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Deoxidant-induced anoxia as a physical measure for controlling spider mites (Acari: Tetranychidae)

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Abstract Tiny agricultural pests such as spider mites (Acari: Tetranychidae) attached to seedlings grown outdoors often invade greenhouses, thereby triggering pest outbreaks. To solve the problem, we examined whether differences in anoxia tolerance between animals and plants would permit the application of an anoxic environment to control spider mites without the aid of acaricides. Under an anoxic environment created by using a commercial deoxidant at 25 °C, the time for 50 % mortality of eggs, non-diapausing adults (summer form), and diapausing adults (winter form) were 6.1, 5.5, and 23.6 h, respectively, for *Tetranychus urticae* Koch and 5.4, 3.9, and 23.2 h, respectively, for *Tetranychus kanzawai* Kishida. With anoxia for 12 h, no eggs and non-diapausing adults survived in either species, whereas most diapausing adults (98 % for *T. urticae* and 88 % for *T. kanzawai*) survived. Under this treatment, host *Phaseolus vulgaris* L. seedlings showed serious physiological disorders in their primary leaves and apical buds, and unusual lateral buds developed in the cotyledon axils. The spider mites acquire anoxia tolerance during diapause, but anoxia can potentially control them during the summer if no negative effects are observed in the treated seedlings.

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Introduction

Transplanting seedlings contaminated with pest insects and mites often induces pest outbreaks in greenhouses, where natural enemies are limited. Therefore, maintaining pest-free seedlings is one of the most important strategies in pest management (van Lenteren 2000). To obtain pest-free seedlings and post-harvest products (e.g., grains, nuts, fruits, timber), chemical fumigation with a broad-spectrum pesticide, methyl bromide, was a commonly used method (Ristaino and Thomas 1997; Mitcham et al. 2006). However, methyl bromide is a significant ozone-depleting substance (Montzka and Reimann 2011); it is also highly toxic to humans. Therefore, the use of methyl bromide has been phased out as a general method for controlling pests in developed countries, and it will be phased out in developing countries by 2015 (Mitcham et al. 2006).

As an alternative, there has been growing interest in atmosphere modification because of its non-chemical pesticidal properties. In previous studies, hypoxia (low O_2) created using elevated CO_2 or N_2 has been used to control arthropod pests (Lay-Yee and Whiting 1996; Held et al. 2001; Neven 2005; Seki and Murai 2012a, b). In addition to a lack of O_2, direct physiological effects of elevated CO_2 and N_2 have been reported. For example, elevated N_2 has an anesthetic action that induces hyperactivity of treated insects, followed by immobility (Fleurat-Lessard 1990). Elevated CO_2 also has an anesthetic action based on its inhibitory effect on the bioelectrical responses of the insect nervous system (Nicolas and Sillans 1989). However, the major cause of insect death is a lack of O_2, which is required for aerobic respiration (Fleurat-Lessard 1990).

Owning to their large range of characteristics, high portability, and low cost, deoxidants that can create anoxia have been utilized to maintain the quality of foods, drugs, and cosmetics (Vermeiren et al. 1999), and for controlling pests of grains and clothing (Ohguchi et al. 1983), as well as in houses (Kamezaki et al. 2005, 2007) and museums (Zhang 2013). However, so far as we know, there have been no reports of the use of deoxidants to control agricultural pest insects and mites.

In this study, we investigated the effects of anoxia caused by a commercially available deoxidant (AGELESS®; Mitsubishi Gas Chemical, Tokyo, Japan), which consists of iron, calcium chloride, and silicon dioxide, on two spider mite species: *Tetranychus urticae* Koch and *Tetranychus kanzawai* Kishida (Acari: Tetranychidae). These mites injure important agricultural crops, including tea and several fruits, vegetables, and ornamentals. After determining the duration of anoxia required to eliminate these pest mites, we also investigated the effects on plant seedlings to determine the suitability of this method as a novel physical control measure for achieving pest-free seedlings in greenhouse culture.

Materials and methods

Mite rearing conditions

Populations of *T. urticae* (green form) and *T. kanzawai* were originally collected from apple (*Malus pumila* Mill. 'Fuji') and red clover (*Trifolium pratense* L.) in Akita and

Hokkaido, Japan, respectively. The populations were then maintained using detached primary leaves of kidney bean (*Phaseolus vulgaris* L. 'Top Crop'; Takii and Company Ltd., Kyoto, Japan). The leaves with each stock population were maintained on water-soaked cotton in polystyrene cups (94-mm inner diameter, 57-mm depth; V-9; As-one, Osaka, Japan), each of which had a polyethylene lid with four ventilation holes (8 mm in diameter) covered with gas-permeable filters (0.45-μm pore diameter; Milliseal; Nippon Millipore, Tokyo, Japan). The cups were held at L:D 16:8 and 25 °C.

Plant cultivation conditions

The bean seedlings (*P. vulgaris* 'Top Crop') were grown in peat pots (Jiffy-7; Jiffy Products International, Stange, Norway) under daily irrigation with tap water, at L:D 16:8, with a photosynthetic photon flux density of 100 μmol m^{-2} s^{-1}, and at 25 °C. The growth duration was 14 days after sowing, including a germination treatment under continuous darkness for 3 days.

Anoxia test of mites

We obtained 30–50 adult females (mothers) of each species from stock populations using a tapered brush (Interlon 1026-3/0; Maruzen Artist Materials & Works, Tokyo, Japan). The mites were transferred onto an area (27 × 27 mm) fenced with water-soaked cotton in a square polystyrene dish (96 × 96 mm) and allowed to lay eggs for 24 h. All mothers and

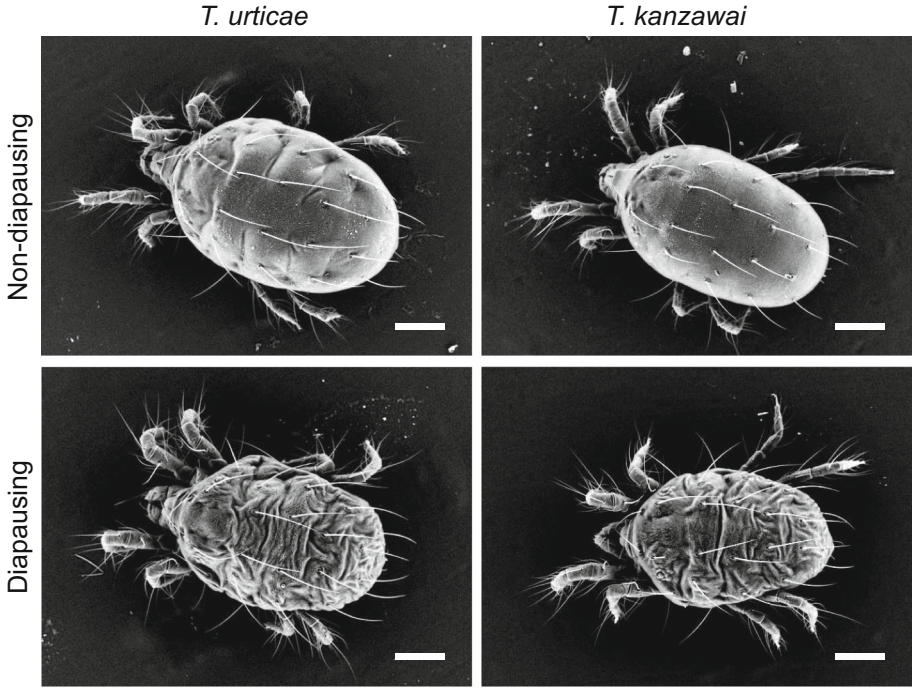

Fig. 1 Electron microscopic images of (*top*) non-diapausing and (*bottom*) diapausing females of *Tetranychus urticae* and *T. kanzawai*. *Scale bars* 100 μm

the water-soaked cotton were then removed from the dishes, and the eggs were used in the anoxia test without transferring them from the dishes.

We obtained 30 adult females (mothers) of each species from stock populations using the previously described brush method, then transferred them onto detached bean leaves placed on water-soaked cotton in the polystyrene cups described earlier in the Methods, and allowed them to lay eggs for 24 h. All mothers were then removed and the eggs were maintained for 5 days under the same conditions. Newly hatched larvae were reared until adulthood in photoperiod chambers (Suzuki and Takeda 2009) under an L:D of 16:8 at 25 °C or an L:D of 8:16 at 18 °C to produce non-diapausing and diapausing adult females, respectively. Adult females were considered to be in diapause if their body color turned from yellowish green (*T. urticae*) or dark brown (*T. kanzawai*) to uniform orange (Takafuji and Morishita 2001). Moreover, images from a scanning electron microscope (TM-1000; Hitachi High-Technologies, Tokyo, Japan) showed that the external morphology of the idiosoma cuticle was much more wrinkled in diapausing females than in non-diapausing females in both species (Fig. 1). Non-diapausing and diapausing females, which were aged 3–5 days and 14–21 days after adult emergence, respectively, were then confined in 1.5-mL polypropylene vials. Each vial had a ventilator (8 mm in diameter) with a gas-permeable filter on the lid. These females (five females per vial) were used in the anoxia test.

The samples (eggs on the dishes and adults in the vials) for a given anoxia duration were placed in a well-closed polycarbonate container (2.5 L; Mitsubishi Gas Chemical) with a sensor for measuring the air temperature and relative humidity (TR-77Ui; T&D, Nagano, Japan), a sensor for measuring the oxygen partial pressure (OXY-1; Jikco, Tokyo, Japan), 40 packages of the iron-based AGELESS® deoxidant (FX-400; Mitsubishi Gas Chemical), and six pieces of filter paper (90 mm in diameter; Advantec No. 2; Toyo Roshi, Tokyo, Japan) with 20 mL of distilled water to increase the relative humidity to 100 %. This is necessary because the deoxidation reaction depends on the presence of water vapor. The containers were placed in the laboratory at L:D 16:8 and 25 °C. A webcam (C920; Logicool, Tokyo, Japan) was used to capture the oxygen sensor's display at 1-min intervals. Although the deoxidant packages required approximately 1.6 h to decrease the oxygen partial pressure inside the container to 0.0 kPa (Fig. 2), we determined the anoxia duration as the time from closing to opening of the container. Because there was no displacement of gas inside the container, the total pressure also decreased from approximately 101 to 80

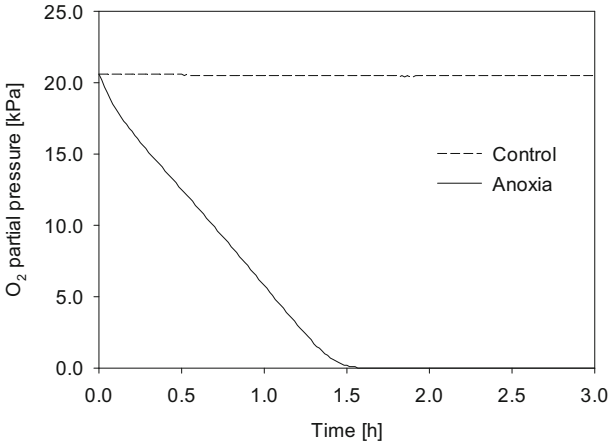

Fig. 2 Time courses of the O_2 partial pressure inside a container with (*solid line*) or without (*broken line*) the deoxidant AGELESS® under vapor-saturated conditions at 25 °C. The deoxidants required approximately 1.6 h after closing the container to achieve an O_2 partial pressure of 0.0 kPa

kPa as the oxygen partial pressure decreased from approximately 21 to 0.0 kPa. The anoxia duration was set at 0 (control), 3, 6, 12, or 24 h. New samples were added to the container for each anoxia duration. After each anoxia duration, the container was opened and the deoxidant packages were removed. After 5 min, the oxygen partial pressure inside the container returned to the ambient level ($\approx$21 kPa) and the container was closed again. Then, 24 h after the first closing of the container, the samples were removed from the container and held at 25 °C and atmospheric pressure. The same setup and conditions (except for the anoxia duration) were used in our test of the effects of anoxia on the plants ("Anoxia test of plants" section).

The samples exposed to each anoxia duration were transferred from the dishes and vials onto leaf disks (12 mm in diameter; 1–10 eggs/disk and 5 adults/disk). The leaf disks were created from the abovementioned primary leaves using a leaf puncher (Fujiwara Scientific, Tokyo, Japan). The egg observations were carried out using a stereomicroscope (SZ61; Olympus, Tokyo, Japan) for 7 days after treatment (DAT). The adult observations were carried out using the stereomicroscope for 2 DAT because our preliminary experiments revealed that adults exposed to anoxia may be paralyzed rather than dead for up to 1 DAT. The anoxia test used four independent replicates in each species using eggs and adults that originated from different mothers to minimize maternal effects.

Anoxia test of plants

Once the anoxia duration for killing the mites had been determined, the effects on the bean seedlings were investigated. The seedlings that had been grown for 14 days after sowing ("Plant cultivation conditions" section) were placed in the container with or without 40 deoxidant packages for the determined duration. The exposed and unexposed seedlings were then transferred into the laboratory and grown under the same conditions described in "Plant cultivation conditions" section. At 7 DAT, seedling aboveground fresh weight was measured using an electronic balance (FZ-500i; A&D, Tokyo, Japan). After the measurement, the aboveground parts were oven-dried (ON-300S; As-one) at 60 °C for 24 h, and then the dry weight was measured.

Data analysis

An arcsine square-root transformation was applied to normalize the hatchability and survival data for eggs and adults in each replicate, and then the means of hatchability and survival were calculated. Significant differences in the mean hatchability of eggs at each DAT, in the mean period of embryonic development, and in the mean survival of non-diapausing and diapausing adult females at 2 DAT between the control and other treatments were analyzed for each species using Dunnett's test after one-way analysis of variance (ANOVA). Significant differences in the survival between non-diapausing and diapausing females from each anoxia duration were identified using the Mann–Whitney U test. We defined the anoxia susceptibility of eggs and adults using the median lethal time (LT_{50}) obtained by probit analysis of the hatchability and survival data at 7 and 2 DAT, respectively. Significant differences in the fresh and dry weights of the bean seedlings between the control and the anoxia treatment were analyzed using the t test.

Statistical analyses were performed using SPSS 11.5J (SPSS, Chicago, IL, USA) for ANOVA and Dunnett's test, R 3.0.1 (R Development Core Team 2013) for Mann–Whitney U test and t test, or PriProbit 1.64 (Sakuma 1998) for the LT_{50} and LT_{90} calculations.

Table 1 Daily changes in the cumulative hatchability and mean period of embryonic development of *Tetranychus urticae* and *T. kanzawai* eggs exposed to anoxia for 0 (control), 3, 6, 12, and 24 h at 25 °C

Species	Anoxia duration (h)	Cumulative hatchability (%)						Mean period of embryonic development (days)
		2 DAT[a]	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT	
T. urticae	0 (control)	0	5 ± 4	81 ± 4	89 ± 2	89 ± 2	89 ± 2	4.05 ± 0.02 (583)[c]
	3	0	0*[b]	72 ± 7	81 ± 3*	81 ± 3*	81 ± 3*	4.11 ± 0.04 (81)
	6	0	0*	39 ± 6***	57 ± 2***	60 ± 3***	60 ± 3***	4.44 ± 0.06*** (105)
	12	0	0*	0***	0***	0***	0***	–[d]
	24	0	0*	0***	0***	0***	0***	–
T. kanzawai	0 (control)	0	26 ± 11	93 ± 2	97 ± 1	97 ± 1	97 ± 1	3.82 ± 0.02 (535)
	3	0	9 ± 3	82 ± 6*	83 ± 6*	85 ± 5*	85 ± 5*	3.93 ± 0.05 (86)
	6	0	0***	1 ± 1***	4 ± 3***	4 ± 3***	4 ± 3***	4.86 ± 0.14*** (7)
	12	0	0***	0***	0***	0***	0***	–
	24	0	0***	0***	0***	0***	0***	–

Data for the cumulative hatchability were collected from four replicates ($N = 20$–197 eggs per replicate) and were arcsine square-root transformed before the statistical analysis. All data are shown as mean ± SE (except data for which SE was zero)

[a] Days after treatment

[b] Values labeled with asterisks differ significantly from the corresponding control: * $P < 0.05$; *** $P < 0.001$ (Dunnett's test after one-way ANOVA)

[c] Values in parentheses indicate the total number of eggs that hatched

[d] Values were not calculated because all eggs died

Results

Egg hatchabilities

In the control (0 h anoxia), *T. urticae* and *T. kanzawai* eggs both started hatching at 3 DAT, and the cumulative hatchabilities were 89 and 97 % at 7 DAT, respectively (Table 1). Under anoxia for 3 h, *T. urticae* and *T. kanzawai* eggs started hatching at 4 and 3 DAT, respectively, and the cumulative hatchabilities were 81 and 85 %, respectively, at 7 DAT; both were significantly lower than those in the corresponding control ($P < 0.05$, Dunnett's test). Although some of the eggs started hatching at 4 DAT after anoxia for as long as 6 h, only 60 and 4 % of the *T. urticae* and *T. kanzawai* eggs hatched at 7 DAT, respectively. After anoxia for 12 and 24 h, no eggs of either species hatched, even at 7 DAT. The cumulative hatchabilities after anoxia for ≥6 h were significantly lower than those in the control for both species ($P < 0.001$).

After anoxia for 3 h, the mean periods of embryonic development were not significantly different from those (4.0 days for *T. urticae* and 3.8 days for *T. kanzawai*) in the control ($P > 0.05$). However, after anoxia for 6 h, the mean periods of embryonic development were significantly longer than those in the control ($P < 0.001$).

Adult survival

In the control, ≥96 % of the non-diapausing and diapausing females survived in both species at 2 DAT (Table 2). Survival of the non-diapausing females became significantly lower than those in the control when *T. urticae* and *T. kanzawai* were exposed to anoxia for 6 and 3 h, respectively ($P < 0.05$, Dunnett's test). No survival was observed in *T. urticae* and *T. kanzawai* after anoxia

Table 2 Survival of non-diapausing and diapausing adult females of *Tetranychus urticae* and *T. kanzawai* at 2 days after the anoxia treatment for 0 (control), 3, 6, 12, and 24 h at 25 °C

Species	Anoxia duration (h)	Survival (%)		P^a
		Non-diapausing	Diapausing	
T. urticae	0 (control)	96 ± 1	99 ± 1	0.037
	3	90 ± 1	100	0.013
	6	7 ± 4***	99 ± 1	0.017
	12	0***	98 ± 2	0.011
	24	0***	39 ± 12***	0.014
T. kanzawai	0 (control)	96 ± 2	98 ± 1	0.19
	3	85 ± 4*	98 ± 2	0.038
	6	0***	100	0.038
	12	0***	88 ± 4	0.014
	24	0***	28 ± 10***	0.013

Data for survival were collected from four replicates ($N = 25$ to 100 adults per replicate) and were arcsine square-root transformed before the statistical analyses. All data are shown as mean ± SE (except data for which SE was zero)

Values labeled with asterisks differ significantly from the control: * $P < 0.05$; *** $P < 0.001$ (Dunnett's test after one-way ANOVA)

[a] Probability obtained from the Mann–Whitney *U* test to identify significant differences in the survival between non-diapausing and diapausing females for each anoxia duration

for ≥12 and ≥6 h, respectively. In both species, no negative effects were observed in diapausing females after anoxia for ≤12 h ($P > 0.05$). However, the survival of both species was significantly lower than those in the controls only after anoxia for 24 h ($P < 0.001$).

Significant differences in survival between non-diapausing and diapausing females were observed under all anoxia conditions ($P < 0.05$, Mann–Whitney U test), except in the control for *T. kanzawai* ($P = 0.19$). Although there was only a slight difference (3 percentage points) between non-diapausing and diapausing *T. urticae* females in the control, it was significant ($P = 0.037$).

Anoxia susceptibilities of the mites

The LT_{50} values for *T. urticae* and *T. kanzawai* eggs were 6.1 and 5.4 h, respectively (Table 3). The LT_{50} values for non-diapausing *T. urticae* and *T. kanzawai* females were 5.5 and 3.9 h, respectively. Diapause increased the times to 23.6 and 23.2 h, respectively. The LT_{90} values for *T. urticae* and *T. kanzawai* eggs were 6.7 and 5.9 h, respectively. The LT_{90} values for non-diapausing *T. urticae* and *T. kanzawai* females were 5.9 and 4.2 h, respectively. Diapause increased the times to 25.7 and 25.2 h, respectively.

Effects of anoxia on the plants

Because the anoxia duration of 12 h killed both eggs and non-diapausing females of both species, we investigated the effects of this exposure on the bean seedlings. In the exposed seedlings, the primary leaves started changing color by 1 DAT and had withered almost completely at 7 DAT (Fig. 3a). The aboveground fresh and dry weights of the exposed seedlings were significantly lower than those in the control (Fig. 3b; $P < 0.001$ and $P < 0.01$, respectively, t test). Although development of the apical buds was prevented, unusual lateral buds developed in the axils of the cotyledons (Fig. 3c).

Discussion

Although management of spider mites has relied heavily on chemical control, the frequent use of chemicals often induces acaricide resistance and eliminates beneficial organisms,

Table 3 Median lethal time (LT_{50}) and 90 % lethal time (LT_{90}) for *Tetranychus urticae* and *T. kanzawai* eggs and adults (non-diapausing and diapausing) exposed to anoxia at 25 °C

Species	Stage (state)	LT_{50} (95 % fiducial interval)	LT_{90} (95 % fiducial interval)
T. urticae	Egg	6.1 (5.9–6.4)	6.7 (6.4–7.0)
	Adult (non-diapausing)	5.5 (5.1–5.8)	5.9 (5.6–6.4)
	Adult (diapausing)	23.6 (23.3–24.1)	25.7 (25.1–26.6)
T. kanzawai	Egg	5.4 (5.0–5.8)	5.9 (5.5–6.3)
	Adult (non-diapausing)	[3.9][a]	[4.2][a]
	Adult (diapausing)	23.2 (22.3–24.1)	25.2 (24.3–26.2)

The LT_{50} and LT_{90} values (with 95 % fiducial intervals; unit: hours) were obtained by probit analysis of the hatchability and survival data at 7 and 2 days after treatment, respectively (Tables 1, 2).

[a] Although non-diapausing *T. kanzawai* adults showed high sensitivity to anoxia (Table 2), the values are shown in square brackets because of difficulties in calculating the fiducial interval

including natural enemies, which promotes pest resurgence (Suzuki 2012). The implementation of integrated pest management—an approach that employs a combination of pest management measures, including timely application of small amounts of chemicals—is urgently needed. Biological measures using commercialized natural enemies have been put to practical use (Yang et al. 2014), whereas physical measures against spider mites have remained unexploited (Suzuki 2012). Although the use of ultraviolet radiation has recently received broad attention due to its miticidal and repellent effects against spider mites (Ohtsuka and Osakabe 2009; Suzuki et al. 2009, 2013, 2014; Sakai and Osakabe 2010; Murata and Osakabe 2014), it may be difficult to apply practically because of the uneven irradiation caused by leaf self-shading. Here, we focused on modification of the atmosphere, which is expected to produce relatively uniform effects throughout the plant because of the rapid diffusion that occurs with gases.

Our results show the possibility of using anoxia as a physical measure for controlling spider mites, using a simple and handy method based on the commercially available deoxidant AGELESS®. The anoxia created by the deoxidant completely killed the eggs and non-diapausing females of both *T. urticae* and *T. kanzawai* at 25 °C when the exposure

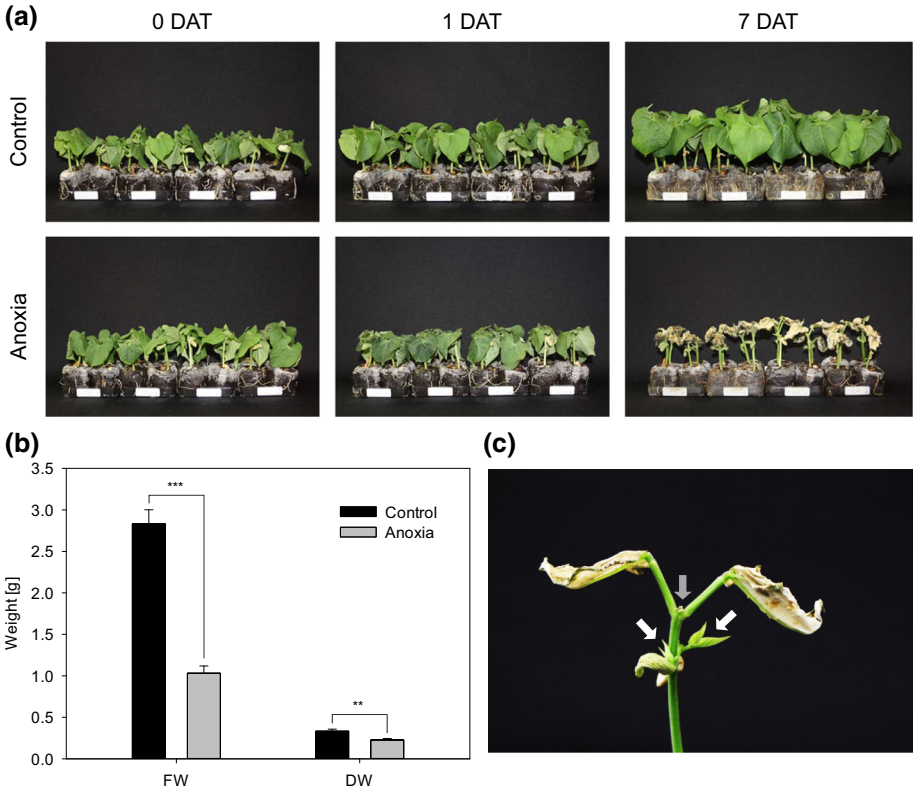

Fig. 3 **a** Bean seedlings that were not exposed to anoxia (control) and those that were exposed to anoxia for 12 h at 0, 1, and 7 days after treatment (DAT). **b** Fresh and dry weights of the control and exposed seedlings at 7 DAT. All data are shown as mean ± SE ($N = 16$). $^{**}P < 0.01$; $^{***}P < 0.001$ (t test). **c** Development of the apical buds was prevented by the anoxia treatment (*gray arrow*), but lateral buds unexpectedly developed in the axils of the cotyledons (*white arrows*) at 7 DAT

duration was ≥12 h (Tables 1, 2). The LT_{50} values show that eggs tolerate anoxia better than non-diapausing females in both species, but that both the eggs and the non-diapausing females of *T. urticae* were more tolerant than those of *T. kanzawai* (Table 3). Ohno et al. (2010) reported higher tolerance to hydroxypropyl starch, which is a spiracle-blocking pesticide, in non-diapausing females of *T. urticae* than in those of *T. kanzawai* inhabiting Okinawa Island. However, the effect of the spiracle-blocking pesticide varied between different *T. urticae* populations on Okinawa Island and Miyako Island and between *T. kanzawai* populations on Okinawa Island and Irabu Island. This suggests that the oxygen demand of *T. urticae* is greater than that of *T. kanzawai* or it differs among populations and thus resulted in apparent differences in anoxia tolerance between the two species used in the present study. Differences in anoxia tolerance among developmental stages and species were previously reported for house dust mites. In the adult females, 48 h of anoxia induced 100 % mortality for *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, but 72 h for *Tyrophagus putrescentiae* (Kamezaki et al. 2005). In *D. farinae* and *D. pteronyssinus*, 48 h of anoxia induced 100 % mortality of the eggs, but almost half of the eggs of *T. putrescentiae* hatched even after 336 h (14 days) of exposure (Kamezaki et al. 2007). In comparison with the house dust mites, *T. urticae* and *T. kanzawai* were more susceptible to anoxia. However, their anoxia tolerance increased during diapause. When exposed to anoxia for 12 h, which killed all eggs and non-diapausing females, 98 % (*T. urticae*) and 88 % (*T. kanzawai*) of the diapausing females survived (Table 2). Even when exposed for 24 h, 39 % (*T. urticae*) and 28 % (*T. kanzawai*) of the diapausing females survived. The LT_{50} values in diapausing females were 4.3 (*T. urticae*) and 5.9 times (*T. kanzawai*) those of non-diapausing females (Table 3).

Diapause is a state of developmental and metabolic arrest in which an organism can survive unfavorable environmental conditions, and it is often observed in arthropods (Tauber et al. 1986). Many physiological, morphological, and behavioral adjustments are associated with the onset of diapause, enabling arthropods to cope with environmental stresses and starvation (Lees 1956; Tauber et al. 1986). Diapausing *Tetranychus* spider mites have high tolerance for low temperatures (Stenseth 1965; Veerman 1986; Popov and Veerman 1996; Khodayari et al. 2012, 2013), heat stress (Waddell and Birtles 1992; Lester et al. 1997), desiccation (Ghazy and Suzuki 2014), gamma radiation (Lester and Petry 1995), ultraviolet radiation (Suzuki et al. 2009), and acaricides (Parr and Hussey 1966; Den Houter 1976). In addition to these stress factors, the present study showed that diapause induces tolerance of anoxia. The anoxia tolerance seems likely to result from the low oxygen demand during diapause. In *T. urticae*, the respiration rate in diapausing females is up to 81 % lower than in non-diapausing females (McEnroe 1961). The reduction of the respiration rate during diapause can be explained by the structure of the mite's gnathosoma: (1) the openings of the peritremes are controlled by adjustment of the stylophore position, which controls the area available for vapor diffusion and respiration through the tracheal system, (2) non-diapausing females are sensitive to a vapor-pressure deficit and are able to reduce the rates of vapor diffusion and respiration by withdrawing their peritremes, and (3) the peritremes of diapausing females are completely withdrawn (McEnroe 1961). In the present study, we created vapor saturation conditions during the anoxia treatment by using wetted filter papers with the goal of enhancing the deoxidation reaction ("Anoxia test of mites" section). Therefore, the peritremes of non-diapausing females under these conditions might have been exposed to anoxia, suggesting that the partial pressure of O_2 in the tracheal system dropped below the lethal level. In contrast, the reduction of respiration caused by the withdrawn peritremes might explain the survival of the diapausing females under anoxia. If diapause of mites is broken by cold rest (i.e.,

chilling) and reactivation in short-night conditions (Veerman 1977; Koveos and Veerman 1994) before the anoxia treatment, it would be worthwhile mentioning that such changes might allow more complete eradication of the mites.

Although the 12-h anoxia treatment eliminated the eggs and non-diapausing females of *T. urticae* and *T. kanzawai* at 25 °C, we observed serious physiological disorders in the primary leaves of the bean seedlings exposed to the same conditions (Fig. 3a, b). In general, anoxia decreases antioxidant activity in plants, and the plants fail to regulate the formation of reactive oxygen species (ROS), leading to the development of oxidative stress (Blokhina et al. 2001). In addition, upon restoration of normoxic conditions, the uncontrolled accumulation of excessive levels of ROS leads to peroxidation of membrane lipids and eventually to cell death (Blokhina et al. 2001). Although development of the apical buds was also eliminated, lateral buds that would normally not develop were observed in the axils of the cotyledons (Fig. 3c). This indicates that the bean seedlings were not completely killed by the anoxia treatment. However, we did not grow the seedlings for long enough to determine whether they would have recovered from the damage caused by anoxia.

Further studies are needed to find more plant-friendly conditions. Because the LT_{99} values were 4.5–7.2 h for the eggs and non-diapausing females of *T. urticae* and *T. kanzawai* (data not shown), it would be worthwhile to examine a shorter duration of the anoxia treatment (e.g., 8 h instead of 12 h) for mitigating the physiological disorders in plants. In addition, attention must be directed toward using anoxia-tolerant plant species or cultivars and changing other environmental factors (e.g., the air temperature). Furthermore, the effects of decreased total pressure on mites and plants should also be investigated. Although we did not measure the total pressure inside the container in the present study, theoretically it would have decreased from approximately 101 to 80 kPa as the oxygen partial pressure decreased (Fig. 2). Finally, investigations of the effects of anoxia on spider mites inhabiting seedlings (i.e., indirect effects through the host plants) and large-scale application of anoxia treatment not only by using deoxidants but also by displacing the gas with deoxygenated air are needed to develop an effective and economically feasible method of preventing mite outbreaks in greenhouses after transplanting.

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References

Blokhina OB, Chirkova TV, Fagerstedt KV (2001) Anoxic stress leads to hydrogen peroxide formation in plant cells. J Exp Bot 52:1179–1190

Den Houter JG (1976) The effect of acaricides on active and diapausing females of *Tetranychus urticae* Koch, the two-spotted spider mite. Z Angew Entomol 81:248–252

Fleurat-Lessard F (1990) Effect of modified atmospheres on insects and mites infesting stored products. In: Calderon M, Barkai-Golan R (eds) Food preservation by modified atmospheres. CRC Press, Boca Raton, pp 21–38

Ghazy NA, Suzuki T (2014) Desiccation tolerance in diapausing spider mites *Tetranychus urticae* and *T. kanzawai* (Acari: Tetranychidae). Exp Appl Acarol 63:49–55

Held DW, Potter DA, Gates RS, Anderson RG (2001) Modified atmosphere treatments as a potential disinfestation technique for arthropod pests in greenhouses. J Econ Entomol 94:430–438

Kamezaki H, Sasaki Y, Ohashi K, Takafuji A (2005) Lethal effects of two types of oxygen absorbers on three house dust mites, *Dermatophagoides farinae*, *D. pteronyssinus* and *Tyrophagus putrescentiae*. J Acarol Soc Jpn 14:127–133 (in Japanese with English summary)

Kamezaki H, Ohashi K, Ishihara K, Sasaki Y, Takafuji A (2007) Lethal effects of two types of oxygen absorbers on the eggs of three house dust mites, *Dermatophagoides farinae*, *D. pteronyssinus*, and *Tyrophagus putrescentiae*. J Acarol Soc Jpn 16:145–151 (in Japanese with English summary)

Khodayari S, Moharramipour S, Kamali K, Javaran MJ, Renault D (2012) Effects of acclimation and diapause on the thermal tolerance of the two-spotted spider mite *Tetranychus urticae*. J Therm Biol 37:419–423

Khodayari S, Moharramipour S, Larvor V, Hidalgo K, Renault D (2013) Deciphering the metabolic changes associated with diapause syndrome and cold acclimation in the two-spotted spider mite *Tetranychus urticae*. PLoS One 8:e54025

Koveos DS, Veerman A (1994) Accumulation of photoperiodic information during diapause development in the spider mite *Tetranychus urticae*. J Insect Physiol 40:701–707

Lay-Yee M, Whiting DC (1996) Response of 'Hayward' kiwifruit to high-temperature controlled atmosphere treatments for control of two-spotted spider mite (*Tetranychus urticae*). Postharvest Biol Technol 7:73–81

Lees AD (1956) The physiology and biochemistry of diapause. Ann Rev Entomol 1:1–16

Lester PJ, Petry RJ (1995) Gamma irradiation for after harvest disinfestation of diapausing two spotted spider mite (Acari: Tetranychidae). J Econ Entomol 88:1361–1364

Lester PJ, Dentener PR, Bennett KV, Connolly PG (1997) Postharvest disinfestation of diapausing and non-diapausing twospotted spider mite (*Tetranychus urticae*) on persimmons: hot water immersion and coolstorage. Entomol Exp Appl 83:189–193

McEnroe WD (1961) The control of water loss by the two-spotted spider mite (*Tetranychus telarius*). Ann Entomol Soc Am 54:883–887

Mitcham E, Martin T, Zhou S (2006) The mode of action of insecticidal controlled atmospheres. Bull Entomol Res 96:213–222

Montzka S, Reimann S (2011) Chapter 1: ozone-depleting substances (ODSs) and related chemicals. In: World Meteorological Organization (ed) Scientific assessment of ozone depletion: 2010, Global Ozone Research and Monitoring Project—Report No. 52. Geneva, pp 1.1–1.108

Murata Y, Osakabe M (2014) The Bunsen–Roscoe reciprocity law in ultraviolet-B-induced mortality of the two-spotted spider mite *Tetranychus urticae*. J Insect Physiol 59:241–247

Neven LG (2005) Combined heat and controlled atmosphere quarantine treatments for control of codling moth in sweet cherries. J Econ Entomol 98:709–715

Nicolas G, Sillans D (1989) Immediate and latent effects of carbon dioxide on insects. Ann Rev Entomol 34:97–116

Ohguchi Y, Suzuki H, Tatsuki S, Fukami J (1983) Lethal effect of oxygen absorber (Ageless®) on several stored grain and clothe pest insects. Jpn J Appl Entomol Zool 27:270–275 (in Japanese with English summary)

Ohno S, Miyagi A, Ganaha-Kikumura T, Kijima K (2010) Effect of several commercial pesticides on the survival of nine *Tetranychus* species (Acari: Tetranychidae) inhabiting Okinawa, southwestern Japan. Kyushu Plant Prot Res 56:58–65 (in Japanese with English summary)

Ohtsuka K, Osakabe M (2009) Deleterious effects of UV-B radiation on herbivorous spider mites: they can avoid it by remaining on lower leaf surfaces. Environ Entomol 38:920–929

Parr WJ, Hussey NW (1966) Diapause in the glasshouse red spider mite (*Tetranychus urticae* Koch): a synthesis of present knowledge. Hortic Res 6:1–21

Popov SY, Veerman A (1996) Behavioural response and winter survival of mated and unmated diapausing females of the *Tetranychus atlanticus–urticae* complex (Acari, Tetranychidae). Exp Appl Acarol 20:167–175

R Development Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Ristaino JB, Thomas W (1997) Agriculture, methyl bromide, and the ozone hole: can we fill the gaps? Plant Dis 81:964–977

Sakai Y, Osakabe M (2010) Spectrum-specific damage and solar ultraviolet radiation avoidance in the two-spotted spider mite. Photochem Photobiol 86:925–932

Sakuma M (1998) Probit analysis of preference data. Appl Entomol Zool 33:339–348

Seki M, Murai T (2012a) Responses of five adult thrips species (Thysanoptera; Thripidae) to high-carbon dioxide atmospheres at different temperatures. Appl Entomol Zool 47:125–128

Seki M, Murai T (2012b) Insecticidal effect of high carbon dioxide atmospheres on thrips eggs oviposited in plant tissue. Appl Entomol Zool 47:433–436

Stenseth C (1965) Cold hardiness in the two-spotted spider mite (*Tetranychus urticae* Koch). Entomol Exp Appl 8:33–38
Suzuki T (2012) Environmental engineering approaches toward sustainable management of spider mites. Insects 3:1126–1142
Suzuki T, Takeda M (2009) Diapause-inducing signals prolong nymphal development in the two-spotted spider mite *Tetranychus urticae*. Physiol Entomol 34:278–283
Suzuki T, Watanabe M, Takeda M (2009) UV tolerance in the two-spotted spider mite, *Tetranychus urticae*. J Insect Physiol 55:649–654
Suzuki T, Kojima T, Takeda M, Sakuma M (2013) Photo-orientation regulates seasonal habitat selection in the two-spotted spider mite, *Tetranychus urticae*. J Exp Biol 216:977–983
Suzuki T, Yoshioka Y, Tsarsitalidou O, Ntalia V, Ohno S, Ohyama K, Kitashima Y, Gotoh T, Takeda M, Koveos DS (2014) An LED-based UV-B irradiation system for tiny organisms: system description and demonstration experiment to determine the hatchability of eggs from four *Tetranychus* spider mite species from Okinawa. J Insect Physiol 62:1–10
Takafuji A, Morishita M (2001) Overwintering ecology of two species of spider mites (Acari: Tetranychidae) on different host plants. Appl Entomol Zool 36:169–175
Tauber MJ, Tauber CA, Masaki S (1986) Seasonal adaptations of insects. Oxford University Press, New York
van Lenteren JC (2000) A greenhouse without pesticides: fact or fantasy? Crop Prot 19:375–384
Veerman A (1977) Photoperiodic termination of diapause in spider mites. Nature 266:526–527
Veerman A (1986) Diapause. In: Helle W, Sabelis MW (eds) Spider mites: their biology, natural enemies and control, vol 1A. Elsevier, Amsterdam, pp 279–316
Vermeiren L, Devlieghere F, Van Beest M, De Kruijf N, Debevere J (1999) Developments in the active packaging of foods. Trends Food Sci Technol 10:77–86
Waddell BC, Birtles DB (1992) Disinfestation of nectarines of two-spotted mites (Acari: Tetranychidae). N Z J Crop Hortic 20:229–234
Yang N-W, Zang L-S, Wang S, Guo J-Y, Xu H-X, Zhang F, Wan F-H (2014) Biological pest management by predators and parasitoids in the greenhouse vegetables in China. Biol Control 68:92–102
Zhang B (2013) The feasibility of using low-oxygen atmospheres to control insect pests for taxidermies in natural history museums. Res J Environ Earth Sci 5:256–261