinese *Monochamus* species were detected: *M. carolinensis*, *M. mutator*, *M. notatus*, *M. s. scutellatus*; *M. obtusus*, *M. clamator*, *M. titillator*, *M. alternatus.*
3. The European trapping system can be effectively employed for early detection of exotic *Monochamus* species at high-risk sites and possibly used in eradication or containment efforts.

Introduction

Pine wilt disease (PWD) is the most serious introduced threat to susceptible coniferous forests worldwide. Since its introduction into Japan via the timber trade over a century ago, PWD spread throughout Asia in the 1980s, and continuous control and containment efforts, as well as implementation of comprehensive regulations and embargoes, have been ineffective in preventing its spread (Commission

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✉ Jean-Claude Grégoire
jcgregoi@ulb.ac.be

Extended author information available on the last page of the article

Decision 2006/133/EC of 13 February 2006 (European Council 2006). In 1999, PWD was reported in Portugal (Mota et al. 1999) and now affects a major part of the country and the island of Madeira. PWD was further discovered in several naturally forested areas of Spain near the border with Portugal in the provinces of Caceres (in 2008 and 2012, EPPO 2010a, b, 2012), Pontevedra (in 2011 and 2016, EPPO 2014), and Salamanca (in 2013, EPPO 2014), where it is officially declared transient, actionable, and under eradication (EPPO 2014).

The PWD is a multipartite system involving intimate relationships between the pathogen, the pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle, its vectors, and symbiotic microorganisms. PWN is native to North America and is transferred between host trees primarily by beetles in the genus *Monochamus* Dejean (Coleoptera: Cerambycidae) (Linit 1988). In North America, all available *Monochamus* species appear to be viable vectors of PWN (Pimentel et al. 2014), and in each newly established region of the world it is vectored by congeneric counterparts (see Humble and Allen 2006). The disease cycle is a positive feedback system, *i.e.* when the pathogen (PWN) infects and subsequently kills its host, it creates a suitable substrate (stressed/dying tree) for oviposition by its vector (Naves et al. 2006), facilitating its establishment and dispersal. The success of PWN as an invasive species is supported by several life history attributes: it is cryptic within its vector, *i.e.* fourth-stage nematode juveniles are transported to susceptible hosts predominantly within the trachea of their vector but also in other parts of their body (Naves et al. 2006); its vectors are cryptic xylophagous beetles which are often undetected while being transported in wood products and wood packaging material; it does not require the establishment of its vector, because after reaching a susceptible host it can establish and subsequently infect native *Monochamus* species. These aspects, combined with the increasing international trade flow, make future invasions inevitable (Brockerhoff et al. 2006). The EU Commission Decision 2012-535-EC (European Council 2012) on emergency measures to prevent the spread of PWN within the Union, as well as earlier EU regulatory documents, require member states to conduct annual surveys in susceptible areas (*e.g.* pine stands, green areas, and logging and wood processing facilities) in which PWN is not known to occur. Due to the obligatory association between the PWN and its insect vectors (Akbulut and Stamps 2012), directing monitoring efforts towards the vector could be an effective means to intercept and prevent the establishment of PWN. However, national surveys and monitoring networks for native and non-native Monochamus require an effective trap and attractant lure system.

Extensive research has led to significant improvements in trap design and lure development. The response of cerambycids to different trap types is often species specific (Morewood et al. 2002; Dodds et al. 2015), but panel traps are generally superior to other trap models (Allison and Redak 2017), especially when coated with Teflon or Fluon (Graham et al. 2010; Allison et al. 2011, 2016; Álvarez et al. 2015). In general, the addition of Teflon or Fluon to cross-vane, panel, and multiple funnel traps significantly increased captures and retention of Cerambycidae, in particular, *Monochamus* species (Graham et al. 2010; Allison et al. 2011, 2016; Álvarez et al. 2015). Additionally, advancements in the understanding of the chemical ecology of *Monochamus* species have led to the development of effective lure combinations which includes both pheromones of *Monochamus* species and kairomones. Monochamol (2-undecyloxy-ethanol) is an aggregation pheromone produced by mature males of *Monochamus* species native to Asia, Europe, and North America, attractive to both sexes (Pajares et al. 2010; 2013; Teale et al. 2011; Allison et al. 2012; Fierke et al. 2012; Ryall et al. 2014; Lee et al. 2017b). Several studies have also confirmed the attractiveness of two pheromones of *Ips* De Geer species (Curculionidae: Scolytinae) (ipsenol and 2-methyl-3-buten-2-ol (methyl butenol)) (de Groot and Nott 2004; Pajares et al. 2010), and their subsequent synergy with monochamol (Miller et al. 2016; Kim et al. 2016; Pajares et al. 2017). Certain host volatiles are also known to indicate suitable hosts to woodborers of conifers, such as ethanol and α-pinene, and are therefore often incorporated into lure blends due to their synergistic activity with ipsenol and ipsdienol (Morewood et al. 2002; de Groot and Nott 2004; Miller and Asaro 2005). In some cases, the addition of α-pinene does not significantly increase captures of *Monochamus*, but it increases captures of associated insects, such as non-target bark and wood borers and their natural enemies (Álvarez et al. 2016). A four-component semiochemical blend (monochamol, ipsenol, methyl butenol, and α-pinene) in combination with a black Teflon-coated cross-vane trap has been shown to be an effective system to capture Cerambycidae, in particular, *M. galloprovincialis* (Pajares et al. 2010; Bonifácio et al. 2012; Álvarez et al. 2013), the main vector of PWN in Europe (Sousa et al. 2001).

Early detection of alien species strongly depends on both the attractiveness of the lures used as bait and trap design (Rassati et al. 2014); it is therefore essential to confirm the efficacy of traps and lures developed for European *Monochamus* species to detect *Monochamus* species non-native to Europe. Here we tested the efficacy of a trapping system developed in Europe towards non-European *Monochamus* species, deploying traps in North America, the area of origin of the PWN, and China, a country where the PWN is established and that represents a significant international trading partner of Europe.

Methods

Four black Teflon-coated cross-vane traps (Crosstrap®, Econex, Murcia, Spain) and four four-component commercially available lures (Galloprotect Pack®, SEDQ, Barcelona, Spain) were distributed to each of the following five locations: Canada (New Brunswick and British Columbia in 2013), USA (Arkansas and Utah in 2013), and China (Nanjing, Jiangsu Province in 2014). Release rates of lure components are as follows: monochamol: 2 mg/day; ipsenol: 2.5 mg/day; 2-methyl-3-buten-1-ol: 10 mg/day; and α-pinene: 500 mg/day. Each lure component was placed at a specific height on the trap: ipsenol and 2-methyl-3-buten-1-ol were hung in the lower level, α-pinene was hung in the centre, and the monochamol was hung in the upper level. Lures were replaced at 6 or 8 week intervals to ensure continuous delivery of volatiles. The attractive lures used in this study were chosen because they were specifically developed in Europe for detecting European *Monochamus* species (Pajares et al. 2010). The host volatile, α-pinene, was included because there were no reports of interference or deterrence towards *Monochamus* species, and it can attract a broader range of xylophagous insects, providing further insights on trapping efficacy.

Traps were deployed in predominantly pine forests with minor components of deciduous and/or other conifer species located in areas known to have populations of *Monochamus* species. Specific site and sampling information is listed in Table S1. Traps were suspended from ropes spanning between two trees, by rope from a branch, or from a free-standing supporting structure so that the collection cup was approximately 0.5 m above ground. Trapped insects were collected weekly in Arkansas, biweekly in New Brunswick, British Columbia, and Utah, and monthly in Nanjing. All locations employed the wet capture method using either monoethylene glycol or saturated salt solution plus detergent to preserve the trapped individuals in the field. Monoethylene glycol does not interfere with identification of PWN for inspection purposes (Berkvens et al. 2017). Collaborators identified *Monochamus* species in their laboratories. Other cerambycid species and some associated insects were identified in New Brunswick and Arkansas. Species of Cerambycidae from British Columbia were identified at the Royal Belgian Institute of Natural Sciences. Voucher specimens of relevant species were provided to ULB from all locations except Nanjing and deposited in the Royal Belgian Institute of Natural Sciences.

Results

Crosstraps baited with Galloprotect Pack were effective in trapping exotic *Monochamus* species at all sites in which they were deployed. Overall, the Crosstraps captured 4550 individuals of seven *Monochamus* species in North America, including *M. carolinensis* (Olivier), *M. clamator* (LeConte), *M. mutator* Leconte, *M. notatus* (Drury), *M. obtusus* Casey, *M. s. scutellatus* Say, and *M. titillator* (Fabricius), and 244 specimens of one species, *M. alternatus* (Hope) in China (Table 1). *Monochamus* species dominated catches in North America [87–99% depending on the site, of all cerambycid species (Tables 1, 2)], whereas in Nanjing, China, the Cerambycidae *M. alternatus* (51.2%) and *Nadezhdiella cantori* (Hope) (48.0%) were the two most abundant species, with minor captures of other species.

Peak flight activity of *Monochamus* species varied depending on site and species (Fig. 1) . In Currie Mountain (New Brunswick, Canada), peak captures of the dominant species *M. notatus* and *M. scutellatus* likely occurred prior to the initiation of the sampling (Fig. 1d), while the dominant species in Sevogle (New Brunswick, Canada), *M. mutator*, appeared to have had two peaks, one prior to the initiation of the sampling and a second in mid-August, but this could be an artefact due to the sampling frequency (Fig. 1c). The other *Monochamus* species in New Brunswick did not have a distinct peak flight activity. In Arkansas (USA), *M. titillator* and *M. carolinensis* peaked in mid-late July (Fig. 1a), as did *M. alternatus* in Nanjing (China) (Fig. 1f). In British Columbia (Canada), *M. scutellatus*, *M. clamator*, and *M. obtusus* peaked in early September (Fig. 1e), as did *M. scutellatus* in Utah (USA) (Fig. 1b).

Table 1 Species and abundance (i.e. total number of individuals) of *Monochamus* captured during international trapping. Abundance is pooled across four traps deployed at each location

Location	*Monochamus* spp.	Abundance
Canada (2013)		
New Brunswick	*M. carolinensis* (Olivier 1792)	17
	M. mutator Leconte in Agassi, 1850	422
	M. notatus (Drury, 1773)	186
	M. s. scutellatus Say, 1824	139
British Columbia	*M. s. scutellatus* Say, 1824	62
	M. obtusus Casey, 1891	45
	M. clamator (LeConte, 1852)	20
USA (2013)		
Arkansas	*M. carolinensis* (Olivier, 1792)	997
	M. titillator (Fabricius, 1775)	2153
Utah	*M. s. scutellatus* Say, 1824	418
China (2014)		
Nanjing	*M. alternatus* Hope, 1842	244

Table 2 Other insects captured in Crosstraps® baited with Galloprotect Pack® in North America (2013) and China (2014)

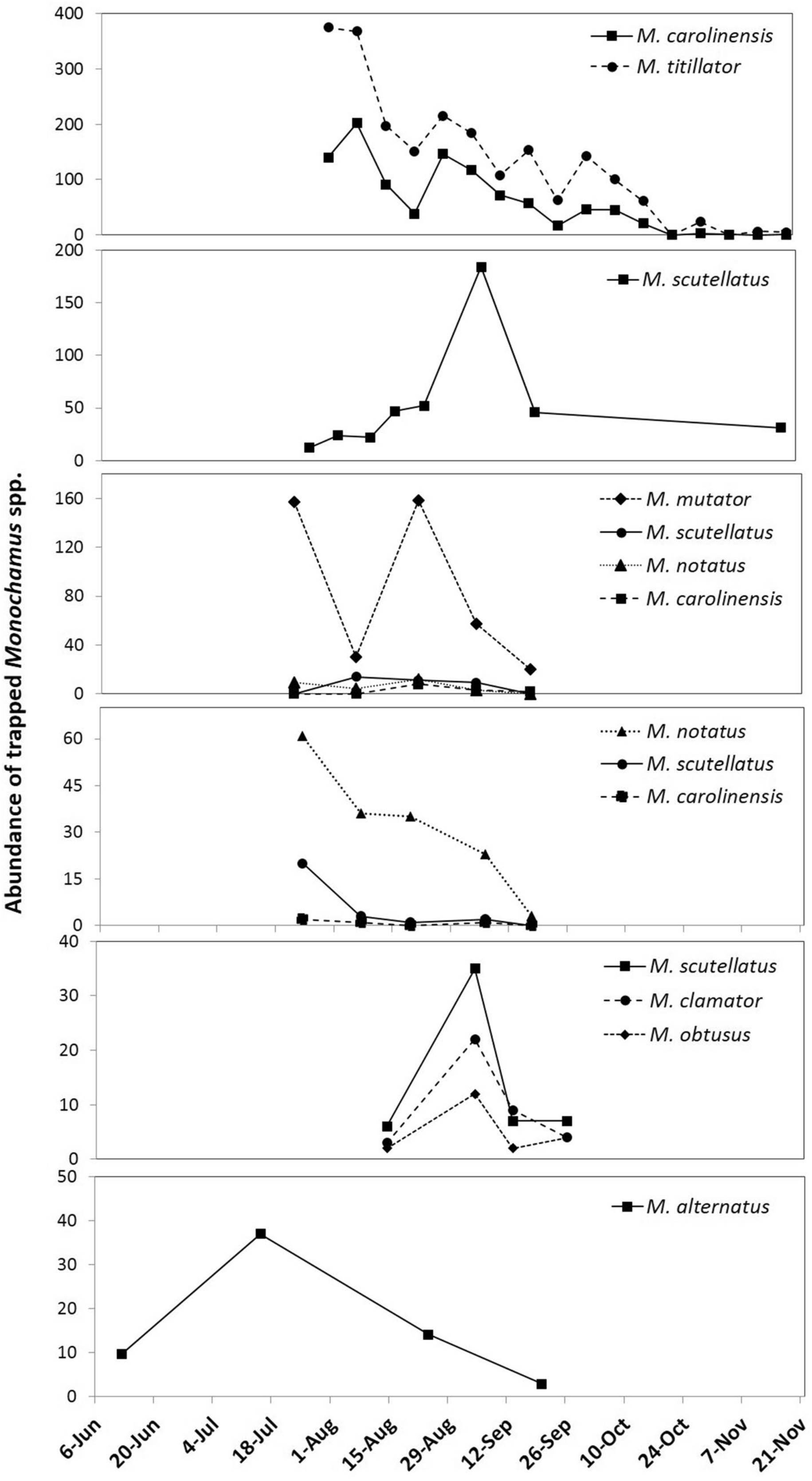
Family	Subfamily or species	Total number	Location
Coleoptera			
Cerambycidae	*Acanthocinus obsoletus* (Olivier)	234	Arkansas
	Megasemum asperum (Leconte)	4	British Columbia
	Nadezhdiella cantori (Hope)	229	Nanjing
	Xylotrechus longitarsis Casey	4	British Columbia
	Xylotrechus sagittus (Germar)	223	Arkansas
	Xylotrechus sp.	1	British Columbia
	Unidentified sp. 1	10	Utah
	Unidentified sp. 2	8	British Columbia
	Unidentified sp. 3	3	Nanjing
Buprestidae	*Buprestis* sp.	196	Arkansas
Cleridae		53	British Columbia
Curculionidae	*Hylobius pales* Herbst	324	Arkansas
	Ips avulsus (Eichhoff)	173	Arkansas
	Ips grandicollis (Eichhoff)	3539	Arkansas
	Scolytinae	563	Nanjing
Elateridae		17	British Columbia
Meloidae		344	Nanjing
Trogossitidae	*Temnochila virescens* (Fabricius)	274	Arkansas
Other		182	Nanjing
Hymenoptera			
Siricidae	*Sirex juvencus* (Linneaus)	1	Utah
	Urocerus albicornis (Fabricius)	3	Utah
Ichneumonidae		8	
Other orders		2	British Columbia
		83	Nanjing

Data on insects captured in select non-cerambycid families are reported for Arkansas, Utah, British Columbia, and Nanjing only

Discussion

The trapping system consisting of Crosstrap® and Galloprotect Pack® developed in Europe, and currently recommended for monitoring *Monochamus* species in the EU, is effective in detecting multiple *Monochamus* species native to North America where PWN is endemic, and as well as *M. alternatus*, the main vector of PWN in Asia. However, Blatt et al. (2017) found that the combination of a North American developed panel trap treated with Fluon, in combination with the European developed Galloprotect Pack, was more effective in capturing two of those species, *M. notatus* and *M scutellatus*, than Crosstraps and North American lures. A plethora of literature exists on the attraction of *Monochamus* species to various lures, including host plant volatiles and pheromones of other xylophagous beetles sharing the same habitat. In various locations throughout North America, Europe, and Asia, blends containing ethanol, α-pinene, and components of *Ips* pheromones, usually ipsenol, and/or ipsdienol (e.g. Allison et al. 2003; de Groot and Nott 2004; Costello et al. 2008; Miller et al. 2013; Hanks and Millar 2013), and/or methyl butenol (e.g. Ibeas et al. 2007; Pajares, et al. 2010; Bonifácio et al. 2012), have been shown to be effective in attracting *Monochamus* species, and the addition of monochamol to these blends increased their attractiveness (Pajares et al. 2010). The consistent captures of the transcontinentally distributed species *M. scutellatus* in this study suggest that it responds positively to the combination of monochamol, ipsenol, methyl butenol, and α-pinene across a broad geographic range in North America. These results are consistent with those of Ryall et al. (2014) who found similar responses of *M. scutellatus* to combinations of monochamol, ipsenol, and α-pinene, and Miller et al. (2015) who showed that several *Monochamus* species in North America were attracted to a lure consisting of alpha pinene, monochamol, and ipsenol. The addition of α-pinene to the combination of monochamol, methyl butenol, and ipsdienol does not, however, significantly increase captures of *M. galloprovincialis*, but is attractive to non-target insects, such as secondary xylophagous insects and bark beetle natural enemies resulting in trapping and killing these species (Álvarez et al. 2016; Jurc et al. 2016). Thus, the inclusion

Fig. 1 Flight activity of *Monochamus* species. captured in *Pinus* species stands in North America and China using Crosstraps baited with Galloprotect Pack®: **a** *P. echinata*, Ozark National Forest, Arkansas 2013; **b** *P. contorta*, Logan Canyon, Utah 2013; **c** *P. banksiana*, Sevogle, New Brunswick 2013; **d** *P. strobus,* Currie Mountain, New Brunswick 2013; **e** *P. contorta*, Southern Interior, British Columbia 2013; **f** *P. massionana*, Xixia Mountains, Nanjing 2014. Data are pooled within locations, except New Brunswick (2 sites)



of α-pinene in blends might be avoided in pine forests where these non-target insects would be impacted, but could continue in imports areas, such as ports and warehouses, where the latter would not be affected.

Most *Monochamus* species exhibited peak flight activity between mid-July and early September, confirming data reported in previous studies (e.g. Hanks and Millar 2013; Miller et al. 2013). Pimentel et al. (2014) found that PWN phoresy in eastern forests was highest on the *Monochamus* species that flew earliest in the year. Conversely, in the south-western USA, where *Monochamus* species are likely multivoltine and have long flight periods, PWN load on adult *Monochamus* beetles was high throughout the season, indicating that PWN can be transmitted to new hosts during most of the year. This suggests that PWN has an ecology that is sufficiently flexible to allow it to exploit different species (and both genders) of *Monochamus*, and to disperse at different times of the year, further contributing to its success in invading Eurasian pine forests (Pimentel et al. 2014). Moreover, seasonal activity, *e.g.* phenology, flight, and voltinism, of adult *Monochamus* beetles can affect both the PWD system and the dynamics of damages in the invaded forests.

Several other insect species of were captured. Regarding the Cerambycidae, some of the most abundant species, such as *Acanthocinus obsoletus* and *Nadezhdiella cantor*, belong to the same subfamily of *Monochamus* species, *i.e.* the Laminae; this is not surprising given the widespread pheromonal parsimony among Cerambycids at varying taxonomic levels (Hanks and Millar 2013). Regarding the other beetle species, it is known that attractant blends for Cerambycidae which include ethanol, α-pinene, and *Ips* pheromones, are also attractive to the guild of saproxylic insects occupying habitats similar to those occupied by cerambycids. Ethanol is released from stressed conifer trees (Kelsey 1996; Kelsey and Joseph 2003, Kelsey et al. 2014), α-pinene is the predominant compound associated with pine trees, and *Ips* species are usually the first colonizers of conifers with compromised defences (Furniss and Carolin 1980). Thus, the combination of these kairomones provides to several xylophagous insects signals indicating suitable host conditions for adult feeding, mating, and oviposition for saproxylic insects and their associates (Miller et al. 2013). Hanks and Millar (2013) suggest, in general, that the host plant volatile α-pinene enhances attraction to conifer specialists while ethanol enhances attraction to hardwood specialists. If, in the future, it is necessary to widen the range of target insects because, *e.g.* new pheromones of some *Monochamus* species are discovered, or other genera are found to vector *B. xylophilus*, the blend used in this study could be complemented with other pheromones, because synergism between these compounds is often observed for some insects, whereas inhibition is rare (Hanks et al. 2012; Miller et al. 2015). In addition to increased catches and the reduction of costs and labour with easier and more rapid sample processing, employing species-specific traps and attractants also reduces the diversity of species that can be trapped. However, a common criticism of semiochemical blends is the removal of beneficial insects from the environment, such as natural enemies of bark beetles which have co-evolved to use the pheromones to locate prey, and, moreover, removal of or interference with rare or threatened species. Conversely, the Crosstrap and Galloprotect Pack trapping system has been shown to detect species of Cerambycidae which have previously been considered rare when monitored using other trapping methods, but are actually present in homogeneous and stable populations, in addition to detection of range expansion of other species (Boone et al. 2015).

Some factors still require clarification before Crosstraps baited with Galloprotect Packs could be put to an optimal use for monitoring exotic *Monochamus* species in Europe. Trapping efficiency can vary among sites (*e.g.* natural areas, ports, and lumber yards) due to shifts in environmental parameters such as population density, competing sources of attraction, and visual cues (Miller 2006). For example, Blatt et al. (2017) caught higher numbers of several *Monochamus* species in Christmas tree plantations than in adjacent forests. Rassati et al. (2012) reported that funnel traps baited with the same components of Galloprotect Pack are effective at low densities of *Monochamus* and can therefore be used as a general tool for beetle surveillance at ports of entry and forests, but also recommend that tree health assessments and trapping should be conducted outside of port areas as well (Rassati et al. 2014). A constraint of this trap/lure combination is that newly emerged *Monochamus* adults require a sexual maturation feeding period, during which they are not attracted to the pheromones, kairomones, or host volatiles included in the Galloprotect Pack (Álvarez et al. 2016), and during which they are able to disperse at least 2 km from the point of emergence (Sanchez-Husillos et al. 2016). Identification and isolation of volatiles attractive to immature *Monochamus* adults should be a focus for the development of a truly comprehensive lure.

A final issue relates to the possibility of using the European trapping protocol for eradicating small, newly established populations of exotic *Monochamus* species. Sanchez-Husillos et al. (2015) reported that the current trap and lure system can effectively remove 95% of a moderate density of a native *M. galloprovincialis* population in Spain, at a trapping density of 0.82 traps per hectare. More conservatively, El-Sayed et al. (2006) suggest that mass trapping has the potential to suppress or eradicate low-density, isolated populations. These prospects need to be further investigated.

Author contributions

JCG, CKB, NB and HC developed the project; CKB coordinated the project; CKB and JCG are the main authors; JCG was the principal investigator for the grant; JS, FS, LM, BB, and BZ coordinated project within their locations; CH and RW conducted projects in New Brunswick; PS and AD verified insect identification.

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Affiliations

Celia K. Boone[1] · Jon Sweeney[2] · Peter Silk[2] · Cory Hughes[2] · Reginald P. Webster[3] · Fred Stephen[4] · Lorraine Maclauchlan[5] · Barbara Bentz[6] · Alain Drumont[7] · Boguang Zhao[8] · Nick Berkvens[9] · Hans Casteels[9] · Jean-Claude Grégoire[10]

Celia K. Boone
ckboone@ymail.com

Jon Sweeney
jsweeney@NRCan.gc.ca

Peter Silk
psilk@nrcan.gc.ca

Cory Hughes
Cory.Hughes@NRCan-RNCan.gc.ca

Reginald P. Webster
reginaldwebster@rogers.com

Fred Stephen
fstephen@uark.edu

Lorraine Maclauchlan
Lorraine.Maclauchlan@gov.bc.ca

Barbara Bentz
bbentz@fs.fed.us

Alain Drumont
adrumont@naturalsciences.be

Boguang Zhao
zhbg596@126.com

Nick Berkvens
nick.berkvens@ilvo.vlaanderen.be

Hans Casteels
hans.casteels@ilvo.vlaanderen.be

[1] Lutte biologique et Ecologie spatiale, Université Libre de Bruxelles, Brussels, Belgium

[2] Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre, Charters Settlement, Canada

[3] 24 Mill Stream Drive, Charters Settlement, NB E3C 1X1, Canada

[4] Department of Entomology, University of Arkansas, Fayetteville, AR, USA

[5] British Columbia Department of Forest, Lands and Natural Resource Operations, Vancouver, Canada

[6] USDA, Forest Service; Rocky Mountain Research Station, Fort Collins, CO, USA

[7] Royal Belgian Institute of Natural Sciences, Brussels, Belgium

[8] Department of Forest Protection, Nanjing Forestry University, Nanjing, China

[9] Institute for Agricultural and Fisheries Research, Plant – Crop Protection – Entomology, Fort Collins, CO, USA

[10] Lutte biologique et Ecologie spatiale, Université Libre de Bruxelles, Avenue F. D, Roosevelt 50, 1050 Brussels, Belgium