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Efficacy of controlling two stored cashew nut insects in 1% O_2 and 99% N_2 at 43°C

Hagit Navarro* and Shlomo Navarro

Green Storage Ltd., Argaman 5, Rishon Letsion, 7570905, Israel.
*Corresponding author's email: hnavarro@green-storage.co.il

Abstract

The efficacy of controlling all life stages of storage insects *Tribolium castaneum* (Herbst) and *Oryzaephilus surinamensis* (L.) were tested at 43°C and in 1% O_2 and 99% N_2 for 48 h of exposure time. For each species and each life stage, four replicates (each replicate consisted of 50 insects) were conducted. Two replicates for each species and each life stage served as control. Two control replicates were compared with treatment, one replicate served as control at 28°C and 60±5% RH and another served as control at 43°C and 60±5% RH. All tests were carried out by introducing insects to 100 g of cashew nuts in gastight 1 L containers. At the beginning and the end of each exposure time O_2 percent was measured. Insect counting was done immediately after exposure treatment and after 7-10 days of incubation at 28°C and 60±5% RH. Cashew nuts obtained were in equilibrium with 56.4% RH at 16.9°C. Increased mortality was observed due to exposure to 43°C in air (control) compared to exposure to 28°C. Adult stage of *O. surinamensis* was the most tolerant one with 99.5 % mortality after 48 h treatment. Larval and pupal stages could not survive the treatment and 100% mortality was recorded. Although in the egg stage in both species some larvae hatch was observed, they could not survive more than 24 h after the treatment. Average egg mortalities were counted as 97.5% for *T. castaneum* and 98% for *O. surinamensis*, the young hatched larvae were found dead immediately after treatment. The pupal and larval stages of both species were more susceptible to the treatment rather than the adult and egg stages. Treating both stored product insects, *T. castaneum* and *O. surinamensis,* at 43°C in 1% O_2 and 99% N_2 atmosphere for 48 h resulted in very effective control treatment. The survival rate was less than 0.5% of adult of *O. surinamensis*.

Keywords: Nitrogen atmosphere, Cashew nuts, *Tribolium castaneum*, *Oryzaephilus surinamensis*, High temperature

Introduction

Cashew (*Anacardium occidentale* L.) belongs to the family Anacardiaceae and is a native of Brazil. Around the 16th century, it reached the Far East where today, Vietnam serves as the largest supplier of cashew nuts to the international market. Cashew is a high economic value crop and is earning considerable foreign exchange for the country. In 2012, about 220,000 tonnes of cashew kernels were exported, with a turnover of over US$1.45 billion (Thai, 2012).

Since the harvest period is short, during storage, cashew nuts must be protected from insect infestation and weight loss. In most countries, food commodities fumigated with phosphine and subsequently aerated are considered to be phosphine free with no regulations attached. The US Food Quality Protection Act (FQPA), which became law in the US in 1996, has set a tolerance for phosphine of 0.01 ppm in processed food stuffs. This is well below the detection level available to most laboratories (Donahaye*,* 2000*).* As concerns about the safety of our food supply increases along with concerns about the impact of agricultural chemicals on our environment and resistant strains of stored product pests to phosphine increase, the development of nonchemical quarantine treatments to meet export requirements become increasingly necessary. Moreover, the increase in consumer demand for organic commodities in recent years has increased.

Therefore, the use of Modified or Controlled Atmospheres (MA/CA) offers a safe and environmentally benign alternative to the use of conventional residue-producing chemical fumigants for controlling insect pests that attack stored grains, oilseeds, processed commodities, and packaged foods (Navarro, 2006) and is increasing in general, and in particular for niche markets such as the treatment of organic commodities. The pre-condition for successful MA treatment is a tight hermetic seal. Sealing techniques (Andrews et al., 1994), and methods of verifying the seal (Navarro, 1999) are well developed, as are the application procedures (Navarro and Donahaye, 1990; Annis and van S. Graver 1991). Modified or Controlled Atmospheres to control insects can be obtained using nitrogen (N_2), provided that a hypoxic atmosphere of ≤ 1% O_2 can be maintained. Generally, the lower the oxygen level, the higher the mortality. For effective control, the O_2 level should be <3% and preferably <1% if a rapid kill is required (Navarro, 1978).

Insect mortality increases more rapidly as temperatures rise and their metabolism speeds up. Cool temperatures slow rates of mortality while lower relative humidity hastens toxic effects, notably in high CO_2 atmospheres because of desiccation of insects (Banks and Fields, 1995).

Sakka et al. (2020) investigated the use of MA on life stages of several storage pests at 40ºC for 2.5 d in commercial nitrogen chambers with phosphine susceptible and resistant populations of *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (L.). Complete mortality of all life stages of the tested pests was obtained with negligible progeny of *O. surinamensis* (0.3% ± 0.3) (Sakka et al., 2020). While Athanassiou et al. (2017) compared insect mortality when nitrogen (1% O_2) was applied at 25ºC, high insect mortality levels of *Tribolium confusum* Jacquelin du Val, *Ephestia elutella* (Hübner) and *O. surinamensis* were noted. However, in most cases there were a number of insects that survived the nitrogen treatment. In contrast, complete control was achieved at 38–43ºC for all insect species and life stages tested, with the exception of *T. confusum* larvae (Athanassiou et al., 2017). Therefore, the objective of the present study was to evaluate the effect of 99% N_2 nitrogen treatment to achieve 1% O_2 on two storage pests at 43ºC in a short exposure time on cashew nuts.

Materials and methods

Control of Temperature and O_2 level within atmosphere of 1% O_2

Insects were exposed to 1% O_2 in 1-L glass jars equipped with two 1/16" i.d. copper tubes soldered to the lid and seal was reassured by applying the half-time pressure decay test to hold a pressure of 6 to 3 mm H_2O for at least 5 min (Navarro, 1999). The temperature and RH in the gastight 1-L volume jars holding 200 g of cashew nuts were tested using data loggers (Elitech RC-4HC, Elitech,

London, UK). The outer ends of the tubes were connected to T-type valves. An electrolytic sensor type oxygen monitor (Oxycheck Bishop, UK) equipped with an internal pump delivering 500 mL/min gas sample was used for monitoring oxygen levels in the jars. To achieve the initial target oxygen concentration, the gastight jar containing 100 g cashew nuts and the insects was purged using N_2 at about 1000 mL/min until the O_2 concentration dropped to about 0.9% in the jar. For testing the gas concentration, the two T-type valves were kept at a position so that the gas flow would be from the O_2 monitor to the exhaust port of the jar. In that position the T-type valves were switched simultaneously to the ports to provide a closed loop gas flow between the gastight jar and the monitor. After 24 h and at the end of the exposure, readings were taken to ensure the gas concentration was maintained during the exposure period of 48 h. Oxygen concentration was measured three times; at the beginning, after 24 h and at the end of each treatment (48 h). Equilibrium relative humidity of 500 g cashew nuts was tested at the beginning of each trial using a Novasina RH monitor (Hygro Mate, Novasina, Switzerland).

Insects

Populations of storage insects *T. castaneum* and *O. surinamensis* were reared at the Green Storage (GS) Ltd. laboratories at 28ºC and 60±5% RH. The efficacy of controlling all life stages of these two species at 43ºC and 99% N_2 for 48 h of exposure time was tested (Fig. 1). There were four replicates for each species and each life stage (eggs, larvae, pupae and adults), and each replicate consisted of 50 insects. Two replicates from each species and each life stage served as control. One replicate served as control at 28ºC and 60±5% RH and the other replicate served as control at 43ºC and 60±5% RH. All tests were carried out by exposing insects to 100 g of cashew nuts in gastight 1 L containers (except the control which was not gastight).

Insect counting was done immediately after each treatment and after 7-10 d of incubation at 28ºC and 60±5%. The reason for the delayed counts was to make ensure there were no surviving insects. All hatched eggs were considered as alive.

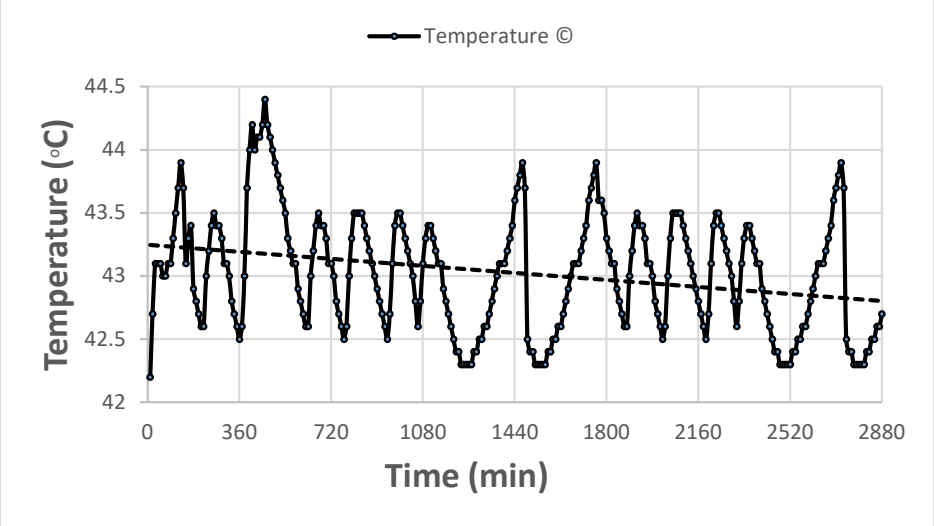

Fig. 1: Temperature obtained within the 1 L container containing 200 g of cashew nuts during a 48-h exposure time at a target temperature of 43ºC.

Results

Equilibrium relative humidity of 500 g cashew nuts was 56.4% at 16.9ºC. Figures 2-3 describe the temperature and RH maintained in the 1 L container during a 48-h exposure. Average temperature was 43.0ºC (Fig. 1) and the average ERH was 58.7 % (Fig. 2).

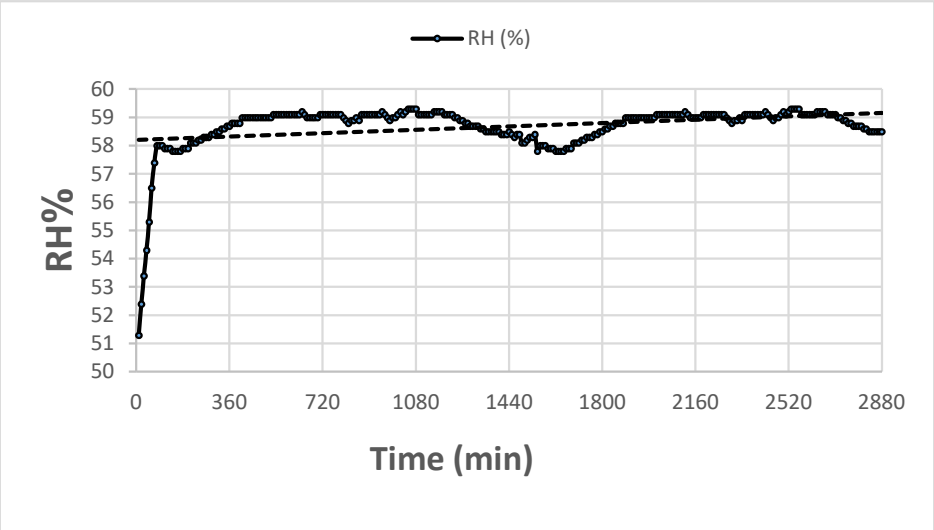

Fig. 2: Relative humidity (%) obtained in the 1 L container containing 200 g of cashew nuts during a 48-h exposure.

Table 1 shows the mortality of all life stages of *T. castaneum* and *O. surinamensis* after 48 h of exposure to 1% O_2 at 43ºC. All larvae were dead during counting (immediately after exposure time).

Table 1. Oxygen concentration (%) and mortality (%) of egg, larva, pupa and adult of *Tribolium castaneum* and *Oryzaephylus surinamensis* after 48 h of exposure to 1% O_2 at 43ºC and its control at 43ºC and 28 ºC.

Average temperature (ºC)	43		43		28	
	Average O_2 (%)	Average mortality (%)	O_2 (%)	Average mortality (%)	Average O_2 (%)	Average mortality (%)
Tribolium castaneum						
Adults	1.30	100.0	20.9	2.0	20.9	0.0
Pupae	1.05	100.0	20.9	42.0	20.9	0.0
Larvae	1.20	100.0	20.9	94.0	20.9	22.0
Eggs	2.35	100.0	20.9	98.0	20.9	76.0
Oryzaephilus surinamensis						
Adults	1.75	99.6	20.9	76.9	20.9	6.0
Pupae	1.25	100.0	20.9	90.9	20.9	62.5
Larvae	1.33	100.0	20.9	72.0	20.9	59.1
Eggs	1.45	100.0	20.9	93.0	20.9	48.0

As mentioned above, controls were carried out at both 43ºC and 28ºC to compare the effect of heat alone on insects. In Table 1, the effect of 43ºC alone in the control of both insects was apparent. Higher mortalities were observed in the controls at 43ºC compared to the controls at 28ºC. The adult stage of *O. surinamensis* was the most tolerant one with 99.5 % mortality on average. Larval and pupal stages could not survive the treatment and 100% mortality was recorded (Table 1).

Although some eggs of both species hatched, they could not survive more than 24 h after the treatment. Average egg mortalities were 97.5% for *T. castaneum* and 98% for *O. surinamensis.* Therefore, the mortalities for both species were considered as 100%.

Discussion

The target temperature of 43ºC, as is shown in Fig. 1, was reached in less than 30 min in the jars. In practice, when handling large commercial volumes, this process may take several hours or days to reach the target temperature at the core of the stack/bags, depending on the volume size (Donahaye et al., 1995).

According to Navarro (2012), to obtain rapid killing when using N_2, the O_2 concentration must be lower than 1%. Timlick et al. (2002) reported that in a sealed commercial storage that maintained less than 1% O_2, insect mortality was completed after 14 d at 17ºC. Therefore, N_2 has been considered unsuitable for bulk commodity treatment at export locations due to the length of time required for 100% mortality.

However, insect mortality increases more rapidly as temperatures rise (Navarro, 2012). In heat treatments carried out in commercial facilities, it was found that the young larvae of *T. castaneum* were the most heat tolerant at 50 to 60ºC within 24 to 36 h (Subramanyam et al., 2011). In our study, the effect of high temperature (43ºC) in the control is more pronounced especially for the egg, larva and pupa of *T. castaneum* which were much more susceptible than the adult (Table 1). In the control of *O. surinamensis,* all development stages were affected by the high temperature. Although eggs hatched to larvae, they were dead one day later.

After exposure to 43ºC in the control, adult *Tribolium castaneum* was the most tolerant stage to this high temperature (<2% mortality), followed by the pupal stage (42% mortality, Table 1). Navarro (1978) reported the significant differences of adult mortality of *T. castaneum* in N_2 which ranged between 2 d to 4.5 d at 0.1 to 1.0% O_2 at 26ºC. However, in these trials, adults of *T. castaneum* at 1% O_2 were much more susceptible due to the high temperatures. This is consistent with Sakka et al. (2020) that a complete mortality was achieved in 2.5 d at 40ºC and 1% O_2 and was emphasized at 28ºC where they obtained mean mortality of 96.7% after 9 d of exposure time.

The pupae and larvae of both species were more susceptible to the treatment than the adults and eggs in both species. Although it is reported that some percentage of eggs did hatch (Fig. 3), the hatched larvae were found all dead immediately after the treatment. The reason for the tolerance of eggs to the treatment might be due to the low respiration rate of the eggs, but the complete control was achieved due to the high temperatures on the hatched eggs which was lethal due to their susceptibility. Fields (2002) reports that at a temperature range of 45-50ºC, death is achieved in less than 24 h. However, at a range of 42-45ºC there is no data.

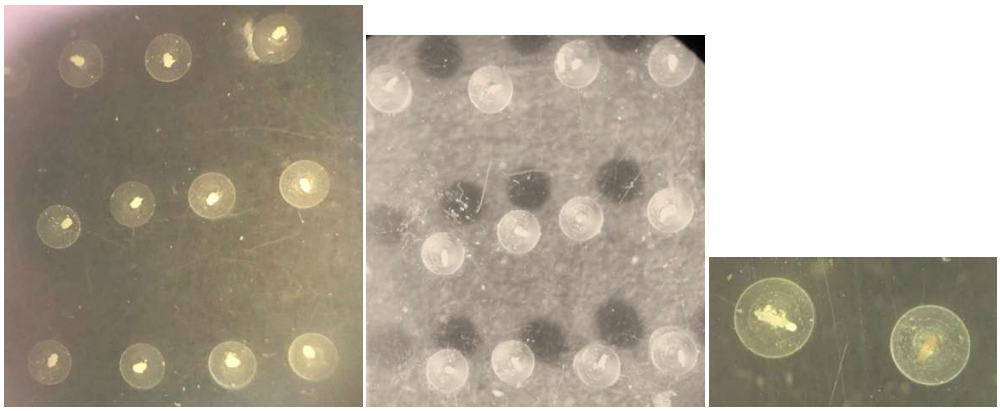

Fig. 3: *T. castaneum* eggs after treatment (left), *O. surinamensis* eggs after treatment (middle), and hatched eggs (right).

Protection of the beneficial qualities of cereals during storage depends on many factors. Among the detrimental factors that reduce the quality of cereals are insects and microflora (Navarro and Donahaye, 2005). Although the main objective of modified or controlled atmospheres is to control insect pests, the use of it enables quality preservation of the product as well, due to the sufficient sealed structure which maintains the vapor pressure (Navarro and Navarro, 2018). Even though in these trials, quality tests were not carried out, it is assumed that no quality deterioration occurred and the organoleptic characteristics were not affected.

Conclusions

Treating both stored product insects, *T. castaneum* and *O. surinamensis* at 43ºC in 1% O_2 using N_2 for 48 h resulted in very effective treatment. The survival rate was less than 0.5 % of adult of *O. surinamensis*. Increased mortality was observed at 43ºC compared to 28ºC. The combination of 43ºC with 1% O_2 in N_2 atmosphere was demonstrated as very effective in the control of all stages of both species. More data should be obtained to fill in the gaps of knowledge as for the tolerant stages of other insect species.

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