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EFFECT OF VOLATILE CONSTITUENTS FROM *Securidaca longepedunculata* ON INSECT PESTS OF STORED GRAIN

THAMARA K. JAYASEKARA,[1] PHILIP C. STEVENSON,[1,2] DAVID R. HALL,[1] and STEVEN R. BELMAIN[1,*]

[1]*Natural Resources Institute, University of Greenwich, Central Avenue Chatham Maritime, Kent ME4 4TB, United Kingdom*

[2]*Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, United Kingdom*

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Abstract—*Securidaca longepedunculata* Fers (Polygalaceae) is commonly used as a traditional medicine in many parts of Africa as well as against a number of invertebrate pests, including insects infesting stored grain. The present study showed that *S. longepedunculata* root powder, its methanol extract, and the main volatile component, methyl salicylate, exhibit repellent and toxic properties to *Sitophilus zeamais* adults. Adult *S. zeamais* that were given a choice between untreated maize and maize treated with root powder, extract, or synthetic methyl salicylate in a four-way choice olfactometer significantly preferred the control maize. Methyl salicylate vapor also had a dose-dependant fumigant effect against *S. zeamais*, *Rhyzopertha dominica,* and *Prostephanus truncates,* with a LD_{100} achieved with a 60 μl dose in a 1-l container against all three insect species after 24 hr of exposure. Probit analyses estimated LD_{50} values between 34 and 36 μl (95% CI) for all insect species. Furthermore, prolonged exposure for 6 days showed that lower amounts (30 μl) of methyl salicylate vapor were able to induce 100% adult mortality of the three insect species. The implications are discussed in the context of improving stored product pest control by small-scale subsistence farmers in Africa.

Key Words—*Securidaca longepedunculata*, Polygalaceae, methyl 2-hydroxybenzoate, methyl salicylate, olfactometer, fumigant toxicity, *Sitophilus zeamais*, *Rhyzopertha dominica*, *Prostephanus truncatus.*

INTRODUCTION

Interest in the discovery of new chemicals for the control of insect pests has continued to increase despite the commercial difficulties of bringing new products

* To whom correspondence should be addressed. E-mail: s.r.belmain@gre.ac.uk

to market (Isman, 1997). Although much of this work is agrochemically driven to find novel compounds and modes of action, other aspects of bioprospecting such as providing low-cost "natural" alternatives to synthetic pesticides, which are safer and less polluting, have also continued. Poor rural farmers in developing countries particularly have difficulty in accessing good quality and affordable synthetic pesticides that are suited to their needs (Belmain and Stevenson, 2001). These farmers, however, have access to local ethnobotanicals and indigenous knowledge systems that could help increase agricultural productivity with minimal human and environmental health hazards that are often experienced when synthetic pesticides are used inappropriately (Belmain, 2002).

Securidaca longepedunculata Fers (Polygalaceae) is commonly used as a medicine in many parts of Africa for the treatment of rheumatic conditions, fever, headache, and various other inflammatory conditions (Oliver-Bever, 1986; Assi and Guinko, 1991; Iwu, 1993). Powdered dried roots are also used as a pest control agent and have potential as a protectant against insect pests in stored grain (Belmain et al., 2001; Boeke et al., 2001). During research to investigate the active principles in *S. longepedunculata*, we analyzed the volatile fraction and found a single major component, methyl salicylate (methyl 2-hydroxybenzoate), accounting for over 90% of volatile material together with two related minor components (Jayasekara et al., 2002). In this report, we evaluate the deterrent and toxic effects of methyl salicylate, the crude root material of *S. longepedunculata,* and a methanol extract of the root material against three major stored product pests, *Rhyzopertha dominica*, *Sitophilus zeamais*, and *Prostephanus truncatus* to determine if methyl salicylate is responsible for use of the plant material as a pest control agent. The implications of these results in storage pest management are discussed.

METHODS AND MATERIALS

Insects and Test Materials. Strains of *Sitophilus zeamais* Motschulsky, *Rhyzopertha dominica* (Fabricius), and *Prostephanus truncatus* (Horn) originally collected in Ghana were reared in the laboratory on organic maize, wheat, and maize, respectively, at 27 ± 5°C, 60 ± 5% RH, and a 12L:12D photoperiod. Roots of *S. longepedunculata* were collected from northern Ghana where they were immediately shade-dried for a period of 2 wk before transport to the UK. The plant roots were ground to a fine powder and extracted in methanol according to the methods described by Jayasekara et al. (2002). Methyl salicylate (Aldrich, Gillingham, Dorset, UK; 98%) was used for all bioassays.

Four-Way Choice Olfactometer Bioassay. Maize grain was treated with *S. longepedunculata* root powder, its methanol extract, methyl salicylate, and methanol only (as the control). The dried methanol extract (0.3 g), prepared according to Jayasekara et al. (2002), was re-dissolved in methanol (5 ml) and mixed with maize (25 g) in a 100 ml glass jar for 1 min. Treated maize was dried for 15 min under a fume hood at room temperature (18°C). Another two sets of maize (25 g each) were treated separately with methyl salicylate (1.4 mg in 5 ml methanol) and methanol only (5 ml) by following the same procedure described above. Three glass tubes (15 cm × 2 cm diam) were filled separately with the maize samples and connected with polythene tubes to three of the four outlets of a four-way choice olfactometer arena as illustrated by Bashir et al. (2001). The fourth outlet of the arena was connected to a tube containing maize (25 g) mixed with *S. longepedunculata* root powder (1.25 g). A continuous stream of air was passed through each treatment and into the arena by using a vacuum pump (DA7C, Charles Austin Ltd, UK). The airflow rate through each treatment into the arena was set at 150 ml/min by using flow meters (D1X640, Meterate GPE, UK). Unsexed *S. zeamais* adults (7–14-days old; 200) that had been deprived of food for 4 days were introduced into the center of the olfactometer arena. The location of each insect was recorded after 12 hr. The bioassay was carried out in a controlled temperature and humidity room (27 ± 5°C and 60 ± 5% RH). Each experiment was repeated ×12, rotating the tubes containing the different maize commodity clockwise to the adjacent delivery pipe between trials to correct for any potential photoresponse behavior. The mean numbers of live adults in each treatment were compared by ANOVA, and means were separated by the LSD test at the 95% confidence level.

Fumigant Potential of Methyl Salicylate. A piece of cotton wool (8 mm × 3.5 mm height) was fixed to the center on the interior surface of a plastic lid to serve as a vapor diffuser inside a glass jar (1 l). Methyl salicylate (10, 20, 30, 40, 50, 60, and 70 μl) was applied with a syringe to the diffusers with 10 replicates per concentration per insect species. Fifty unsexed *S. zeamais*, *R. dominica*, and *P. truncatus* adults (7–14-days old) were introduced separately into glass jars (1 l), and the jars were capped with the lids holding the diffusers. After 24 hr, the dead adults in each jar were removed to separate clean jars and kept for a further 24 hr to confirm whether they were dead or alive, at which time the numbers of dead adults were recorded. The numbers of dead adults were subjected to Probit analysis to estimate the LD_{50} values of methyl salicylate.

To evaluate the effect of prolonged exposure of adult insects to low concentrations of methyl salicylate, the above experiment was repeated using 10 and 30 μl methyl salicylate and an untreated control. Dead adults in each jar were removed at 2-day intervals up to 6 days and collected in separate clean jars. As previously described, the total number of dead adults in each clean jar was recorded after a further 24 hr. The mean numbers of dead adults in each treatment were analyzed by ANOVA with the significant differences between means compared by using the LSD test. All statistical analyses were carried out using the program SPSS v.10 (SPSS Inc., Chicago, IL 60606).

RESULTS

The data from the choice olfactometer bioassay showed that the number of live *S. zeamais* adults found in maize treated with the *S. longepedunculata* root powder, methanol extract of the roots, and methyl salicylate was significantly lower than the number of insects present in the control (methanol only) maize (LSD, $P < 0.05$, Figure 1). Approximately half of all "non-participating" insects remaining in the arena were recorded as dead at the end of the trial. Living insects in the arena that had not made a choice at the time of data collection had usually settled along the edges of the arena. There were no significant differences in the number of insects present among the three treatments, suggesting the repellent effects of the treatments were similar.

The fumigant effects of methyl salicylate were evident and showed that insect mortality increased with increasing concentration (Figure 2). Doses of methyl salicylate above 10 μl in the 1-l jar increased insect mortality in all three insect species when compared to the untreated controls (LSD, $P < 0.05$). The observed mortality rates were dose-dependent, and 100% mortality occurred at 24 hr when there was 60 μl of methyl salicylate in the jars. Linear and nonlinear regression analyses of the data showed cubic polynomial relationships for each insect species with R^2 values of 0.98, 0.97, and 0.97 for *S. zeamais*, *R. dominica*, and *P. truncatus*, respectively. The LD_{50} and LD_{90} values, estimated by Probit analysis, showed similar values for all three insect species (Table 1).

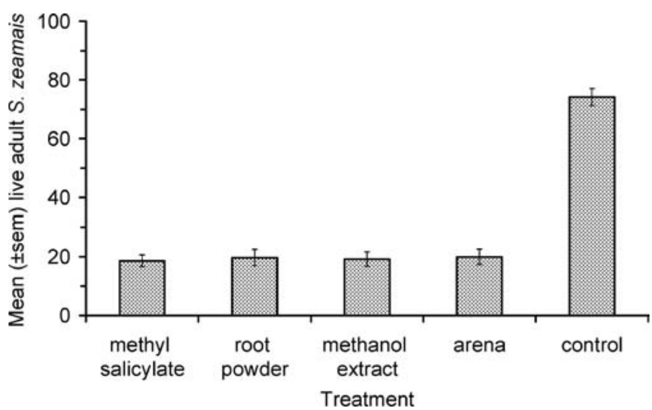

FIG. 1. Mean number of adult *S. zeamais* present in different treatments of maize grain after a period of 12 hr in a continuous airflow multiple choice olfactometer. Error bars represent the standard error of 12 means when 200 insects were initially introduced into the arena.

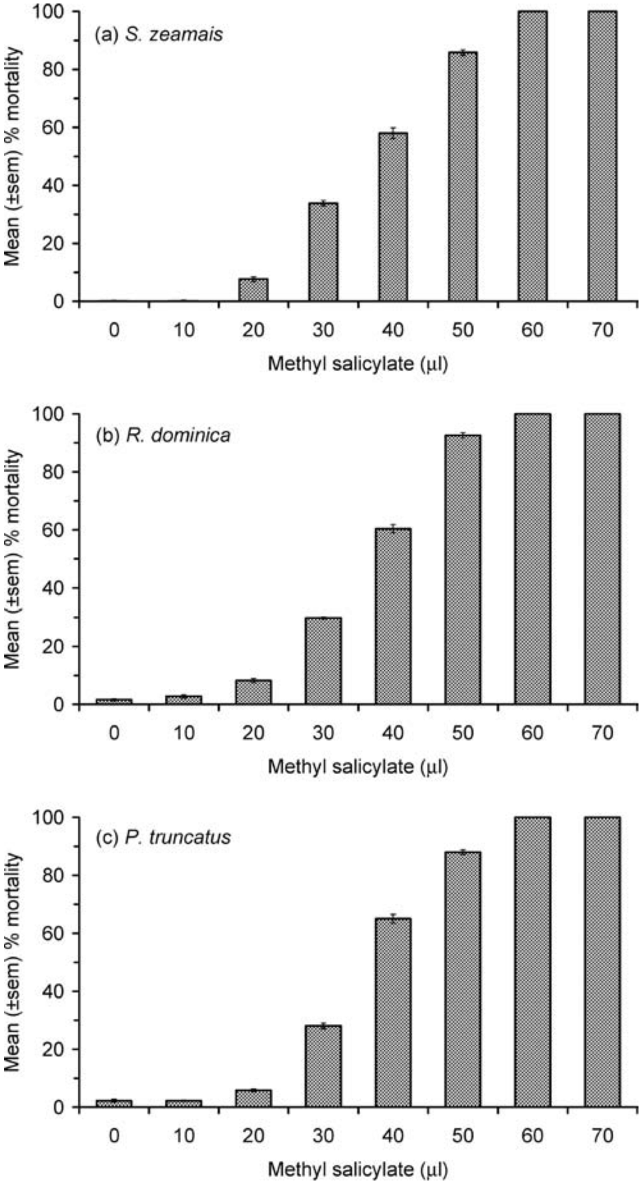

FIG. 2. The effect of dose (μl in 1-l jar) of methyl salicylate vapor on the adult mortality of (a) *S. zeamais*, (b) *R. dominica,* and (c) *P. truncatus* after a period of 24 hr. Error bars represent the standard error of 10 means when 50 insects were initially introduced into the fumigation chamber.

TABLE 1. PROBIT REGRESSION ANALYSIS ESTIMATES OF LETHAL DOSES OF METHYL SALICYLATE AGAINST *S. zeamais*, *R. dominica,* AND *P. truncatus*

		Dose (μl)		
Insect species	Probit Estimation	Lethal dose	Lower 95%	Upper 95%
S. zeamais	LD_{50}	36.17	35.46	36.88
	LD_{90}	51.27	50.23	52.41
R. dominica	LD_{50}	35.12	34.41	35.83
	LD_{90}	50.19	49.15	51.33
P. truncatus	LD_{50}	35.61	34.90	36.32
	LD_{90}	50.88	49.83	52.02

Amounts of methyl salicylate below the LD_{50} value resulted in high insect mortality with prolonged exposure times (Figure 3). After 2 days of exposure, 30 μl of methyl salicylate increased mortality compared to the control (LSD, $P < 0.05$), and 100% mortality was observed when insects were exposed to 30 μl of methyl salicylate vapor for 6 days. However, 10 μl of methyl salicylate did not show a strong effect on adult mortality even after 6 days of exposure.

DISCUSSION

Previous research has shown that methyl salicylate comprises more than 90% of the volatile components found in the roots of *S. longepedunculata* (Jayasekara et al., 2002). Methyl salicylate is a ubiquitous aromatic ester that is a well-known plant stress signal (Wees et al., 2000; Cardoza et al., 2002) acting as a semiochemical to recruit insect predators (Shimoda et al., 2002; James, 2003; De Boer and Dicke, 2004), is involved in host orientation and selection (Ninkovic et al., 2003), and has insect repellent properties (Hardie et al., 1994). Its antimicrobial and antifungal properties are well-established (Meiller et al., 2001; Papandreou et al., 2002), and these have been exploited in liniments, ointments, and toothpaste, and it has been widely exploited by the flavor and fragrance industry (Clark, 1999). The absorption of high concentrations of methyl salicylate in some traditional medicinal preparations can be fatal (Chan, 1996).

In this study, adult *S. zeamais* demonstrated clear orientation choices between the volatiles generated by untreated maize and maize that had been mixed with *S. longepedunculata* root powder, a methanol extract of the powder, or its main volatile component, methyl salicylate. This suggests that *S. zeamais* are able to detect methyl salicylate through olfaction and avoid it when given the choice. This could explain, at least in part, how the application of powdered roots of *S. longepedunculata* protects grain from insect infestation.

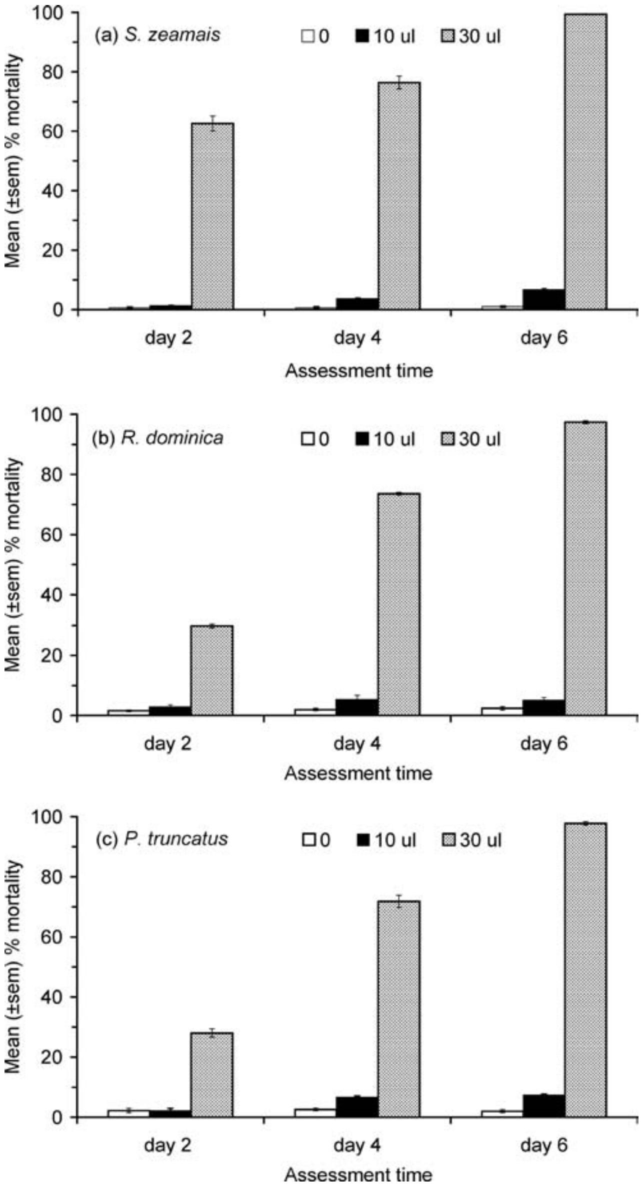

FIG. 3. The effect of prolonged exposure to methyl salicylate vapor on the adult mortality of (a) *S. zeamais*, (b) *R. dominica,* and (c) *P. truncatus* over a period of 6 days. Error bars represent the standard error of ten means when 50 insects were initially introduced into the fumigation chamber.

A number of stored product insects are attracted to host volatiles (Barrer, 1983; Phillips et al., 1993; Bashir et al., 2001). Pike et al. (1994) showed that *S. zeamais* is attracted to maize volatiles, and identified the main volatile components as hexanoic acid, nonanoic acid, nonanal, decanal, 2-phenylethanol, and vanillin. Methyl salicylate has not been previously reported to occur in the seeds of other grains or legumes, although it is emitted by damaged potato plants, and as a component of the blend attractive to the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera; Chrysomelidae) (Dickens, 2000). It would be interesting to test the effect of methyl salicylate on a species such as *P. truncatus* that does not orientate towards host volatiles and apparently relies on an aggregation pheromone (Hodges et al., 1998). However, the relative immobility of *R. dominica* and *P. truncatus* and their inability to walk through plastic tubing in comparison to *S. zeamais* prevented their inclusion in this experimental design. A different bioassay would be required to evaluate whether these species were similarly able to detect and avoid the volatile components of *S. longepedunculata* (Bashir et al., 2001). Methyl salicylate has been shown to induce plant defense responses (Ozawa et al., 2000; Wees et al., 2000), and it is possible that the avoidance of maize grains treated with methyl salicylate by *S. zeamais* is not due to the methyl salicylate itself but to other defense mechanisms induced by the methyl salicylate. This is considered to be unlikely due to the slow rate of metabolism of the grains, but the possibility cannot be excluded.

Avoidance of methyl salicylate vapors by *S. zeamais* was accompanied by a toxic effect on the insects. Dose- and time-dependant mortality effects were recorded when adult insects were in a sealed chamber containing the volatile compound. The low mortality recorded by 10 μl methyl salicylate in the 1-l container over 6 days may suggest that insects can tolerate low levels of methyl salicylate and potentially develop resistance to higher concentrations (Rajendran and Gunasekaran, 2002). However, this result may be an artifact of the experimental methodology because the fumigation chamber was opened every second day to remove dead insects, resulting in a partial loss of methyl salicylate from the chamber and diffuser.

Although there will be relatively minor volatile components present in the experimental treatments using the crude powder of *S. longepedunculata* roots and its methanol extract, our study suggests that it is the main volatile compound to which *S. zeamais* adults were responding. This is indicated by the similar numbers of insects found in the maize treated with the crude root powder containing approximately 4 mg of methyl salicylate in the experimental treatment (Jayasekara et al., 2002), the methanol extract of the root powder that also contained approximately 4 mg methyl salicylate, and the synthetic methyl salicylate at 1.4 mg per experimental treatment.

This research provides evidence that the indigenous strategy used by African farmers of mixing *S. longepedunculata* with their stored grain to protect it from storage pests is valid. The study also helps elucidate the mode of action of the botanical through the effects of repelling and killing adult insects. For methyl salicylate to act as a true grain fumigant, the compound would have to possess sufficient grain penetrative properties to kill the insect developmental stages inside the grain (Shaaya et al., 1997; Lee et al., 2001). As with all fumigants, insect mortality will only be achieved if the material is applied within a sealed environment, ensuring the parameters of concentration and duration are sufficient to achieve 100% mortality (Rajendran, 2000; Reed and Pan, 2000). Using a volatile or essential oil compound to protect grain stocks may be more acceptable to some consumers than, for example, applying a dilute dust or powder that may result in higher residue levels (Shaaya et al., 1991; Isman, 2000). Nonvolatile, bioactive compounds present in *S. longepedunculata* have been recently characterized, and they also contribute to its observed efficacy in protecting stored grain (Jayasekara et al., 2003).

Evidence from other recent studies on *S. longepedunculata* indicates that there can be considerable variability in its bioactivity depending on the origin of the plant (Boeke et al., 2004). Therefore, potential phytochemical variability across African regions should be more adequately understood before the plant is promoted widely for pest control by small-scale African farmers. Although some preliminary evaluations of the potential vertebrate toxicity of *S. longepedunculata* have indicated that low concentrations of the material should be safe (Belmain et al., 2001), further investigations are essential to confirm the safety of mixing the botanical with stored grain.

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