Artificial Feeding Site To Investigate Emigration of Nitidulid Beetles from Dried Fruits

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ABSTRACT Experiments were conducted to investigate the influence of different treatments on nitidulid beetle emigration from infested dried fruits. In the search for a sensitive method for measuring emigration, an artificial feeding site was devised. Emigration from feeding sites was compared with emigration from infested fruit when treated with methyl bromide, low pressure (100 mm mercury), carbon dioxide (CO_2) in air, or 2.8% oxygen (O_2) in nitrogen. Emigration from fruit and artificial feeding site treated with methyl bromide, low pressure (100 mm mercury), or 2.8% O_2 were significantly greater than from fruit and site treated with CO_2 or control. No significant differences were recorded in emigration from fruit or artificial feeding site.

KEY WORDS *Carpophilus* spp., dried fruits, feeding site

DRIED FRUITS are frequently infested with insect pests, and in particular with several species of nitidulid beetles. Because these beetles are both field and storage pests, infestation is a source of contamination of fruit in storage and in packing houses, and necessitates control by fumigation of all fruit immediately after harvest (Carmi & Donahaye 1971).

Fumigation of dried fruits with methyl bromide upon arrival at the packing stations effectively controls infestation, and also causes a high proportion of larvae and adults to emigrate from the fruit before they succumb (Anonymous 1986). The mechanism of this emigration is not clear; however, methyl bromide causes mortalities, and its mode of action is different from most other fumigants that have an anesthetic effect and are termed indifferent narcotics (Price 1985).

Emigration from infested fruit is no less important than the toxic effect of the treatment because established tolerances set minimum acceptance levels for the presence of both dead and live insects.

In this study, the effectiveness of a number of treatments and methyl bromide in causing emigration of *Carpophilus* spp. larvae from dates was compared. Initial experiments were undertaken on naturally or artificially infested fruit in which comparisons were made between different treatments; however, the material available was insufficient and was too heterogeneous to enable us to conduct investigations on the effect of parameters such as exposure time and concentration on treatments. Because of these difficulties, a series of experiments using artificial feeding sites was devised to determine whether larvae on artificial sites reacted to treatments similarly to larvae on fruit.

Materials and Methods

Test Insects. Larvae of *Carpophilus hemipterus* (L.) and *C. mutilatus* Erichson were reared on media described by Donahaye & Navarro (1989). Cultures were obtained by placing adult beetles in 200 ml jars that contained ≈150 g of food medium chopped and mixed with sawdust. After allowing 2 d for oviposition, the adults were removed and larvae were reared in these jars until required. Cultures were held in a room at 26 ± 1°C and 75 ± 5% relative humidity (RH).

To obtain infested dates, mixed populations of *C. hemipterus* and *C. mutilatus* larvae were used. Dates were moistened in the laboratory to ≈25% moisture content, placed in 2-liter jars, larvae of both species were added, and cultures were incubated at 30°C for ≈1 mo.

The artificial feeding sites designed to simulate the dates consisted of cardboard rectangles placed on media in petri dishes. Circles (9.5 cm diameter) were cut from sheets of polyethylene film (0.1 mm thickness), and these were used to line the lids of 9-cm diameter plastic petri dishes. The food medium was reheated and diluted as required to obtain a consistency at which it could be poured over the polyethylene

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film to form a layer 5 mm deep. Cardboard rectangles (2 by 4 cm) were placed on top of the food medium, four rectangles to each petri dish. The lid sections were then covered by the lower section of the dish and stored until the media had resolidified. Then the petri dishes were opened and 30 larvae (4–5 d old) were introduced. These larvae wandered over the food medium and penetrated the food medium beneath the cardboard rectangles to form feeding sites. These feeding sites were removed by cutting out the rectangles with scissors 24 h after the larvae were introduced, so that each rectangle contained food medium bounded on its upper surface by cardboard and on its lower surface by polyethylene film. The proportion of larvae in feeding sites beneath the cardboard averaged 46% of the number placed in the petri dishes 24 h earlier. Each feeding site generally held two to four larvae.

All treatments were carried out at 26 ± 1°C. Humidity of the microenvironment within the dates could not be controlled due to the heterogeneity of the experimental material, but ranged from 70 to 80% RH, as determined by equilibrium RH measurements recorded in a sealed chamber using a humidity sensor (Nova-Sina, Model JEL-20, sensor Enmbrf-3, Novasina AG, Zurich, Switzerland).

Larvae in feeding sites were exposed to different treatments in 2.54-liter desiccators. Each treatment was exposed for 4 h.

The first treatment consisted of a dose of 16 mg/liter methyl bromide. Dosage calculations were converted to the gaseous phase (Anonymous 1981) and the required volume of methyl bromide gas was removed from a 25-ml screw-cap septum vial (Mininert valves, Model 13074, Pierce, Rockford, Ill.) using a Pressure-Lok syringe (Series A-2, Model 050031, Dynatech Precision Sampling Corp., Baton Rouge, La.). The gas was then injected into the desiccator via a section of latex tube attached to the top of the desiccator lid and clamped at its distal end. The desiccator was then placed on a magnetic stirrer for 30 min to obtain a uniform gas concentration.

The second treatment consisted of a 20% concentration of carbon dioxide (CO_2) in air. This mixture was delivered from an apparatus described by Donahaye (1990). The gas mixture was delivered to the desiccator through a capillary tube and the CO_2 concentration at the outlet was monitored by a gas meter calibrated for CO_2 (Model 20-600, Gow Mac, Bridgewater, N.J.). The gas mixture was stirred during delivery, as described previously. When the 20% CO_2 level was reached, the desiccator was detached from the apparatus. The time needed to obtain 20% CO_2 in the desiccator was ≈15 min.

The third treatment was a low pressure of 100 mm Hg. The low pressure was obtained using a laboratory vacuum pump and measured with a mercury manometer. For these experiments, pressure within the desiccator at the end of the exposure period was remeasured, and if a rise of >25 mm Hg above the initial pressure was recorded, the treatment was discarded.

The fourth treatment was an atmosphere of 2.8% oxygen (O_2) in nitrogen. This mixture (equivalent to the partial pressure of oxygen in air at 100 mm Hg) was obtained by evacuating the desiccator to 100 mm Hg, followed by restoration of atmospheric pressure using nitrogen.

The final treatment and control was ambient air at atmospheric pressure and 26 ± 1°C.

The desiccators were fitted with false Perspex floors into which were drilled 5-mm holes that separated each desiccator into upper and lower compartments. Before treatment, the dates and the feeding sites were cleaned of any external infestation and placed separately on the Perspex floor. For experiments with the fruit, each desiccator was loaded with ten dates taken at random from the infested date supply. The dates were then exposed to treatment as described above, and upon completion of the exposure period they were removed from the desiccators and the larvae (dead or alive) present on the surface of the dates and at the base of the desiccators were counted. Each date was then opened lengthwise using a scalpel and the adults and larvae (dead or alive) still present in each date were counted.

For experiments with artificial feeding sites, ten cardboard rectangles containing food medium and larvae were placed in each desiccator and the appropriate treatment was applied. During exposure the desiccators were held in the dark at 26 ± 1°C and 75 ± 5% RH.

Each treatment was replicated at least four times and for each set of experiments, a control desiccator was exposed to the normal atmosphere for the same time period.

The proportion of insects found outside the dates or the feeding sites was used to measure response. Data were analyzed using a two-way analysis of variance and significance of differences between the means was analyzed by least significant degree test (Steel & Torrie 1980).

Results and Discussion

The influence of different treatments on emigration from artificially infested dates and artificial feeding sites is shown in Fig. 1. Analysis of variance for site effect showed that emigration from dates did not differ than the artificial feeding sites ($F = 1.752$; df = 1; $P = 0.19$). Whereas the difference between treatments were highly significant ($F = 167.822$; df = 4; $P = 0.00$). Results of analysis of variance did not reveal a significant interaction between site effect and treatments ($F = 41.050$; df = 4; $P = 0.87$). This

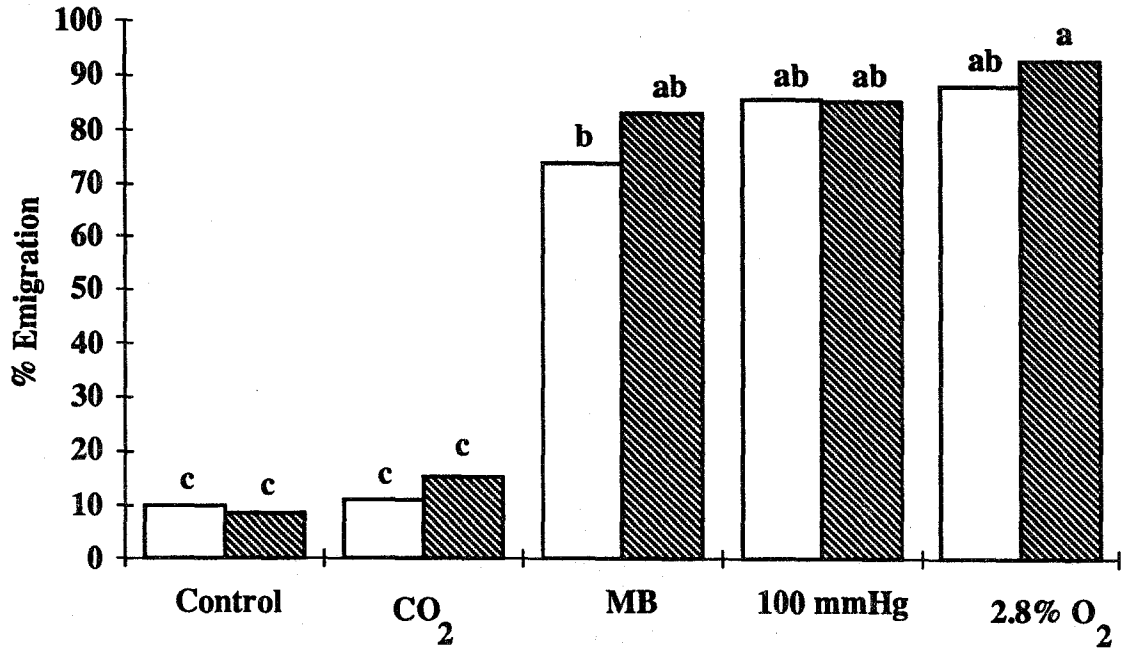

Fig. 1. Comparison of the influence of methyl bromide (MB) (16 mg/L), low atmospheric pressure (100 mm Hg), 2.8% oxygen (O_2), and carbon dioxide (CO_2) on the disinfestation of artificially infested dates and artificial feeding sites after 4 h of exposure at 26°C. Emigration rates assigned a common letter do not differ significantly at $P < 0.05$. □, dates; ▨, artificial feeding sites.

indicates a consistent lack of significant difference on emigration levels obtained between the dates and artificial feeding sites of control groups, or when emigration was tested for different treatments.

Emigration under the influence of methyl bromide, low atmospheric pressure, and low O_2 concentration were similar for the treatments carried out on dates and artificial feeding sites. Results of these treatments differ significantly from the control and the CO_2 treated groups. These experiments demonstrate the potential for using artificially feeding sites to conduct simulation studies of emigration from dried fruits by nitidulid beetles. The *Carpophilus* larvae appear to seek out and penetrate their food substrate through cracks and crevices and then remain largely sedentary, thus creating a niche where they feed until they abandon it when they begin to wander before pupation. Premature emigration from the feeding sites takes place under exposure to methyl bromide, low pressures, and low O_2 concentration. These treatments place the larva under stress, which interrupts feeding and causes them to wander. The similarity in the response of insects in the artificially infested dates and in the artificial feeding sites demonstrate the validity of using these sites instead of dried fruit for further studies of the emigration effect.

A common characteristic of all the treatments tested in the present work is their capacity to cause eventual mortality, depending on the length of exposure. The treatments at the tested exposure periods in this study permit high levels of insect survival, and only exposure times considerably longer than 24 h would cause mortality.

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