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The influence of low temperatures on two species of *Carpophilus* (Col., Nitidulidae)[1]

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Abstract

The effect of exposure to 0 °C, −5 °C, −10 °C and −18 °C was investigated on all developmental stages of *Carpophilus hemipterus* L. and *C. mutilatus* Er.

Exposure to 0 °C caused relatively slow kill. Lethal exposure time (LT_{99}) was longest (317.30 h) for larvae of *C. hemipterus*. At −5 °C, exposure times required to control both species were also prolonged. Pupae were the most resistant stage, about 90 h being needed to produce 99 % kill of both species. At −10 °C, mortality of both species was rapid, pupae being again the most resistant stage, 10.35 h was required for LT_{99}. Exposure to −18 °C caused very rapid kill of both species. LT_{99} of all stages being obtained within 2.25 h.

1 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 field infestations, and are then stored until processing, usually in cold storage to maintain date quality. This initial fumigation, formerly done with ethylene dibromide and more recently with methyl bromide (MB), serves a twofold purpose of killing the insect population and also disinfesting the dates by stimulating the active insect stages (larvae and adults) to abandon the dates before they succumb. Studies have shown (Navarro and Dias 1984) that non-toxic treatments using modified atmospheres and low pressures were also effective in achieving disinfestation of *Carpophilus* larvae and adults, and it was also demonstrated that sub-lethal doses of MB are highly efficient in disinfesting the dates (Donahaye and Navaro 1989). However, to enable such treatments to replace the initial fumigation, subsequent control is necessary both of eggs and pupae as well as of any active stages still present in the dates. It has been suggested that storage at low temperatures is sufficient to control such infestations. The packing houses that are 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 for quality control.

There is considerable information in the literature on the influence of temperatures close to 0 °C and of sub-zero temperatures on stored product insects, Adler 1960;

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Burges 1956; Cline 1970; Jacob and Fleming 1986; Mullen and Arbogast 1979; Solomon and Adamson 1955; Ushatinskaya 1950), 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 the best of our knowledge, the sensitivity of *Carpophilus* species to zero and sub-zero temperatures (°C) has not been examined.

This investigation was carried out to examine the influence of low temperatures on the mortality of all stages of *Carpophilus* insects in dates.

2 Materials and method

2.1 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 × 20 mm) placed over each other and separated from each other by a strip of paper 10 mm wide and ca 50 mm long to which they were glued; this produced a slit 0.2 mm 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 7 days after egg hatch. Pupae were exposed to the treatments 1 to 2 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.

2.2 Treatments

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

Exposure to 0 °C was carried out in a chamber set at 0 ± 1 °C. The experiments were carried out in open petri dishes, 50 mm diameter, placed in a covered desiccator of 3.4 liters 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). Adults were pre-chilled for 5 min in closed petri dishes, to prevent their escape before transfer in open dishes to the desiccator.

Exposure to −5 °C, −10 °C and −18 °C were carried out in closed petri dishes containing a wad of saturated filter paper. The dishes were placed on a shelf in a chamber thermostatically controlled to maintain the test temperatures at a range of ± 1 °C.

2.3 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 by muslin squares before being placed in the post-exposure desiccator. Time intervals were selected to cover the different ranges of mortalities of the stages of the two species.

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 representative of the microenvironment within the dates. It was based on measurement of relative humidities in equilibrium with moisture contents of several date varieties taken from cold storage in the Zemach packing station in the Jordan Valley, Israel. Moisture-humidity equilibrium measurements of the dates were made using an electronic humidity sensor (Nova Sina).

All experiments were done in three replicates. 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. 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).

3 Results and discussion

3.1 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. From table 1 it can be seen 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 smore resistant to 0 °C than was *C. mutilatus*. For *C. hemipterus*, the times required to produce 99 % kill (LT_{99}) ranged from 50.16 h (eggs) to 317.3 h (larvae); for *C. mutilatus*, the LT_{99} was 51.74 h for eggs and 148.73 h for pupae.

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) which need exposure of more than 2 months to produce complete mortality, but are closer to the sensitivity of *Oryzaephilus surinamensis* (OBRETENCHEV 1983; JACOB and FLEMING 1986), which is killed after 21 to 26 days at 0 °C.

Table 1. **Time (in hours) required to kill *Carpophilus hemipterus* and *C. mutilatus* at 0 °C** (99 % confidence limits)

Insect species	Stage	LT_{99}	limits lower	limits upper	Slope	SE	chi-square
C. hemipterus	egg	50.16	34.7–	99.4	2.50	0.40	21.02
	larva	317.30	178.1–	2522.5	3.15	0.89	37.70
	pupa	199.50	136.4–	530.7	4.65	1.65	23.68
	adult	169.66	125.6–	294.3	4.03	0.62	28.87
C. mutilatus	egg	51.74	42.3–	68.3	2.96	0.28	21.03
	larva	140.03	104.1–	253.1	4.22	0.75	38.88
	pupa	148.73	110.7–	301.6	4.46	0.94	27.59
	adult	78.70	48.8–	200.1	2.47	0.45	28.87

3.2 Sensitivity to −5 °C

The sensitivities of the two *Carpophilus* species to − 5°C are given in table 2. The pupal stage of both species was by far the most resistant of all the development stages, with the LT_{99} ~ 89 h 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 h (eggs) to 89.7 h (larvae); for *C. mutilatus* the LT_{99} was 10.25 h for adults and 89.26 h 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 ~18 h, whereas for eggs of *Callosobruchus maculatus* the same level of control was obtained only after 46 h of exposure. In another study (OBRETENCHEV 1983) complete mortality of all development stages of *O. surinamensis* was obtained after 60 h of exposure to −5 °C.

3.3 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 3. At −10 °C the order to sensitivity was adult > larva > egg > pupa for *C. hemipterus*, and adult > larva > egg > pupa for *C.*

Table 2. **Time (in hours) required to kill *Carpophilus hemipterus* and *C. mutilatus* at −5 °C** (99 % confidence limits)

Insect species	Stage	LT_{99}	limits lower	limits upper	Slope	SE	chi-square
C. hemipterus	egg	13.92	9.3–	3.3	3.34	0.60	35.17
	larva	17.65	8.6–	77.3	1.69	0.30	34.41
	pupa	89.70	59.4–	179.8	2.96	0.39	31.41
	adult	24.32	12.81–	123.3	2.80	0.61	33.92
C. mutilatus	egg	27.90	17.4–	61.7	2.03	0.30	46.90
	larva	17.55	8.03–	172.6	1.79	0.43	26.68
	pupa	89.26	42.9 –	511.9	2.03	0.42	33.92
	adult	10.25	6.65–	23.53	2.94	0.52	41.38

mutilatus. At −10 °C all stages of both species were killed within 10.35 h. RASSMANN (1980) found that complete mortality of *Lasioderma serricorne* larvae was obtained at −12 °C after 14.5 h 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 h 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 h, respectively, were sufficient, whereas longer exposures were required for the same mortality level of eggs of *L. serricorne* (28 h) and *C. maculatus* (62 h).

Table 3. **Time (in hours) required to kill *Carpophilus hemipterus* and *C. mutilatus* at −10 °C** (99 % confidence limits)

Insect species	Stage	LT_{99}	limits lower	limits upper	Slope	SE	chi-square
C. hemipterus	egg	3.85	2.83–	6.44	3.05	0.44	43.77
	larva	2.85	1.31–	54.21	1.85	0.54	33.92
	pupa	4.34	3.51–	6.51	6.07	1.00	36.42
	adult	1.60	1.11–	3.45	3.82	0.72	36.41
C. mutilatus	egg	5.67	3.71–	21.98	4.77	1.25	33.92
	larva	2.44	1.45–	10.53	2.85	0.70	35.17
	pupa	10.35	6.4 –	32.0	2.61	0.45	46.19
	adult	0.84	0.68–	1.32	8.03	1.55	25.00

3.4 Sensitivity to −18 °C

At −18 °C, all stages of both species are killed within 2.25 h (table 4). 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 eggs of *L. serricorne* and *C. maculatus*, among the five species of stored product insects tested, were most resistant to −20 °C and exposure in excess of 1 h 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.

Table 4. **Time (in hours) required to kill *Carpophilus hemipterus* and *C. mutilatus* at −18 °C** (99 % confidence limits)

Insect species	Stage	LT_{99}	limits lower	limits upper	Slope	SE	chi-square
C. hemipterus	egg	1.39	0.79	7.32	3.26	0.78	25.00
	larva	1.43	0.93	4.63	3.50	0.70	27.59
	pupa	2.25	1.53	4.80	3.56	0.60	26.30
	adult	0.48	0.36	0.94	6.37	1.32	16.92
C. mutilatus	egg	1.37	0.82	6.64	3.70	0.93	25.00
	larva	0.77	0.34	4.00	1.47	0.27	38.89
	pupa	1.72	1.30	2.57	3.39	0.36	16.92
	adult	0.64	0.45	1.54	4.34	0.99	23.68

4 Conclusions

The extreme differences in rates of mortality between exposure to 0 °C and −18 °C indicate that storage at 0 °C and −5 °C is relatively inefficient for control of the *Carpophilus* species, particularly since 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 centre of the date container reaches these temperatures, complete control will be 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 which were not removed during the disinfestation treatment.

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Zusammenfassung

Zum Einfluß tieferer Temperaturen auf zwei Carpophilus-Arten (Col., Nitidulidae)

Es wurden die Wirkungen der Temperaturstufen 0, −5, −10 und −18 °C auf die Entwicklungsstadien der Vorratsschädlinge *Carpophilus hemipterus* L. und *C. mutilatus* Er. untersucht.

Ein relativ langsames Absterben verursachte die Einwirkung von 0 °C. Die letale Einwirkungszeit (LT_{99}) war hier für die Larven von *C. hemipterus* am längsten (317,3 h). Bei −5 °C erwiesen sich die Puppen als Stadien höchster Kälte-Resistenz: etwa 90 h waren nötig, um 99 % der Puppen beider Arten abzutöten. Bei −10 °C erfolgte das Absterben beider Arten rapide, wobei die Puppen wiederum die stärkste Resistenz zeigten: 10,35 h für die LT_{99}. Die tiefste Temperatur von −18 °C führte bei beiden Käferarten sehr schnell zum Tod. Hier wurde die LT_{99} für alle Stadien bereits innerhalb von 2,25 h erreicht.

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