Navarro, S. and Donahaye, E. [Eds.](1993) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Grain Storages, Winnipeg, Canada, June 1992, Caspit Press Ltd., Jerusalem, pp. 389-398.

INTEGRATION OF CONTROLLED ATMOSPHERE AND LOW TEMPERATURE FOR DISINFESTATION AND CONTROL OF DRIED FRUIT BEETLES

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ABSTRACT

Nitidulid beetles are important pests of dried fruits, particularly dates at the time of harvest. Upon arrival at the packing stations, dates are disinfested by fumigation. This treatment serves a twofold purpose of stimulating the active insect stages (larvae and adults) of field infestations to abandon the fruits and also killing all stages of the insect population. Experiments were carried out to investigate the effect of various modified atmospheres (MAs) and low pressures alone in prompting the emigration of Nitidulid beetles from infested dried fruit, for which dates served as a model. The most effective treatments were pressure of 100 mm mercury (Hg) and 2.8% oxygen (O_2) in air, both of which caused over 80% of the initial insect populations to emigrate from the fruit after a 4-hr exposure. However, the MA treatments required longer exposure times to achieve complete mortality as compared with standard fumigation procedure. An alternative approach to controlling the insects after disinfestation by MAs was by storage at low temperatures. Exposure to -5°C caused relatively slow kill. Pupae were the most resistant stage, necessitating approximately 90 hr to produce 99% kill (LT_{99}) of *Carpophilus mutilatus* Er and *Carpophilus hemipterus* L. Exposure to -18°C caused very rapid kill of both species, LT_{99} of all stages being obtained within 2.25 hr.

INTRODUCTION

Nitidulid beetles, *Carpophilus mutilatus* Er and *Carpophilus hemipterus* L. in particular, are the most important pests of dates in Israel at harvest. Upon arrival at the packing stations, the dates are fumigated to control field infestations, and are then stored until processing, usually in cold storage facilities to maintain date quality. This initial fumigation, done formerly with ethylene dibromide and more recently with methyl bromide

(MB), serves a dual purpose of disinfesting the dates by stimulating the active insect stages (larvae and adults) to emigrate from the fruit before they succumb, and also killing the insect population (Anon, 1986). The mechanism of this emigration effect that results in "disinfestation" is not yet clear.

The disinfestation effect of the treatment is no less important than the toxic control effect enabling the producer to conform with minimum acceptance levels for the presence of insect contamination. With this approach in mind the possibility was considered that other treatments, such as the use of modified atmospheres (MAs) or low pressures, may be effective in causing disinfestation of dried fruits (Navarro and Dias, 1984). It was also demonstrated that sub-lethal doses of MB are highly efficient in disinfesting the dates (Donahaye and Navarro, 1989). However, for such treatments to replace the initial fumigation, subsequent control, both of eggs and pupae as well as of any active stages still present in the dates is necessary. It has been suggested that storage at low temperatures is sufficient to control such infestations and indeed the packing houses that are equipped with cold storage facilities, attempt to maintain a temperature of -18°C for date storage prior to processing. The effect of 0°C on mortality of *Carpophilus* species has also been questioned. This is of interest as it has been shown (Rygg, 1975) that the date variety "Deglet Noor" maintains its quality for up to a year at 0°C, and for this variety sub-zero temperatures may be superfluous for quality control.

There is considerable information in the literature on the effect of near 0°C temperatures and of sub-zero temperatures on stored-product insects (Ushatinskaya, 1950; Solomon and Adamson, 1955; Burges, 1956; Adler, 1960; Cline, 1970; Mullen and Arbogast, 1979; Jacob and Fleming, 1986), while the mechanisms of adaptation to cold have been reviewed by Smith (1974). Cangardel (1981) found that for *C. hemipterus* and *Carpophilus ligneus*, young larvae survived at 5°C for 15 days only, while the threshold for development to the prepupal stage was 10°C. Porter (1986) showed that for *Carpophilus dimidiatus*, eggs failed to hatch at 15°C. On this basis, an investigation was initiated to examine the effect of low temperatures on the mortality of all stages of *Carpophilus* insects in dates (Donahaye *et al.*, 1991).

This paper demonstrates the possibility of integrating two non-chemical insect control methods, namely, MAs for disinfestation, and low temperatures for the control of insect pests of dried fruits at packing houses.

MATERIALS AND METHODS

Experiments for testing effectiveness of MAs on insect emigration

Test insects

These experiments were carried out using dates infested with Nitidulid beetles (mixed populations of *C. hemipterus*, and *C. mutilatus*, with a few individuals of *Haptoncus luteolus*).

Treatments

All treatments (5) were carried out at 26±1°C. The experiments were conducted in 2.54-L desiccators. For each treatment, an exposure time of 4 hr was employed. The treatments and methods of application were as follows:

1) Application a dose of 16 mg/L MB. Dosage calculations were converted to the gaseous phase (Anon., 1981).
2) Treatment consisting of a 20% concentration of carbon dioxide (CO_2) in air. This mixture was delivered from an apparatus described by Donahaye (1990).
3) Maintenance of a low pressure of 100 mm Hg.
4) Creation of an atmosphere of 2.8% oxygen (O_2) in nitrogen (N_2). This mixture (equivalent to the partial pressure of O_2 in air at 100 mm Hg) was obtained by evacuating the desiccator to 100 mm Hg. followed by restoration of atmospheric pressure using N_2.
5) Ambient air at atmospheric pressure and 26±1°C (control).

Experimental procedure

Desiccators were fitted with raised perspex floors drilled with 5-mm holes that separated each desiccator into upper and lower compartments. Each desiccator was loaded with 20 dates taken at random from the infested date supply. The dates were then exposed to the treatments as described above, and upon completion of the exposure period, they were removed from the desiccators and free insect stages (adult and larva, dead or alive) present on the surface of the dates and at the base of the desiccators, were counted. Then, each date was opened lengthwise with a scalpel and the numbers of adults and larvae (dead or alive) still present in each date were counted.

The ratio of the number of insects found outside the dates to the total number of insects (including adults and larvae) still present in the dates was used to give the percentage of insects found outside the dates and termed "per cent disinfestation".

Each treatment, except MB alone, was carried out at least ten times and for each set of experiments, a control desiccator was exposed to the normal atmosphere under the same ambient conditions as the treated dates and for the same time period. Results of the experiments were analyzed by

the SAS program for completely randomized design of analysis of variance, and significance of differences between the means was analyzed by Duncan's Multiple Range Test (Freund and Littell, 1985).

Experiments for testing effects of low temperatures on insect mortality

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 a synthetic food medium (SFM) (Donahaye and Navarro, 1989).

Eggs were obtained by placing 20 adults in a petri dish containing a wad of filter paper saturated with water, a blob of SFM, and an oviposition chamber. The chamber consisted of two microscope cover slips (20 x 20 mm) placed over each other and separated from each other by a strip of paper 10 mm wide and approximately 50 mm long to which they were glued; this produced a 0.2 mm-wide slit along 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 counted conveniently, and emergence recorded, under a binocular microscope.

Larvae were taken from culture jars 7 days after egg hatch. Pupae were exposed to the treatments 1-2 days after pupation, and 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 following four temperatures: 0°, -5°, -10°, and -18°, all within the range of ± 1°C.

Experimental procedure

For larvae, pupae, and adults, 20 insects were exposed in each petri dish, while for eggs, an oviposition chamber containing no fewer than 20 eggs was used for each petri dish. The adults and pupae were transferred to 200-ml jars containing food medium, and covered with muslin squares before being placed in the post-exposure desiccator.

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%). An air humidity of 75% was chosen as being representative of the microenvironment within the dates.

All experiments were done in three replicates. For each experiment, petri dishes containing insects were maintained 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. Failure of eggs to hatch, or of pupae to produce adults, were criteria of mortality for the non-active stages. Results were analyzed by probit analysis using the program of Daum (1979).

RESULTS AND DISCUSSION

Emigration of beetles from dates

Results of disinfestation of infested dates exposed to the treatments for 4 hr expressed as "per cent disinfestation" are given in Fig. 1. For the 4 hr exposure, the 2.8% O_2 treatment was most effective, followed by 100 mm Hg, although these did not differ significantly (Fig. 1). No significant differences in disinfestation were obtained between 100 mm Hg and MB alone. Also, MB alone did not differ significantly from 2.8% O_2. Exposure to CO_2 for 4 hr was less effective than the other treatments and did not differ significantly from the control.

Data in Fig. 1 show that treatments other than MB are just as, and some are more effective in inducing insects to abandon the fruits. The reasons for this emigration effect have not yet been studied, but as all the treatments in the present study had an adverse effect on insect survival it is postulated that the principal reason for emigration is the stress exerted on the insects.

The enhanced disinfestation effect of a low pressure of 100 mm Hg was evident; however, it was less effective than, though not significantly different from, the equivalent partial pressure of O_2 in nitrogen at atmospheric pressure. This indicates that disinfestation was not due to low pressure *per se* but to the low-O_2 partial pressure resulting from this treatment.

Effects of low temperatures on insect mortality

Sensitivity to 0°C

The sensitivities of the two *Carpophilus* species to 0°C as recorded from regression analysis of log-time against probit mortality, are given in Table 1. Table 1 demonstrates that for both species the egg stage was most sensitive, followed by the adult. For *C. hemipterus* the larva was more resistant than the pupa, whereas for *C. mutilatus* the pupa was more resistant than the larva. Except for the egg stage, *C. hemipterus* was more resistant to 0°C than was *C. mutilatus*. For *C. hemipterus*, the times required to produce 99% kill (LT_{99}) ranged from 50.16 hr (eggs) to 317.3 hr (larvae); for *C. mutilatus*, the LT_{99} range was 51.74 hr (eggs) and 148.73 hr (pupae).

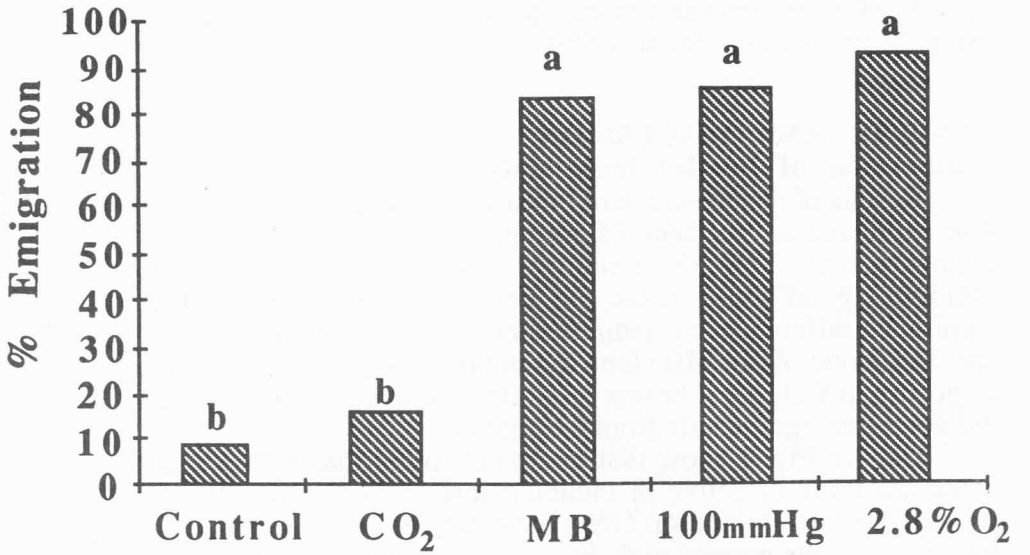

Fig. 1: Emigration of insects expressed as percentage of insects found outside the dates infested by Nitidulid beetles after exposure to various treatments for 4 hr at 26°C. Disinfestation levels assigned the same letter do not differ significantly at $p<0.05$.

These results show a far greater sensitivity to 0°C than that of several other stored-product insects investigated (namely *Sitophilus granarius, Plodia interpunctella,* and *Ephestia kuehniella*) (Mullen and Arbogast, 1979) that need an exposure time of more than 2 months to produce complete mortality, but are closer to the sensitivity of *Oryzaephilus surinamensis* (Obretenchev, 1983; Jacob and Fleming, 1986), that is killed after 21 to 26 days at 0°C.

Sensitivity to -5°C:

The sensitivities of the two *Carpophilus* species to -5°C are given in Table 1. The pupal stage of both species was by far the most resistant of all the development stages, with the LT_{99} approximately 89 hr for both species. At -5°C, the egg stage was no longer consistently the most sensitive. For *C. hemipterus,* LT_{99} values ranged from 13.92 hr (eggs) to 89.7 hr (pupae); for *C. mutilatus* the LT_{99} was 10.25 hr for adults and 89.26 hr for pupae. The order of sensitivity was: egg>larva>adult>pupa for *C. hemipterus*, and adult>larva>egg>pupa for *C. mutilatus*. Mullen and Arbogast (1979) demonstrated that the LT_{95} for *Tribolium castaneum* eggs exposed to -5°C was approximately 18 hr, whereas for eggs of *Callosobruchus maculatus* the

same level of control was obtained only after 46 hr of exposure. In another study (Obretenchev, 1983), complete mortality of all development stages of *O. surinamensis* was obtained after 60 hr of exposure to -5°C.

Sensitivity to -10°C

The times required to produce complete kill of the different stages of the two species of *Carpophilus* exposed at -10°C are given in Table 1. At -10°C, the order of sensitivity was adult>larva>egg>pupa for *C. hemipterus*, and adult>larva>egg>pupa for *C. mutilatus*. at -10°C all stages of both species were killed within 10.35 hr. Rassmann (1980) found that complete mortality of *Lasioderma serricorne* larvae was obtained at -12°C after 14.5 hr when exposed within boxed cigars. As for *O. surinamensis,* investigated by Obretenchev (1983), complete mortality of all development stages was obtained after exposure for 3 hr and 55 min at -10°C. Results obtained by Mullen and Arbogast (1979) on eggs of *O. surinamensis, T. castaneum,* and *Ephestia cautella* showed that to obtain LT_{95}, exposures of 7, 8, and 9 hr, respectively, were sufficient, whereas longer exposures were required for the same mortality level of eggs of *L. serricorne* (28 hr) and *C. maculatus* (62 hr).

Sensitivity to -18°C

At -18°C, all stages of both species are killed within 2.25 hr (Table 1). The order of sensitivity was adult>egg>larva>pupa for *C. hemipterus*, and adult>larva>egg>pupa for *C. mutilatus* . In work carried out by Mullen and Arbogast (1979) it was shown that among the five species of stored-product insects tested, eggs of *L. serricorne* and *C. maculatus* ,were most resistant to -20°C and exposure in excess of 1 hr was required to obtain LT_{95}, whereas to control all development stages of *O. surinamensis,* Obretenchev (1983) found that 47 min was required at -15°C.

CONCLUSIONS

The experiments were carried out to compare the effectiveness of a number of treatments including MB in inducing emigration of *Carpophilus* spp. larvae and adults from dates. Our study of emigration from infested fruit was subject to several constraints including the presence of different sizes and compositions of insect populations among the various batches of dates, use of various date varieties at different moisture contents, and non-uniformity of developmental stages of insects among batches. Many larvae emerged from control dates, all of these being mature larvae in the wandering stage before pupation. In spite of these limitations our study revealed significant differences between the treatments in causing emigration of insects, though even for the most effective treatments there still remained a small residual insect population within the fruit.

Table 1: LT 99 values (in hours) required to kill *Carpophilus hemipterus* and *C. mutilatus* at different temperatures.

Insect species	Stage	Temperature (°C)			
		0	-5	-10	-18
C. hemipterus	egg	50.16	13.92	3.85	1.39
	larva	317.30	17.65	2.85	1.43
	pupa	199.50	89.70	4.34	2.25
	adult	169.66	24.32	1.60	0.48
C. mutilatus	egg	51.74	27.90	5.67	1.37
	larva	140.03	17.55	2.44	0.77
	pupa	148.73	89.26	10.35	1.72
	adult	78.70	10.25	0.84	0.64

There were extreme differences in rates of mortality between exposure to 0°C and -18°C. Our results indicate that storage at 0°C and -5°C is relatively inefficient for control of the *Carpophilus* species, particularly as rates of cooling of the dates, and the form and size of packaging, must be taken into consideration. Conversely, mortality at -10°C and -18°C is extremely rapid, and shortly after the center of the date container reaches these temperatures, complete control is assured. In situations where cold penetration is rapid, as in the case of unpacked dates, this treatment would be sufficient to control any field infestations by these two *Carpophilus* species that were not removed during the disinfestation treatment.

These studies show that integration of MAs and low temperatures offers an alternative to methyl bromide fumigation to achieve disinfestation and control of dried fruit pests.

ACKNOWLEDGMENTS

This research was supported in part by Grant No. I-1095-86R from the United States-Israel Binational Agricultural Research and Development Fund (BARD).

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