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Effectiveness of modified White's solution at removing ascomycetes associated with the bark beetle *Ips pini*

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Summary

Modified White's solution (1 g $HgCl_2$/l H_2O) is widely used to surface disinfest bark beetles of their phoretic fungi. We investigated the effectiveness of this solution at disinfesting adult *Ips pini* from its associated ophiostomatoid fungi. A treatment for 1, 4 or 8 min does not completely rid beetles of phoretic fungi, but does substantially reduce the amount of fungi they carry externally. Sterilizing with modified White's solution caused limited mortality (< 16%).

1 Introduction

Bark beetles (Coleoptera: Scolytidae) and the fungi they transport cause severe damage to coniferous forests in the United States. Bark beetles inoculate host trees with various microorganisms during colonization. Ophiostomatoid fungi are of particular importance because they are the most consistent associates and because some species are pathogenic to trees (Paine et al. 1997). Thus, there is considerable interest and debate concerning the nature of bark beetle–ophiostomatoid fungal relationships, which have been interpreted as ranging from mutualistic to antagonistic (Klepzig et al. 2001). There is a need for a method that provides phoront-free beetles, particularly when trying to characterize the net impact of symbionts on beetle fitness. The most commonly used method for surface disinfecting beetles consists of rinsing beetles with a modified White's solution (1 g $HgCl_2$/l H_2O) (Barras 1972; Goldhammer et al. 1990; Six and Paine 1998). We tested the effectiveness of modified White's solution at removing phoront fungi from *Ips pini* (Say) (Coleoptera: Scolytidae) and its effect on beetle survival.

2 Materials and methods

Groups of 20 adult *I. pini* were subjected to one of four treatments. For the modified White's solution treatments, beetles were immersed in a series of four beakers, that in sequence, contained sterilized distilled water, modified White's solution, sterilized distilled water, and sterilized distilled water. The three sterilized distilled water rinses were conducted for 1 min. each. The immersion in modified White's solution was for 1, 4 or 8 min, depending on treatment. The 4-min exposure to modified White's solution was the same as used by Barras (1972). For the control treatment, beetles were washed with sterilized distilled water for 1, 4, 1 and 1 min. After the beetles were exposed to their respective treatment, half were crushed and half were left intact. Each beetle was then rolled onto 1% v/w malt extract agar (MEA) plates amended with 0.2% v/w cycloheximide and 0.1% v/w streptomycin sulfate, which preferentially selects for ophiostomatoid fungi

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(Harrington 1992). Plates were incubated at 22°C in darkness for 5 days. Fungal species found in each plate were assigned a coverage value using the Daubenmire (1959) method, which included six classes: 0, 0–5, 5–25, 25–50, 50–75 and 75–100%. Data were ranked prior to statistical analysis (PROC RANK), because variance was heterogeneous across treatments. Ranked data were analyzed using anova (PROC MIXED; Littell et al. 1996). Mean values and standard errors (prior to any transformation) were calculated using the PROC MEANS procedure and are reported for each sterilization × beetle treatment combination.

3 Results and discussion

Overall, modified White's solution reduced, but did not eliminate phoretic fungi. Three fungi were found consistently during the study; a yeast and two *Ophiostoma* [*O*. sp. A (most likely *O. ips* or *O. nigrocarpum*) and an unidentified species (*O*. sp. B)]. The occurrence of yeast was affected by both the disinfestant and beetle treatments (Table 1). White's solution reduced the occurrence of yeast on intact but not in crushed beetles relative to their respective control (Fig. 1). Intact beetles in the 1- and 4-min disinfestant treatments had low occurrences of yeast, and no yeast was detected in the 8-min treatment. Exposure to modified White's solution, regardless of duration, reduced the presence of *O*. sp. A (Table 1, Fig. 1). There was little difference in occurrence of *O*. sp. A between intact and crushed beetles. *Ophiostoma* sp. A was not detected on intact beetles exposed to 4 and 8 min of modified White's solution. The occurrence of *O*. sp. B depended on the duration of exposure to modified White's solution and whether beetles were crushed (Table 1). Intact beetles had lower occurrence of *O*. sp. B after 1 and 4-min exposure than did those in the control treatment. Beetles in the 8-min exposure, however, had similar amounts of *O*. sp. B as did those in the control treatment. Among crushed beetles, modified White's solution did not significantly reduce levels of *O*. sp. B relative to those in the control. *Ophiostoma* sp. B was present under all conditions, but occurrence was least frequent among intact beetles exposed to White's solution for 4 min. Beetle survivorship was greater than 84% for all treatments.

Modified White's solution was most effective at externally disinfesting beetles, with beetles exposed for 4 min having the lowest incidence of fungi. Eight-minute exposure completely eliminated yeast and *O*. sp. A. We are not certain, however, as to why the 8-min exposure did not affect levels of *O*. sp. B. Nevertheless, increasing the exposure time to modified White's solution would likely have completely eliminated all three species of fungi from the beetle's exterior. Additionally, amending modified White's solution with a surfactant (e.g. Tween-80) may improve efficacy by increasing ability of the solution to penetrate into the sutures and pits on the beetle's exoskeleton.

For crushed beetles, we found little difference between the occurrence of fungi between treatments for yeast and *O*. sp. B. The disinfestant, however, reduced the presence of *O*. sp. A. These results suggest that modified White's solution does not disinfest beetles internally and that *O*. sp. A is primarily carried externally. Our data agree with that of Furniss et al. (1995), who found that *I. pini* carries the pathogenic fungus *O. ips* both

Table 1. **Summary of *p*-values for the effects of sterilization and beetle treatments on fungi. Analysis based on ranked data.**

Main effects and interaction	Yeast	*O*. sp. A	*O*. sp. B
Sterilization treatment	< 0.001	< 0.001	0.399
Beetle treatment	< 0.001	0.036	0.019
Two-way interaction	0.005	0.008	0.013

Beetle treatment = intact or crushed beetles.

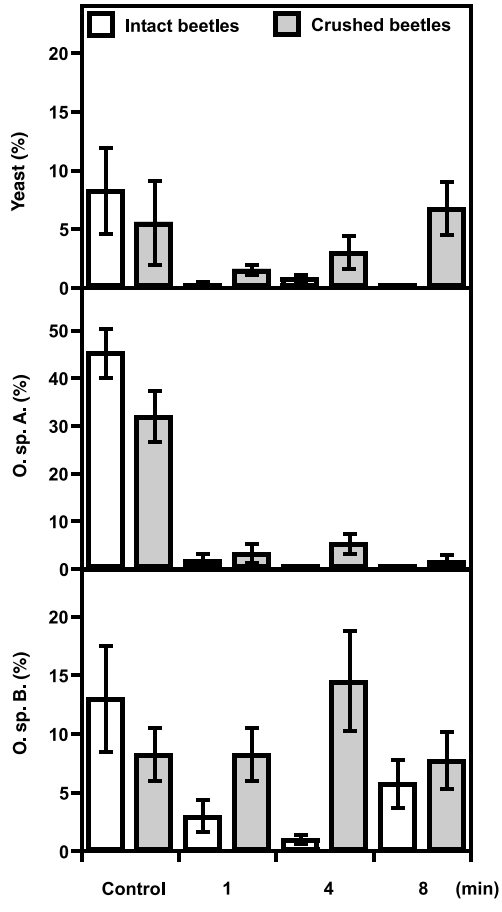

Fig. 1. Effectiveness of modified White's solution as a disinfestant. Reported are the average coverage values of the three fungi found on *Ips pini*. Error bars indicate ±1 standard error.

externally (head, prothorax and elytra) and internally (alimentary canal). Spores of yeasts and other fungi have also been found in the gut of *I. avulsus* (Gouger et al. 1975) and *I. typographus* (Leach et al. 1934; Furniss et al. 1990).

In conclusion, modified White's solution did not completely disinfest beetles of all phoretic fungi, but greatly reduced the amount of yeast and ophiostomatoid fungi carried on the beetles' exterior. Moreover, modified White's solution caused minimal mortality (< 16%) even after 8 min of exposure, which is similar to levels reported by Barras (1972). While modified White's solution does not provide completely axenic beetles, it can be useful when externally clean beetles are required for experimental purposes.

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Résumé

Efficacité d'une solution de White modifiée pour supprimer les champignons ascomycètes associés au scolyte Ips pini

La solution modifiée de White (1 g $HgCl_2$ / L eau) est largement utilisée pour désinfecter les scolytes de leur champignons phorétiques. Nous avons étudié l'efficacité de cette solution pour désinfecter des adultes d'*Ips pini* de leurs champignons associés du groupe des *Ophiostomatales*. Un traitement de 1, 4 ou 8 minutes réduit significativement la quantité de champignons phorétiques, sans les éliminer complètement. La désinfection avec la solution de White modifiée a causé une mortalité réduite (< 16%).

Zusammenfassung

Wirksamkeit einer modifizierten Lösung nach White zur Entfernung von Ascomyceten, die mit dem Borkenkäfer Ips pini assoziiert sind

Eine modifizierte Lösung nach White (1 g $HgCl_2$/L H_2O) wird häufig zur Desinfektion von Borkenkäfern von ihren phoretischen Pilzen verwendet. Wir untersuchten die Wirksamkeit dieser Lösung bei *Ips pini* gegen die mit diesem Käfer assoziierten ophiostomatoiden Pilze. Die Behandlung (1, 4 oder 8 Minuten) befreite die Käfer nicht völlig von den phoretischen Pilzen, reduzierte den Befall der Käferoberfläche jedoch deutlich. Die Behandlung verursachte nur eine beschränkte Mortalität (unter 16 %).

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