THE INFLUENCE OF DIFFERENT TREATMENTS CAUSING EMIGRATION OF NITIDULID BEETLES

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Experiments were carried out to investigate the influence of different modified atmospheres, low pressures alone, methyl bromide (MB) alone, and MB in combination with CO_2 or low pressure, in causing nitidulid beetles to emigrate from infested dried fruit for which dates served as a model. All the treatments at 4 h and 16 h exposure and at 26°C, had a marked influence in causing insects to abandon the infested fruit. The most effective treatments at the two exposures were pressures of 50 mm Hg, and 1.4% O_2 in air, both of which caused over 87% of the initial insect populations to emigrate from the fruit. At 4 h exposure, 2.8% O_2 was less effective than 100 mm Hg or MB. At 16 h exposure, MB+CO_2 was third in effectiveness and 100 mm Hg was one of the least effective treatments.

KEY WORDS: Dried fruit; infestation; nitidulid beetles; *Carpophilus*; *Haptoncus*; modified atmospheres; methyl bromide; carbon dioxide; atmospheric pressure.

INTRODUCTION

Fumigation of dried fruits with methyl bromide (MB) upon arrival at packing stations effectively controls infestation, and has been shown to cause a high proportion of the mobile stages (larvae and adults) to emigrate from the fruit before they die (2). The mechanism of this emigration effect which results in 'disinfestation' is not yet clear. However, with MB delayed mortalities are experienced, and its mode of action is different

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from most other fumigants which have an anesthetic effect and are termed indifferent narcotics (8).

Clearly the disinfestation effect is no less important than the toxic effect of the treatment in respect to the minimum acceptance levels for the presence of insect contamination. With this approach in mind it was considered possible that other treatments, such as the use of modified atmospheres (MAs) or low pressures, may also be effective in producing disinfestation of dried fruits.

In this study the effectiveness of MB and a number of altered environmental treatments in causing emigration of nitidulid beetle larvae from dates, was compared.

MATERIALS AND METHODS

Test insects

The experiments were carried out using dates infested with nitidulid beetles (mixed populations of *Carpophilus mutilatus* Erichson, *C. hemipterus* L., *Haptoncus luteolus* Er. and *Urophorus humeralis* F.). Field studies (6,7) indicate that *C. mutilatus* is normally the prevalent species in the Bet She'an and Jordan valleys during the harvest season and that the three other species occur in much smaller numbers. Our observations on picked dates corroborate these findings although *U. humeralis* was absent from the majority of samples. Most of the infested dates (var. 'Hadrawi') were obtained during the harvest season. Other dates for the tests were obtained by moistening stored dates and exposing them during the following spring and summer to natural infestations in the date groves. Lastly, dates were moistened in the laboratory to approx. 25% moisture content (m.c.), placed in 2-*l* jars, artificially infested with adults of *C. mutilatus* and *C. hemipterus* (approximately 100 individuals of each species), and incubated at 30°C.

Treatments

All treatments were applied at 26±1°C. The humidity of the micro-environment within the dates could not be controlled due to heterogeneity of the experimental material, but ranged from 70% to 80% r.h. as determined by m.c.-r.h. equilibrium measurements recorded in a sealed chamber using a humidity sensor (Nova Sina meter).

The experiments were carried out in 2.54-*l* desiccators. For each treatment, exposure times of 4 h and 16 h were employed. The treatments and methods of application were as follows:

1. Methyl bromide: For the 16-h exposure a dose of 5 mg/*l* was used (equivalent to one-third the locally recommended dose for fumigation chamber treatments). For the 4-h exposure the dose was 16 mg/*l*. Dosage calculations were converted to the gaseous phase (1), and the required volume of MB gas was removed from a 25 ml screw-cap septum vial using a Pressure-Lok syringe (Pierce). This was then injected into the desiccator *via* a section of latex tube attached to the tap 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.

2. Methyl bromide plus 20% CO_2: MB concentrations for 16 h and 4 h were 2.5 and 8.0 mg/l, respectively. The 20% CO_2 concentration in air was delivered from an apparatus designed to produce MAs and described by Donahaye (3). The gas mixture was delivered to the desiccator through a capillary tube and CO_2 concentration at the outlet was monitored by a gas meter calibrated for CO_2 (Gow-Mac). When the 20% CO_2 level was reached, the desiccator was detached from the apparatus, the appropriate dose of MB was injected through the latex tubing, and the gases were mixed on the stirrer as described previously. The time to obtain 20% CO_2 in the desiccator was approx. 15 min.

3. A 20% concentration of CO_2 alone, as described above.

4. Methyl bromide at 100 mm Hg: Fumigations were carried out at doses of 2.5 and 8 mg/l for 16 h and 4 h, respectively. The low pressure was obtained using a laboratory vacuum pump and measured with a mercury manometer. Doses of MB were then measured and injected, and the gases mixed as described previously.

5. A low pressure of 100 mm Hg: Obtained as described above.

6. A pressure of 50 mm Hg: Obtained as described above.

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

8. An atmosphere of 1.4% O_2 in nitrogen: Equivalent to the partial pressure of O_2 in air at 50 mm Hg, and obtained as above.

9. Normal ambient air: A control group from each batch of infested dates was kept at normal air composition and atmospheric pressure at 26±1°C. These dates were examined after 4 h and 16 h.

For the experiments under reduced pressure, the pressure within the desiccator at the end of the exposure period was remeasured, and if a rise of more than 25% of the initial pressure was recorded, the treatment was discarded.

Experimental procedure

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 were cleaned of any external infestation and placed individually on the Perspex floor. Each desiccator was loaded with 20 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 free insect stages (adult and larva, dead and alive) present on the surface of the dates and at the base of the desiccators were counted. Then each date was opened lengthwise using a scalpel and the numbers of adults and larvae (dead and alive) still present in each date were counted.

The ratio of the number of insects found outside the dates to the total number of

insects (including adults and larvae still present in the dates) was used to describe the percentage of insects found outside the dates and was termed 'percent disinfestation'. The ratio of the number of dates containing insects to the total number of dates in each treatment, was termed 'percent infested dates'.

In all cases the entire insect populations counted consisted of adults and larvae only, since pupation normally takes place outside the dates, and eggs were too small to be revealed by unaided visual examination. Each treatment, except for percent disinfestation treatments for MB alone and for MB+100 mm Hg, was carried out at least ten times and for each set of experiments a control desiccator was exposed to the normal atmosphere under the same ambient conditions as the treated dates and for the same time period. Results of the experiments were analyzed by the SAS program for completely randomized design of analysis of variance and significance of differences between the means was analyzed by Duncan's multiple range test (5).

RESULTS AND DISCUSSION

Nitidulid beetle populations in dates

The efficacy of the different treatments is best expressed as the percent of insects that abandon the dates, yet routine analysis for determining sanitation levels is based on the percent of infested fruit. In order to relate between these two measurements, the control group of each experiment was analyzed separately to reveal the relationship between the untreated infestation by nitidulid beetles expressed as number of insects/10 dates and the level of infested dates as percent infested dates. The curve of calculated infestation levels in Figure 1 shows that a significant correlation exists ($R^2 = 0.6199$). The information in Figure 1 is given to provide a base-line to assess the significance of emigration of beetles from the dates under the influence of the various treatments. Since each treatment has a different influence on emigration of the beetles, clearly its efficacy as expressed in the final percent of infested dates is dependent on the initial level of infestation. These results cover a wide range of infestation levels that may occur in infested dates. However, it was found that typical field infestation levels recorded directly after harvest, lie in the lower segment of the curve (Fig. 1). For example, from a study of natural field infestations (2), a typical average number of insects/10 dates of 0.296 (S.E. ± 0.0764, n=20) gave an equivalent average percent infested dates of 9.89 (S.E. ± 3.0661, n=20).

Emigration of beetles from dates

Results of disinfestation of infested dates exposed to the treatments for 4 h and 16 h expressed as 'percent disinfestation' are given in Figures 2 and 3, respectively.

For the 4-h exposure the 1.4% O_2 and 50 mm Hg treatments were most effective, followed by 100 mm Hg, although these did not differ significantly (Fig. 2). No significant differences in disinfestation were obtained between 100 mm Hg and MB

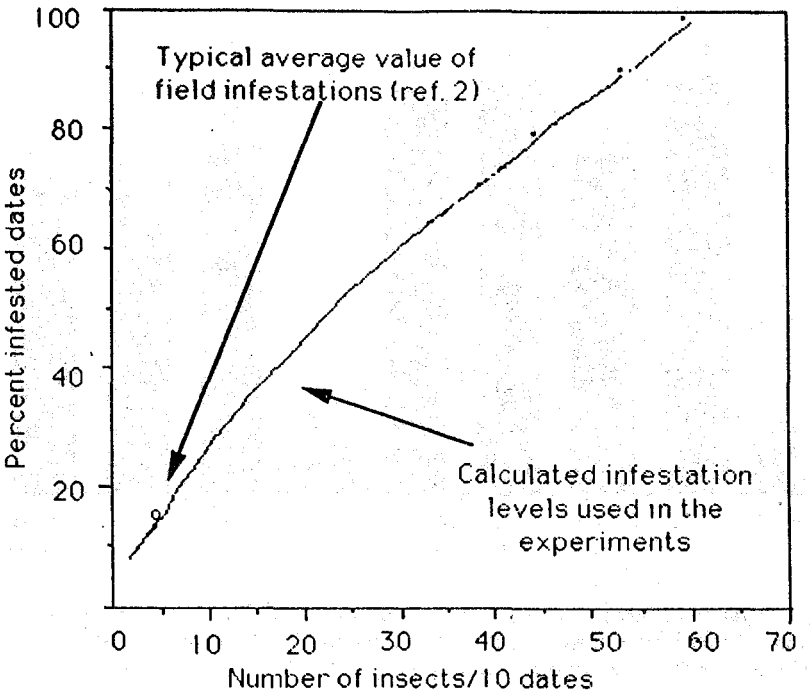

Fig. 1. Calculated curve to demonstrate the relationship between the number of insects/10 dates found in the control group after 4 h and 16 h exposure, and the percent of infested dates at 26°C ($y = 3.8530 + 2.2289x - 0.01095x^2$; $R^2 = 0.6199$).

alone. Also MB alone was not significantly different from 2.8% O_2, MB+CO_2, or MB+100 mm Hg. Exposure to CO_2 for 4 h was less effective than the other treatments and not significantly different from the control.

For the 16-h exposure (Fig. 3), the 50 mm Hg treatment was most effective followed by the 1.4% O_2 treatment and MB+CO_2, although these did not differ significantly from each other. As with the 4-h exposure, 2.8% O_2, MB+100 mm Hg, 100 mm Hg and MB alone were less effective than the former treatments in producing disinfestation. No significant differences in disinfestation were obtained among MB+100 mm Hg, MB+CO_2, 2.8% O_2, and CO_2; nor did 100 mm Hg, MB alone and CO_2 differ significantly, although all of these had a significant disinfestation effect in comparison with the control.

It should be noted that for MB, different concentrations were used at the two

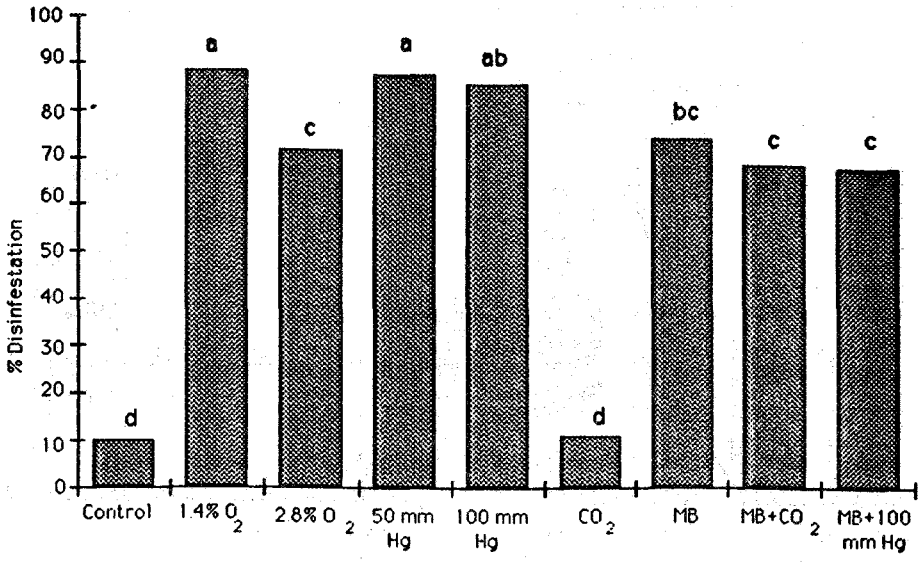

Fig. 2. Emigration of insects expressed as percent of insects found outside the dates infested by nitidulid beetles after exposure to different treatments for 4 h at 26°C. Disinfestation levels assigned the same letter do not differ significantly at $P<0.05$.

exposure times to conform with insect response to fumigant Ct product. However, in contrast to other treatments, disinfestation by MB at 4 h was more effective than at 16 h, and this seems to indicate that disinfestation response is not dependent upon Ct product

Data in Figures 2 and 3 show that treatments other than MB are as effective as, or more so, in causing insects to abandon fruit. The reasons for this emigration effect have not yet been studied, but since all the treatments in the present work have an adverse effect on insect survival, it is postulated that the principal reason for emigration is the stress exerted on the insects.

The enhanced disinfestation effect of low pressures, particularly at the 50 mm Hg level, was evident for both exposure periods. Yet it was less effective than, although not significantly different from, the equivalent partial pressure of O_2 in nitrogen at atmospheric pressure. This indicates that disinfestation was not due to low pressure *per se* but to the low O_2 partial pressure — resulting from this treatment.

Disinfestation of dates

To obtain enough insects in each replicate, these experiments were generally carried out on heavily infested batches of dates. Consequently, the levels of infestation expressed as percent infested dates were very high and depended on the initial number of insects in

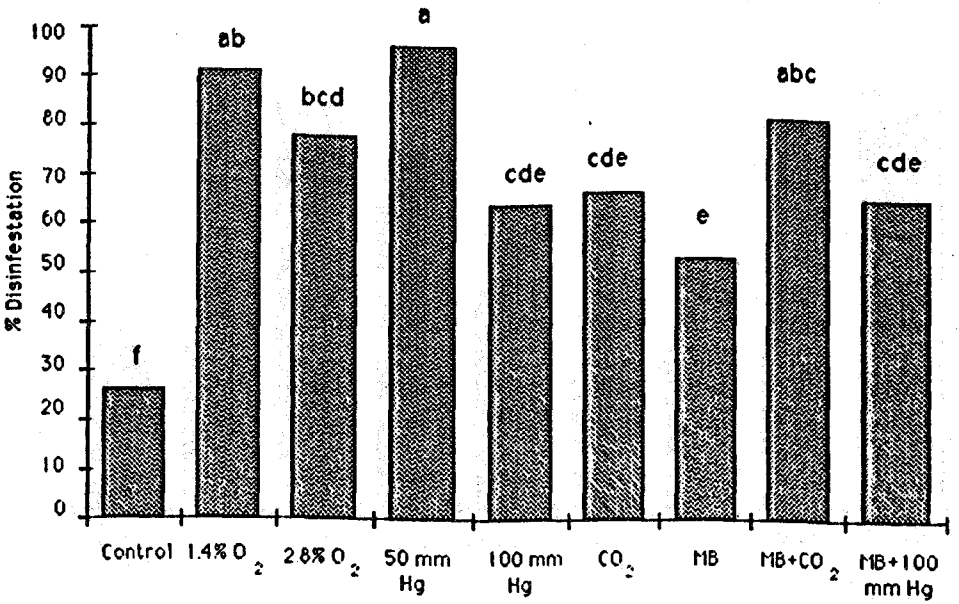

Fig. 3. Emigration of insects expressed as percent of insects found outside the dates infested by nitidulid beetles after exposure to different treatments for 16 h at 26°C. Disinfestation levels assigned the same letter do not differ significantly at $P<0.05$.

each batch. As a result of the large initial number of insects, treatments could not produce absolute zero levels of percent infested dates, since despite large numbers of emigrating insects, any date with an insect remaining inside it was still infested. Results of the different treatments on percent infested dates for 4 h and 16 h are shown in Figures 4 and 5, respectively.

The level of infested dates after treatment with MB for 4 h was not significantly different from the control (Fig. 4). Examination of the specific batch of dates used in the series of experiments with MB alone revealed an average infestation of 89 insects/10 dates. From the data in Figure 2, 4 h exposure to MB caused 73.8% of the insects to emigrate from the dates. This would result in a reduction in the insect population from an average of 89 insects/10 dates to an average of 23 insects/10 dates. The insect population shown in Figure 1 is equivalent to 54.9% infested dates, which is comparable to the actual value of 57.8% shown in Figure 4. Overlooking the discrepancies in the results obtained due to the variations in the initial number of insects in each trial, results given in Figures 4 and 5 demonstrate the influence of various treatments in causing the insects to emigrate and thereby reduce the level of infested dates. In this series the most effective treatments at 4-h exposure were: 1.4% O_2, 2.8% O_2, 50 mm Hg, MB+CO_2, and MB+100 mm Hg; and at 16 h exposure 1.4% O_2, 2.8% O_2 and 50 mm Hg

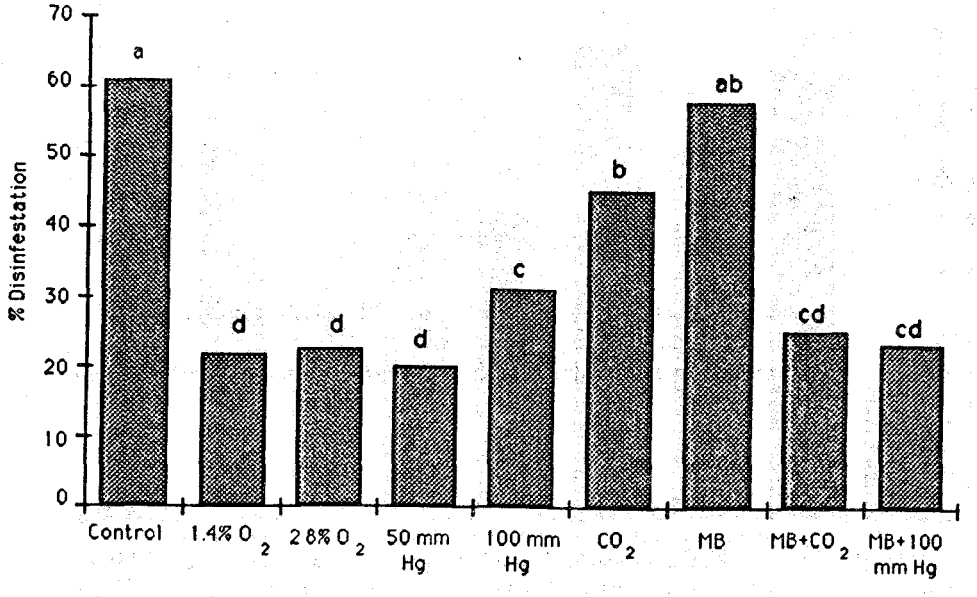

Fig. 4. Percent of infested dates after exposure to different treatments for 4 h at 26°C. Disinfestation levels assigned the same letter do not differ significantly at $P<0.05$.

TABLE 1
ANALYSIS OF VARIANCE OF RESULTS GIVEN IN FIGURES 2, 3, 4 AND 5

Figure No.	*Source*	*d.f.*	*Mean square*	*F ratio*	*Significance level (P)*
2: Emigration - 4 h	Treatments	8	10408.9	61.41	<0.01
	Error	76	169.5		
3: Emigration - 16 h	Treatments	8	6282.8	22.4	<0.01
	Error	96	280.6		
4: % Infested dates - 4 h	Treatments	8	3118.9	12.5	<0.01
	Error	107	248.7		
5: % Infested dates - 16 h	Treatments	8	1387.0	7.8	<0.01
	Error	98	176.9		

(Figs. 4 and 5). Here, too, the effect of CO_2 alone was more pronounced after exposure for 16 h (Fig. 5).

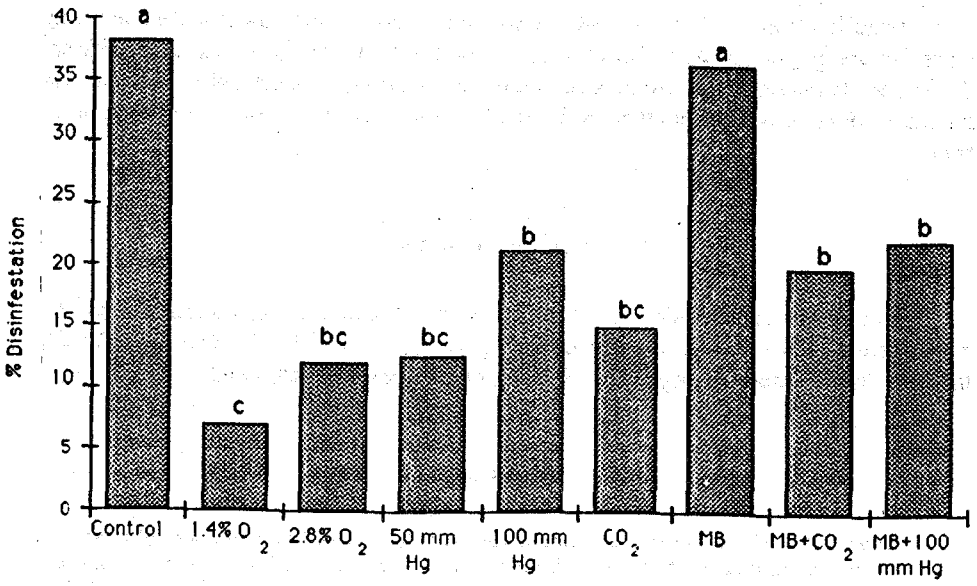

Fig. 5. Percent of infested dates after exposure to different treatments for 16 h at 26°C. Disinfestation levels assigned the same letter do not differ significantly at $P<0.05$.

Table 1 gives the results of the analysis of variance obtained from the data expressed in Figures 2 to 5. This information indicates the large variation in percent infested dates (Figs. 4 and 5), as compared with the data on percent disinfestation (Figs. 2 and 3) under the influence of each treatment.

The MB and MB+CO_2 treatments, although less effective than the low O_2 concentrations and low pressures, still have the advantage of producing complete mortality at these concentrations and exposure periods (4). Clearly the CO_2 treatment alone at 20% was of only intermediate effectiveness compared with the influence of MB.

CONCLUSIONS

The experiments were carried out to compare the effectiveness of a number of treatments including MB in causing emigration of nitidulid larvae and adults from dates. Our attempts to study emigration from infested fruit were subject to numerous limitations. These included the presence of different sizes and compositions of insect populations between the different batches of dates, use of different varieties of dates at different moisture contents, and non-uniformity of developmental stages of insects between batches. Also many larvae emerged from control dates, and in fact almost all

larvae recorded from the base of control desiccators were mature larvae in the wandering stage before pupation. In spite of these limitations our study revealed significant differences between the treatments in causing emigration of insects, although even for the most effective treatments there still remained a residual insect population within the fruit.

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