Integration of monoterpenoids with low pressure simulating vacuum for control of diapausing Indian meal moth larvae and red flour beetle adults

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Abstract

Monoterpenoids as well as low pressure simulating vacuum, when applied alone have been demonstrated to cause mortality of stored-product insect pests. The current report explored the possibility of integrating these two control methods in the management of stored-product insects. The insects used for this study were the adults of the red flour beetle, *Tribolium castaneum*, and diapausing larvae of the Indian meal moth, *Plodia interpunctella*. The monoterpenoids investigated were *E*-anethole, estragole, *S*-carvone, linalool, *L*-fenchone, geraniol, γ-terpinene and *DL*-camphor. Exposure of the insects to all the monoterpenoids alone, with the exception of camphor, at a concentration of 66.7µL/1L of volume required more than 24 h to generate 100% mortality at 28.0 ± 0.8°C and 70 ± 2.5 r.h. However, exposure of the insects to camphor alone generated 100% mortality with 3 h exposure in *T. castaneum*. Exposure of the insects to low pressure at 36.5 mm Hg generated 100% mortality in beetles with 24 h exposure and in the diapausing *P. interpunctella* larvae with 48 h exposure. However, combination of the monoterpenoids with low pressure reduced exposure periods (3-24 h) required to generate 100% mortality in both diapausing larvae of *P. interpunctella* and the adult beetles of *T. castaneum*. In all cases *T. castaneum* showed signs of weakness faster than diapausing *P. interpunctella* larvae.

Keywords: Low pressure, *DL*-camphor, Estragole, γ-terpinene, Carvone

1. Introduction

Physical methods, such as controlled atmospheres or extreme temperatures, are attractive for post-harvest commodities because they do not leave chemical residues on food, but many are expensive, damaging to the commodity, or impractical. Low-oxygen controlled atmospheres from the application of a vacuum to achieve low pressure have potential for effective postharvest insect control in some applications. Back and Cotton (1925) and Bare (1948) were among the first to study the use of low pressure for controlling stored-product insects. Insect mortality under low pressure is predominantly a result of the low oxygen concentration affecting key cell physiological processes such as glycolysis, and not to low pressure per se (i.e., physical pressure effects) or dehydration from lowered water concentrations under vacuum (Navarro and Calderon,1979; Friedlander and Navarro, 1983). Some studies considered the activity of vacuum alone applied to stored-product insects (Back and Cotton, 1925; Bare, 1948; Calderon et al., 1966; Calderon and Navarro, 1968), while other studies investigated the effects of temperature and pressure level on different life stages of post-harvest insects (Mbata and Phillips, 2001; Mbata et al., 2004). Other studies investigated the combination of low pressure with increases of other atmospheric gases or addition of fumigants (Calderon and Leesch,1983; Donahaye and Navarro, 1989; Locatelli and Daolio, 1993). Mbata et al. (2009) investigated the integration of low pressure with bruchid resistant cowpea varieties for the control of the cowpea weevil, *Callosobruchus maculatus* (F.). Controlled atmosphere has also been combined with essential oils for the control of *Liposcelis bostrychophila* Badonnel (Wang et al., 2001). Vacuum and fumigation have been used together in commercial practice for several decades (Bond, 1984), but we are unaware of any reports of procedures using combination of monoterpenoids with low pressures.

Monoterpenoids are 10-carbon, secondary plant chemicals that are major components of essential oils extracted from leaves or fruits of herbs such as *Eucalyptus*, *Ocimum* spp., *Carum carvii* L. (caraway), *Coriandrum sativum* L., and many others (Rice and Coats, 1994; López et al., 2008). The monoterpenoids are believed to aid plants in chemical defense against phytophagous insects and are now being exploited as insecticides. Monoterpenoids that have been investigated for insecticidal actions

include *E*-anethole, estragole, *S*-carvone, linalool, *L*-fenchone, geraniol, γ-terpinene and *DL*-camphor (Lee et al., 2002; Pascual-Villalobos and Ballesta-Acosta, 2003; Pascual-Villalobos et al., 2004; Lopez et al., 2008). Many of these monoterpenoids have been found effective against several post-harvest insects (López et al., 2008). It is hypothesized that combining low pressure with monoterpenoids will accelerate the mortality of exposed insects and also shorten the exposure period required to generate 100% mortality compared to when either low pressure or monoterpenoid is used alone.

The objective of the research reported herein was to investigate in the laboratory the effects of exposure of adults of *Tribolium castaneum* (Herbst.) and diapausing larvae of *Plodia interpunctella* (Hübner) to combinations of monoterpenoids and low pressure on their mortality. From these experiments we intended to determine monoterpenoids that can be applied simultaneously with low pressure to achieve 100% mortality of post-harvest insect pests in commodities within short exposure periods.

2. Materials and methods

2.1. Insects

Diapausing larvae of *Plodia interpunctella* (Lepidoptera: Pyralidae), and adults of *Tribolium castaneum* (Coleoptera: Tenebrionidae) were obtained from laboratory colonies. Laboratory colonies of insects were maintained at 28.0 ± 0.8°C and 70 ± 2.5 r.h. and reared using standard methods (Mbata,1985; Howe, 1991).

Diapausing *P. interpunctella* were generated by rearing larvae up to fourth instar (12 days following egg hatch) at 28.0 ± 0.8°C and 70 ± 2.5% r.h. and transferring them to a chamber maintained at 16ºC, 67% r.h. and a photoperiod of 8 h:16 h L:D (Mbata, 1987). Up to a third of the transferred larvae entered diapause within 4 weeks. Diapausing larvae were identified based on their extended larval developmental period, big sizes and yellowish color of larvae due to accumulated fat (Bell, 1977, Mbata 1987).

Adults of *T. castaneum* used for the study were sieved from the food as pupae and placed in a 500 mL jar for eclosion of adults. Following eclosion, adult red flour beetles were kept for 3 days before use in experiments. Both *T. castaneum* adults and the *P. interpunctella* diapausing larvae were separately placed in glass vials containing 2.0 g of rearing food, milled maize for the red flour beetle and moth rearing medium (Mbata and Osuji, 1983) for *P. interpunctella*. The insects were: five adult red flour beetles or five *P. interpunctella* diapausing larvae per vial and five vials were set up for each species.

2.2. Monoterpenoids

E-anethole (99%), Estragole (98%), *S*-carvone (98%), Linalool (97%), *L*-fenchone (98%), Geraniol (99%), γ-Terpinene (98%) and *DL*-Camphor (96%) were obtained from ACROS Organics BUBA/SPRL.

2.3. Experimental protocol

Three trials were carried out for each treatment and the treatments included exposing the insects to monoterpenoids alone, monoterpenoids and low pressure together, and low pressure alone. Exposure of test insects to low pressure was conducted by placing vials of test insects into 1L, thick-walled Erlenmeyer vacuum flasks with a side arm. Rubber stoppers were fitted with dial-type pressure gauges that had been previously calibrated to a mercury column manometer and placed in the flask opening. The side-arm outlet of the flask was connected to a vacuum pump (Budget Dyna-Pump; Fisher, Pittsburgh, PA) via a Tygon vacuum hose (4.76 mm i.d. and 1.59 mm wall thickness) equipped with a screw-type hose clamp. The air in the flasks was evacuated with the vacuum pump to an absolute pressure of 36.5 mmHg. Once the target pressure was attained the vacuum hose was clamped, the pump shut off, and for treatments requiring the addition of monoterpenoids, the chemical was injected with a 100 µL syringe through the rubber stopper. The concentration of the monoterpenoids used was 66.7µL/L. The syringe was removed by pulling from the needle, and the point of needle insertion through the rubber stopper was sealed with a glue to prevent the loss of pressure. The flasks were placed in an environmental chamber maintained at 28.0 ± 0.8°C and 70 ± 2.5% r.h. for given time periods needed for experiments. Untreated control flasks were set up with insects in the same way as treated flasks, but were vented so they were at ambient pressure and maintained in a chamber at 30°C. Approximately 30 vacuum flasks were available for use on any given day of experiments.

Vacuum flasks were set up with 5 vials for insect species, so there were 10 vials per flask for each trial. Treatment flasks were held at low pressure for five or more time periods that were 0.5, 1, 2, 3, 6, 12, 24, 48, 72, 96 h after evacuation. Flasks were removed from controlled temperature chambers at the end of the exposure period, vented to ambient pressure and placed in an environmental chamber at 28°C for an appropriate period of recovery. *T. castaneum* adults and larvae of *P. interpunctella* were observed for mortality after 2 d of recovery.

3. Results and discussion

Tables 1 and 2 summarize the results of our experiment. Less exposure time is required to kill *T. castaneum* (24 h) than *P. interpunctella* (48 h) at low pressure.

Table 1 Exposure time required to generate 100% mortality of *T. castaneum* adults exposed to low pressure, monoterpenoids, or their combinations[a].

Monoterpenoids	**Exposure period (h)**	
	Monoterpenoids[b]	**Monoterpenoids & low pressure[b]**
E-anethole	96	24
Estragole	24	6
S-carvone	48	12
Linalool	48	12
L-fenchole	72	12
Geraniol	48	24
γ-terpinene	24	3
DL-camphor	3	3
None	-	24

[a] complete mortality was achieved at >96 h of control (red flour beetles exposed to neither low pressure nor monoterpenoids); [b] The concentration of the monoterpenoids used was 66.7 µL/L.

Table 2 Exposure time required to generate 100% mortality of diapausing larvae of *P. interpunctella* exposed to low pressure, monoterpenoids, or their combinations[a].

Monoterpenoids	**Exposure period (h)**	
	Monoterpenoids[b]	**Monoterpenoids & low pressure[b]**
E-anethole	96	24
Estragole	24	12
S-carvone	>96	24
Linalool	72	24
L-fenchole	>96	24
Geraniol	>96	24
γ-terpinene	48	24
DL-camphor	24	6
None	-	48

[a] complete mortality was achieved at >96 h of control (diapausing larvae of *P. interpunctella* exposed to neither low pressure nor monoterpenoids); [b] The concentration of the monoterpenoids used was 66.7 µL/L.

The volatile toxic effects of monoterpenoids alone on both insects were faster (3-48 h) when *DL*-camphor, γ-terpinene or Estragole were used. The time required to kill 100% *T. castaneum adults* was at least 96 h when exposed to *E*-anethole, while exposure of diapausing *P. interpunctella* to Geraniol, *L*-fenchole, *S*-carvone and *E*-anethole required more than 96 h to achieve 100% mortality.

When monoterpenoids were used in combination with low pressure, the exposure periods required to generate 100% mortality were shortened. For instance, exposure periods were shortened from 24 h to 3 h with *DL*-camphor and γ-terpinene against *T.castaneum* or from 48 h to 6-12 h with *DL*-camphor and Estragole against diapausing *P. interpunctella*.

Rajendran and Sriranjini (2008) reviewing information on plant products as fumigants for stored-product insects documented that essential oils proved effective in mixture with carbon dioxide or ethyl formate.

CO_2 accelerate the penetration of fumigants by keeping the respiratory spiracles open. It is also probable that low pressure simulating vacuum will enhance the penetration of essential oils by keeping the spiracles open.

Some authors have focussed on enhancing the toxicity of gases, for example with a 95:5% v/v mixture of ethyl formate: carvone against *Sitophilus oryzae* (L.) (Waterford et al., 2004) or by combining carbon monoxide with carbon dioxide against *T.castaneum* (Wang et al., 2009). Ethyl formate efficacy against *Carpophilus hemipterus* (L.) can be increased (Rouzes et al., 2008) if applied at low pressure simulating vacuum.

Others have reported an enhancement of the toxicity of essential oils in the presence of controlled atmospheres (Wang et al., 2001) or of allyl acetate with carbon dioxide (Leelaja et al., 2007).

Besides enhancing the toxicity of monoterpenoids, their application at low pressure reduced considerably the long exposure times usually needed to kill the insects, either at exposure to low pressure alone or to monoterpenoids alone.

Our preliminary results indicate that *DL*-camphor, γ-terpinene and estragole at 66.7 µL/L have the potential to be applied simultaneously at low pressure (36.5 mmHg) to reduce the exposure periods required to achieve stored product pests mortality. But optimum combinations for each pest species have to be established.

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