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Original article

Field trials of potential attractants and inhibitors for pine shoot beetles in the Yunnan province, China

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Abstract – Funnel traps and trap log bundles were used to test potential attractants and inhibitors for a new species of pine shoot beetle in the genus *Tomicus* (Coleoptera: Scolytidae) in the Yunnan province, China. The trap log bundles were attractive to beetles, and untreated bundles were heavily attacked. Verbenone, either alone or in combination with non-host volatiles (3 green leaf C_6 alcohols and 1 bark C_8 alcohol), significantly reduced attacks on the bundles. The non-host volatiles alone did not inhibit attack. Neither of the two monoterpene blends tested in funnel traps caught many beetles, possibly because the traps were run during the period of beetle movement to the trunks for breeding.

pine shoot beetle / Yunnan pine / semiochemical / verbenone / non-host volatile

Résumé – Tests de terrain de substances potentiellement attractives et répulsives pour les scolytes des pousses de pins dans la province chinoise du Yunnan. Des pièges à entonnoir et des rondins-pièges ont été utilisés afin de tester l'effet de substances potentiellement attractives ou inhibitrices pour les scolytes des pousses de pins, *Tomicus* spp. (Coleoptera: Scolytidae), dans la province du Yunnan en Chine. Les rondins-pièges étaient attractifs pour les scolytes, et les rondins non traités ont été sévèrement attaqués. La verbénone, seule ou en combinaison avec des substances volatiles non produites par les pins-hôtes (3 alcools en C_6 de type feuillage vert et 1 alcool en C_8 de type écorce), a significativement réduit l'attaque des rondins. L'utilisation des seules substances volatiles non-hôtes n'empêche pas l'attaque. Aucun des deux mélanges de monoterpènes testés dans les pièges à entonnoir n'a capturé un nombre important d'insectes. Ce résultat pourrait être dû au fait que les pièges ont été utilisés durant la période où les scolytes se déplacent vers les troncs pour la reproduction.

scolyte des pousses de pins / pin du Yunnan / composé semiochimique / verbénone / susbtances volatiles non-hôtes

1. INTRODUCTION

A new species of pine shoot beetle, *Tomicus* n. sp. [6], has caused extensive mortality of Yunnan pines, *Pinus yunnanensis* Franch., in the Yunnan province of China, affecting over 200 000 ha of pine plantations [14, 15]. Ye and Lieutier [30] and Långström et al. [11] noted behavioral differences between this species and *Tomicus piniperda* (L.), a major pest in Europe, North America, and other parts of China. After emergence, both species engage in maturation feeding on the shoots before attacking the tree bole [1, 8–10, 16, 25]. For *T. piniperda*, shoot feeding generally occurs in the vicinity of the brood trees [10, 19]. Aggregation occurs when trunk attacks are initiated, and the beetles usually do not select shoot-attacked trees for trunk attack [10, 19].

In contrast, Ye and Lieutier [30] found that pine shoot beetles in the Yunnan province aggregate during maturation feeding. Emerging adults fly to the shoots starting in March, with peak flight in mid-June [13]. A long period of maturation feeding ensues, lasting six to eight months [25]. Trunk attacks occur from December through May, peaking from January-March. Trunk attacks begin in the crown and spread down the bole, often on the same trees affected by severe shoot-feeding [27]. Recent studies have shown that shoot feeding can weaken the host trees, leading to tree mortality if followed by trunk attacks [9, 14, 28].

The identification of the new species of *Tomicus* makes the interpretation of previous work difficult. *Tomicus piniperda* and *T. minor* have been reported from the Yunnan province [11, 30], but it is unclear how much of the damage and mortality attributed to these species in the literature was actually caused by *Tomicus* n. sp. As *Tomicus* n. sp. and *T. piniperda* are morphologically undistinguishable [6], effective lures for *Tomicus* n. sp. would help researchers decipher the ecological roles of *Tomicus* species present in the Yunnan province.

Tomicus piniperda does not produce an aggregation pheromone, but it is readily attracted to freshly cut pine logs and stumps, as well as stressed, dying, or recently killed pines [10,

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Table I. Content and release rates of attractants and inhibitors tested against *Tomicus* sp. in Yunnan province, China, 2001.

Yunnan Blend (YB) 15 mL in wicked polyethylene bottle release rate: 125–150 mg/d at 22 °C	65% α-pinene (75% (+)) 15% (–)-β-pinene 15% terpinolene 5% myrcene
Jilin Blend (JB) 15 mL in wicked polyethylene bottle release rate: 125–150 mg/d at 22 °C	40% α-pinene (75% (+)) 20% nonanal 20% Δ3-carene 20% terpinolene
Non-host volatiles 15 mL in wicked polyethylene bottle release rate: 15–30 mg/d at 22 °C	25% 1-hexanol[a] 25% (*Z*)-3-hexen-1-ol[a] 25% (*E*)-2-hexen-1-ol[a] 25% (±)-3-octanol[b]
Verbenone 5 mL in 1.5 mil polyethylene pouch release rate: 25 mg/d at 20 °C	100% verbenone (80%(–))

[a] Green leaf C_6 alcohols.
[b] Bark C_8 alcohol.

Table II. Combinations of potential attractants (YB: Yunnan Blend, JB: Jilin Blend) and inhibitors (VN: verbenone, NHV: non-host volatiles) tested for pine shoot beetles in funnel trap or trap log bundles studies in Yunnan province, China, 2001.

Funnel Trap (FT) test	Trap Log Bundle (TLB) test
FT only	TLB only
FT + YB	TLB + NHV
FT + YB + NHV	TLB + NHV + VN
FT + YB + NHV + VN	TLB + VN
FT + YB + VN	
FT + JB	

22]. This species utilizes the host monoterpenes α-pinene, terpinolene, and Δ3-carene to locate suitable hosts [4, 12, 24]. Low levels of ethanol also may attract *T. piniperda* or synergize the attractiveness of the host monoterpenes [5, 23, 24]. Verbenone and non-host volatiles have been shown to inhibit the attractiveness of host kairomones to *T. piniperda* [3, 17, 20–22]. Unlike *T. piniperda*, *T. minor* females produce a pheromone [12].

Recent studies indicate that *Tomicus* n. sp. does not readily respond to the monoterpene combination of attractants described above [31, 32]. The development of species-specific lures for *Tomicus* n. sp. would help researchers identify and ascertain which species were involved in the aggressive tree-killing behavior observed in the Yunnan province [13, 25, 26]. The objectives of this study were to test the attractiveness of two blends of host volatiles for *Tomicus* n. sp. in the Yunnan province, and to determine if the inhibitors utilized for *T. piniperda* also deter the response of the new species.

2. MATERIALS AND METHODS

2.1. Study site

The experiment was conducted in a 200 ha plantation of Yunnan pine, located on a small mountain near Qujing in the Yunnan province. The trees were 25 years old and ranged from 10–15 m in height and 10–15 cm in diameter. Trees appeared stressed and many yellow needles were observed. Larvae of the pine caterpillar, *Dendrolimus houi* L., were feeding at the time of the study, adding to the tree stress. Moderate damage by pine shoot beetles was evident in the plantation, and no control measures had been implemented by the onset of the study.

2.2. Attractants and inhibitors

A blend of high (+)-α-pinene, terpinolene, Δ3-carene, and nonanal was used to increase trap catch of *T. piniperda* in a trapping study in the Jilin province (Steve Burke, pers. comm., Phero Tech Inc., Canada) This Jilin blend (JB) and a new Yunnan blend (YB) were tested as attractants. The YB lure design was based on a profile of volatiles from cones collected in the Yunnan province (A. Roques, pers. comm., INRA-CRF, Orléans, France). This blend of monoterpenes was used because it was the only lure readily available at the time of the study that was manufactured based on host volatiles from Yunnan pine. Verbenone and non-host volatiles (3 green leaf C_6 alcohols and 1 bark C_8 alcohol) were tested as inhibitors. Specific data on the attractants and inhibitors are given in Table I. All experimental materials were provided by Phero Tech Inc. (Delta, B.C, Canada).

2.3. Experimental design

Two experiments were conducted from February 14–March 14, 2002. In the first, trap log bundles were used to test the effectiveness of the inhibitors. Each bundle was composed of 3 logs, 2 m long and approximately 10 cm in diameter. No bundle contained two logs from the same tree. All trees selected for constructing the bundles were free of beetle attacks (no beetle entrance holes evident). Two logs formed the base of the bundle with the third log on top. One end of the bottom logs was placed on another short log to raise the ends and to increase available surface area for attack. The ends of the logs were waxed to prevent desiccation. Four treatments were tested (Tab. II), with ten replicates per treatment. The bundles were placed at 50 m intervals, and treatment assignment was random. The inhibitors were attached to the middle of the upper log of the bundle. The number of beetle entrance holes per trap bundle was counted at the end of one month.

In the second study, 30 Lindgren funnel traps (8-funnel model) were used to test both lures and inhibitors. The traps were arranged at 50 m intervals in a 6 × 5 grid, with six treatments (Tab. II) replicated 5 times each. The traps were suspended from poles so that the collection cups were 2 m above the ground. A 2 × 2 cm piece of pesticide strip was placed in the collection cup to kill the captured beetles and prevent escape. Treatment assignment was random, and the lures and inhibitors were attached between the 2nd and 3rd funnel from the top of the trap. Trap catches were collected weekly and the treatments then randomly reassigned to the traps. The funnel trap and trap log bundle studies were located on opposite sides of the mountain, separated by over 100 m.

2.4. Data analysis

Data were checked for normality and a log transformation performed when necessary. Data were subjected to an analysis of variance (ANOVA) using SAS GENMOD [18]; followed by a Tukey's HSD test, $P \leq 0.05$.

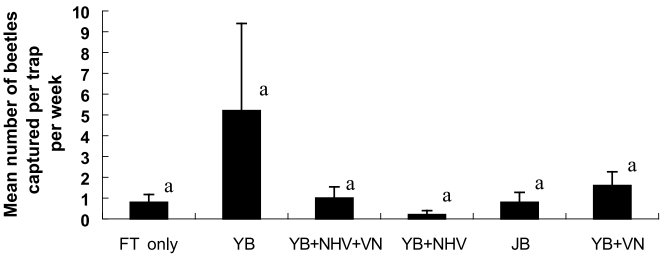

Figure 1. Weekly mean trap catch (± SE) of *Tomicus* sp. in Lindgren funnel traps baited with various combinations of potential attractants and inhibitors, 5 replications per treatment. FT: funnel trap, YB: Yunnan Blend, JB: Jilin Blend, NHV: non-host volatiles, VN: verbenone (see Tab. I for detailed information on attractants and inhibitors). Means with the same letter are not significantly different (ANOVA, Tukey's HSD test, $P \geq 0.05$).

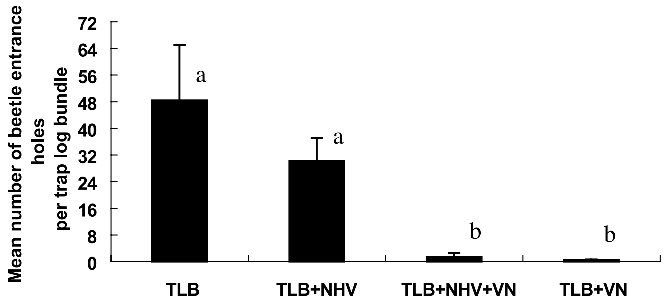

Figure 2. Mean attacks (± SE) by *Tomicus* sp. on trap log bundles treated with combinations of potential inhibitors, 10 replications per treatment. TLB: trap log bundle, NHV: non-host volatiles, VN: verbenone (see Tab. I for detailed information on inhibitors). Means with the same letter are not significantly different (ANOVA, Tukey's HSD test, $P \geq 0.05$). Data transformed by log transformation prior to analysis.

3. RESULTS

In the trap log bundle test, attack rates were significantly lower on trap log bundles treated with verbenone alone or in combination with non-host volatiles (Fig. 1). Seven of the ten bundles in each of the treatments with verbenone had no sign of beetle attack. Of the attacked bundles in the 2 treatments with verbenone, one bundle had 12 attacks, while the remainder had 2 or fewer attacks. The non-host volatiles tested did not significantly reduce beetle attack, and their application in combination with verbenone did not inhibit attacks in comparison with verbenone application alone.

Neither the Jilin nor Yunnan monoterpene blends appeared attractive to the pine shoot beetles in Yunnan, as beetle catch in traps baited with either lure did not differ significantly from unbaited traps (Fig. 2). Very few *Tomicus* were captured in any of the funnel traps. Untreated trap log bundles placed in the field during the same time period as the funnel traps were heavily attacked by pine shoot beetles (Fig. 1). The number of attacks on the trap log bundles indicates that sufficient numbers of beetles were present and dispersing to provide a valid test of the attractants and repellents.

The low beetle catches in the funnel traps did not allow an additional evaluation of the effectiveness of verbenone and non-host volatiles as inhibitors.

4. DISCUSSION

Trunk attacks by *Tomicus* n. sp. are a major cause of tree mortality in the Yunnan province [25]. Our results indicate that verbenone applications on pines may effectively reduce the number of *Tomicus* attacks. However, this and a previous study [20] only tested verbenone for the prevention of *Tomicus* attacks on logs. The protection of living trees may be more difficult. The intensive, aggregated shoot-feeding in the Yunnan province appears to predispose pines for subsequent trunk attacks [11], and may occur during a single generation [30] or over several generations [11]. Also, trunk attacks begin in the crown on Yunnan pines [29]. Studies investigating the appropriate number and placement of verbenone elution devices on individual trees and the timing of the applications are necessary for designing effective prevention treatments.

The low number of beetles collected in the traps baited with the JB lure suggests that *T. piniperda* was not present in the area. These results provide further evidence that *Tomicus* n. sp. primarily is responsible for the tree mortality observed in the Yunnan province.

The monoterpene blends tested in this study would not be useful for monitoring *Tomicus* populations in the Yunnan province, at least during the period when the adults were moving from the shoots to the trunks. Recent work by Borg-Karlson et al. [2] and Fäldt [7] documented differences between the monoterpene content of Yunnan pine and other pine species, and between the trunk xylem and twig xylem and phloem of Yunnan pines. Unlike *Pinus sylvestris* L., a major host of *T. piniperda* in Europe, the trunk xylem of *P. yunnanensis* contained very little Δ3-carene [2], which may be a reason the JB lure was not attractive. The YB monoterpene blend in this study was derived from an analysis of the cones. As in the YB lures, twigs and the trunk of *P. yunnanensis* contained significant percentages of α-pinene and (-)-β-pinene [2]. However, only the twigs had myrcene and terpinolene as main components, suggesting any attractiveness of the YB lures may be in the late spring-early summer, when the beetles are dispersing to the shoots. Our results and the profiles of monoterpene composition of Yunnan pines [2, 7] could be used to guide the development of new lures for *Tomicus* n. sp.

The timing of the study also may have affected the negative results of the tests with the non-host volatiles. While avoiding non-host volatiles would have adaptive value in the search for suitable hosts for maturation feeding [17], Schlyter et al. [21] suggest that non-host volatiles may not be ecologically relevant to *Tomicus* in their search for breeding sites. Further testing of the effects of non-host volatiles as a deterrent to maturation feeding is necessary to evaluate their potential in *Tomicus* management in Yunnan province.

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