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Mutual interactions between an invasive bark beetle and its associated fungi

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Abstract

Interactions between invasive insects and their fungal associates have important effects on the behavior, reproductive success, population dynamics and evolution of the organisms involved. The red turpentine beetle (RTB), *Dendroctonus valens* LeConte (Coleoptera: Scolytinae), an invasive forest pest in China, is closely associated with fungi. By carrying fungi on specialized structures in the exoskeleton, RTB inoculates fungi in the phloem of pines (when females dig galleries for egg laying and when males join them for mating). After eggs hatch, larvae gregariously feed on the phloem colonized by the fungi. We examined the effects of five isolates of RTB associated fungi (two from North America, *Leptographium terebrantis* and *L. procerum*, and three from China, *Ophiostoma minus*, *L. sinoprocerum* and *L. procerum*) on larval feeding activity, development and mortality. We also studied the effects of volatile chemicals produced in the beetle hindgut on fungal growth. *Ophiostoma minus* impaired feeding activity and reduced weight in RTB larvae. *Leptographium sinoprocerum*, *L. terebrantis* and *L. procerum* did not dramatically influence larval feeding and development compared to fungi-free controls. Larval mortality was not influenced by any of the tested fungi. Hindgut volatiles of RTB larvae, verbenol, myrtenol and myrtenal, inhibited growth rate of all the fungi. Our results not only show that *D. valens* associated fungus, *O. minus*, can be detrimental to its larvae; but, most importantly, they also show that these notorious beetles have an outstanding adaptive response evidenced by the ability to produce volatiles that inhibit growth of harmful fungus.

Keywords: antagonism, invasive insect, insect-fungus interactions, *Dendroctonus valens*, *Ophiostoma minus*, *Leptographium* spp.

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Introduction

Invasion of ecosystems by exotic species is one of the greatest threats to biodiversity and community structure (Elton, 1958). When introduced to new habitats by humans, invasive species interact with indigenous species in a wide range of habitats (Mills *et al*., 2004; Rodriguez, 2006). Most studies of biological invasions have focused on the negative effects of exotic species on native biota (Human & Gordon, 1996; Callaway & Aschehoug, 2000). On the other hand, some studies show that indigenous species might either assist or resist invasion of exotic species (Lu *et al*., 2010; McGlone *et al*., 2011). In some cases, when invasions are made by insect species, microorganisms can contribute to the success of the invasion (Jiu *et al*., 2007; Lu *et al*., 2010).

Invasive insects often carry a variety of fungal associates; and, in some cases, fungi can have intimate interactions with

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its insect vector (Lu *et al.*, 2008; Lu *et al.*, 2009a). Relationships between fungi and their insect vectors can be broadly grouped into mutualism (Chapela *et al.*, 1994; Farrell *et al.*, 2001; Aanen *et al.*, 2002) commensalism and antagonism (Madelin, 1966; Hofstetter *et al.*, 2006). These interactions can have important effects on the behavior, reproductive success, population dynamics and evolution of both invasive insects and their fungal associates. Whether or not fungal associates play a significant role in facilitating or inhibiting the invasion of insect vectors remains to be answered.

The red turpentine beetle (RTB), *Dendroctonus valens* LeConte (Coleoptera: Curculionidae, Scolytinae), is an invasive pine-killing bark beetle in China that was introduced from North America (Yan *et al.*, 2005). Over six million Chinese pines, *Pinus tabuliformis* Carriére, have been killed by RTB since 1999 in Shanxi Province (Li *et al.*, 2001). One of the most striking characteristics of RTB is its widespread association with ophiostomatoid fungi (Wingfield, 1983; Lu *et al.*, 2009a). Adult RTBs carry fungi on their exoskeleton; pioneer beetles select a suitable pine host, bore into trees and release pheromones to recruit conspecifics; afterwards, the host is killed by mass attack (Zhang & Sun, 2006; Zhang *et al.*, 2006; Lu *et al.*, 2010). Adult beetles mate, construct egg galleries, lay eggs and inoculate fungi in the phloem layer of the tree (Smith, 1971). Fungi hyphae then spread in the phloem adjacent to eggs. When eggs hatch, larvae gregariously feed on the phloem colonized by fungi (Zhang *et al.*, 2002). In North America, *Leptographium procerum* and *L. terebrantis* have been isolated from RTB (Harrington & Cobb, 1983; Wingfield, 1983; Owen *et al.*, 1987). In China, *Ophiostoma minus*, *L. sinoprocerum* and *L. procerum* have been isolated from cuticule and galleries of *D. valens* (Lu *et al.*, 2008; Lu *et al.*, 2009a). We have isolated several species of fungi, including *O. minus*, *L. sinoprocerum* and *L. procerum*, from phloem adjacent to RTB larvae at different developmental stages in the field (Wang *et al.*, unpublished data). Fungi benefit from the association by being transported to new plant resources (i.e. pine trees) by adult beetles and also may facilitate invasion of RTB by contributing to overcome host defense (Lu *et al.*, 2009b; Lu *et al.*, 2010). However, the influence of these fungal associates on RTB larvae has not been studied. Volatile chemicals produced by host pine trees can inhibit fungal growth (Lu *et al.*, 2010), but the effect of volatile chemicals produced by beetles on fungal growth is unknown. Volatile chemicals released by beetles are produced via detoxification of host defensive phytochemicals and often function as aggregation pheromones (Renwick *et al.*, 1973; Shi & Sun, 2009).

The goal of this study was to determine whether RTB fungal associates from native and invaded ranges affect feeding activity, development and survival of larvae, and to explore whether chemicals produced by adult beetles affect fungal growth. We also investigated if chemicals produced by adult beetles were also produced in different larval instars. We tested five RTB fungal isolates, two from North America, *L. terebrantis* and *L. procerum*, and three from China, *O. minus*, *L. sinoprocerum* and *L. procerum*. Understanding the interactions between invasive and indigenous species is important because these relationships can have deep ecological consequences, including cascading effects through trophic levels, changes in the community structure and evolutionary changes. Studying these interactions can also open new windows to understand the biology of invasion and to develop new control methods for *D. valens* and many other notorious pests.

Table 1. Fungal isolates tested in the experiments. All isolates were recovered from *Dendroctonus valens* adults.

Species	Isolate no.	Host	Origin
Ophiostoma minus	[a]CMW 26254	*Pinus tabuliformis*	Shanxi, China
Leptographium terebrantis	[a]CMW 1767	*P. contorta*	Canada
L. sinoprocerum	[b]MUCL 46352	*P. tabuliformis*	Hebei, China
L. procerum	[a]CMW 10217	*P. strobes*	VT, USA
L. procerum	[a]CMW 25626	*P. tabuliformis*	Shanxi, China

[a] CMW, Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.
[b] MUCL is a part of the Belgian Coordinated Collections of Microorganisms, BCCMTM.

Materials and methods

Beetle collection and rearing

RTB larvae were collected from infested Chinese pine, *P. tabuliformis*, in the Tunlanchuan Forestry Preserve (N 37°48′, E 111°44′; average elevation 1400 m), Gujiao City, Shanxi Province in September 2008. These larvae were used in the 'Effects of fungi on RTB larvae' experiment (below). Approximately 4000 larvae were collected from two infested pine trees. Two thousand 2nd to 3rd instar larvae were selected and reared on artificial medium (water 200 ml, phloem 100 g, agar 7.5 g) at 25°C. Medium composition was adapted after Wallin & Raffa (2000) and Kopper *et al.* (2005). Phloem was scraped from healthy mature *P. tabuliformis*, freeze-dried (Christ Alpha 2–4 LD) for 48 h, and ground with a laboratory blender (model 24CB10C, Waring Commercial, Torrington, CT, USA). Larvae were fed on the artificial medium for one week prior to experiments. Lethargic larvae were excluded. Larvae collected in September 2010 at the same location were used in the 'RTB larvae hindgut volatiles extraction and analysis' experiment (below); these larvae were collected from 16 pine trees.

Fungal isolates tested

Ophiostoma minus, *L. terebrantis* and *L. procerum* isolates came from the culture collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa (Lu *et al.*, 2009a). *Leptographium sinoprocerum* is a newly described species associated with RTB in China (Lu *et al.*, 2008), and its isolates came from the culture collection of the Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry. Information on the fungal isolates used is shown in table 1.

Effects of fungi on RTB larvae

A different artificial medium (water 400 ml, phloem 15 g, agar 15 g) was used to test the effects of fungi on RTB larvae boring, weight change and mortality. To make the medium, ground phloem and the agar-water mixture were autoclaved separately for 30 min at 126°C and 0.14 mPa of pressure, and

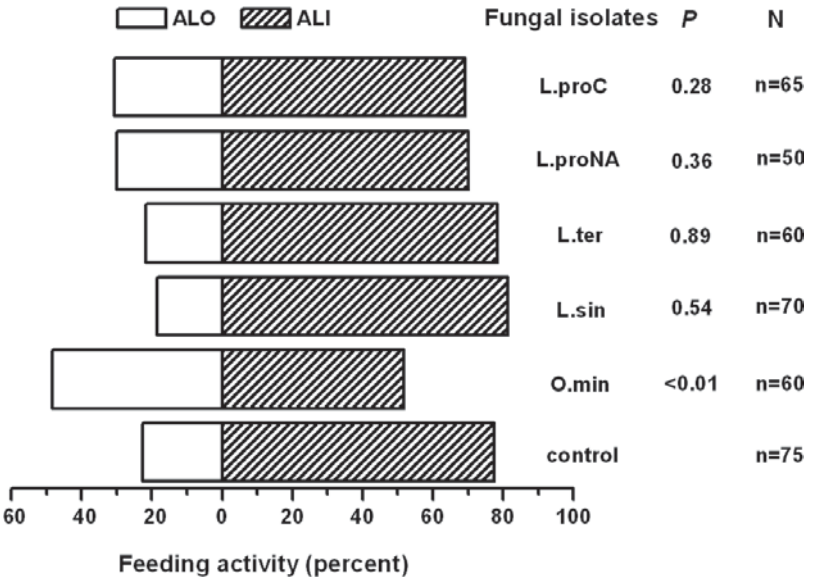

Fig. 1. Percent of *Dendroctonus valens* larval feeding activity on phloem-agar media inoculated with fungi and fungi-free control at 6, 12, 24, 48 and 96 h. ALI, average number of larvae inside the media over all the time periods; ALO, average number of larvae ouside the media over all the time periods. Pearson's chi-square test was used to compare boring percent between each fungal colonized media and fungal-free control; control, fungal-free media; O.min, *Ophiostoma minus*-colonized media; L.sin, *Leptographium sinoprocerum*-colonized media; L.te, *L. terebrantis*-colonized media; L. proNA, *L. procerum* North American isolate-colonized media; L. proC, *L. procerum* Chinese isolate-colonized media.

then mixed together in a 500 ml conical flask; 15 milliliter of the mixture were poured in Petri dishes (65 mm diameter × 15 mm high). After cooling, each agar-phloem disk was removed from the base of the Petri dish and transferred to a larger Petri dish (75 mm diameter × 15 mm high), thus providing a gap surrounding the medium to accommodate beetles. Fungal isolates were individually inoculated on the surface of the medium and incubated at 25°C in darkness for 20 days. Although fungal growth rate on the medium was different, all the media discs were fully covered by fungi after 20 days.

Five 2nd to 3rd instar larvae were randomly chosen, weighed together and placed into the gap surrounding the medium in the Petri dish. The Petri dish was sealed with parafilm (Parafilm M, Pechiney Plastic Packaging, Menasha, WI, USA) to maintain moisture. The assays were conducted in a dark growth chamber (model LRH-250, Shanghai Yiheng Biotech Co., Ltd.) at 25°C. At least ten replications were done for each treatment (media with fungus). A group of 14 petri dishes with fungi free media were used as controls.

Effects of fungal isolates on larval feeding activity, larval development and larval mortality were evaluated. Total number of larvae inside the media and larvae outside of the media for each treatment and control at 6, 12, 24, 48 and 96 h were recorded. For data analysis, average number of larvae inside the media (ALI) and the average number of larvae outside the media over all time periods (ALO) for each treatment and control was calculated. Five larvae in each Petri dish were weighed together after six days. Weight change was calculated by subtracting the recorded weight after six days from the recorded weight at the beginning of the experiment. Petri dishes with dead beetles were excluded. Number of dead larvae was recorded 18 days later for each treatment and control.

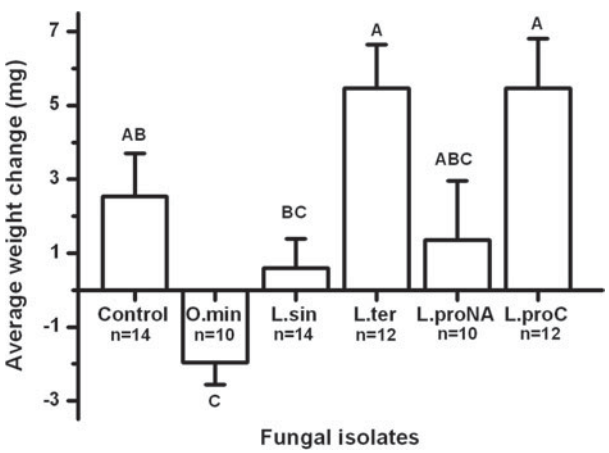

Fig. 2. Average weight change of five *Dendroctonus valens* larvae over six days of feeding on phloem-agar media inoculated with fungi. Bars indicate standard error. Bars with a different letter are significantly different ($\alpha = 0.05$) (LSD multiple comparisons). Abbreviations for fungal names are the same as those in fig. 1.

RTB larval hindgut volatiles extraction and analysis

Due to the difficulty of precisely distinguishing larval instars, larvae were grouped as first/second, second/third, third/fourth, and fourth/fifth instar by comparing head capsule size. Five larvae in each group were randomly chosen, then dissected, and hindguts were immediately immerged into a 250-µl vial insert that was inside a 2-ml glass vial (Agilent, vial insert, 250 µl glass with polymer feet); 20 µl of hexane were used as solvent and 100 ng heptyl acetate

Table 2. Mean (±SE) of hindgut volatiles extracted from different stages of *Dendroctonus valens* larvae.

Hindgut volatiles	Quantity of volatiles (nmol)			
	First/second instar larvae	Second/third instar larvae	Third/fourth instar larvae	Fourth/fifth instar larvae
verbenol	0.0000 ± 0.0000^{c}	0.0018 ± 0.0005^{b}	0.0084 ± 0.0015^{a}	0.0086 ± 0.0010^{a}
verbenone	0.0191 ± 0.0024^{c}	0.0703 ± 0.0162^{b}	0.1805 ± 0.0104^{a}	0.1283 ± 0.0306^{a}
myrtenol	0.0425 ± 0.0025^{c}	0.1356 ± 0.0190^{b}	0.2145 ± 0.0381^{a}	0.0541 ± 0.0125^{c}
myrtenal	0.0014 ± 0.0004^{c}	0.0084 ± 0.0013^{b}	0.0138 ± 0.0010^{a}	0.0000 ± 0.0000^{d}

In each row, values with the same letter in superscript are not significantly different ($\alpha = 0.05$; LSD multi-comparison).

Table 3. One-way ANOVA analyzing effects of four volatiles (verbenone, verbenol, myrtenol, and myrtenal) in three concentrations on linear growth rate of five fungal isolates.

Fungal species	Volatile concentration (mol l^{-1})	$F_{4,20}$	P
Ophiostoma minus	0.1	12.4	<0.001
	0.5	7.7	<0.001
	1	199.3	<0.001
Leptographium sinoprocerum	0.1	391.0	<0.001
	0.5	168.4	<0.001
	1	391.0	<0.001
L. terebrantis (Canada)	0.1	3.7	<0.05
	0.5	5.4	<0.01
	1	81.8	<0.001
L. procerum (China)	0.1	20.1	<0.001
	0.5	81.1	<0.001
	1	185.1	<0.001
L. procerum (North America)	0.1	43.7	<0.001
	0.5	177.3	<0.001
	1	477.2	<0.001

(TCI Shanghai Development Co., Ltd. purity 95%) were added as internal standard (Shi & Sun, 2009). Each instar group had five replications (five vials). Vials were stored at −20°C before chemical analyses.

Extracts were identified on a Hewlett-Packard 6890 gas chromatograph-mass spectral detector (GC-MS) equipped with DB-WAX column (60 m × 250 µm × 0.15 µm). The temperature program was 50°C for 1 min, 5°C min^{-1} to 95°C, 2°C min^{-1} to 110°C, 5°C min^{-1} to 220°C, 10°C min^{-1} to 230°C. The flow of nitrogen was 1.0 ml min^{-1}. Aliquots of extracts (2 µl) were injected splitless at 250°C. Compounds were identified by comparing retention times and mass spectra of synthetic standards. Chemicals used as standards were (s)-*cis*-verbenol (Sigma-Aldrich, Inc, purity 95%), (+)-verbenone (Acros Organics, purity 94%), (1R,-)-myrtenol (Acros Organics, purity 95%), and (-)-myrtenal (Acros Organics, purity 98%).

Extracts were quantified on an Agilent 7890 GC-FID equipped with the same DB-WAX column mentioned above. Compound quantification was based on the internal standard (IS 100 ng of heptyl acetate in each sample; assuming similar or identical response factors between the analytes and the IS). Nitrogen was used as the carrier gas at a constant flow of 1.0 ml min^{-1}, and the injector and detector temperatures were 220°C and 300°C, respectively. Column temperature was 50°C for 1 min, 5°C min^{-1} to 95°C, 2°C min^{-1} to 110°C, 5°C min^{-1} to 220°C, 10°C min^{-1} to 230°C.

Effects of RTB hindgut volatiles on fungal growth

For each fungus, four chemicals (verbenol, verbenone, myrtenol and myrtenal) in three concentrations (0.1, 0.5 and 1 mol l^{-1}), and n-hexane controls were tested. Each treatment and a control had five replications. The method was modified from Hofstetter *et al.* (2005). A 5-mm diameter disk of 2% malt extract agar (MEA) medium colonized by one of the five test fungal isolates (incubated at 25°C in darkness for three weeks) was placed on the center of a 60 × 15 mm Petri dish of 2% MEA. The four volatiles tested in the experiment were (s)-*cis*-verbenol, (+)-verbenone, (1R,-)-myrtenol and (−)-myrtenal. Each compound was dissolved in n-hexane (Beijing Chemicals Works) to get a concentration of 1 mol l^{-1}, 0.5 mol l^{-1} and 0.1 mol l^{-1}.

For each treatment, 20 µl of test solution were absorbed onto a sterile filter paper (15 × 15 mm) and the paper was placed on the lid of a Petri dish containing fungal culture. Plates were sealed with parafilm, placed upside down and incubated in darkness at 25°C. Linear fungal growth was measured in four directions (0°, 90°, 180°, 270°) at 24-h intervals. Tests were continued until one of the fungal treatments reached the edge of the Petri dish. Fungal growth in the four directions was averaged for each replicate.

Statistical analysis

In the 'Effects of fungi on RTB larvae' experiment, we tested for fungal effects by comparing feeding activity (proportion of ALI and ALO) from all the treatments, including the control, with a Pearson's chi-square test (two-tail, $\alpha = 0.05$) (i.e. null hypothesis $fungi_A = fungi_B = fungi_C = fungi_D = fungi_E = control$). Differences in feeding activity between each treatment and the control were also tested with a Pearson's chi-square test (two-tail, $\alpha = 0.05$) (i.e. null hypotheses $fungi_A = control$, $fungi_B = control$, etc.) (Rosner & Sun, 2004). Average weight change of five larvae in each Petri dish among five fungal isolates and a control was compared using one-way ANOVA followed by a LSD multi-comparison. Larval mortality among fungal isolates was compared by Fisher's exact test (two-tail $\alpha = 0.05$) (Rosner & Sun, 2004).

In the 'RTB larval hindgut volatiles extraction and analysis' experiment, quantity of larval hindgut volatiles was compared by one-way ANOVA (two-tail, $\alpha = 0.05$), followed by LSD multi-comparison for each chemical among larval instars.

In the 'Effects of RTB hindgut volatiles on fungal growth' experiment, for each fungal isolate, average fungal linear growth rate (fungal growth divided by number of days cultured) was compared at three specific concentrations separately by one-way ANOVA (chemicals as factor) followed by a Dunnett's multi-comparison with hexane as control.

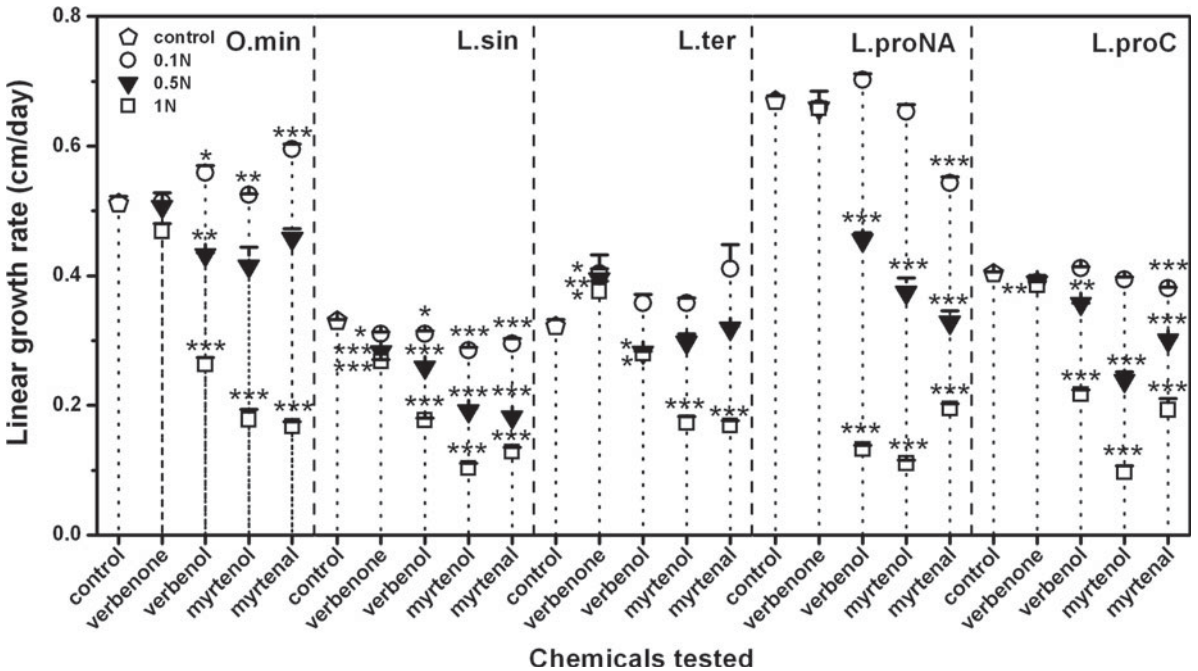

Fig. 3. Linear growth rate (±SE) for each fungus on 2% malt extract agar in the absence (control) or presence of different concentrations of *Dendroctonus valens* hindgut volatiles. For each fungal species/isolate, average fungal linear growth rate (fungal growth divided by number of days cultured) was compared at three specific concentrations separately by one-way ANOVA (chemicals as factor) followed by Dunnett's multi-comparison with hexane as control. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. Abbreviations for fungal name are the same as those in fig. 1.

All tests were performed with the statistical software SPSS for windows 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Effects of fungi on RTB larvae

The feeding activity among treatments, including the control, was significantly different (Pearson's $\chi^2 = 17.92$, df = 5, $N = 380$, $P < 0.01$). In *O. minus* colonized-media, larvae had a low feeding activity (52%), significantly different from the control (77%) (Pearson's $\chi^2 = 9.78$, df = 1, $N = 135$, $P < 0.01$). Feeding activity of *L. sinoprocerum* (81%) (Pearson's $\chi^2 = 0.37$, df = 1, $N = 115$, $P = 0.54$), *L. terebrantis* (78%) (Pearson's $\chi^2 = 0.02$, df = 1, $N = 135$, $P = 0.89$), *L. procerum* (North America, 70%) (Pearson's $\chi^2 = 0.85$, df = 1, $N = 125$, $P = 0.36$) and *L. procerum* (China, 69%) (Pearson's $\chi^2 = 0.80$, df = 1, $N = 140$, $P = 0.28$) was not significantly different from the control (fig. 1).

Mean weight change was significantly affected by fungal isolates after six days ($F_{5,66} = 6.00$, $P < 0.001$). Mean weight change on media discs colonized by *O. minus* decreased and was significantly different from that of *L. terebrantis* ($P < 0.001$), *L. procerum* (Chinese isolate, $P < 0.001$) and control ($P < 0.01$) (fig. 2).

Total larval mortality was 12%, and no significant differences were found in mortality among the treatments, including control (Fisher's exact test = 10.00, $P = 0.07$).

RTB larval hindgut volatiles extraction and analysis

We identified verbenol, verbenone, myrtenol and myrtenal from the hindgut extracts of RTB larvae. From these volatiles, verbenone and myrtenol were major components and verbenol and myrtenal were minor components in all larval stages. Verbenol and myrtenal were not detected in first/second and fourth/fifth instar larvae (table 2).

There were significant differences among larval stages in volatile contents verbenone ($F_{3,16} = 34.07$, $P < 0.001$), myrtenol ($F_{3,16} = 24.48$, $P < 0.001$), verbenol ($F_{3,16} = 40.53$, $P < 0.001$) and myrtenal ($F_{3,16} = 87.92$, $P < 0.001$).

Effects of RTB hindgut volatiles on fungal growth

For each fungus, all the three concentrations of volatile chemicals significantly affected linear growth rates (table 3). Multi-comparison with the hexane control showed that *O. minus* was significantly inhibited by verbenol, myrtenol and myrtenal at 1 mol l^{-1}. Myrtenal caused the lowest linear growth rate, followed by myrtenol and verbenol. However, growth of *O. minus* was enhanced by verbenol, myrtenol and myrtenal at low concentration (0.1 mol l^{-1}) and was significantly different from control. *Leptographium sinoprocerum* was significantly inhibited by all four volatiles in the three concentrations compared to the control. *Leptographium terebrantis* was only significantly inhibited by verbenol, myrtenol and myrtenal at 1 mol l^{-1}. For the Chinese isolate *L. procerum*, growth rate was inhibited by verbenol, myrtenol and myrtenal at 0.5 mol l^{-1} and 1 mol l^{-1}; myrtenal significantly inhibited growth rate at 0.1 mol l^{-1}. The North American isolate *L. procerum* was significantly inhibited by verbenol, myrtenol and myrtenal at 0.5 mol l^{-1} and 1 mol l^{-1}; myrtenal also significantly inhibited the fungal growth rate at 0.1 mol l^{-1} (fig. 3).

Discussion

RTB larvae fed on *O. minus* had reduced larval boring activity as well as a decrease in body weight. The mechanisms by which *O. minus* caused this detrimental effect remains to be studied, but one way in which the fungus could achieve this includes a change in the nutritional characteristics of the diet, which may involve reduced protein and caloric content. In North America, *O. minus* is not reported as an associate of RTB, but in China this fungus is closely related to both larvae (Wang *et al.*, unpublished data) and adults (Lu *et al.*, 2009a). By affecting larval performance, *O. minus* may have negative effects at the population level, and this may have constituted an obstacle to its invasion in China. *Leptographium* (from native ranges and invaded ranges) fungi had no significant effects on RTB larvae. Boring percent and body weight change were not significantly different between larvae feeding on *L. sinoprocerum*, *L. terebrantis* and *L. procerum* (both isolates) and control. Larvae exhibited low mortality, suggesting that these fungi may not lead directly to death. Previous work suggests that the most important role of these fungi is to assist RTB to overcome tree defense (Lu *et al.*, 2010). In China, *L. sinoprocerum* and *L. procerum* (Chinese isolate) are most likely fulfilling this role. Further studies should focus on the establishment dynamics of fungal associates in RTB galleries along with the development of larvae.

Fungi growth, in all isolates, was dramatically inhibited by verbenol, myrtenol and myrtenal at high concentration ($1\,mol\,l^{-1}$). Inhibition by these chemicals decreased at 0.5 mol l^{-1}. At $0.1\,mol\,l^{-1}$, these chemicals caused little or no growth inhibition in all isolates, with the exception of *O. minus*, whose growth was stimulated. Verbenol and myrtenol have also been detected in pine trees (Fernandez *et al.*, 2009); therefore, the enhanced growth of *O. minus* may reflect an adaptation of this fungus to host pines and vector beetles. Myrtenol is the dominant compound in the hindgut of RTB in China but is not present in the hindgut of North American beetles (Luxova *et al.*, 2007). Myrtenol is an efficient fungal growth inhibitor and might be an unknown chemical weapon of RTB to restrain growth of detrimental fungi. Although most fungi are only inhibited at high concentrations, we suggest that these chemicals may have a significant ecological function by regulating fungi prevalence in RTB galleries. Verbenol, myrtenol and myrtenal are constantly produced by all larval instars; and, thus, they could have a relatively high concentration in the galleries, which are small spaces. These volatiles may also synergistically affect fungal growth.

Although invasive species can thrive in introduced ranges, the invaded communities may resist invasion through a variety of ecological mechanisms, including predation, competition or disease (Hunt & Yamada, 2003; Levine *et al.*, 2004; Parker & Hay, 2005). Our results show a mutually antagonistic relationship between an invasive bark beetle, *D. valens*, and an indigenous fungal associate, *O. minus*, in China, which might reflect a natural defense of a native species against an exotic invader. RTB probably adapted to overcome this defense mechanism by suppressing detrimental fungus growth and regulating fungal abundance in the galleries using a chemical arsenal. Detecting these particular interactions is not only crucial to comprehend the complexity of insect multitrophic interactions but also to understand the mechanisms of invasion in organisms as aggressive as *D. valens*, with over 500,000 ha of invaded forest (Yan *et al.*, 2005). From this perspective, our study may also set the base to develop innovative control and management strategies in this and other systems.

Acknowledgements

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