Indian Journal of Agricultural Sciences **81** (6): 555–9, June 2011

Time-mortality relationships for *Cadra* (*Ephestia*) *cautella* infesting walnut (*Juglans regia*) using thermal treatments

MANJU THAKUR[1], SHASHI BHALLA[2], SUDHIR RAWAT[3], SUBADAS SINGH[4] and R K KHETARPAL[5]

National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110 012

Received: 15 January 2010; Revised accepted: 14 March 2011

ABSTRACT

Laboratory experiments were conducted at National Bureau of Plant Genetic Resource, New Delhi during 2008–09 to determine the time-mortality response relationship of different developmental stages of *Cadra* (*Ephestia*) *cautella* (Wolker), viz egg, early larva, late larvae, pupa and adult infesting kernel and in-shell walnut kernel exposed to thermal treatment at 50°C for different durations. Mortality of each stage increased with the increase in exposure time. Egg was found to be the most tolerant stage, followed by late larva, early larva, pupa and adult. The 99% (LT_{99}) mortality of early larva, late larva and pupa in kernel and in-shell walnut kernel was achieved within ≤41 min. of exposure period at 50°C, while for the egg (most tolerant stage) in in-shell walnut kernel LT_{99} was 121.97 min. and LT_{50} was 34.03 min. Adult was the most susceptible stage to heat and complete mortality was achieved within 20 min of exposure. No aberrations occurred in organoleptic test and also there was no significant difference in the moisture content after 45 days of storage of kernel in shell and walnut kernel kept in airtight containers at 10°C. These results provide a basis for successful use of thermal treatments for management of all the life stages of *C. cautella* in kernel and in-shell walnut kernel.

Key words: *Cadra* (*Ephestia*) *cautella*, In-shell walnut kernel, Kernel, Phytosanitary, Quarantine, Thermal treatments, Walnut

Walnut (*Juglans regia* L.), is a potential export commodity and has become increasingly popular for consumers due to its nutritional advantages over cereal products. A major concern in its storage and marketing is the infestation of insect pests (Buranasompob *et al.* 2007). Seventeen pests are reported to affect walnut during storage worldwide, of which nine are reported from India (CABI 2007). Of these, the almond moth, *Cadra* (*Ephestia*) *cautella* (Walker) (Lepidoptera: Pyralidae) is an important pest of walnut causing direct damage and thereby reducing the product quality through larval feeding and webbing, contamination with faecal material which favours mold growth and product degradation. Post-harvest management of insects in nuts is essential to meet the quarantine and phytosanitary requirements of many importing countries. Methyl bromide (MB) fumigation is applied for various tree nuts including walnuts for the control of pests. However, MB has been designated as an ozone depleting substance under Montreal Protocol (UNEP 2006) and is to be eventually phased out. India ratified Montreal Protocol and its subsequent amendments in March 2003 and is legally committed to phase out the use of MB except for pre-shipment and quarantine purposes by 2015. Sulfuryl fluoride may be effective for post-harvest disinfestation of walnuts; however there is an interest for developing non-chemical alternatives (Monzon *et al.* 2006). Alternative disinfestation treatments for walnuts include irradiation (Thayer and Harlan 1983; Johnson and Marcotte 1999), cold storage (Moffit and Burditt 1989), controlled atmospheres (Toba and Moffit 1991), radiofrequency (Mitcham *et al.* 2004) and thermal treatments (Johnson 2004). The thermal treatments have emerged as an effective treatment for control of post-harvest insects in dried fruits and nuts. They are based on the fact that temperature determines the rate of metabolism, growth, development, reproduction, general behaviour and distribution of insect pests. Each insect pest and stage thereof has a development

[1]Research Associate (e mail: manju.thakur@gmail.com), NAIP, KAB II, Pusa, New Delhi 110 012;
[2]Principal Scientist (e mail: sbhalla@nbpgr.ernet.in) Division of Plant quarantine, NBPGR, New Delhi 110 012;
[3]Associate Professor (e mail: skrawatanjana@rediffmail.com), Government PG College, Rishikesh;
[4]Senior Research Fellow (e mail: khwaigi_das@gmail.com), NBPGR, New Delhi 110 012;
[5]Country Director (e mail: r.khetarpal@cabi.org), CABI South Asia, India

threshold within which it completes its activities. Any temperature lower/higher than that will affect the insect activities (Bhalla *et al*. 2009). Therefore, thermal treatments using temperatures higher and lower than this range have been exploited for pest control by various workers. Both low and high temperatures have a significant effect on the food consumption and fecundity of insects.

High temperatures may be obtained with forced hot air, vapour heat, hot water dips, microwaves or radiofrequencies. The use of hot air methods to develop post-harvest disinfestation of agricultural commodities has been reported by earlier workers (Beckett and Morton 2003, Mahroof *et al.* 2003, Johnson *et al.* 2003, 2004). The literature contains numerous reports on lethal limits for various post-harvest insects of dried fruits and nuts. High temperatures for quarantine treatments have been used effectively in various commodities. Quarantine treatments against insects commonly require Probit 9 mortality (99.9968%) while also minimizing or avoiding damage to the product (Bhalla *et al*. 2009). It is therefore, important to examine the effect on walnut quality of treatment times and temperature that control the target pests. Quality of dried fruits and nuts is often found to be less affected by temperature extremes than fresh products. Because some drying techniques for dried fruits and nuts include high temperatures, product disinfestation may be achieved by relatively minor changes in current practices. High temperatures have also been found effective when combined with vacuum and controlled atmosphere (Al -Azwai *et al.*1984; Navarro *et al.* 2003; Soderstrom 1992). Several studies have reported that the temperatures over 50°C or above are efficient for controlling even the most heat resistant stage of the major stored-product insect pests infesting walnut (Wang *et al.* 2002, Johnson *et al.* 2003, 2004). In view of this the temperature of 50°C was chosen in this study. Moreover, developing a successful thermal treatment relies on a thorough knowledge of the temperature-time-mortality relationships of the target insects. The present studies were undertaken to standardize a thermal disinfestation treatment against *C. cautella* and stages thereof infesting walnut both in shell and kernels and its impact on quality. This is to evaluate efficacy of heat treatments (forced hot air) as potential quarantine disinfestation treatment, which besides being effective would be economically feasible and environmentally safe.

MATERIALS AND METHODS

The almond moth (*C. cautella*) was reared on walnut (both kernel and in-shell walnut) at 28±1°C and 65–70% relative humidity in the BOD incubator in the Entomology Laboratory of Plant Quarantine Division, National Bureau of Plant Genetic Resources, New Delhi during 2008–09. Twenty to twentyfive freshly emerged adults were released for each 200 g of kernel in the rearing jars. Successive larval cultures were set up to obtain larvae and pupae of known ages to be used for the experiments. Larvae that reached their appropriate age interval for experiments were separated from the cultures. Pupae were obtained by means of polythylene transparent tubes of 2.0–2.5 mm id × 7 mm long, placed in the rearing jars at the time when larvae begin to pupate. For collecting the eggs of *C. cautella*, a number of healthy pupae were kept in beakers until adult eclosion inside inverted jars and eggs were collected daily from the Petri-plates inside these jars.

Infestation of in-shell walnut/kernel with different stages of Cadra cautella

One pair of adult/walnut was released in the 100 ml sample container for egg laying for 24 hr. The walnuts were infested through a small predrilled hole in the shell with different stages, viz early larva (7–10 days), late larva (18 –22 days), pupa (1–3 days) at the rate of two larvae/pupae/walnut and holes were sealed with a cello-tape to prevent escape of stages thereof from the walnuts. Similarly, walnut kernel was infested with different developmental stages, viz egg (by releasing one pair of adult for 20 g of walnut kernel for 24 hr), early larva (7–10 days), late larva (18 –22 days), pupa (1–3 days) at the rate of two, larvae/pupae/20 g walnut kernel. The infested material was incubated at 28±1°C and 65–70% relative humidity for 24 hr for acclimation.

Treatment of infested in-shell walnut/kernel

Ten walnuts infested with each of the different developmental stages of *C. cautella* were exposed to thermal treatment at 50°C ±1°C in a forced hot air oven in a single layer for different durations, viz 30, 60, 90,120 min. Ten walnuts were used in each replicate and each treatment was replicated 10 times. Also, 20 g walnut kernel infested with different developmental stages of *C. cautella* were exposed to the same thermal treatments as above and also replicated ten times. *Adult Treatment*- 5 Adults/walnut/20 g of kernel were exposed to the thermal treatment at 50°C ±1°C in a forced hot air oven for different durations of varying lengths, viz 10, 20, 30 min. Infested walnut/kernel as above with no thermal treatment served as control.

Evaluation of insect mortality

After treatment, samples were incubated under controlled conditions at 28±1°C and 65±5% relative humidity. The treated material infested with egg stage was evaluated on the basis of the parameter of larval emergence. Similarly, treated material infested with larval stage was evaluated on the basis of the pupal formation and pupae on the basis of adult emergence. The larval stage was monitored for any movement or starting to pupation. After two weeks the moribund larvae were checked under a microscope for any internal movement. Mortality of each stage was calculated as the percentage of dead insects relative to total treated insects for each exposure period. The adults treated for different durations were observed for their survival, moribund state and mortality immediately after exposure and at regular intervals after every two hour to ensure complete mortality with no revival**.**

Statistical analysis

Treatment mortality was corrected for control mortality using the Abott (1925) formula. After calculating the corrected mortality for time-mortality responses, treatment means were subjected to probit analysis of SPSS version 14.0. Data was analyzed as log-transformed dosage mortality regression lines in order to calculate the regression estimates and LT_{50} and LT_{99} values.

Determination of the moisture content of in-shell walnut/ kernel

The moisture contents of walnut shell, whole nut and kernel of heated and unheated samples was determined using the standard oven method. For this, 20 walnuts were crack opened and kernel was separated from shell and converted into small pieces to facilitate water loss. The initial weight (W1) of the kernels and shells was determined before heating and then the samples were heated at 70°C in an oven until there was no reduction in weight of the samples (Wang *et al.* 2002). The per cent water loss (weight loss) was determined by subtracting the total initial weight (W1) with the total final weight (W2) for each treatment. Moisture per cent was calculated as:

Moisture% = (W1-W2)/W1 ×100

Sensory evaluation

A limited organoleptic taste of the treated and untreated in-shell walnuts and walnut-kernel was evaluated by 10 panelists in terms of flavour and degree of liking for each sample. A separate group of walnuts and walnut kernel were used for this sensory analysis. The walnuts and walnut kernel were heat-treated at 50°C for 30, 60, 90 and 120 min. and untreated walnut kernel was kept as control. After giving treatment the samples were stored for 45 days at 10°C in air-tight containers as cold storage at 10°C is observed as a protective treatment (Johnson *et al.* 2009) and then the in-shell walnuts were shelled by hand and the treated kernel samples were used for the test. All the treatments were sampled and labeled and provided to the panelists. The quality grades were based on the 1–5 scale. 1=poor, 2=fair, 3=satisfactory, 4=good and 5 =very good.

RESULTS AND DISCUSSION

The results comprise the effect of thermal treatment on adult and different immature stages of the pest and the quality of walnut.

Insect mortality

After correcting for control mortality, time-mortality data were subjected to probit analysis to obtain 50% and 99% mortality (LT_{50} and LT_{99}) values for eggs, larvae (early and late), pupae and adults infesting kernel as well all stages in-shell walnut. The regression estimates obtained after probit analysis are presented in Table 1. The mortality of all the stages increased with an increase in exposure time. Egg was found to be the most tolerant stage, followed by late larva, early larva, pupa and adult. Adult was found to be the most susceptible stage. The same trend was also observed for the life stages of *Cadra* (*Ephestia*) *kuhniella* exposed to abnormally high temperatures by Mansbridge (2008). The adult response to treatments revealed complete mortality of the moths within 20 min. of exposure period at 50°C. Therefore, mortality responses of adult stage were not subjected to probit analysis and thus the results are not included in Table 1. The results show that 99% of the most tolerant stage of *C. cautella*, ie eggs may be controlled after 121.97 min of exposure when treating in in-shell walnut and after 120.16 min when treating in kernel. The LT_{50} values for egg, early larva, late larva and pupa in in-shell walnuts were 34.03, 24.87, 26.59 and 24.39, respectively and in kernel

Table 1 Time-mortality regression estimates for *Cadra cautella* life stages exposed for different durations in walnut kernel and in-shell walnut kernel at 50°C

Life-stage	R^2	Heterogeneity χ^2 (df)	Intercept	Slope	LT_{50} (fiducial limits)* (min.)	LT_{99} (fiducial limits)* (min.)
Kernel						
E (egg)	1.0	1.678 (2)	–6.57	4.29	33.87 (24.73–58.60)	120.16 (100.64–130.36)
EL (early larva)	0.99	6.055 (2)	–10.0	7.5	24.78 (21.78–27.24)	33.34 (31.67–75.55)
LL (late larva)	1.0	8.229 (2)	–21.0	15.0	25.58 (25.14–26.37)	36.43 (34.04–41.00)
P (pupa)	0.97	6.203 (2)	–60.0	4.20	21.51 (20.57–22.43)	28.89 (27.48–33.76)
In-shell walnut kernel						
E (egg)	0.99	2.32 (2)	–6.57	7.14	34.03 (16.26–44.14)	121.97 (106.92–142.96)
EL (early larva)	0.95	9.99 (2)	–4.0	0.15	24.87 (10.17–30.15)	39.52 (31.67–75.55)
LL (late larva)	0.97	4.792 (2)	–14.49	9.99	26.59 (24.78–25.96)	40.42 (32.69–122.812)
P (pupa)	1.0	4.395 (3)	–39.11	27.7	24.39 (16.06–32.33)	30.99 (29.79–32.90)

*95% confidence limits. Fiducial limits are calculated at $P \leq 0.05$ level of significance

LT_{50}, Lethal time for 50% kill; LT_{99}, lethal time for 99% kill

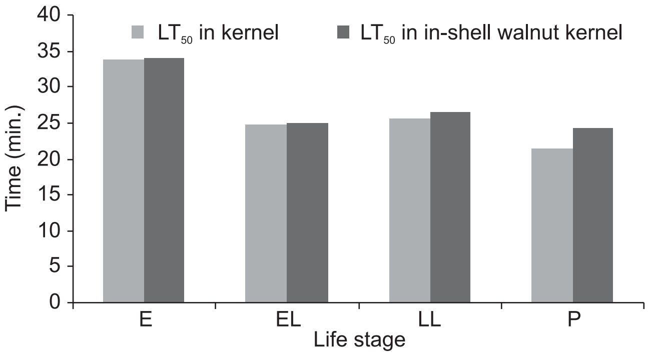

Fig 1 Comparative LT_{50} values for *Cadra cautella* life stages exposed for different durations in kernel and in-shell walnut kernel at 50°C.

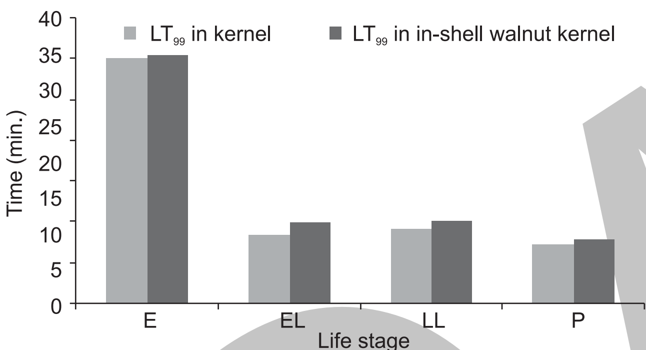

Fig 2 Comparative LT_{99} values for *Cadra cautella* life stages exposed for different durations in kernel and in-shell walnut kernel at 50°C.

were 33.87, 24.78, 25.58 and 21.51, respectively. The LT_{99} values for egg, early larva, late larva and pupa in in-shell walnuts were 121.97, 39.52, 40.42 and 30.99, respectively and in kernel were 120.16, 33.34, 36.43 and 28.89, respectively. Although not much difference was found in the lethal times for both the cases (kernel and in-shell kernel) because the eggs were outside the shell/kernel. There was no significant difference in the LT_{50} values of all the life stages treated in kernel and in-shell walnut kernel (Fig 1), whereas a slight difference was observed in the LT_{99} values of the treatment of all life-stages in kernel and in-shell walnut kernel (Fig 2). Denlinger and Yocum (1999) also suggested that the lethality in insects at high temperature depends on both high temperature and time of exposure. Temperature and exposure time to achieve a certain percentage of insect kill are inter-related. At high temperatures insect cuticular wax becomes compromised allowing loss of water. This affects water balance in insects, leading to death and dessication. High temperature exposure denatures proteins, affects haemolymph ionic balance and *p*H and adversely affects enzyme activity (Neven 2000). High temperatures are also reported to adversely affect the reproduction if not causing 100% insect kill. Mahroof *et al.* (2005) reported that when pupae and adults of the red flour beetle [*Tribolium castaneum* (Herbst)] were exposed to 50°C for 39 and 60 min., respectively the surviving insects after exposure these showed significant reduction in oviposition, egg-adult survival rate and progeny production.

Quality evaluation of walnuts

Any disinfestation method is acceptable or practically feasible only if it kills the insects without adversely affecting the quality of the walnut. Table 2 summarizes the results of the moisture contents of kernel, shell and whole nut before and after different time and temperature combinations. The average moisture content of shell was higher than that of whole nut and kernel. The observations conform to the findings of earlier workers. The moisture content of the kernel was 2.3% and that of the whole nut was 5.0%, after 120 min. of exposure period, which was not significantly different from their respective controls (P<0.05), whereas the moisture content of the kernel is significantly different from that of shell and whole nut (P>0.05). In particular, the moisture content of the shell, whole nut and kernel was reduced by 0.1% after 120 min. after treatment. These results indicate that the original quality of the walnuts and kernel is still maintained after 2 hr (120 min.) of exposure period at the temperature of 50°C. Lurie and Mitcham (2007) also supported that only small sensory changes were observed at water content of 3.1–4.0% in kernel to ensure optimal storage. Taste tests did not suggest any aberrations in the taste of nuts as well as its kernel when the nuts were heated to final maximum temperature of treatment after 120 min. when compared to untreated kernels. In the organoleptic test the taste of the treated in-shell walnuts was ranked very good to good and kernel was ranked good to fair by the panelists. These results indicate that there is no alteration in the quality of kernel and in-shell walnut quality even after exposure for 120 min. at 50°C and after 45 days of storage. The results were in agreement with the findings of that hot air heating at 55°C for 1 hr showed no increase in rancidity after nine months of accelerated storage at 20°C. Mitcham *et al.* (2004) also observed no taste aberrations when the nuts were heated and final kernel temperatures were around 75°C after radio frequency treatments. These results clearly demonstrate the effectiveness of thermal treatments as a non-chemical disinfestation method for management of *C. cautella* in kernel

Table 2 Moisture content (Mean ±SD) of kernel, shell and whole nut after heating at different durations at 50°C

Treatment (min.)	Moisture content (%)		
	Shell	Kernel	Whole nut
30	5.2 ±0.8a*	2.4 ±0.6b	5.0 ±0.9c
60	5.1 ±0.6a	2.4 ±0.8b	5.0 ±0.7c
90	5.1 ±0.7a	2.3 ±0.7b	5.0 ±1.5c
120	5.0 ±0.9a	2.3 ±1.7b	4.8 ±0.3c
0 (control)	5.2 ±1.2a	2.4 ±0.8b	5.0 ±1.3c

*Different letters within a row indicate that means are significantly different (P<0.05) and in-shell walnut.

REFERENCES

Abbott W S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomol*ogy **18**: 265–7.

Al-Azawi A F, El-Haidari, H S, Aziz F M, Murad A K and Al-Saud H M. 1984. The effect of high temperatures on the dried fruit beetle *Carpophilus hemipterus* L., a pest of stored dates in Iraq. *Date Palm Journal* **3**: 327–36.

Beckett S J and Morton R. 2003. Mortality of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) at grain temperatures ranging from 50°C to 60°C obtained at different rate of heating in a sprouted bed. *Journal of Stored Product and Research* **39** (3):313–2.

Bhalla S, Gupta K, Lal B, Kapur M L, Singh S, Thakur M and Khetarpal R K. 2009. Novel techniques for disinfestations of insects in quarantine. (*in*) *Biosecurity and Biosafety Policies, Procedures and Issues*, pp 293–9. VC Chalam, Kavita Gupta, Shashi Bhalla, Rajan, RK Khetarpal) (Eds). National Bureau of Plant Genetic Resources, New Delhi.

Buranasompob A, Tang J, Powers, JR, Reyes J, Clark S, Swanson B G. 2007. Lipoxygenase activity in walnuts and almonds. *LWT* **40**: 893–9.

CABI. 2007. *Crop Protection Compendium*. Wallingford, UK: CAB International.

Denlinger D L and Yocum G D. 1999. Physiology of heat sensitivity. (*in*) *Temperature Sensitivity in Insects and Application in Insect Pest Management*, pp 6–53. Hallman GJ, Denlinger DL (Eds), Westview Press Boulder, CO, USA.

Johnson J A and Marcotte M. 1999. Irradiation Control of insect pests of dried fruits and walnuts. *Food Technology* **53** (6): 46–53.

Johnson J A, Wang S and Tang J. 2003. Thermal death kinetics of fifth instar *Plodia interpunctella* (Lepidoptera: Pyralidae). *Journal of Economic Entomol*ogy **96**: 519–24

Johnson J A. 2004. Dried fruit and nuts: United States of America. (*in*) *Crop Post-Harvest Science and Technology*, pp 226–35, Vol. 2: Durables, K Hodges and G. Farrell (Eds). Oxford, UK: Blackwell Science..

Johnson J A, Valero K A, Wang S and Tang J. 2004. Thermal death kinetics of red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Economic Entomol*ogy **97**: 1868–73.

Johnson J A, Mitcham E, Tang J, Wang S. 2009. *Methyl Bromide Alternatives for Postharvest Insect Disinfestation of California Walnuts*. (*in*) *6th International Walnut Symposium*, 25–27 February 2009, Melbourne, Australia.

Lurie S and Mitcham E J. 2007. Temperature measurement: physiological responses of agricultural commodities to heat treatments. (*in*) *Heat treatments for Post Harvest Pest Control: Theory and Practice.* 349pp Juming Tang, Elizabeth Mitcham, Shaojin Wang (Eds)). CAB International.

Mahroof R, Subramanyam B, Throne J E and Menon A. 2003. Time–mortality relationships for *Tribolium castaneum* (Coleoptera: Tenebrionidae) life stages exposed to elevated temperatures. *Journal of Economic Entomology* **96** (4):1345–51.

Mahroof R, Subramanyam Bh and Flinn P. 2005. Reproductive performance of *Tribolium castaneum* (Coleoptera: Tenebrionidae) exposed to the minimum heat treatment temperature as pupae and adults. *Journal of Economic Entomology* **98**: 626–33.

Mansbridge G H MA. 2008. Experiments on the resistance of the flour moth (*Ephestia kuhniella* Zell.) to abnormally high temperatures. *Annals of Applied Biology* **23** (4): 803–21.

Mithcham E J, Veltmam RH, Feng X, Castro E, Johnson J A, Simpson T L, Biasi W V, Wang S and Tang J. 2004. Application of radio frequency treatments to control insects in in-shell walnuts. *Postharvest Biology and Technology* **33**: 99–100.

Moffit H R and Burditt A K. 1989. Effect of low temperature on three embryonic stages of the codling moth (Lepidoptera: Tortricidae). *Journal of Entomology* **82**: 1379–81.

Monzon M E, Biasi B, Simpson T L, Johnson J, Feng X, Slaughter D C and Mitcham E J. 2006. Effect of radio-frequency heating as a potential quarantine treatment on the quality of "Bing" sweet cherry fruit and mortality of codling moth larvae. *Postharvest Biology and Technology* **40**:197–203.

Navarro S, Finkelman S, Sabio G, Isikber A, Dias R, Rindner M and Azrieli A. 2003. Enhanced effectiveness of vacuum or CO_2 in combination with increased temperatures for control of storage insects (*in*) *Advances in Stored Product Protection, Proceedings of the 8th International Working Conference on Stored-product Protection*, pp. 818–22. Credland P F, Armitage D M, Bell C H, Cogan P M, Highley E (Eds). CAB International, Oxon, UK.

Neven L G. 2000. Physiological responses of insects to heat. *Postharvest Biology and Technology* **21**:103–11.

Soderstrom E, Curtis C, Brandl D, Mackey B and Vail P. 1992. Alternative treatments for quarantine disinfestation of walnuts. *Walnut Research Reports*, pp 148–55. Walnut Marketing Board.

Thayer D W and Harlan J W. 1983. Status of the USDA food irradiation programs. *Food Technology* **37**: 46–7.

Toba H H and Moffitt H R. 1991. Controlled atmosphere storage and quarantine treatment for non-diapausing codling moth larvae in apples. *Journal of Entomol*ogy **84**: 1316–9.

UNEP (United Nations Environmental Programme). 2006. *Handbook for the Montreal Protocol on Substances that Deplete the Ozone Layer*, 7th edn, UNEP Ozone Secretariate, Nairobi, Kenya. http://ozone.unep.org/Publications/Handbooks/MP_Handbook_2006.pdf.

Wang S, Tang J, Johnson J A, Mitcham B, Hansen J D, Cavalieri R P, Bower J and Biasi B. 2002. Process protocols based on radiofrequency energy to control field and storage pests in in-shell walnuts. *Postharvest Biology and Technol*ogy **26**: 265–73.