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Effect of monoterpenoids on oviposition and mortality of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) under hermetic conditions

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ABSTRACT

Monoterpeniods have been shown to cause mortality in certain stored-product insect pests. The current report investigated the prospects of using monoterpenoids as oviposition deterrents of the cowpea beetle, *Callosobruchus maculatus* (Fabricius), as well as in the management of populations of the beetle. The monoterpenoids investigated include *E*-anethole, estragole, *S*-carvone, linalool, *L*-fenchone, geraniol, γ-terpinene and *DL*-camphor, and at the concentrations of 66.7, 33.3, 16.7, 8.33 and 0 μL/L. Exposure of the life stages of the beetle, which included eggs, young larvae (first instar), 4th instar, pupae and adults to different concentrations of the monoterpenoids over 24 h period caused varying levels of mortality. The stages of the beetle that were the least susceptible to the monoterpenoids were the 4th instar, and the pupae, which required high concentrations of the monoterpenoids to achieve 99% mortality. The adults and the eggs exhibited the highest susceptibility to the monoterpenoids. Mated *C. maculatus* females that were offered cowpea seeds upon treatment with low doses (8.33 μL/L) of the monoterpenoids did not lay eggs, while control female beetles offered untreated seeds laid several eggs. However, mated *C. maculatus* females laid eggs on cowpea seeds treated with monoterpenoids 3 weeks before to the day of experimentation. The monoterpenoids did not exhibit residual toxicity to the cowpea beetles. These monoterpenoids could be further investigated for the postharvest management of seed beetles of grain legumes.

1. Introduction

The cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), is a worldwide pest of cowpea, *Vigna unguiculata* (L.) Walps. Infestation of cowpea by this bruchid commences in the field before mature seeds are harvested (Huignard et al., 1985). Infestation level of cowpea is very low at harvest and may sometimes be undetectable (Huignard et al., 1985). The cowpea weevil multiplies rapidly in storage, produces a new generation every month, and may cause losses up to 30% in 3 months of storage (Ouedraogo et al., 1996). Complete loss of cowpea could occur within 6 months of storage if this pest is not controlled (Caswell, 1961). The most effective pest management tool used in the disinfestation of commercial quantities of cowpea is fumigation with methyl bromide or phosphine (Mbata, 2004). Methyl bromide use, which continued in developed countries under the critical use exemptions is scheduled to end worldwide by 2015 under the terms of the Montreal Protocol (United Nations Environment Programme, 1998). The phosphine gas is known to cause fire if it gets wet and could react with copper thus corroding electrical fittings (Mbata, 2004). Uses of some insecticides, such as aluminum phosphide, in stored-products are facing restriction, and a strain of *C. maculatus* has exhibited higher tolerance than normal to some insecticides such as dimethoate, permethrin, carbosulfan and malathion (Bogamuwa et al., 2002). Several traditional measures for protecting harvested cowpea are in use in subsistence agriculture, but their efficacy is often unverified (Alebeek, 1996). Physical methods, such as controlled atmospheres (Mbata and Reichmuth, 1996), are effective in controlling bruchid pests of cowpea and they do not leave chemical residues on products, but many are expensive or impractical. Monoterpenoids, which are volatiles from plants (López et al., 2008), are being proposed here for the management of *C. maculatus* populations in postharvest storage of cowpeas.

Monoterpenoids are ten- carbon, secondary plant chemicals that are major components of essential oils extracted from leaves or fruits of herbs such as *Eucalyptus* spp., *Ocimum* spp., *Carum carvi* L. (caraway), *Coriandrum sativum* L. and other plants (Rice and Coats, 1994; López et al., 2008). These monoterpenoids are

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believed to aid plants in chemical defense against phytophagous insects and are now being exploited for their insecticidal properties (Pascual-Villalobos and Ballesta-Acosta, 2003). Adoption of monoterpenoids as an integrated pest management (IPM) tool in the management of postharvest insect pests will depend on further toxicology experiments that will determine if these materials pose a problem in terms of mammalian toxicity. The possible adsorption of volatiles from monoterpenoids has not been exhaustively studied. However, an unpublished study shows that residual odors from monoterpenoids disappear after 3–5 d, taste and other quality parameters of treated rice were not affected (M. Pascual-Villalobos personal communication).

Monoterpenoids that have been investigated for insecticidal actions include *E*-anethole, estragole, *S*-carvone, linalool, *L*-fenchone, geraniol, γ-terpinene and *DL*-camphor (Mbata et al., 2012; López et al., 2008; Pascual-Villalobos et al., 2004; Pascual-Villalobos and Ballesta-Acosta, 2003). Many monoterpenoids have been found to be effective against several postharvest insects (López et al., 2008). Pascual-Villalobos and Ballesta-Acosta (2003) observed that essential oil extracts from *Ocimum basilicum* L. had both mortality and anti-oviposition effect on *C. maculatus* because the essential oils from varieties of this plant contained monoterpenoids. However, Pascual-Villalobos and Ballesta-Acosta (2003) only investigated mortality of adult *C. maculatus* but did not consider other life stages. In addition, the effect of individual monoterpenoids on life stages of *C. maculatus* has not been previously studied.

The current study investigated toxicity of synthetic monoterpenoids on life stages of *C. maculatus* and effect of low doses on egg-laying by mated females of *C. maculatus*. The authors hypothesize that most of these monoterpenoids would deter oviposition in exposed mated females of *C. maculatus* and cause mortality of exposed life stages of *C. maculatus*.

2. Materials and methods

2.1. Insects – C. maculatus

The *Callosobruchus maculatus* colony used in this study was obtained from the rearing facility of USDA, ARS, Center for Grain and Animal Health Research, Manhattan, Kansas, USA, and has been maintained for 10 years at the Department of Biology, Fort Valley State University, Georgia. The beetles were reared on cowpea seeds in 1-L wide-mouth, glass jars at 30 ± 0.5 °C, 70 ± 5% relative humidity (r.h.), and a photoperiod of 12:12 h (L:D) (Shu et al., 1996). A fresh culture was started every 2 weeks by placing 60 pairs (0–3 d old) of adult bruchids in 1-L jars containing 250 ml cowpea seeds of the "California Black Eye" variety. The beetles were allowed 24 h to mate and lay eggs after which they were removed using an aspirator. Infested cowpea seeds were held in the 1-L jars at the same conditions of temperature, photoperiod and humidity specified above until adult emergence.

2.2. Experimental protocol

2.2.1. Mortality of life stages of C. maculatus exposed to different concentrations of monoterpenoids

Eggs (6–24 h old), 1st and 4th instars, pupae and adults (24 h old) were used in these experiments. Females of *C. maculatus* glue their eggs on cowpea seeds and the eggs are easily discernable on surface of cowpea seeds. Seeds bearing 1–2 eggs were sorted to obtain a total of 30 eggs per 1-L rearing jar. The larvae of *C. maculatus* feed and develop internally, and could not be discerned externally by observing the surface of seeds. Radiography was used previously to follow larval and pupal development in this beetle at 30 ± 0.5 °C, 70 ± 5% r.h. (Mbata and Reichmuth, 1996; Mbata et al., 2000), and these developmental schedules were used to estimate the life stages present in infested seeds tested in this study. Eggs hatch into first instar after 2 d. Following hatching, the color of eggs changed from clear to cream-white because of frass deposition in the eggshell. Fourth instar is attained between 14 and 17 d while the pupal stage is attained between 18 and 21 d from the day eggs were laid. Thus, in these experiments, 3 d old and 16 d old developing individuals were assumed to be 1st and 4th instars, respectively. The number of eggshells with the appearance of successful hatching was used to estimate the number of larvae in seeds used in experiments requiring larval stages. Infested seeds from *C. maculatus* cultures of desired ages bearing first and 4th instars were sorted as described for the eggs and placed in 1-L jars with each jar containing 30 larvae. The pupae used in the experiments were from infested seeds that were 19–20 d old, and could be seen through opaque pre-emergence "windows" in the cotyledons of the seed. The pre-emergence window is created by the feeding of the developing larva and the adult emerges from it at the completion of development. Seeds bearing 30 pupae were placed in 1-L jars as described above for eggs. Adult beetles used were 36 h old and 30 adults comprising 15 males and 15 females were placed in 1-L jars containing 30 cowpea seeds. Filter papers held in place with metal rings were used as covers for jars prior to treatments with monoterpenoids. The monoterpenoids investigated were *E*-anethole, estragole, *S*-carvone, Linalool, *L*-fenchone, geraniol, γ-terpinone and DL-camphor (Sigma–Aldrich Co. LLC, St. Louis, MO 63178, USA). The concentrations of monoterpenoids investigated for mortality of life stages of the beetles were 66.7, 33.3, 16.6, 8.3, 0 μl/L. Developing life stages of the beetle that were not exposed to monoterpenoids (0 μl/L) served as the control. The monoterpenoids were injected with 100 μl syringes through the filter papers into the jars containing experimental *C. maculatus* individuals. As soon as the monoterpenoids were introduced into the system, the filter paper lids were replaced with airtight metal lids. In the process of replacing the filter paper with a metal lid, some volatiles might have escaped but the replacement of lids was done quickly to minimize potential loss of volatiles. Each trial consisted of a total of 200 jars representing 5 life stages of the beetle, 5 different concentrations of the 8 monoterpenoids investigated. Eight replications were repeated out over time. The treatment jars were placed in a chamber maintained at 30.0 ± 0.5 °C and 70 ± 5% r.h. for 24 h. Afterward, the airtight lids were replaced with filter paper lids until the surviving life stages of the beetles completed development. Seeds bearing eggs, larvae, and pupae were observed daily for adult emergence. The individuals that failed to emerge 2 weeks following emergence of the last adult from control were considered dead. Treated adults were considered dead if they were immobile 2 d after treatment. Mortality values were recorded and analyzed as percentages.

2.2.2. Evaluation of residual effect of monoterpenoids on oviposition by C. maculatus

The concentration of monoterpenoids used for the investigation of oviposition by adult beetles on treated cowpea seeds was 8.3 μl/L. Eighteen 1-L jars, each containing 30 uninfested seeds, were set up for each of seven trials. A set of 8 jars, 1 for each monoterpenoid, was treated with 8.3 μl/L of the same monoterpenoid on seeds in the jars. An additional jar served as a control in which only the hexane solvent was applied. Four pairs of mixed-sex adults were placed in each jar within 12–36 h of eclosion. Jars were closed with filter papers and metal rings. Another set of 8 jars containing seeds that were similarly treated with monoterpenoids 21 d earlier were also dispensed with 4 pairs of approximately 12–36 h old adult beetles. An additional jar was set up with cowpea seeds and 4 pairs