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Integration of Modified Atmospheres for Disinfestation of Dried Fruits

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C. Abstract

The effect of reduced atmospheric pressures alone, methyl bromide (MB) alone, and combinations of MB with CO₂ and with reduced pressures to increase sensitivity of all development stages of Carpophilus hemipterus and Carpophilus mutilatus was investigated.

The effectiveness of non-chemical treatments for removing Carpophilus species larvae from naturally and artificially infested dates was tested by exposing them to: low pressures, high temperatures, and modified atmospheres (MAs), while MB served as a chemical disinfestation treatment for comparison.

A method based on the use of artificial refuges was devised to simulate the infested dates. It was demonstrated that both the tested chemical and non-chemical treatments have the potential to exert a state of stress on the insects which appears to cause Carpophilus larvae to abandon the host.

The low oxygen atmospheres and the low pressures tested were the most effective non-chemical disinfestation methods in expelling larvae in relatively short exposure times. Disinfestation at low pressures was caused principally by the low partial pressure of oxygen in the tested atmospheres.

A series of field trials was carried out to demonstrate the effectiveness of reduced MB dosage in combination with CO₂ to disinfest and control Nitidulid beetles populations in freshly harvested dates within a shortened exposure time of 4 h. Also the effectiveness of a large scale application of CO₂ alone to disinfest dates was demonstrated.

D. Objectives of the Original Research Project

The original research project had two principal objectives. The first was that of finding alternatives to existing chemical methods such as ethylene dibromide (EDB) and Methyl bromide (MB), either based on MAS alone or in combination with high temperatures and low pressures, for the disinfestation of dried fruits. The second was to reduce the residue risks of existing chemical control methods by integrating them with MAS and other physical treatments.

The fumigant MB served as a reference gaseous treatment for evaluating the effectiveness of disinfestation by non-chemical methods. Over the period during which the investigation was carried out to examine the influence of various treatments on field infested dates, heterogeneity of infestation level and heterogeneity of composition of the insect populations led to attempts being made to use artificially infested dates. However it was also difficult by this procedure to obtain a continuous supply of artificially infested dates of known moisture content. To infest dates with known Nitidulid species, date moisture requires to be adjusted in advance to 75% equilibrium relative humidity. This procedure was both laborious and prolonged. Furthermore the need to evaluate levels of date infestation before each test, rendered this portion of the study even more difficult to accomplish. In view of the fact that the project was limited to one year, a method based on the use of artificially infested refuges was devised. These refuges actually simulated the infested dates.

Because of the complexity of the subject related to the mechanism through which the insects abandon the fruit, it was decided to concentrate on two representative Nitidulid species: Carpophilus hemipterus and Carpophilus mutilatus. Since observations made on insect populations of freshly harvested fruits showed that the larval stage was dominant of the two Nitidulid species. Also, since from the disinfestation aspect,

larvae are more resistant to treatments than adults, it was decided to concentrate the investigation on the larval stage of the two Nitidulid species. Among the non-chemical treatments it was proposed originally to investigate the influence of elevated temperatures. For two reasons the influence of elevated temperatures was only investigated to a limited extent. The first was that elevated temperatures and extended exposure times increased the risk of rendering the freshly harvested dates susceptible to deterioration. The second was the low effectiveness of elevated temperature in comparison with other treatments, especially MAs and low pressures.

2. Effect of low pressures on the mortality of Nitidulidae beetles

Introduction

The effect of reduced pressures on insect pests has been studied in the past. More than three centuries elapsed between the observations first made by Boyle (1870) on the activity of insects and other animals exposed in partial vacuum, and the time when entomologists began to pay serious attention to this factor. The effect of reduced pressures on several insect pest were studied by Cole (1906) and Nagel (1921). Back and Cotton (1925) investigated the effect of reduced pressures on the mortality of a large number of stored product insects species at different stages of development, and listed the sensitivity of different insects to low pressures. Bare (1948), studied the effects of prolonged exposures to low pressures on Ephestia elutella and Lasioderma serricorne and his mortality results were within the range given by Back and Cotton (1925). Calderon et al. (1966) obtained complete mortality of adults of five stored product insect species kept at 10-20mm Hg for 120 hours. Navarro and Calderon (1979), demonstrated the influence of low atmospheric pressures and low O₂ concentrations on E. cautella pupae. Another approach was the use of reduced pressures before or during fumigation to enhance fumigation treatments and this was investigated by Cotton (1932), Lepigre (1949), Monro et al. (1962, 1966), Dumas et al. (1969) and Calderon and Leesch (1983). This vacuum fumigation method, by which better penetration into the commodity could be achieved, became widely accepted and enabled the establishment of shorter fumigation schedules.

In the present work the effect of reduced pressures on the survival of different developing stages of two Nitidulid beetles, namely Carpophilus hemipterus (L.) and Carpophilus mutilatus (Er.) was studied.

Materials and Methods

Exposure chambers

Insects were exposed to different low pressures in 500 ml Erlenmayer suction flasks equipped with stopcocks to withstand low pressures. A relative humidity of 75% in the flasks was achieved by means of a saturated solution of NaCl (Solomon, 1951). Reduced pressures of 25, 50 and 100mm Hg were obtained using a vacuum pump. The pressures were measured by a mercury manometer at the beginning and end of each exposure period. A slight increase in pressure was observed between the two measurements. Whenever this pressure increment was greater than 10% of the initial pressure, the experiment was discarded.

Test insects: Insect species used in the experiments were Carpophilus hemipterus (L.) and Carpophilus mutilatus (Er.). Both species were obtained from laboratory cultures reared on synthetic food medium (SFM), (Donahaye and Navarro, 1989), in a rearing room at $26 \pm 1^{\circ}\text{C}$ and $70 \pm 5\%$ relative humidity (r.h.). For each replicate, groups of 50 larvae, or pupae, or adults, were placed before exposure in plastic tubes covered with gauge 200 nylon mesh. After exposure the insects were transferred to Erlenmayer flasks containing a blob of SFM, and these were held in the rearing room until mortality was determined.

Treatments of eggs of the two species differed from those of the other stages. A strip of paper 15mm wide was sandwiched between two microscope glass cover slips each 18x18mm, and lightly glued to both by adhesive. Adult females laid eggs along the sides in the slit between the two glass cover slips. Four such oviposition "plates" chambers were placed in Petri dishes containing 120 adult insects (mixed males and females) 1-2 weeks old, together with about 0.5 g SFM for a period of 24 hours. Plates containing eggs were (counted under a binocular microscope and then) placed in 500 ml Erlenmayer suction flasks and exposed to different reduced pressures for different exposure periods. To avoid cannibalism on eggs following exposure, food was supplied to freshly hatched larvae. This was carried out after separating the

two cover glasses from each other and placing them on SFM in Petri dishes together with moist absorbent paper and covered to prevent desiccation. The Petri dishes were held in the rearing room and were checked twice daily for a period of 8 days after exposure, when hatched larvae were counted. Final mortality counts of the treatments with larvae and pupae were made two weeks after the exposure time. Larvae that failed to pupate and pupae that failed to emerge as adults were recorded as dead. Mortality of adults was recorded two days after the end of each exposure period. For each group of treated insects a corresponding non-treated control was held at normal atmospheric pressure at the same temperature and r.h. conditions. Results were analyzed by calculating LT_{50} and LT_{99} values on a computer handling a probit analysis program (Daum, 1979).

Results and Discussion

Results of exposure of the different development stages of the two *Carpophilus* species to 25, 50 and 100 mm Hg pressures are shown in Tables 1, 2 and 3. In all treatments, adults of the two species were found to be the most susceptible stage to the tested reduced pressures. *C. mutilatus* adults were always less sensitive than adults of *C. hemipterus* to corresponding low pressures. Reducing the pressure increased susceptibility of adults of both species. LT_{99} values for *C. mutilatus* adults exposed to 25, 50 and 100 mm Hg were obtained after 8, 28 and 81 hours respectively. While LT_{99} for *C. hemipterus* adults exposed to the same low pressure were obtained after 3.5, 4, and 18 hours, respectively.

Mortality responses of the other development stages to the tested pressures generally required longer exposure times than those required for adults. However, lower pressures did not always result in higher susceptibilities. Pupae and eggs of *C. mutilatus* showed higher tolerance at 25 and 50 mm pressure than larvae and adults. Pupae of both species appeared generally the most tolerant to reduce pressures except for eggs of *C. mutilatus*.

at 25 and 50 mm Hg. C. hemipterus eggs were more sensitive than C. mutilatus.

Back and Cotton (1925) who listed the lethal effect of reduced pressures, also observed at 25 and 50 mm Hg that exposure times required to produce complete mortality varied with species and developmental stage. In our study the only consistently susceptible stage of both insect species examined, was the adult which was more susceptible than egg, pupal, and larval stages at all pressure tested. Tolerance of the four developmental stages of C. hemipterus to pressures of 25 and 50 mm Hg was in decreasing order; pupa>egg>larva>adult. For C. mutilatus tolerance in

Table 1 Response of four developmental stages of Carpophilus hemipterus and Carpophilus mutilatus exposed to 25 mm Hg absolute pressure at 75% relative humidity and 26°C.

Species	Development stage	LT ₅₀	LT ₉₉	chi square	Slope ± SE
<u>Carpophilus hemipterus</u>	larvae	5.84 (5.36-6.31)	13.09 (10.74-18.98)	30.14	6.64 ± 1.01
	pupae	19.10 (16.15-22.02)	99.19 (69.65-183.29)	27.59	3.25 ± 0.46
	adults	1.15 (0.95-1.34)	3.46 (2.53-6.81)	21.03	4.86 ± 0.87
	eggs	15.84 (13.93-17.98)	82.53 (61.94-124.57)	28.87	3.24 ± 0.30
<u>Carpophilus mutilatus</u>	larvae	7.71 (6.48-9.28)	110.57 (64.60-256.81)	31.41	2.01 ± 0.22
	pupae	18.23 (13.90-20.80)	48.64 (38.34-87.76)	27.59	5.46 ± 1.16
	adults	2.57 (2.24-2.91)	8.38 (6.35-13.56)	22.36	4.53 ± 0.60
	eggs	24.22 (20.17-27.62)	86.26 (64.03-155.31)	54.57	4.21 ± 0.71

decreasing order was: larva>egg>pupa>adult at 25 mm Hg. and egg>pupa>larva>adult at: 50 mm Hg. At 100 mm Hg the order of tolerance for both species was again different, being pupa>larva>egg>adult for C. hemipterus and egg>pupa>larva>adult for C. mutilatus.

From the results obtained above, it is clear that at 100 mm Hg pressure, exposures of 137 hours are required to produce 99% mortality of all developing stages of the two species while lower pressures require considerably shorter exposure times. The results indicate the feasibility of using low pressure to control

Table 2 Response of four developmental stages of Carpophilus hemipterus and Carpophilus mutilatus exposed to 50 mm Hg absolute pressure at 75% relative humidity and 26°C.

Species	Development stage	LT ₅₀	LT ₉₉	chi square	Slope $\pm$ SE
<u>Carpophilus hemipterus</u>	larvae	10.52 (9.20-11.94)	26.37 (21.65-35.41)	23.68	5.83 $\pm$ 0.64
	pupae	28.77 (27.25-30.49)	67.88 (58.81-82.76)	31.41	6.24 $\pm$ 0.51
	adults	1.77 (1.58-1.96)	4.23 (3.61-5.38)	21.03	6.16 $\pm$ 0.66
	eggs	15.51 (12.36-19.99)	44.54 (34.03-79.47)	26.30	5.07 $\pm$ 0.95
<u>Carpophilus mutilatus</u>	larvae	12.12 (10.75-13.51)	48.70 (39.78-64.10)	33.92	3.85 $\pm$ 0.32
	pupae	27.13 (19.90-33.19)	183.20 (104.73-783.31)	25.00	2.80 $\pm$ 0.60
	adults	7.42 (6.09-8.86)	28.32 (20.60-48.98)	18.31	4.00 $\pm$ 0.52
	eggs	32.25 (26.91-37.11)	172.67 (117.10-362.65)	26.30	3.19 $\pm$ 0.49

infestations by Carpophilus spp. though exposure times required are extremely long even at 25 mm Hg.

In Carpophilus spp. treatment programs a clear distinction must be made between control and disinfestation treatments. In the latter treatment the aim being removal of insects from the commodity before they succumb. This aspect of disinfestation has been studied separately and given in section 3 of this report. Low pressures for the control of Carpophilus spp. are characterized by long exposure times to become effective. For this reason low pressures alone for the control of insects in dried fruits should be implemented on commodities where the exposure time is not a constraint.

Table 3 Response of four developmental stages of Carpophilus hemipterus and Carpophilus mutilatus exposed to 100 mm Hg absolute pressure at 75% relative humidity and 26°C.

Species	Development stage	LT ₅₀	LT ₉₉	chi square	Slope $\pm$ SE
<u>Carpophilus hemipterus</u>	larvae	21.74 (20.58-22.82)	53.00 (48.10-63.00)	23.68	5.89 $\pm$ 0.47
	pupae	42.27 (36.79-47.52)	136.59 (108.35-198.29)	23.68	4.56 $\pm$ 0.54
	adults	6.29 (5.79-6.80)	17.63 (14.64-23.27)	35.17	5.19 $\pm$ 0.52
	eggs	16.25 (14.76-17.66)	51.38 (42.84-66.92)	30.14	4.65 $\pm$ 0.44
<u>Carpophilus mutilatus</u>	larvae	29.26 (26.75-32.27)	86.35 (67.22-134.64)	18.31	4.92 $\pm$ 0.60
	pupae	49.80 (41.10-55.41)	85.90 (73.34-128.20)	15.50	9.82 $\pm$ 2.04
	adults	22.06 (20.73-23.53)	81.12 (65.21-111.21)	22.36	4.11 $\pm$ 0.39
	eggs	31.49 (30.04-32.91)	132.32 (121.56-145.72)	30.14	3.73 $\pm$ 0.14

3. DISINFESTATION OF DATES INFESTED BY CARPOPHILUS SPECIES (COLEOPTERA: NITIDULIDAE)

3.1. Disinfestation from naturally and artificially infested dates using methyl bromide, low pressures and modified atmospheres.

Introduction

In Israel, dates at the time of harvest are generally infested by insect pests, and in particular by several species of Nitidulid beetle. Since these beetles are both field and storage pests, this infestation is a source of contamination of both the dates in storage, and of the packing houses, and has necessitated routine control treatments by fumigation of all dates immediately after harvest (Carmi and Donahaye, 1970).

Fumigation of dates with methyl bromide (MB) upon arrival at the packing stations effectively controls infestation, and has also been shown to cause a high proportion of the active stages (larvae and adults) to abandon the dates before they succumb (Anon, 1986b). The mechanism of this "disinfestation" effect is not yet clear but is common to both MB and ethylene dibromide. With these fumigants, delayed mortalities are experienced, and their modes of action are different from most other fumigants which have an anesthetic effect and are termed indifferent narcotics (Price, 1985).

Clearly the disinfestation effect is no less important than the toxic effect of the treatment since tolerances established by importing countries set minimum acceptance levels for the presence of insect contamination. Furthermore, since dates arriving at the packing houses are held in cold storage until processing in order to maintain quality, any remaining infestation of sessile stages is likely to be killed or its development arrested until processing.

With this approach in mind it was considered possible that other, non-toxic treatments, such as the use of modified

atmospheres (MAs) or low pressures, may be equally or more effective than MB for disinfestation of the dates. These could be employed together with reduced concentrations of MB, or alone, thus relieving the industry of its dependence on MB to which carcinogenic effects on rodents has been attributed recently. (Anon 1980a, Danse et. al. 1984), and for which residue levels are at present under revision (Anon 1988).

This study was made to compare the effectiveness of a number of non-toxic treatments, and MB in their efficacy for removing Carpophilus spp. larvae from dates.

Materials and Methods

The experiments were carried out using dates infested with Nitidulid beetles (mixed populations of C. hemipterus, C. mutilatus, and Haptoncus luteolus). Most of the dates, heavily infested with field infestations were obtained at the Zemach date packing station in the Jordan valley over the harvest seasons of 1986/7. Other dates 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 app. 25% m.c., placed in 2 l jars, artificially infested with adults of C. hemipterus and C. mutilatus, and incubated at 30°C.

All the treatments were carried out at 26±1°C. 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 moisture content-relative humidity equilibrium measurements (Donahaye and Rindner, 1983) recorded in a sealed chamber using a humidity sensor (Nova Sina meter).

The experiments were carried out in 2.54 l desiccators (Fig. 1). For each treatment, exposure times of 16 h and 4 h were employed.

Fig. 1- Apparatus for exposure of dates to different gaseous treatments.

The results of exposure for 16 h reflect the treatment routine as undertaken at present at the packing houses permitting one turn-over per day of the fumigation chambers. This poses the main bottle-neck for date reception during the picking season. The 4 h exposure time was used to explore the possibility of enabling 2 or even 3 treatments per fumigation chamber per 24 h.

The treatments and methods of application were as follows:

1. **Methyl bromide:** For 16 h exposure a dose of 5 mg/litre was used (equivalent to one third the locally recommended dose for fumigation chamber treatments). For 4 h exposure the dose was 16 mg/litre. Dosage calculations were converted to the gaseous phase (Anon 1981), 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% carbon dioxide:** MB concentrations for 16 h and 4 h were 2.5 mg/litre and 8.0 mg/litre respectively. The 20% carbon dioxide concentration in air was delivered from an apparatus designed to produce modified atmospheres and described by Donahaye (1985). The gas mixture was delivered to the desiccator through a capillary tube and CO₂ concentration at the outlet was monitored by a gas meter calibrated for CO₂ (Gow Mac). When the 20% CO₂ 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% CO₂ in the desiccator was about 15 minutes.

3. **A 20% concentration of CO₂ alone,** as described above.

4. **Methyl bromide at 100 mm Hg:** Fumigations were carried out at doses of 2.5 and 8mg/litre 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

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.

7. **An atmosphere of 2.8% oxygen (O₂) 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.

8. **An atmosphere of 1.4% O₂ in nitrogen.** Equivalent to the partial pressure of O₂ in air at 50 mm Hg, and obtained as above.

The desiccators were fitted with false floors of perspex drilled with 5 mm holes, that separated each desiccator into upper and lower compartments. Before treatment, the dates were cleaned of any external infestation and placed separately 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) present on the surface of the dates and at the base of the desiccators were counted. Then each date was opened lengthwise and the numbers of adults and larvae still present in each date were counted. The number of "insect free dates", and number of dates still infested after treatment were recorded. 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 un-aided visual examination.

Each treatment was carried out at least 10 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 on the VAX 750/11 computer of the ARO, and significance of differences between the means was analyzed by Duncan's multiple range test.

Discussion and Results

Results of disinfestation of infested dates exposed to the treatments for 4 and 16 hours are given in Figures 1 and 2 respectively.

Fig. 2 - Percentage of insects found outside the dates infested by Nitidulid beetles after exposure to different treatments for 16h at 26°C. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

From the figures it can be seen that for 16 h exposure the 50 mm Hg treatment was most effective followed by the MB+CO₂ treatment. No significant differences in disinfestation were obtained between MB+100 mm Hg, 100 mm Hg, MB, and CO₂, though all these had a significant disinfestation effect when compared with control.

For the 4 h exposure, it can be seen that the 1.4% O₂ exposure was most effective, followed by 50 mm Hg., though these did not differ significantly. As at the 16 h exposure, 2.8% O₂, MB+100 mm Hg, 100 mm Hg and MB alone were less effective in producing disinfestation, while exposure to CO₂ for 4 h was even less effective and not significantly different from control.

The enhanced disinfestation effect of low pressures, particularly at the 50 mm Hg level, was evident for both exposure periods. However it was less effective though not significantly

Fig. 3 - Percentage of insects found outside the dates infested by Nitidulid beetles after exposure to different treatments for 4h at 26°C. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

different from the equivalent partial pressure of O₂ in nitrogen at atmospheric pressure. This indicated that disinfestation was not due to low pressure per se but to the low oxygen partial pressure - resulting from this treatment.

The MB and MB+CO₂ treatments, although less effective than the low O₂ concentrations and low pressures, still have the advantage of producing complete mortality at these concentrations and exposure periods (Donahaye and Navarro 1989), whereas from data provided in section E.2. of this report, for exposure of C. hemipterus larvae to 50 mm Hg, LT₉₉ is 26 h, and for exposure to 100 mm Hg, LT₉₉ is 53 h. Those treatments that do not produce a complete kill would require a subsequent treatment such as cold storage at sub-zero temperatures to control insects remaining within the dates. Clearly the CO₂ treatment alone at 20% was only of intermediate effectiveness at 16 h and not effective at 4 h

exposure, and at the tested concentration (20%) did not provide a suitable alternative for insect disinfestation.

Conclusions

This experiment, aimed at simulating commercial treatments of dates, suffered from a number of drawbacks among which should be mentioned: different sizes and composition of insect populations within the dates between different batches, use of different varieties of dates at different moisture contents, and non-uniformity of developmental stages of insects between batches. Also the relatively large number of larvae emerging from control dates may be explained by the fact that mature larvae generally leave the dates before pupation, and in fact all larvae recorded from the base of control desiccators were mature larvae in the wandering stage.

In spite of the above limitations the experiment clearly demonstrated the relative effectiveness of the different treatments in disinfesting the dates. However, it was concluded that before recommendations could be made to the date industry on new disinfestation treatments requiring modifications of existing fumigation chambers and systems, further information on insect response to different concentrations (both of MB, MAS and combinations of both), to different exposure periods and to different low pressures, must be obtained. This would require a more sensitive experimental method and was the subject of separate studies (sections E.3.2. and 3.3.).

3.2. A comparison of the influence of methyl bromide, modified atmospheres and low pressures on the disinfestation of artificially infested dates and artificial refuges

Introduction

The importance of the disinfestation effect on dates caused by the fumigation treatment undertaken immediately after harvest has been discussed previously. This phenomenon was investigated initially on naturally and artificially infested dates to evaluate the levels of disinfestation produced by fumigation with methyl bromide (MB), modified atmospheres (MAS), MB and MA mixtures, and low pressures. The findings showed the effectiveness of employing all three types of treatment for date disinfestation, and revealed significant differences between the different treatments and non treated dates. However the high variance in infestation levels, differences in date variety and condition, and mixed populations of at least 3 Nitidulid species, all necessitated a large number of replicates in order to obtain significant results. Also the availability of infested dates was limited, naturally infested dates being only available during the short picking season, and the experimental method was not sensitive enough to enable an examination of response to different concentrations (MB and MAS), exposure periods, or low pressures. In order to design experiments aimed at revealing optimum treatments for producing disinfestation, as well as investigating the mechanism through which the insects abandon the host, an artificial refuge was devised and this was used to undertake the above mentioned evaluations. In the experiments described here, the behavior of Carpophilus larvae, when subjected to treatments in artificial refuges, was compared to that in dates in order to ascertain the validity of using refuges instead of dates for the subsequent experiments.

Materials and Methods

Test insects:

The test insects consisted of larvae of Carpophilus hemipterus and C. mutilatus. These were reared on an synthetic food medium (SFM)(Donahaye and Navarro, 1989).

Cultures were obtained by placing adult beetles in 200 ml jars containing about 150 g of SFM chopped up and mixed with sawdust. After 2 days for oviposition, the adults were removed and the larvae were reared in these jars until required for experiment. Cultures were held in a room at $26 \pm 1^{\circ}\text{C}$ and $75 \pm 5\%$ relative humidity (r.h.).

The refuge:

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 disposable Petri dishes placed in an inverted position. The SFM was reheated and diluted as required to obtain a consistency at which it could be poured over the polyethylene film to form a layer 5mm deep. Cardboard rectangular plates (2 x 4 cm) were then placed on top of the SFM, 4 plates to each Petri dish. The inverted lid sections were then covered by the lower section of the dish and stored until the SFM had re-solidified. Then the Petri dishes were opened and 30 4-5 day old larvae were introduced. These larvae wandered over the food medium and then penetrated the SFM to form "feeding cells" beneath the cardboard plates. Refuges were obtained 24 h after introducing the larvae by cutting out the plates with scissors, so that each plate contained SFM bounded on its upper surface by cardboard, and its lower surface by polyethylene film. The proportion of larvae underneath the refuges averaged 46% of the number inserted into the Petri dishes 24 h earlier. Also, the uniformity of infestation was relatively good, each refuge generally holding from 2 to 4 larvae.

Artificially infested dates:

The experiments were carried out using dates artificially infested with mixed populations of C. hemipterus, and C. mutilatus. Dates were moistened in the laboratory to approximately 25% m.c., placed in 2 L jars, artificially infested with adults of C. hemipterus and C. mutilatus, and incubated at 30°C.

Treatments:

All the treatments were carried out at 26±1°C. 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 moisture content-relative humidity equilibrium measurements recorded in a sealed chamber using a humidity sensor (Nova Sina meter).

All experiments were carried out in 2.54 l desiccators as described in the trials with infested dates (see section E.3.1.). For each treatment, an exposure time of 4 h was employed.

The treatments were as follows:

1. Methyl bromide: For 4 h exposure a dose of 16 mg/litre was used (equivalent to one third the locally recommended dose for fumigation chamber treatments).

2. A 20% carbon dioxide concentration in air.

3. A low pressure of 100 mm Hg.

4. An atmosphere of 2.8% oxygen in nitrogen.

The required gas concentrations and reduced pressures were obtained by the same procedures as described in Section E.3.1.

Experimental procedure:

For artificially infested dates, experimental procedure was the same as that described in section E.3.1.

For experiments with refuges, 10 refuges were placed in each desiccator and the appropriate treatment then applied. During exposure the desiccators were held in the dark at 26±1°C and 75±%

r.h. Methods of treatment application were as described previously.

Each treatment was carried out at least 4 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 or refuges, and for the same time period. Results of the experiments were analyzed by the SAS program for completely randomized design of analysis of variance on a VAX 750/11 computer, and significance of differences between the means was analyzed by Duncan's multiple range test.

Results and Discussion:

The influence of different treatments on disinfestation levels obtained from artificially infested dates and refuges are shown in Fig. 1. The figure shows that disinfestation does not differ significantly between the control groups, or between them and the 20% CO₂ treatments.

Disinfestation levels under the influence of methyl bromide, low atmospheric pressure, and low oxygen (O₂) concentration gave very similar results for the treatments carried out on artificially infested dates and infested refuges. Results of these treatments differ significantly from the control and the CO₂ treated groups.

These experiments clearly demonstrate the potential for using artificially infested material for conducting simulation studies on disinfestation of dried fruits specifically 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 in which they feed until they abandon it when fully grown as they begin to wander prior to pupation. The premature abandonment of the feeding sites takes place under exposure to methyl bromide, low pressures, and low oxygen concentration. All these treatments clearly place the larva under stress, it interrupts feeding and

begins to wander. Although CO_2 at high concentrations also has a similar influence, the tested 20% concentration appeared to be insufficient to stimulate the insect to abandon the dates or the refuges.

The similarity in the response of insects in the artificially infested dates and in the refuges demonstrate the validity of using artificial dates instead of refuges.

Fig. 1- Comparison of the influence of methyl bromide (16mg/L), low atmospheric pressure (100 mm Hg), 2.8% oxygen, and 20% CO_2 on the disinfestation from artificially infested dates and artificial refuges after 4 h exposure at 26°C . Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

3.3. The influence of time and concentration on the effectiveness of treatments that induce Carpophilus hemipterus larvae to abandon artificial refuges.

Introduction

A comparison between the effect of both chemical and physical treatments on the disinfestation of dates, and of artificial refuges, showed no significant differences. It was shown that for the same treatments, artificial refuges were abandoned by Carpophilus spp. at the same intensity as they abandoned the infested dates (see section 3. 2 of this report).

The importance of the disinfestation effect on dates caused by the fumigation treatment undertaken immediately after harvest has been discussed previously. This phenomenon was investigated initially on naturally and artificially infested dates to evaluate the levels of disinfestation produced by fumigation with methyl bromide (MB), modified atmospheres (MAs), MB and MA mixtures, and low pressures. The findings showed the effectiveness of employing all three types of treatment for date disinfestation, and revealed significant differences between the different treatments and non treated dates.

In this section the influence of time and concentration of MB (which is the best known fumigant causing disinfestation) was examined, on the rate at which C. hemipterus larvae abandon their feeding sites (artificial refuges reported on in the previous section). This served as a reference for studies on the influence of time and concentration of MB and MA mixtures, of MAs alone, of time and pressure levels of reduced pressure treatments, and of time on high temperature treatments, on the disinfestation phenomenon.

Exposure times of 4 and 6h received special attention since at these exposures, operative procedure of the treatment chambers would enable two treatments per day to be carried out.

Materials and Methods

Test insects:

The test insects consisted of larvae of Carpophilus hemipterus. These were reared on an synthetic food medium and cultures were obtained as explained in section E.3.2.

The refuge:

Consisted of the same refuges used as in the experiments reported in previous section E.3.2.

The experimental procedure:

All experiments were carried out in 2.54 L desiccators as described in the trials with infested dates (see section E.3.1). For all experiments, 10 refuges were placed in each desiccator and the appropriate treatment then applied. During exposure the desiccators were held in the dark at $26 \pm 1^{\circ}\text{C}$ and $75 \pm \% \text{ r.h.}$ Methods of treatment application were as described previously (see section E.3.1.)

Treatments:

The following treatments were carried out in three replicates for each experiment unless stated otherwise:

Methyl bromide (MB) alone

1. Exposure for 4 h to 14 different concentrations of MB ranging from 0.25 mg/L to 50 mg/L.

MB+CO₂

For these series of treatments eight different concentrations of MB ranging from 0.25 mg/L to 30 mg/L in combination with the following CO₂ concentrations, and for an exposure of 4 h were used.

2. MB+5%CO₂.
3. MB+10%CO₂.
4. MB+20%CO₂.

Carbon dioxide in air

5. Exposure for 4 h to nine concentrations ranging from 5% to 45% CO₂.
6. Exposure for 6 h to six concentrations ranging from 10 to 35% CO₂.
7. Exposure to 20%, 30%, 40% and 50% CO₂ at exposure times of 10, 20, 30 minutes, 1, 4, and 24 hours. Six replicates were carried out at each concentration and each exposure time.

Low pressures

- 8 .Exposure for 2 and 4 h to nine low pressures ranging from 50 to 300 mm Hg; single replicates were carried out at different pressures for Probit analysis.
9. Exposure to 50 mm Hg for periods of 10, 20, and 30 minutes. Six replicates were carried out for each exposure period.
- 10.Exposure to 100 mm Hg for exposure periods of 10, 20 and 30 minutes with 12 replicates for each exposure period.

Oxygen in nitrogen

- 11.Exposure for 2 and 4 h to eight concentrations of O₂ in nitrogen ranging from 1.1 to 10.0%O₂.

High temperature

- 12.Exposure for 2 and 4 h to a temperature of 40°C was investigated. Four replicates were carried out for each exposure period and the controls for the same exposure times were maintained at 26°C. Before each treatment exposure chambers were maintained at 40°C until their temperature was stabilized and this process lasted for a few hours. Then the refuges containing the insects were introduced into the exposure chambers.

Results of all above experiments except numbers 8, 11, and 12 were analyzed separately by one way analysis of variance with Duncans Multiple Range test using the SAS ANOVA procedure. Results

of experiments 8, 11, 12 were analyzed by probit analysis (Däum, 1979).

Results and Discussion

Methyl bromide alone

The influence of MB alone at different concentrations for 4 h fumigations is given in Fig. 1. From the figure it can be seen that the disinfestation effect peaked at 5 mg/L with a gradual decrease in effect at concentrations above 15 mg/L. The value of this finding is that since there is an optimum concentration that induces peak response in the range of 5 to 15 mg/L, dosage in practice should be adjusted to obtain this concentration range within the dates if maximum disinfestation is the objective.

Fig. 1: The influence of different concentrations of MB on the disinfestation from artificial refuges after 4 h exposure at 26°C. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

MB+CO₂

The influence of MB at different concentrations plus 5% CO₂ on disinfestation during 4 h exposure, is given in Fig. 2, and those of MB plus 10% and 20% CO₂ are given in Figs. 3 and 4.

Fig. 2: The influence of different concentrations of MB plus 5% CO₂ on the disinfestation from artificial refuges after 4 h exposure at 26°C. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

These findings indicate that for 4 h exposure, the same phenomenon of peak concentration inducing disinfestation occurs with MB+CO₂ as with MB alone. Although significant differences in disinfestation effect were not recorded between 2.5 and 30 mg/L MB for any of the CO₂ concentrations, there was a peak in disinfestation for every CO₂ concentration at the 10 mg/L level. Analysis of variance of all four experiments 1-4 together, failed to show significant differences in disinfestation effect between MB alone or MB+CO₂ at any of the MB concentrations above 1 mg/L. This indicates that for 4 h exposure there is no synergistic disinfestation effect between MB and CO₂. Analysis of variance of

Fig. 3: The influence of different concentrations of MB plus 10% CO₂ on the disinfestation from artificial refuges after 4 h exposure at 26°C. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

Fig. 4: The influence of different concentrations of MB plus 20% CO₂ on the disinfestation from artificial refuges after 4 h exposure at 26°C. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

each separate experiment showed that although significant differences in disinfestation effect were not recorded between 2.5 and 30 mg/L MB for any of the CO₂ concentrations, there was a peak in disinfestation for each CO₂ concentration at the 10 mg/L MB level.

Clearly for 4 h exposure, the main advantage in using a MB/CO₂ mixture is to enable reduction in MB dosage that still achieves complete kill (Donahaye and Navarro, 1989).

Carbon dioxide in air

The effect of exposure for 4 h and 6 h to different CO₂ concentrations on disinfestation from the refuges are given in Figs. 5 and 6.

Fig. 5: The influence of different concentrations of CO₂ on the disinfestation from artificial refuges after 4 h exposure at 26°C. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

The above findings clearly indicate that for both exposure periods disinfestation reached a peak at 30% CO₂ though differences in disinfestation effect at concentrations of 25% CO₂ and above were not significant.

Fig. 6: The influence of different concentrations of CO₂ on the disinfestation from artificial refuges after 6 h exposure at 26°C. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

The experiments on disinfestation at fixed concentrations of 20, 30, 40 and 50% CO₂ for time periods ranging from 10 minutes to 24 hours are given in Figures 7 to 10. In this series of experiments at exposure for 4 h, differences between exposure to 20% CO₂ and control are significant (Fig. 4). However, in experiments reported in section 3.2 (the influence of 20% CO₂ on disinfestation of dates and of artificial refuges were compared, p.28, Fig. 1) they were not. This might be explained by the fact

that in the former experiment the insects were mixed populations, whereas in the latter they were C. hemipterus alone. It is known that Carpophilus infestation is often accompanied by fermentation with CO₂ production, and therefore tolerances between species may differ, though this was not investigated. This insect may be more susceptible to CO₂ than C. mutilatus.

Fig. 7: The influence of exposure period on the disinfestation of C. hemipterus larvae from artificial refuges, caused by exposure to 20% CO₂. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

From figures 7 to 10 it can be seen that for all the concentrations tested, there was a gradual increase in disinfestation effect over the range of exposure periods, with a 70% disinfestation level for the 30% CO₂ concentration at 4 h exposure, and above 90% disinfestation for the 30% 40% and 50% CO₂ concentrations at 24 h exposure. However, the differences in

Fig. 8: The influence of exposure period on the disinfestation of *C. hemipterus* larvae from artificial refuges, caused by exposure to 30% CO₂. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

Fig. 9: The influence of exposure period on the disinfestation of *C. hemipterus* larvae from artificial refuges, caused by exposure to 40% CO₂. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

Fig. 10: The influence of exposure period on the disinfestation of C. hemipterus larvae from artificial refuges, caused by exposure to 50% CO₂. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

disinfestation between the 4 h and 24 h periods were only significant at the 40 and 50% CO₂ levels, while shorter exposures were significantly less effective in causing insect disinfestation.

Low Pressures And Oxygen in Nitrogen

In view of the very rapid and high levels of disinfestation produced by both low pressures and low oxygen concentrations, results of experiments 8, and 11 are given in comparative form in fig. 11.

This comparison was made to elucidate whether the disinfestation effect was produced by subjecting larvae to low

Fig. 11: Probit lines expressing regression of percent disinfestation of larvae against log oxygen (O_2) concentration and log pressure (LP) for different time periods (2 and 4 h). Values on x axis were calculated equivalent oxygen concentrations at low pressures.

pressures, or to the reduction in partial pressure of oxygen that accompanies low pressure treatments. Figure 11 shows that subjecting the larvae to low O_2 concentrations is slightly more efficient in causing them to abandon the refuges than subjecting them to low pressures at the equivalent partial pressure of oxygen. Although examination of confidence bands of the probit lines fails to reveal these differences as significant at the 0.05 level of probability (Table 1), there is an indication that the low pressure alone may even have an inhibitory effect on disinfestation.

As stated above, the disinfestation process in these experiments was observed to be very rapid. Therefore, to evaluate the order of magnitude of the exposure time necessary for the larvae to abandon the refuges, experiments were carried out at 50 and 100 mm Hg. for 10, 20 and 30 min. exposure. Results shown in Figures 12 and 13 demonstrate that 10 min. exposure was sufficient to obtain a marked disinfestation level. Values obtained at 10 min. did not differ significantly from those at 30 min. exposure. Comparison of Fig 12 and Fig 13 also indicates that at 50 mm Hg a higher disinfestation level was obtained than at 100 mm Hg.

Table 1 Response of Carpophilus hemipterus larvae to low pressures and low oxygen concentrations expressed in terms of 50% disinfestation (DD_{50}) at 26°C and 75% relative humidity (values in brackets are confidence limits at ≤ 0.05).

Treatment	DD_{50}		Chi square	Slope + SE
	%O ₂ concentration	absolute pressure (mm Hg)		
Low pressure	2h 4.03* (3.374-4.883)	146 (122.1-176.7)	18.3	-3.49±0.617
	4h 4.70* (4.258-5.291)	170 (154.1-191.5)	12.6	-3.59±0.388
Low O ₂	2h 5.35 (4.307-7.800)	194** (155.9-282.3)	12.6	-3.32±0.554
	4h 6.35 (5.156-8.061)	230** (186.6-291.7)	23.7	-3.61±0.646

* Numbers are calculated equivalent O₂ concentrations at low pressure.

** Numbers are calculated equivalent low pressure at O₂ concentrations.

Fig. 12: The influence of exposure period on the disinfestation of C. hemipterus larvae from artificial refuges, caused by exposure to 50 mm Hg. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

Fig. 13: The influence of exposure period on the disinfestation of C. hemipterus larvae from artificial refuges, caused by exposure to 100 mm Hg. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

High temperature

The influence of exposure to 40°C on disinfestation of C. hemipterus larvae is shown in Fig. 14. Essentially no significance was found between the 2h and 4h exposure at 40°C but both treatments gave significantly higher disinfestation than the control at 26°C. The levels of disinfestation obtained at 2 and 4h (Fig. 14) are lower than those achieved by other treatments such as low pressure (Fig. 12 and 13), low O₂ concentrations, or even MB. Nevertheless this aspect of disinfestation using high temperatures, deserves further investigation.

Fig. 14: The influence of exposure period on the disinfestation of C. hemipterus larvae from artificial refuges, caused by exposure to 40 °C. Disinfestation levels assigned the same letter are not significantly different at $p \leq 0.05$.

Conclusions

Information gathered on methods of causing Carpophilus hemipterus larvae to abandon the artificial refuges, demonstrate that physical treatments are as effective as the conventional chemical treatment using MB. In the present work MB served as a reference treatment for comparison with disinfestation levels obtained by other methods. MB alone, even at concentrations as low as 3g/m^3 was effective in causing larvae to abandon the refuges, though the maximum level of disinfestation was obtained at concentrations between 5 to 10 g/m^3 .

The presence of 5 to 20% CO_2 in the atmosphere in combination with different MB concentrations did not improve the efficiency of disinfestation of MB alone. For CO_2 alone, in order to obtain a level of disinfestation comparable with that of MB alone, concentrations of 25 to 35% CO_2 in air were required. Investigations of the exposure time required to obtain maximum disinfestation at a given CO_2 concentration showed that for the 20% CO_2 concentration, prolonging the exposure period above 1h. did not significantly increase disinfestation levels. However, since maximum disinfestation was obtained at concentrations between 30 to 50% CO_2 , the best results were obtained after 24h of treatment. Clearly optimization of treatment would call for an exposure time of less than 24 h for which results are not yet available.

Exposure of insect to low pressures and low O_2 concentrations demonstrated that both treatments were equally effective in causing disinfestation. Probit analysis also showed the dependence of disinfestation levels on the partial pressure of O_2 in the atmosphere. From results obtained so far it appears that low pressures are among the most effective methods of obtaining disinfestation; the lower the atmospheric absolute pressure (or the partial O_2 pressure) the higher the level of disinfestation.

Among the physical environmental factors the influence of heat at 40°C was also tested. It was demonstrated that this high

temperature was less effective than the other non-toxic treatments and MB.

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 are known to permit high levels of insect survival, and only exposure times considerably longer than 24h would cause mortality. It is postulated that the tested treatments have the potential of exerting a state of stress on the larvae. This stress in turn induces the larvae to abandon the artificial refuge. From results obtained so far it can be concluded that any treatment with the potential to place the larvae under stress would cause them to abandon their refuge, provided the treatment does not have a rapid knock-down effect.

4. TRIALS ON DISINFESTATION OF DATES IN COMMERCIAL FUMIGATION CHAMBERS

Introduction

This study was undertaken in order to apply in practice results obtained in the laboratory on the disinfestation of dates infested by Nitidulid beetles. The objectives of the trials were fourfold: 1 - to reduce the dosage of methyl bromide (MB) used during the initial fumigation of dates arriving at the packing houses during the harvest season, by fumigation in a carbon dioxide enriched atmosphere; 2 - to obtain an optimum gas mixture concentration for insect disinfestation instead of insect control; 3 - to reduce the treatment time to a minimum of 4 hours thus permitting a turnover of two treatments per fumigation chamber per day; and 4 - to develop the application method to a stage where treatments can be undertaken on a routine footing.

The final goal which was not realized in this study would be to phase out the use of MB by substituting the treatment with non-toxic gas mixtures for disinfestation, based on the fact that at most packing stations, subsequent to treatment on arrival, all dates are cold stored at -18°C , under which conditions complete control of all stages of Nitidulid beetles should be obtained.

Materials and Methods - General

The Fumigation Chamber

The trials were carried out in two fumigation chambers at the Date packing station of Zemach. Each chamber was constructed of a concrete floor and roof with concrete block walls, lined on the inside with hard cement and epoxy paint. Dimensions were: length 12.1 m, width 3 m, and heights (chamber 1) 2.99 m, and (chamber 2) 2.88 m, giving total volumes of 108.5 m^3 and 104.5 m^3 , respectively. The door formed one end of the chamber and consisted of galvanized sheet metal on a wooden frame installed as a lift door with counterweight. The door gasket consisted of an endless

rubber tube positioned around the periphery of the door that was inflated to seal the gap between door and door-frame. The ventilation system consisted of an axial fan that could be operated to provide closed circuit ventilation and a centrifugal fan to exhaust the chamber after fumigation. The chamber was equipped with application equipment for MB measurement and discharge (Fig.1).

Fig. 1: Fumigation chamber used for disinfestation trials

The Treatments

Ten fumigation trials were undertaken (Table 1). Fumigants used were Methyl bromide (MB) plus carbon dioxide (CO_2), and both MB and CO_2 separately, according to the following regimes.

The initial treatment was 20% CO_2 in air plus 12 g/m^3 MB. This mixture was based on preliminary laboratory findings on disinfestation for 4 h exposure (20% CO_2 + MB at 8 mg/L), and laboratory findings for control of larvae of two Carpophilus species which indicated an LD_{95} of between 6 and 12 mg/L MB plus 20% CO_2 for a four hour exposure.

Table 1 - Summary of Fumigation Trials - Zemach

TRIAL No.	CO ₂ (%)		MB g/m ³	DURATION hrs	TEMP °C	LOAD
	Calculated	actual				
1	19	7.4		0.9		Full
2	22.5	19	5	2.5		Full
3	21	14	12	4		18%
4	23.6	20	12	4	29-29.5	Full
5	35	33	-	8	21-24	62%
6	-	-	16	8	21-24	43%
7	10	12	12	4	24	62%
8	20	21	12	4	24	43%
9	15	16	12	4	22	Full
10	10	9	12	4	22	Full

Trials 1 and 2:

Methods

These trials were undertaken to determine the most appropriate method for application of CO₂ into the fumigation chamber. The projected exposure period of 4 h necessitated a rapid purge with CO₂. Therefore it was decided to test delivery of CO₂ into the chamber in liquid form using cylinders equipped with siphons, so as to eliminate the problem of super-cooling and blockage of the delivery line. Three 1 inch sampling ports were set into the side wall of chamber No 1, and were used for delivery of CO₂ into the chamber and monitoring of gas concentrations. In the second trial one port was used to deliver the CO₂ into the chamber at floor level; the second was used to purge air from the ceiling level of the chamber and prevent pressure build-up during delivery, while the third was used to distribute gas sampling tubes to different parts of the chamber in order to monitor gas

concentrations and to record pressure within the chamber during gas delivery (Fig. 2).

Chamber load:

In these trials the chamber was loaded with 13 pallets (81% loading capacity) each containing 135 5 kg sealed boxes of packed dates comprising a total of about 9,100 kg. The gas sampling points were placed in the positions indicated in Fig. 2.

CO₂ dosage:

The calculated weight of CO₂ to produce a 20% CO₂ atmosphere within the chamber was 42.8 kg. Delivery was by manual adjustment of the cylinder tap. In the first trial, pressure was adjusted to 25 mm water, however this caused the gasket to slide off the door

Fig. 2: Gas inlet and outlet ports and sampling points in trials 1 and 2

rim and pressure was subsequently reduced and door clamps inserted. In the second trial, delivery was maintained at a pressure within the chamber of not more than 5 mm of water as measured by an inclined manometer. This pressure was well within the stated pressure rating of 25 to 50 mm water as given by the door manufacturer. In the first trial a total of 39.4 kg CO₂ was delivered intermittently within 10 min over a total time of 51 min. In the second trial siphon cylinders were not available and delivery of 47.4 kg CO₂ from normal cylinders took about 30 minutes over a total time of 2.5 h with intermittent closed circuit ventilation of 15 min.

Results

CO₂ concentrations:

In the first trial final CO₂ concentrations were uniform at 7.4% after closed circuit ventilation, the loss being due to leakage as a result of excess pressure during delivery. In the second trial final CO₂ concentrations were uniform at all 7 sampling points at 18.5-19% CO₂.

MB dosage:

MB was delivered in trial 2 at the end of the CO₂ delivery period at a dose of 5 g/m³, followed by closed circuit ventilation for 5 additional minutes. A halide leak detector was used to test for leaks, and a number of faults so revealed around the door seal were repaired in the interim period before trial 3.

Trial 3

In this trial, test insects and infested dates were placed in the chamber to examine the effect of treatment on insect disinfestation and mortality of all stages of Carpophilus

hemipterus. The CO₂ dosage was as in the previous two trials, and MB dosage was 12 g/m³.

Methods

Chamber load:

The chamber was loaded with three pallets (18% load capacity) each consisting of 78 crates of loose dry dates (var: Hadrawi), comprising a total of about 780 kg per pallet, or a total of 2,340 kg. The dates occupied a volume of app. 1.5 m³ or 1.4% of the chamber space (app. 20% of pallet volume). The gas sampling points were placed in the positions indicated in Fig. 2.

Insect cages:

Prior to the trial, adults, pupae and larvae of Carpophilus hemipterus reared under standard conditions in the laboratory, were placed, each stage separately, in groups of 20 individuals per cage. The cages consisted of PVC capped tubes 25 mm long and 15mm i.d. with circles of closely woven brass mesh heat sealed to the tube to replace the lid and floor. In addition eggs were prepared in "oviposition plates" (see Section E.2.) and held in 20 ml glass jars containing wads of saturated filter papers and covered by strips of muslin. Each chamber contained not less than 20 eggs. Seven sets of cages and jars containing each stage were placed before treatment at the gas sampling points given in Fig. 2. Three sets of cages and jars were held outside the chamber and served as control. Upon completion of treatment the chamber was opened, the cages were retrieved and returned to the laboratory, where mortality counts were made as described in Section E.2.

Infested dates:

This trial was carried out before the harvest season when naturally infested dates were not available. Therefore, to evaluate the disinfestation effect of the treatment, infested dates were obtained from two sources:

1 - by moistening dates of the previous season and exposing them in crates for two weeks in the date groves. This material became heavily infested by both adults and larvae of several Nitidulid species;

2- dates, (var: Zaidi) moistened in the laboratory and artificially infested by adults of C. hemipterus and C. mutilatus.

The field infested dates were weighed out into five lots of about 2kg each and placed in date trays lined with polyethylene sheeting. These trays were then placed at points 1, 2, 4, 6, and 7, as shown in Fig 2. After exposure, each sample was wrapped in its liner and returned to the laboratory for evaluation. The laboratory infested dates were divided into four groups of about 1 kg each, placed on deep plastic trays and positioned at points 1, 2, 6, and 7. At the end of treatment the dates were removed from the trays, weighed, and placed in plastic bags for laboratory examination while all insects that had fallen onto the trays were collected and held separately.

Treatment conditions:

CO₂ and MB Dosage: CO₂ was delivered from siphon cylinders continuously. During the period after the first two trials, the chambers had been fitted with 10cm diameter pipes at ceiling level and sealable from the outside with ball-valves to permit CO₂ purging without pressure build-up. Dosage was 45.6 Kg delivered over 11 minutes at a chamber pressure of 10 mm water. This was followed immediately by delivery of 1.29 kg (=2.84 lb) MB to give a dosage of 12 g/m³. MB delivery lasted 2 min. and this was followed by 15 min. closed circuit ventilation. Fumigation temperature as measured by thermo-couple cable inserted in the chamber attached to an electronic thermometer was 31.5°C throughout the treatment.

Post treatment procedure:

Upon completion of the 4 h exposure, the ventilation system was used to exhaust the chamber of the gas mixture. The insect samples and infested date samples were then collected and removed to the laboratory for examination.

Results and Discussion

CO₂ and MB concentrations:

Initial dosage was calculated to give 20% CO₂ with an additional 6% (2.8 kg) to account for sorption into the dates. Initial CO₂ measurements as measured by a Gow Mac thermal-conductivity meter, gave readings ranging from 1.1-1.3% (points 1, 3, 6, at ceiling level) to 40% (points 2, 5, 7, at floor level). After completion of ventilation, all points gave a uniform reading of 14.0% CO₂. This can only be explained by strong initial sorption of CO₂ by the dates and is born out by an observation of the manometer before ventilation of a slight negative pressure within the chamber. At the end of the 4 h exposure period, CO₂ concentrations at the 7 points ranged from 12.0 to 14.0%. Gas samples taken from the same sampling points for MB determination in the laboratory by gas chromatography failed to give accurate results apparently due to leakage from the ampoules during transport. However measurements with a drager detector indicated at least 250 ppm MB within the chamber (equivalent to 1 g/m³).

Insect Control:

From Table 2 it can be seen that complete control of all stages of C. hemipterus was obtained at all 7 sampling points, whereas mortality of eggs larvae, pupae and adults in the control cages were 15.8%, 5.1%, 28.9%, and 19.3%, respectively.

Date disinfestation:

Results of the treatment on date disinfestation are given in Table 3. From the table it can be seen that although the "disinfestation" level in the control dates was high (44.6%), this

possibly being due to handling the dates and the presence of many mature larvae in the samples, this level of disinfestation was lower than all the exposed samples of both laboratory and field infested dates, which ranged from 50% to 100% disinfestation. For all the exposed dates, complete mortality was recorded.

Table 2: Mortality of developmental stages of C. hemipterus placed at 7 sampling points during Trial 3. (Dead insects are given in brackets.)

Sampling Point	Eggs	larvae	pupae	adults
1	*+(146)	0 +(20)	0+(20)	0+(20)
2	*+(86)	0+(20)	0+(20)	0+(20)
3	*+(121)	0+(20)	0+(20)	0+(20)
4	*+(38)	0+(20)	0+(21)	0+(6)
5	*+(82)	0+(7)	0+(19)	0+(20)
6	*+(75)	0+(20)	0+(19)	0+(20)
7	*+(44)	0+(20)	0+(21)	0+(20)
Controls	245+(46)	55+(3)	49+(20)	50+(12)

* Partial emergence occurred prior to exposure. Some dead larvae were observed while other freshly emerged larvae had left the oviposition chambers and mortality for these individuals could not be ascertained.

Trial 4

Methods

This trial was similar to trial 3, with the difference that a full load of dates was treated. The chamber was loaded with two rows 8 pallets measuring 115 x 110 x 190 cm (l x w x h) each made up of stacked date trays. This represents about 35.5% of the total volume of the chamber. Gas sampling points and test insects were placed in similar positions to those in trial 3. Infested dates

were not employed for disinfestation evaluation and only insect control was monitored.

Treatment conditions:

The treatment routine was as in trial 3. A total of 50.5 kg CO₂ was delivered from two cylinders over a 17 minute period immediately followed by 2.84 lb MB, and then closed circuit ventilation for 10 min. Treatment temperature was 29 to 29.5°C. Previous to this trial, airflow rate of the ventilation system was measured using a velometer and calculated as 66.3 m³/min or about one complete circulation of chamber volume every 2 m in.

Table 3: Effects of the treatment in Trial 3 on disinfestation of laboratory (L) and field (F) infested dates.

(results adjusted to express infestations as insects/kg inside and outside dates. Dead insects are given in brackets.)

Sampling Point		Number of insects				Percent dis-infestat- ation	Percent mortality
		inside dates		outside dates			
		larvae	adults	larvae	adults		
1	F	0+(6)	0+(19)	0+(7)	0+(32)	60.1	100
	L	0+(39)	0+(6)	0+(289)	0+(35)	86.1	100
2	F	0+(0)	0+(7)	0+(34)	0+(0)	83.2	100
	L	0+(125)	0+(9)	0+(353)	0+(53)	75.2	100
4	F	0+(10)	0+(21)	0+(79)	0+(37)	78.5	100
6	F	0+(0)	0+(14)	0+(0)	0+(14)	50.0	100
	L	0+(213)	0+(28)	0+(284)	0+(29)	56.5	100
7	F	0+(0)	0+(0)	0+(12)	0+(6)	100	100
	L	0+(87)	0+(0)	0+(283)	0+(8)	77.0	100
Control		137+(0)	7+(0)	109+(0)	7+(0)	44.6	0

Results

CO₂ and MB concentrations:

Initial CO₂ concentrations after circulation were a uniform 20.0%. At the end of the 4 h exposure period these concentrations had decreased to 17 -17.5%. Although facilities were not available to make accurate MB measurements, readings with a Drager sampler after having made appropriate dilutions, indicated an initial MB concentration of 1250 to 1500 ppm (4.8 - 5.8 g/m³) and a final concentration of 1000 ppm, (3.8 g/m³).

Insect control:

Test insects (larvae, pupae and adults) gave complete control of all three stages as in trial 3.

Trials 5 and 6

Methods

Trials 5 and 6 were carried out to determine the effects of carbon dioxide (35%) alone and MB (16mg/m³) alone on insect disinfestation. Fumigation time was 8 hours (overnight) with chamber temperatures ranging from 21 to 24 °C. Initial random samples withdrawn from trays in Trial 5, (fumigation in chamber 1 containing mainly varieties Hadrawi and Deri and filled with 14 pallets each containing 13 - 14 layers of trays), showed percentage of infested dates ranging from 0 to 13%. Trial 6 was carried out in chamber 2 containing variety Omri (5 pallets consisting of 13 layers of trays and 2 pallets each consisting of 3 layers of crates). Here too initial infestation ranged from 0 to 13%. Gas application techniques and gas sampling of both these trials were as in previous trials.

Date Disinfestation:

These trials and subsequent ones were carried out during the harvest season. The technique used for measuring the disinfestation levels was to remove samples of 60 dates "at random" from the trays or crates at points 1, 2, 4, 6, and 7, and place them in trays as described in Trial 3. At the end of the treatment these were collected, the insects on the plastic liners were counted immediately while the dates were taken to the laboratory for examination of residual internal infestation.

Results

Results are given in Tables 4 and 5.

Table 4: Effects of treatment with 35% CO₂ for 8 hours on Date infestation (Trial 5)

(results based on "random" samples of 60 dates from each sampling point. Dead insects are given in brackets.)

Sample Point	Number of insects				Percent dis- infest ation	Percent mort- ality	Percent infest -ed dates
	inside dates		outside dates				
	larvae	adults	larvae	adults			
1	41+(0)	0+(0)	15+(0)	8+(0)	39.6	0	13.3
2	22+(0)	0+(0)	11+(2)	5+(6)	46.6	17.3	11.7
4	5 +(0)	0+(0)	6+(0)	25+(0)	100	0	0
6	0 +(0)	0+(0)	0+(0)	3+(0)	100	0	0
7	47+(0)	0+(0)	11+(0)	3+(0)	22.9	0	3.3
AV.					61.8	3.4	5.7

Examination of the chamber floor after fumigation revealed large numbers of dead insects lying on the floor. Three "random" counts of 10 x 10 cm areas revealed the following numbers:

<u>Area</u>	<u>Dead larvae</u>	<u>Dead adults</u>
1	280	29
2	166	24
3	87	35

Table 5: Effects of MB at 16g/m3 for 8 hours on Date infestation
(Trial 6).

(results based on "random" samples of 60 dates. Dead insects are given in brackets.)

Sample Point	Number of insects				Percent dis- infest ation	Percent mort- ality	Percent infest -ed dates
	inside dates		outside dates				
	larvae	adults	larvae	adults			
1	0+(2)	0+(0)	0 +(6)	0+(3)	81.8	100	3.3
2	0+(0)	0+(2)	0 +(62)	0+(84)	98.6	100	3.3
4	0+(1)	0+(0)	0 +(10)	0+(27)	97.3	100	1.7
6	0+(18)	0 +(0)	0+(110)	0+(22)	88	100	15
7	0+(6)	0 +(0)	0 +(10)	0+(0)	62.5	100	5
Av.					85.6	100	5.7

Trials 7 and 8

Methods

These trials were carried out to compare the disinfestation effect of the MB/CO₂ mixture between dosages of CO₂ at the 10% (Trial 7) and 20% (Trial 8) level for 4 hours exposure. Application of MB and CO₂, gas monitoring were as in previous trials. Insect counts were as in trials 5 and 6.

Results

Results are given in Tables 6 and 7.

Trials 9 and 10

The objective of these trials was to pinpoint the most suitable combination of CO₂ with 12g/m³ MB for 4 hours fumigation. All methodology was as in previous trials. Results are given in Tables 8 and 9.

Conclusions

On the basis of the above ten trials it was concluded that the application of 20%CO₂+12 g/m³ MB mixture for a four hour exposure period, produced complete kill of Carpophilus, and was highly effective in removing the active stages from the dates (disinfestation) at 31°C. Date handling at the packing station during the harvest season indicated the advantage of undertaking a 4 - 6 hour treatment in the morning (8 am to 12 p.m. or 2 pm) followed by an overnight treatment of 16 hours (3 pm to 7 am).

Clearly for the longer fumigation periods, the other MB/CO₂ combinations could be used, and even the 35% CO₂ treatment alone could be envisaged for removal of insects provided that the fumigated dates are transferred directly to the cold storage rooms upon completion of the treatment.

Consequently recommendations were made for the adaptation of the fumigation chambers at the Zemach packaging station to enable CO₂/ MB applications to be carried out at the coming date harvest season.

Table 6: Effects of MB at 12g/m³, CO₂ at 10% for 4 hours on date infestation (Trial 7)

(results based on random samples of 60 dates. Dead insects are given in brackets.)

Sample Point	Number of insects				Percent dis- infest ation	Percent mort- ality	Percent infest -ed dates
	inside dates		outside dates				
	larvae	adults	larvae	adults			
1	50+(4)	0+(0)	20+(14)	1+(1)	40	21.1	5
2	138+(2)	0+(0)	16+(8)	0+(6)	17.6	9.4	3.3
4	4+(6)	0+(1)	4 +(16)	1+(1)	66.6	73	11.7
6	26+(7)	0+(11)	14+(16)	15+(8)	55	43	11.7
7	1+(0)	0+(0)	5+(4)	0+(3)	92	54	1.7
Av.					54.3	40.1	6.7

Table 7: Effects of MB at 12g/m³, CO₂ at 20% for 4 hours on date infestation (Trial 8).

(results based on random samples of 60 dates. Dead insects are given in brackets.)

Sample Point	Number of insects				Percent dis- infest ation	Percent mort- ality	Percent infest -ed dates
	inside dates		outside dates				
	larvae	adults	larvae	adults			
1	41+(11)	0+(0)	28+(18)	0+(5)	49.5	33	10
2	8+(18)	0+(0)	8+(18)	2+(25)	67.1	77	6.7
4	12+(11)	0+(1)	0+(1)	0+(1)	7.6	53.8	5
6	0+(11)	0+(0)	0+(1)	0+(0)	8.3	100	1.7
7	0+(0)	0+(0)	2+(1)	1+(6)	100	70	0
Av.					46.5	66.7	3.97

Table 8: Effects of MB at 12g/m3 and CO2 at 15%, for 4 hours on date infestation (Trial 9)

(results based on random samples of 60 dates. Dead insects are given in brackets.)

Sample Point	Number of insects				Percent dis- infest ation	Percent mort- ality	Percent infest -ed dates
	<u>inside dates</u>		<u>outside dates</u>				
	larvae	adults	larvae	adults			
1	0+(0)	0 +(1)	0+(0)	8+(0)	88.9	11	1.7
2	25+(0)	1 +(0)	34+(0)	0+(12)	63.8	16.6	8.3
4	4+(0)	0 +(0)	10+(0)	2+(0)	75	0	5
6	7+(0)	1 +(0)	54+(0)	0+(2)	87.5	3.1	3.3
7	0+(0)	0 +(0)	1+(6)	0+(0)	100	85.7	0
Av.					83	23.3	3.7

Table 9: Effects of 12g/m3 MB and CO2 at 10%, for 4 hours on date infestation (Trial 10)

(results based on random samples of 60 dates. Dead insects are given in brackets.)

Sample Point	Number of insects				Percent dis- infest ation	Percent mort- ality	Percent infest -ed dates
	<u>inside dates</u>		<u>outside dates</u>				
	larvae	adults	larvae	adults			
1	0+(1)	0+(0)	0+(19)	0+(0)	95	100	1.7
2	0+(0)	0+(0)	0+(2)	0+(4)	100	100	0
4	169+(0)	0 +(0)	0+(90)	0+(0)	34.7	35	15
6	0 +(0)	0+(0)	0+(3)	0+(4)	100	100	0
7	0 +(0)	0+(0)	0+(8)	0+(38)	100	100	0
Av.					85.9	87	3.3

5. The Influence of Low Temperatures on Two Species of Carpophilus (Coleoptera: Nitidulidae)

Introduction

Nitidulid beetles, and in particular Carpophilus mutilatus Er. and C. hemipterus L. are the most important pests of dates in Israel at the time of harvest. Upon arrival at the packing stations the dates are fumigated to control these field infestations and are then stored until processing, usually in cold storage to maintain date quality. This initial fumigation previously with ethylene dibromide, and more recently with methyl bromide (MB) served a two-fold purpose of killing the insect population and also disinfecting the dates by stimulating the active insect stages (larvae and adults) to abandon the dates before they succumb.

Studies have shown (see section 3.1; 3.2 and 3.3 of this report) that non-toxic treatments using modified atmospheres and low pressures were also effective in causing disinfection of Carpophilus larvae and adults and it was also demonstrated that sub-lethal doses of MB are highly efficient in disinfecting the dates (Donahaye and Navarro, 1989). However to enable such treatments to replace the initial fumigation, subsequent control is necessary both of eggs and pupae as well as any active stages still present in the dates. It has been suggested that the storage at low temperatures is sufficient to control such infestations. Those packing houses equipped with cold storage facilities attempt to maintain a storage temperature of -18°C . However during the harvest season the daily introduction of fresh material creates higher ambient temperatures within the cold chambers. Also the rate of penetration of cold into the date crates is relatively slow and the effectiveness of this method for insect control is still unclear. The influence of 0°C on mortality of Carpophilus species has also been questioned. This is of interest since it has been shown (Rygg 1975) that Deglet Noor maintains its quality for

up to a year at 0°C, and for this variety sub-zero temperatures may be superfluous. This investigation was carried out to examine the influence of low temperatures on the mortality of all stages of Carpophilus insects in dates.

There is considerable information in the literature on the influence of temperatures close to 0°C and sub-zero temperatures on stored product insects, (Adler, 1960; Burges, 1956; Cline 1970; Cotton, 1956; Evans, 1987; Jacob and Fleming 1986; Mathlein, 1961; Mullen and Arbogast, 1979; Solomon and Adamson, 1955), while the mechanisms of adaptation to cold have been reviewed by Smith (1974). Cangardel (1981) found that for C. hemipterus and C. ligneus young larvae at 5°C survived for 15 days only, while the threshold for development to the prepupal stage was 10°C. Porter (1986) showed that for C. dimidiatus, eggs failed to hatch at 15°C. However, to our knowledge the sensitivity of the genus Carpophilus to zero and sub-zero temperatures (°C) has not been examined.

Materials and Methods

Test Insects

All stages of C. hemipterus and C. mutilatus were obtained from cultures reared at 26°C and 70% relative humidity (r.h.) on an artificial diet (Donahaye and Navarro 1989).

Eggs were obtained by placing 20 adults in Petri dishes containing a wad of filter-paper saturated with water, a blob of food medium and an "oviposition chamber". This consisted of two microscope cover slips (18 x 18 mm) placed over each other and separated one from the other by a strip of paper 10mm wide and about 50mm long to which they were glued; this produced a slit 0.2mm wide around their periphery. Females inserted their eggs into this slit. The oviposition chambers could be handled using the projecting strip of paper and eggs could be conveniently counted, and emergence recorded under a binocular microscope.

Larvae were taken from culture jars seven days after egg hatch. Pupae were exposed to the treatments one to two days after pupation and were obtained by daily removal of pupae from culture jars. Newly emerged adults were collected daily and held separately on culture medium for 7 days before exposure to the treatments.

Treatments

All stages of both species of Carpophilus were exposed to the two following temperatures:

1. Exposure to 0°C was carried out in an incubation chamber set at 0±2°C. The experiments were carried out in open Petri dishes, 50 mm diameter, placed in a covered desiccator containing a saturated solution of sodium chloride in its lower section. In this way an ambient r.h. of 76% at 0°C was obtained within the desiccator (Hickman, 1970). For larvae, pupae, and adults, 20 insects were exposed in each Petri dish, while for eggs an oviposition chamber containing not less than 20 eggs was used for each Petri dish. Adults were pre-chilled for 5 minutes in closed Petri dishes before transfer in open dishes to the desiccator to prevent their escape. Exposure times of each stage were from 24 h to 120 h at intervals of 24 h. After exposure, food medium was placed in the Petri dishes of the larvae, and Petri dishes of larvae and eggs were transferred to a desiccator containing saturated sodium chloride held at 26°C (to produce an ambient r.h. of 75%) (Solomon, 1951). The adults and pupae were transferred to 200 ml jars containing food medium, and covered by muslin squares before being placed in the post-exposure desiccator. All experiments were replicated 3 times. For each experiment, Petri dishes containing insects were held at 26°C and 75% r.h. inside a desiccator to serve as controls. Pupae were examined for mortality 10 days after exposure while all other stages were examined after 7 days.

The air humidity of 75% was chosen as representative of the micro-environment within the dates. It was based on measurement of relative humidities in equilibrium with moisture contents of

several date varieties taken from cold storage of the Zemach packing station in the Jordan valley. Moisture-humidity equilibrium measurements of the dates were made using an electronic humidity sensor (Nova Sina).

2. Exposure to -18°C was carried out in closed Petri dishes containing a wad of saturated filter paper. The dishes were placed on a shelf of a chamber thermostatically controlled to maintain a temperature of $-18 \pm 2^{\circ}\text{C}$. All stages were exposed for time periods ranging from 10 minutes to 60 minutes at intervals of 10 minutes, with dishes held at 26°C serving as controls. After exposure the insects were returned directly to 26°C and were held at this temperature until examined for mortality. Petri dishes containing larvae and adults received blobs of food medium immediately after exposure and the filter paper in each dish was wetted periodically. Mortality was determined as described previously.

For exposure to 0°C , results were analyzed by probit analysis using the program of Daum (1979). However, lethal times at -18°C were extremely short and data was heterogeneous. Consequently these results are expressed as times required to produce total mortality (LT_{100}).

Results and Discussion

Sensitivity to 0°C

The sensitivities of the two Carpophilus species to 0°C as expressed by regression lines of log-time against probit mortality are given in Figures 1 and 2 and Table 1.

From the figures it can be seen that for both species the egg stage was most sensitive followed by adult, larva and pupa. C. mutilatus was more sensitive to 0°C than C. hemipterus. For C. mutilatus times required to produce 95% kill (LT_{95}) ranged from 30 h (eggs) to 104 h (pupae); for C. hemipterus the LT_{95} for eggs was 89.5 h and for pupae, 142 h (6 days). Although a high heterogeneity of response was recorded which may be partly attributed to the temperature range of $0 \pm 1^{\circ}\text{C}$, these results indicate that to produce total mortality of C. mutilatus an

Fig. 1 - Regression lines to demonstrate the influence on % mortality (probit scale) of exposure time in h, (log scale) at 0°C, of Carpophilus hemipterus (Fig. 1A), and Carpophilus mutilatus (Fig. 1B).

Table 1: Times (h) required to kill Carpophilus hemipterus and C. mutilatus at 0°C (limits designate 95% confidence limits).

species	<u>C. hemipterus</u>				<u>C. mutilatus</u>			
stage	LT50	limits	LT95	limits	LT50	limits	LT95	limits
egg	19.8	13-40	89.5	49-442	8.49	7-10	30.48	26-36
larva	58.1	35-76	192.9	127-783	39.4	30-47	96.6	77-144
pupa	63.1	49-78	142.4	99-286	44.8	31-54	104.6	85-164
adult	44.9	36-53	114.9	91-186	9.05	4-13	41.7	28-76

exposure of about 250 h (10 days) would be required, whereas for total kill of all stages of C. hemipterus an exposure of about 350 h (15 days) would be necessary. The results show a far greater sensitivity to 0°C than that of several stored-product insects investigated so far (namely Sitophilus granarius Plodia interpunctella and Ephestia kuehniella) (Ushatinskaya, 1950; Mullen and Arbogast, 1979) which require more than 2 months to produce complete mortality, but are similar to the sensitivity of Oryzaephilus surinamensis (Obretenchev, 1983; Jacob and Fleming 1986) which is killed after 21 to 26 days at 0°C.

Sensitivity to -18°C:

The times required to produce complete kill of the different stages of the two species of Carpophilus are given in Table 2.

From the table it can be seen that all stages of both species are killed within one hour. This indicates that in situations where cold penetration is rapid such as in the case of unpacked dates, this treatment would be sufficient to control any field infestations by Carpophilus which were not removed during the disinfestation treatment.

Table 2: Times required to produce complete mortality of all stages of C. hemipterus and C. mutilatus exposed to -18°C.

STAGE	LT 100 in minutes	
	<u>C. hemipterus</u>	<u>C. mutilatus</u>
egg	30	40
larva	90	10
pupa	60	60
adult	25	20

Conclusions:

The extreme differences in rates of mortality between exposure to 0°C and -18°C indicate that storage at 0°C is a relatively inefficient means of control, particularly since rate of penetration into the date crates is a factor that must be taken into consideration. Conversely mortality at -18°C is extremely rapid. Clearly this aspect should be studied further to determine mortality rates at intermediate temperatures, as well as rates of cooling within different storage containers, so that exposure schedules could be prepared to account for ambient temperatures within the cold chambers and the packaging used.

6 Influence of Storage in a Carbon Dioxide Enriched Atmosphere on Color Conservation of Deglet Noor Dates

Introduction

The date harvest in the southern Arava valley of Israel in 1987 was 1200 tons. In coming years the annual harvest is expected to rise considerably and in 1992 anticipated production will be in the order of 2500 tons. A similar increase in production is also predicted for the Northern Arava valley. It has been accepted practice in Israel to store dates at the packing houses in refrigerated storage. However, the existing cold-storage capacity is limited, and even at present, refrigerated-storage space is insufficient to store all dates reaching the packing houses during the picking season.

Furthermore, the cost of refrigerated storage of dates is high (about US\$ 8/ton/month at 0°C, and US\$ 15/ton/month at -18°C), while the cost of construction of new refrigerated stores was rated in 1987 at about US\$ 1000 per cubic meter (personal communication, Ardom date packing house).

The objective of this study was to examine the feasibility of storing dates, and maintaining their quality at ambient temperatures and thereby providing a solution to the problem of limited cold-storage capacity.

In the United States and other date producing countries it is accepted practice to store Deglet Noor variety under ambient conditions. In Israel, fruit harvested late in the season is stored in crates under black polyethylene sheeting in the open and undergoes periodic fumigation with methyl bromide to control insect infestations. This fruit is the first to be processed so that it is held for a minimum time, and under ambient winter conditions.

The date variety Medjool and other invert sugar varieties are normally stored in Israel at -18°C. No information is available for these varieties on storage at ambient temperatures (Rygg, 1942; Fattah, 1927; Gensla and Weers, 1973).

Rygg (1957) showed that browning of Deglet Noor depends on two factors, namely: moisture content of the fruit, and temperature during storage. The higher either one of these factors, the more rapid the rate of browning of the fruit. Therefore for prolonged storage it is important to use dry fruit and store at low temperatures.

For storage of date varieties containing invert sugars, such as Saidy, Medjool, and Khadrawy, the main problem when dates are stored at temperatures above -10°C is that of sugar granulation (Rygg, 1942). The granules develop as white spots on or below the date skin and this blemish imparts a disagreeable appearance to the date. Here too, factors influencing granulation were shown to be the moisture content and temperature at which the fruit is stored. It was found that granulation is particularly high at the intermediate range of m.c.s. of 21-30%. Fruit at m.c.s above 30% and below 21% failed to produce spotting.

In addition to the problem of quality conservation of the dates, there is that of insect infestations that can continue to develop throughout the storage period when the fruit is stored at ambient temperatures. The fumigant presently employed to control infestations is methyl bromide. This fumigant is highly toxic and dangerous to operators, imparts a toxic residue to the fruit, (Annon, 1986a) while repeated fumigations under sheeting within the packing house area render its use both inconvenient and expensive with no effective protection against renewed infestation after the fumigant has been released. As an alternative to fumigation, the use of modified atmospheres (MAs) has been developed as an insect control method that leaves no toxic residue in the fruit and is much safer to the user. Furthermore, the MA method has potential to improve maintenance of fruit quality.

Studies made to determine the effect of MAs on the major insect pests of dates showed that effective control can be obtained by exposure to high concentrations of carbon dioxide (CO_2), (Navarro and Dias, 1984; Le Torc'h, 1983; Soderstorm and Barndl, 1984). However, information is lacking on the retention

of quality of dates held for prolonged periods in CO₂ enriched atmospheres, though its influence on quality of stored products has been studied (Banks, 1981; Banks and Gras, 1982; Eguiazu, 1984; Narziss and Hellich, 1971; Slay, 1980). Since the browning effect is at least partly dependent upon the availability of oxygen, it is reasonable to suppose that CO₂ enriched atmospheres will retard the browning process.

The objective of this study was to evaluate quality of dates stored for prolonged periods in CO₂ enriched atmospheres.

Materials and Methods:

Experiments to determine the rate of browning in a MA of 80% CO₂ in air, were carried out on dates var. Deglet Noor, harvested at about 14% m.c. from the Southern Arava valley in mid October 1987. The dates were stored at 0°C at the regional packing station until mid November when they were divided into two lots. One lot was then hydrated to raise its m.c. to about 18%. Both moist and dry lots were then stored at -18°C until transferred to the Stored Products Department of the ARO at Bet Dagan in early December.

Date moisture was measured by sampling 10 fruit at random from each lot. The dates in each sample were chopped up and then divided into 4 sub-samples each weighing about 25 g. Moisture content was determined according to the AOAC method (Anon, 1980b) in a vacuum oven with introduction of dried air for 48h at 70°C. The dates were then weighed out into groups of 550 g, and each group was placed in a 1 litre "Kilner type" screw capped jar. These jars served as containers for storage under the experimental MA. The metal screw-lid was fitted with a foam-rubber seal to provide a hermetic closure between jar and lid. A copper tube (3mm e.d) was inserted through the lid and soldered in place, so that about 2.5 cm projected above the lid. This tube served for introduction of the CO₂ to obtain the required MA, and also for periodic sampling for gas concentration analyses.

To obtain the desired MA of 80% CO₂ in air, air was removed from the jars using a vacuum pump attached via a connecting tube,

to the copper tube, until the pressure within the jar, as indicated by a manometer, was 20% of the original atmospheric pressure. Then the vacuum pump was disconnected and the connection tube was switched to a supply of CO₂ that was allowed to flow into the jar until the original pressure was restored. The connecting tube was then removed and the copper tube was capped with a rubber septum that served for subsequent withdrawal of samples by syringe for gas composition analysis. This was carried out using a gas chromatograph equipped with a thermal-conductivity detector.

Dates placed in Kilner jars, and hermetically sealed by the screw lid without introduction of CO₂, served as control.

The jars containing both moist and dry dates, both under modified atmosphere, and the control jars (normal atmosphere), were held at four temperatures, namely: 15, 20, 25 and 30°C. Control jars were also held at -18°C for comparison with rates of browning at the higher temperatures.

Four storage periods of one, two, three, and five months were investigated. Each treatment was carried out with three repetitions, where each jar consisted of one repetition.

Carbon dioxide concentrations in each jar were recorded monthly, and for jars in which the CO₂ concentration had dropped to below 70%, additional CO₂ was added to restore the 80% level.

Color of the dates was measured for each treatment at the end of each storage period using a Gardner (Gardner Laboratory Inc. Bethesda, Maryland U.S.A.) digital color difference meter. This instrument contains three filters which fit over three photocells, giving three different readings on color: L (lightness) - the higher this value the paler the color, and the lower the value the darker the color; a (red-green) - red gives a positive value (+), and green a negative value (-); b (yellow-blue) - yellow gives a positive value (+) and blue a negative value (-). (Pomeranz, 1978; Basker, 1988). The instrument was calibrated using a yellow reflectance standard with values of L=74.9, a=1.6 and b=49.3.

Meter readings: After removal of the calibration standard source from the sample aperture plate, a sample holder consisting of an aluminum ring with a 1cm diameter hole was placed over the opening and the instrument was adjusted to produce a narrow beam. The dates to be measured were placed over the sample holder and each date was measured on both sides. When there were differences in color between the two sides distinguishable by the naked eye the palest area and the darkest area were tested. From each jar 10 dates were sampled at random, the average of the two readings for each date was recorded, and the average value of the 10 dates was recorded.

Results were analyzed by factorial analysis of variance using the SAS program, and significance was determined using Duncan's multiple range test. (Duncan, 1955; Sokal and Rohlf (1981)).

Results and Discussion

Changes in quality of Deglet Noor dates during storage have been documented by Rygg and co-workers 35 years ago (Rygg et al., 1953; Rygg, 1956; Rygg, 1957). They showed that Deglet Noor at and below 18% moisture content (MC), and at 23°C, can be stored for 6 months without significant changes in quality, while color remains pale. Commercial scale trials were carried out by Norman et. al. (1975; 1976) on Deglet Noor dates held outdoors in 360 kg crates in California over a 5 month period from November till April. Using dates at 16-20% m.c. they recommended that storage under these conditions be as short as possible and limited to the driest fruit. The principal change recorded during storage was browning due to increase in soluble pigment content.

In our experiments moisture content determination of the two lots of dates showed that the average MC of the dry dates used in the experiment was 13.94% and that of the hydrated dates was 17.5% m.c.

Figure 1 shows the influence of 80% CO₂ on the maintenance of pale (amber) color of Deglet Noor dates at two moisture contents and four temperatures during storage. It should be noted

from the initial level of paleness that the moist dates were darker than the dry dates. Accordingly, in the figure it can be seen that the dry dates were significantly paler than the moist dates at all temperatures and storage periods. The statistical analysis confirmed that at all the temperatures for a given storage duration, and for all storage periods at the same temperature, there were almost always significant differences in color between moisture contents, the moist dates being darker than the dry ones. Also the dates held in the 80% CO₂ atmosphere were paler than the controls for any given temperature or storage period, except for isolated treatments such as moist dates at 20°C after 2 and 3 months storage and at 30°C after one month storage. Also, for dry dates, the effect of the MA was not significant at one and two months storage at 25°C, and at one month storage at 30°C, but at other temperatures and for the other time periods it was, dates held under MA being paler than control dates. From this it is evident that for the majority of treatments, and particularly for prolonged storage of 3 to 5 months, date conservation in the CO₂ enriched atmosphere was superior to that of the controls.

Modified atmospheres in storage are being used principally to control stored product insect pests in cereal grains. For this purpose atmospheres of 1% oxygen in nitrogen, or 60% CO₂ in air are recommended (Banks, 1981).

The above data provides a basis for design of date storage in CO₂ enriched atmospheres both for the control of potential insect infestation and preservation of date quality. The mode of action of CO₂ enriched atmospheres on date pigments is not known from the literature. However it may be presumed that the superior coloration of dates obtained in these trials were largely due to reduction in partial pressure of oxygen in the MA to 4%, and the consequent retardation of any browning process of the pigments due to oxidation.

Fig. 1 - Influence of 80% CO₂ on lightness of Deglet Noor dates at two moisture contents and four temperatures during storage of up to 5 months. Plus an additional control group kept at -18°C.

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F - DESCRIPTION OF COOPERATION

At all stages of the project, cooperation existed between Dr. Jay, USDA Savannah, Georgia, and the Israeli investigators. This cooperation was expressed at the initial planning stages of the proposal and over the actual undertaking of the research, during the work, mainly by correspondence. Since the research originally was limited to one year, the proposal was deprived of mutual foreign visits. Although the principal investigator had the opportunity to visit the Savannah laboratory privately, a counter-visit of Dr. Jay to Israel would have better contributed to the cooperation. However, Dr. Jay met the principal investigator at an international Conference, and this provided an additional opportunity to discuss different aspects of the project and to supply information of mutual interest concerning the research achievements. The ideas exchanged and the professional approaches adopted by both countries' investigators formed the basis for the amassment of the results presented in this report.

G - EVALUATION OF THE RESEARCH ACHIEVEMENTS WITH RESPECT TO THE AIMS OF THE ORIGINAL RESEARCH PROPOSAL, EMPHASIZING THE BENEFIT TO AGRICULTURE.

- Evaluation of the research achievements

Serious doubts on the possibility of using alternative methods to toxic chemicals for disinfestation of dried fruits, were expressed by the reviewers at the evaluation stage of this project. These doubts were pertinent to two different aspects of the research: the low credit given to potential MAS to have a pronounced expelling effect; and the limited time-frame in the proposal given to the elucidation of the mechanism by which insects self separate from dates.

For commercial treatments, as an initial phase of the research, a means of reducing MB dosages was demonstrated by exposing the dates to a mixture of MB+CO₂. These treatments achieved both reduced exposure time and concentration of MB required for disinfestation and control. This section of the project was carried out to supply the date industry with a tool for immediate use until the non-chemical treatment method is fully developed and implemented on a large scale.

Two of the alternative non-chemical disinfestation methods were proven to be significantly and markedly more effective than currently practiced fumigation using MB. This disinfestation technique may be viewed as the principal breakthrough of this research. In terms of practicality and economic feasibility both low oxygen concentrations in nitrogen, and CO₂ enriched atmospheres were found to be effective in expelling insects from dates, though CO₂ was only effective at prolonged exposures. For short term treatments the application of low oxygen atmospheres was proven very effective in disinfestation. We suggest that this could be achieved in existing fumigation chambers either through flushing with on-site generated nitrogen or by recirculating the fumigation chamber air through catalytic converters - though this aspect has yet to be investigated. Another method requiring more

expensive installations is the use of low pressures. Since some packing houses may have such equipment for vacuum fumigation with MB, the use of vacuum alone without the toxic residue-leaving fumigant should be encouraged as a disinfestation treatment.

Results of this research provide important tools for alternative solutions to the time worn reliance upon toxic fumigants. It clearly indicates that the food market can be supplied with dried fruits free from undesired insect contamination and above all, free from toxic fumigant residues.

The limited time-frame allotted to this research was a direct outcome of the skepticism expressed by the reviewers to the original proposal. Consequently the revised proposal of this project was influenced by its one-year limitation and focussed on the need to demonstrate the possibility of using non-chemical alternative treatments for disinfestation. After the initial successful results, the investigators requested extension of the project, and this was approved with additional financial support.

The approach adopted in this project for disinfestation of fruits without chemicals should be accompanied with subsequent measures to control both eggs and pupae as well as any active stage still present in the dates. Those packing houses equipped with cold storage facilities attempt to maintain a storage temperature of -18°C , suitable for invert sugar date varieties. This temperature is potentially lethal to storage insects. However, for dry date varieties which can be stored at ambient temperatures, a possible solution could be storage in a MA environment. Further aspects on the influence of low temperatures as well as, and especially the influence of storing dry date varieties (i.e. Deglet Noor) in a MA environments deserves further investigation.

The physiological studies relevant to the mechanism through which insects abandon the dates were not accomplished. The investigators were able to demonstrate that any state of stress exerted on the insects causing them to stop feeding and move restlessly, was capable of expelling them from the dates. This

finding by itself was of value in that it indicated the importance of the end effect rather than the physiological mechanism induced by the treatment. Still the elucidation of this mechanism by using different techniques (such as video taping with slow motion techniques as suggested by one of the reviewers) remains of important scientific value. The importance of this mechanism could receive further emphasize if it were to direct our search toward other non-toxic methods of causing insects to alter their behavior from one of feeding to wandering.

- Benefit to agriculture

Improvements in the keeping quality of dried fruits during storage by lowering or eliminating pesticide residues is an important objective of agricultural production. Development of non-chemical methods for disinfestation of dried fruits is, we believe, an important new avenue of approach for replacing the time worn fumigation techniques for which until now, no alternatives had been available. The benefits of this development will contribute directly to the trade of both countries and to the health of their citizens.

Results of this report clearly indicate the possibility of supplying "non fumigated dried fruits" which are at the same time disinfested at a higher level than the currently used fumigation techniques. In the environment orientated world of today, and in view of the genuine deep concern of both countries regarding the presence of toxic chemical residues in food, and specifically in this case, of MB which is now under scrutiny, we consider the results presented in this report to be a significant contribution to the benefit of the agriculture of both countries.

H - List of publication

Donahaye, E. and Navarro, S. (1989) Sensitivity of two dried fruit pests to methyl bromide alone, and in combination with carbon dioxide or under reduced pressure. Trop. Sci. 29: 9-14.