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ORIGINAL RESEARCH PAPER

Insecticidal effect of high carbon dioxide atmospheres on thrips eggs oviposited in plant tissue

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Abstract Thrips are damaging crop pests, but their eggs are difficult to detect for farmers and agricultural inspectors. We investigated the insecticidal effects of an elevated carbon dioxide atmosphere on thrips eggs oviposited within plant tissues. Percent mortality of *Frankliniella occidentalis* (Pergande), *Frankliniella intonsa* (Trybom), *Thrips tabaci* Lindeman, and *Thrips palmi* Karny exposed to 60 % CO_2 was evaluated at different temperatures (20, 25, 30, and 34 °C) and durations. Egg mortality of all four species increased with CO_2 exposure duration at each temperature, and the time required to achieve 100 % mortality decreased as the temperature increased between 20–30 °C. Exposure to 60 % CO_2 at 30 °C for 12 h is considered to be 100 % lethal to most thrips pests of fresh agricultural produce. Our findings suggested that CO_2 treatment could be used to propagate thrips-free plants in horticultural nurseries.

Keywords Thrips · Controlled atmosphere · CO_2 exposure time · Mortality · Temperature

Introduction

Fumigation using high levels of carbon dioxide (CO_2), in what is referred to as controlled atmosphere (CA) treatment, has been widely used in Europe, the USA, and Australia since the late twentieth century (Field and White. 2002). This technique has attracted much attention as a non-chemical alternative to fumigants such as methyl bromide (MeBr) for controlling insect pests in agricultural products (Mitcham et al. 2006). Carbon dioxide is a by-product of existing industrial processes, such as the production of hydrogen gas from petroleum. Therefore, CO_2 fumigation does not itself add to the "greenhouse effect" (Newton 1993).

Several thrips species are major agricultural pests; they cause feeding damage on flowers and vegetables and transmit tospoviruses (Wijkamp et al. 1995; Nault 1997; Kato et al. 2000; Doi 2003; Uekusa 2006). In recent years, horticultural seedlings infected with thrips have been transported throughout Japan, causing major expansions of plant virus distributions. In addition, several thrips species such as *Thrips tabaci* Lindeman and *Frankliniella occidentalis* (Pergande) were frequently found in imported fresh agricultural products at plant quarantine stations in Japan. Species of Thysanoptera intercepted on imported plants by Japanese plant quarantine have been reported several times since 1991 (Hayase 1991; Masumoto et al. 2005; 2012).

Because thrips in the suborder Terebrantia, which includes the genera *Frankliniella* and *Thrips*, lay eggs within plant tissue, the eggs are difficult for inspectors and farmers to find. Ideally, all plants to be transported could be subjected to a non-toxic treatment that would kill thrips and their eggs. We previously reported that high CO_2 levels had insecticidal effects on both adults and larvae of *F. occidentalis* and suggested that CO_2 treatment could potentially be used in nurseries to produce thrips-free seedlings (Seki and Murai 2011). We subsequently examined how exposure to 60 % CO_2 at different temperatures and durations affected adult female mortality of five thrips species—*F. occidentalis*, *Frankliniella intonsa* (Trybom), *T. tabaci*,

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Thrips palmi Karny, and *Thrips parvispinus* (Karny)—and proposed that 60 % CO_2 treatment at 30 °C could be used in nurseries as an effective and practical means of producing thrips-free seedlings (Seki and Murai 2012). However, further detailed studies on the insecticidal effects of carbon dioxide on thrips eggs are required before CA treatment can be used more widely.

In this study, we have clarified the relationship between thrips egg stage and mortality due to exposure to elevated CO_2 levels at different temperatures and durations using four thrips species: *F. occidentalis*, *F. intonsa*, *T. tabaci,* and *T. palmi*.

Materials and methods

Insect cultures

The thrips used in this study were obtained from stock cultures. *Frankliniella occidentalis* was collected on *Gerbera* spp. and *T. tabaci* on onions (*Allium cepa* L.) at Izumo, Shimane Prefecture, western Japan, in 1995. *Frankliniella intonsa* was collected on clover at Utsunomiya, eastern Japan, in 2006. *Thrips palmi* was collected on eggplant in Okayama, western Japan, in 1993. Thrips were reared on germinated broad bean (*Vicia faba* L.) seeds as described in Murai and Loomans (2001) and maintained in an incubator (MIR-141, Sanyo, Japan) at 20 °C under a photoperiod of 16:8 h light:dark.

Preparation of eggs

A ring cage (50 ml) and kidney bean leaves (*Phaseolus vulgaris* L.) were used for thrips oviposition. A fresh kidney bean leaf atop wet tissue paper was set on the bottom of a ring cage by stretched laboratory film (Parafilm 'M', Bemis Co., Inc., Neenah, WI, USA), and the top of the cage was covered with stretched film. For each thrips species, about 50 adult females were introduced into a cage and incubated for 24 h at 25 °C under a 16:8 h light:dark photoperiod. Then, kidney bean leaves with eggs were transferred to wet filter paper in petri dishes (8.5 cm diameter) and kept under the same conditions for durations of 0–3 days.

Experimental procedures

All experiments were conducted in airtight jars (10 cm diameter × 13 cm high, 1.0 l) made of an acrylic cylinder equipped with gas injection and sampling ports, a temperature probe, and air-inlet and exhaust valves as Seki and Murai (2011) described. The CA treatments involved replacing the atmosphere in the airtight jars with premixed gas (60 % CO_2, 8 % O_2, 32 % N_2). Unmanipulated air in the laboratory was used as a control treatment for these experiments. Thrips eggs oviposited into kidney bean leaves were incubated in the jars for different durations at different temperatures (as described below). The humidity in the jar was maintained at 100 % RH using wet tissue paper on the jar bottom. After treatment, the eggs in the leaves were transferred to fresh laboratory air at 25 °C for hatching. The number of hatched eggs was recorded every day for 7 days after treatment. As untreated eggs hatched within 5 days at 25 °C, eggs that had not hatched by 7 days after treatment were assumed to be dead. About 60 eggs were tested in each treatment. The number of eggs in each leaf was counted under a microscope using lighting from below.

Exposure times and temperatures

One- to 2-day old eggs of each species were exposed to 60 % CO_2 for: 4, 8, 12, 16, 20, 24, and 28 h at either 20 or 25 °C. At 30 °C, exposure durations were 2, 4, 8, 12, 16, and 20 h. Exposure durations at 34 °C were 4, 6, 8, 10, 12, 16, and 20 h. Zero- and 3-day-old eggs were exposed to 60 % CO_2 for 24 h at 25 °C.

Data analysis

The mortalities of 1–2-day-old eggs were transformed to probit values. Mortalities on tested treatment durations among temperatures and among thrips species were compared by analysis of covariance, and the median lethal time (LT_{50}) was estimated by linear regression analysis using JMP version 9 (SAS Institute, Cary, NC, USA).

Results

Elevated (60 %) CO_2 had an insecticidal effect on the egg stages of all four thrips species. The percent mortality of 1–2-day-old thrips eggs increased with exposure duration at all temperatures tested (Table 1). The time required to achieve 100 % mortality differed among species from 28 h for *F. intonsa* to 16 h for *T. tabaci* at 20 °C. For three thrips species, except for *T. palmi*, the exposure duration required for insecticidal activity decreased as temperature increased. There were significant differences in treatment duration required for mortality among temperatures ($F_{3,41} = 7.24$, $P = 0.0005$) and among thrips species ($F_{3,41} = 5.76$, $P = 0.0022$).

The LT_{50} values of three thrips species at 20, 25, and 34 °C were estimated (Table 2). Those of four thrips species at 30 °C, *T. tabaci* at every temperature and *F. occidentalis* at 34 °C were not estimated, because there were

Table 1 Mortality of 1–2-day-old eggs of four species of thrips subjected to elevated CO_2 at different temperatures and durations

Thrips	Temp. (°C)	Mortality (%) at different durations of CO_2 treatment (h)										
		0	2	4	6	8	10	12	16	20	24	28
Frankliniella intonsa	20	7.8		0.0		8.8		56.0	78.6	60.5	87.7	100.0
	25	0.0		21.2		24.5		77.7	87.5	96.0	100.0	100.0
	30	0.0	0.0	8.9		53.1		100.0	100.0	100.0		
	34	0.0		9.5	50.0	77.8	85.7	100.0	100.0	100.0		
Frankliniella occidentalis	20	5.5		39.8		37.9		68.0	79.6	89.3	100.0	100.0
	25	0.0		10.0		24.2		84.3	97.5	100.0	100.0	100.0
	30	0.0	6.1	54.7		100.0		100.0	100.0	100.0		
	34	4.2		36.7	100.0	100.0		100.0	100.0	100.0		
Thrips palmi	20	9.2		9.0		8.1		86.4	74.9	100.0	100.0	100.0
	25	0.0		20.9		60.2		98.1	100.0	100.0	100.0	100.0
	30	0.0	20.0	93.9		100.0		100.0	100.0	100.0		
	34	0.0		37.5	96.2	95.3	90.7	100.0	100.0	100.0		
Thrips tabaci	20	0.0		0.0		15.7		74.7	100.0	100.0	100.0	100.0
	25	8.5		4.0		28.6		100.0	100.0	100.0	100.0	100.0
	30	0.0	16.7	56.8		100.0		100.0	100.0	100.0		
	34	8.3		38.9	89.2	100.0	100.0	100.0	100.0	100.0		

Values were calculated using Abbott correction for natural mortality (0 h)

Table 2 Estimated 50 % lethal time (LT_{50}) for eggs of three thrips species exposed to 60 % CO_2 at different temperatures

Thrips species	50 % lethal time (h)		
	20 °C	25 °C	34 °C
Frankliniella intonsa	14.5	9.5	6.7
Frankliniella occidentalis	10.6	9.1	–
Thrips palmi	9.0	6.6	2.4

LT50 of *F. occidentalis* at 34 °C was not estimated, because there was no significant linear correlations between mortality and treatment duration

no significant linear correlations between mortality and treatment duration. The LT_{50} values of *F. intonsa* were longer than those of other thrips species at every temperature tested.

No zero- or 3-day-old eggs of any of the four species hatched after 24 h of 60 % CO_2 treatment at 25 °C. No damage to fresh kidney bean leaves subjected to 60 % CO_2 treatment was observed at any of the tested temperatures.

Discussion

We investigated the effect of CA treatment on the egg stage of four thrips species at different temperatures and exposure durations. We used 1–2-day-old eggs to evaluate the insecticidal effects of elevated CO_2 at different temperatures and treatment durations. Because thrips eggs hatch quickly, 1–2-day-old eggs were considered ideal to investigate egg control in the field.

The percent mortality of 1–2-day-old eggs of each species at 30 °C was proportional to exposure time. Furthermore, the exposure duration required for 100 % mortality differed among species and temperatures. All egg ages (0, 1–2, and 3 days) of all four thrips species were killed by 60 % CO_2 treatment for 24 h at 25 °C; therefore, this treatment is considered to be insecticidal throughout the thrips egg stage. The exposure durations required to achieve 100 % egg mortality of all four thrips species at all temperatures tested were longer than those required to kill the adult stages (Seki and Murai 2012). The longer survival of eggs probably occurred because the eggs were oviposited into plant tissue and thus exposed to atmospheric CO_2 indirectly, whereas adults and larvae were exposed directly.

Mitcham et al. (1997) reported that CA treatment of *F. occidentalis* with 45 % CO_2 at 5 °C resulted in an LT_{99} for eggs of approximately 4 days and for larvae and adults of about 5 days. Furthermore, CA treatment using 45 % CO_2 at 5 °C for 10–15 days on grapes did not significantly affect berry firmness, total soluble solids, titratable acidity, shatter, decay, browning, or consumer preference. Those studies were conducted at low temperatures during plant quarantine. Consequently, relatively long treatment durations were required to achieve 100 % mortality of the target pests.

We previously reported that exposure durations to achieve 100 % mortality against adults of *F. occidentalis*,

F. intonsa, *T. tabaci*, *T. palmi,* and *T. parvispinus* in a CA treatment using 60 % CO_2 were <24 h at 20 °C, <16 h at 25 °C, <8 h at 30 °C, and <4 h at 34 °C (Seki and Murai 2012). In this study, we found that the effectiveness of 60 % CO_2 treatment in killing thrips eggs varied among species. To achieve 100 % egg mortality of *F. intonsa*, the species with the longest survival times of those tested, required 28 h at 20 °C, 24 h at 25 °C, and 12 h at 30 °C. Our findings showed that mortality of all four species increased with CO_2 exposure times and with treatment temperature between 20–30 °C. Sixty percent CO_2 was sufficient to control the thrips species at temperatures below 30 °C. Thus, treatment conditions may vary for target insect pests. Indeed, studies on other insect pests showed that the time required to achieve 100 % mortality in an atmosphere of 60 % CO_2 at 30 °C was less than 4 h for the green peach aphid, *Myzus persicae* (Sulzer) and 24 h for two mealybug species, *Planococcus kraunhiae* (Kuwana) and *Pseudococcus comstocki* (Kuwana) (Seki and Murai 2012).

Use of 60 % CO_2 atmospheres at 30 °C is believed to be 100 % lethal within 24 h (Carpenter et al. 1996; Seki and Murai 2011, 2012) to most pests of fresh agricultural produce. Thus, this CA technique is considered suitable for use by import and export facilities to ensure the safety of both fresh agricultural produce (e.g., flowers and fruits) and the natural environment. Toda et al. (2011) reported that high concentrations of CO_2 fumigation may be applicable to certain varieties of apple and for certain pest species. In addition, this pest control technique could be used to produce pest-free seedlings for greenhouse cultivation. Further detailed studies on aspects such as flower bud formation and the quality of agricultural produce are required before CA treatment methods can be used more widely.

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