www.czasopisma.pan.pl PAN POLSKA AKADEMIA NAUK www.journals.pan.pl

JOURNAL OF PLANT PROTECTION RESEARCH Vol. 54, No. 3 (2014)

DOI: 10.2478/jppr-2014-0040

Effects of low ozone concentrations and short exposure times on the mortality of immature stages of the Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae)

Ensieh Keivanloo[1]*, *Hussein Sadeghi Namaghi*[1], *Mohammad Hossein Haddad Khodaparast*[2]

[1] Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, 9177948974 Iran
[2] Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, 9177948974 Iran

Received: January 22, 2013
Accepted: July 24, 2014

Abstract: In Iran, the Indian meal moth, *Plodia interpunctella* (Hübner), is one of the most important pests of such stored products as date fruits and pistachio nuts. Ozone was applied as a gas at four concentrations (0, 2, 3, and 5 ppm) for four different periods (30, 60, 90, and 120 min) on the immature stages of *P. interpunctella*. The results indicated that by increasing the concentration and exposure time, the rate of mortality increased for all tested stages. This study showed that 12-day-old larvae were more susceptible than other stages when exposed to 5 ppm ozone for 120 min. The next in order of susceptibility were pupae, then 5-day-old larvae, and 17-day-old larvae had the highest sensitivity to ozonation. At the highest concentration of ozone, for the longest time, the least mortality rate was recorded for one-day-old eggs. According to the results, a reduction in the population density of *P. interpunctella* in laboratory experiments is promising. However, validation studies will be necessary to fully determine the potential of ozone as a replacement for the current post harvest chemical control of *P. interpunctella* on either pistachio nuts or date fruits.

Key words: ozonation, *Plodia interpunctella*, post harvest pests

Introduction

For thousands of years, insects have been a problem when trying to store surplus food products. Contaminated products can cause financial, health, legal, and aesthetic problems. Financial losses can result from the presence of live or dead insects in products and containers, presence of odors, webbing and feces in products and containers, and direct loss in weight which is the result of insect feeding (Shadia and Abd El-Aziz 2011). According to Pimentel (1991), insect infestation in stored grain can result in economic losses of up to 9% in developed countries and 20% in developing countries.

In recent years, *Plodia interpunctella* (Hübner) is considered to be the most important pest of stored pistachios in Iran. This pest causes severe qualitative and quantitative losses in the pistachio crop (Shojaaddini *et al*. 2005). Larvae are able to penetrate and infest a wide range of packaged foods (Cline 1978). Infestation can have a great economic impact due to direct product loss. Indirect factors, such as the cost of pest control and loss of sales from consumer complaints (Sauer and Shelton 2002) also must be taken into consideration.

Fumigation of stored pistachios with methyl bromide or phosphine is the usual method used to control infestations of post-harvest pests, especially *P. interpunctella* (Johnson *et al*. 1996). But, chemical control methods using fumigants are restricted because of development of pest resistance (Zettler *et al*. 1989), health hazards, and risk of environmental contamination. Therefore, several other control methods, such as low temperature storage and heat treatment (Na and Ryoo 2000; Sauer and Shelton 2002), pheromone-baited traps (Mullen and Arbogast 1979), a change in the photoperiod (Shojaaddini *et al*. 2005), and essential oil from *Carum copticum* (L.) Link seeds (Shojaaddini *et al*. 2008) are being suggested as alternatives to fumigants.

Ozone is an oxidant that has long been used in food processing as a water treatment to disinfect as well as eliminate odors, taste, and color (Kim *et al*. 1999; EPA 1999). Recently, the feasibility of using ozone to manage stored product pests has received increased attention. Several studies have been carried out on the efficacy of gaseous ozone against stored product insect pests (Erdman 1980; Mason *et al*. 1997; Kells *et al*. 2001; Sousa *et al*. 2008; Bonjour *et al*. 2011; Hansen *et al*. 2012). Ozone also has the potential for controlling insect strains that are resistant against phosphine (Zhangui *et al*. 2003; Sousa *et al*. 2008). According to Hansen *et al*. (2012), full control of the majority of tested insects can generally be obtained with 35 ppm for 6 days. Full mortality of the internal stages of *Sitophilus* spp. and *Rhyzopertha dominica* F. required approximately 135 ppm for 8 days.

Ozone is not only used to control insect pests of stored products, but also to inactivate microorganisms and deg-

*Corresponding address:
Ekeivanloo@yahoo.com

radation of aflatoxin. The influence of ozone treatments on microbial flora as well as insets associated with some edible horticultural commodities is of great importance. The moth *P. interpunctella,* is an important pest of stored products like date fruits and pistachio nuts. In their study, Habibi Najafi and Hadad Khodaparast (2009) found that a one-hour minimum ozone treatment at 5 ppm can successfully reduce microbial populations associated with fresh date fruits. If such a concentration of ozone is to be applied against microbial flora of date fruits, what would be the expected effects on the immature stages of *P. interpunctella*? To address this question, we evaluated the influence of ozone gas at the same concentration and exposure time used in the above 2009 study by Habibi Najafi and Hadad Khodaparast, on the mortality of the immature stages of *P. interpunctella*.

Materials and Methods

Test insect

A stock culture of *P. interpunctella* was established using adults collected in a food warehouse in Mashhad (Khorasan Razavi province), in 2011. The larvae were raised on a diet consisting of 160 g yeast, 200 ml glycerol, 200 ml honey, and 800 g wheat barn (Sait *et al*. 1997). The larvae were kept in transparent plastic containers which were 25 cm in diameter and 30 cm in height. The top of the containers were covered with muslin cloth to keep the moths from escaping. For each trial, a group of 20 adults were transferred to an oviposition funnel, 22 cm in diameter and 20 cm in height. The funnel was covered with a 20 mesh cloth net and was then placed, reversed, on a black plastic sheet. After 24 h, the adults were removed, and the eggs which had been laid on the plastic sheets were collected and used in the experiments. For rearing other life stages needed in further trials, four groups were used, of which each group included at least 180–200 eggs of the same age (deposited during a 24 h period). These eggs were incubated in the same conditions that were used for rearing. All cultures were maintained at 28±1°C, 13 : 11 h (light : : dark) and 65±5% relative humidity (RH) in an incubator (provided by Razi Company Inc., Mashhad-Iran).

Experimental setup

The experimental setup for ozone application consisted of an ozone generator, monitor-controller and ozone detector, which were obtained from Ozoneab Company Inc., Iran (http://www.ozoneab.com under the license of Tech Trade International, Australia). Ozone in the form of gas, was generated using a laboratory corona discharge ozone generator (Model AS – 1200 M) from purified extra dry oxygen feed gas. The output of the generator was 8 g/h. The amount of ozone in the fumigation chamber was controlled by a monitor-controller having a plug-in sensor on board, which was changeable for different ranges of ozone concentration. A wheel in the monitor-controller allowed us to control the concentration in a selected range. The ozone monitor was placed outside the treatment chamber and the fan of the ozone monitor drew the air from inside the chamber. An incubation chamber, with a 50-litre volume, was used for the ozone treatment. An extra check of the ozone concentration in the treatment chamber was measured by a portable ozone detector (Model OZO21ZX) in the range between 0 and 10 ppm with the accuracy of 0.01.

Bioassays

For each growth stage of the insect in our study, 16 combinations of ozone concentration and exposure time were tested. Eggs which had been laid on the piece of plastic sheet, pupae, and larvae (5, 12, and 17 days old) were placed in 130-ml jars. The center of the jar lids were removed and covered with a 40 mesh cloth net. The eggs, larval stages and pupae of *P. interpunctella* were then exposed to ozone concentrations of 0, 2, 3, and 5 ppm for 30, 60, 90 and 120 min. For each insect stage, the experiment was replicated six times by using at least 10 specimens of the same age per replicate. After each treatment, eggs, larvae and pupae were held at 28±2°C and 65±5% RH using the same diet, until examined for mortality. Mortality counts were determined for larvae that had failed to pupate 9 days after exposure. Pupal mortality was based on those pupae that failed to produce adults 9 days after exposure. Egg hatch was counted 7 days after treatment.

Data analysis

Statistical analyses were performed using the General Linear Models (GLM) procedure in SAS version 9.1 (Institute, 2002, Cary NC, USA). Data were first transformed using the arcsine square-root transformations to stabilize variances and then mortality (%) analyzed separately for each life stage of *P. interpunctella* using one-way analysis of variance (ANOVA). Tukey's studentized range (Honestly Significant Difference – HSD) test (at $\alpha = 0.05$) was used to compare means among treatments.

Results

Data in table 1 show that all immature stages of *P. interpunctella* were susceptible to ozone. Susceptibility depended on the life stage, concentration of ozone used, and the length of time the insect was exposed. The results of comparing the means by HSD, showed that differences in the mortality percentage of each life stage at different ozone treatments were significant. Generally, for all three life stages tested (eggs, larvae, and pupae) mortality ratios increased with an increasing ozone concentration and exposure time.

Egg

Egg mortality ranged from zero (the control) to 56.66±4.94 and 56.66±6.14% at ozone concentrations of 5 and 3 ppm, respectively, for an exposure time of 120 min. ANOVA results for eggs were ($F = 2.51$; $df = 9, 95$; $p = 0.0139$). In comparison with other life stages tested, mortality ratios of eggs at different concentration × exposure time combinations were generally lower than the other stages of *P. interpunctella* (Table 1).

Table 1. Effects of ozone concentration and exposure time on mortality (mean ±SE) of *P. interpunctella* in different life stages

Life stage	Exposure time [min]	Ozone concentration [ppm]			
		0	2	3	5
1 day old eggs	30	0.00±0.00 e	20.00±0.00 d	35.00±2.23 cd	36.66±4.21 bc
	60	10.00±0.20 e	20.00±0.00 d	38.33±3.07 bc	45.00±4.28 abc
	90	10.00±0.20 e	31.66±3.07 cd	46.66±3.33 abc	51.66±3.07 ab
	120	0.00±0.00 e	33.33±3.33 cd	56.66±6.14 a	56.66±4.94 a
5 days old larvae	30	0.00±0.00 e	28.33±4.77 d	30.00±3.65 d	36.66±2.10 cd
	60	0.00±0.00 e	38.33±7.03 cd	53.33±9.18 bcd	56.66±10.54 bc
	90	0.00±0.00 e	65.00±5.00 ab	70.00±5.16 ab	73.33±5.57 ab
	120	0.00±0.00 e	83.33±4.94 a	85.00±5.62 a	90.00±3.65 a
12 days old larvae	30	0.00±0.00 g	20.00±0.00 fg	51.66±4.77 de	58.33±4.77 cd
	60	0.00±0.00 g	26.66±3.33 f	70.00±2.58 bcd	76.66±6.14 abc
	90	0.00±0.00 g	36.66±3.33 ef	85.00±5.62 ab	86.66±4.21 ab
	120	0.00±0.00 g	73.33±8.02 bc	88.33±4.77 ab	95.00±3.41 a
17 days old larvae	30	0.00±0.00 g	20.00±0.00 fg	51.66±4.01 cde	71.66±3.07 abc
	60	0.00±0.00 g	30.00±2.58 ef	55.00±5.00 cd	68.33±5.42 abcd
	90	0.00±0.00 g	56.66±4.94 bcd	66.66±6.14 abcd	78.33±4.77 ab
	120	0.00±0.00 g	46.66±7.60 de	63.33±3.33 abcd	85.00±9.21 a
Pupae	30	0.00±0.00 f	35.00±5.62 e	43.33±4.21 cde	50.00±6.83 cde
	60	0.00±0.00 f	38.33±5.42 de	53.33±5.57 bcde	56.66±4.94 abcde
	90	20.00±5.40 f	58.33±7.49 abcde	61.66±3.07 abcd	65.00±8.85 abc
	120	0.00±0.00 f	76.66±6.14 ab	78.33±6.00 ab	80.00±6.83 a

For each life stage separately, means followed by different superscript letters are significantly different ($p < 0.05$) by HSD test. ANOVA results for eggs, 5-days larvae, 12-days larvae, 17-days larvae and pupae were ($F = 2.51$; $df = 9, 95$; $p = 0.0139$), ($F = 5.82$; $df = 9, 95$; $p \leq 0001$), ($F = 9.79$; $df = 9, 95$; $p \leq 0001$), ($F = 4.07$; $df = 9, 95$; $p = 0.0002$), ($F = 2.72$; $df = 9, 95$; $p = 0.0080$), respectively

Larvae

P. interpunctella larvae were more susceptible to ozone than other immature stages. However, there was a variation in susceptibility of different aged larvae to ozone. For 5-day-old larvae, mortality ratios at ozone concentrations of 2, 3, and 5 ppm for a 2 h exposure time were 83.33±4.94, 85.00±5.62, and 90.00±3.65, respectively. These results were significantly higher than other concentration × time treatment combinations. In the case of 12 days old larvae, the highest mortality ratio (95.00±3.41) was achieved at an ozone concentration of 5 ppm for a 2 h exposure time, which was significantly higher than other treatments. For 17-day-old larvae, mortality ratios at different ozone concentration × exposure time treatments were significantly different (Table 1). The highest mortality (85.00±9.21%) was observed at an ozone concentration of 5 ppm for 2 h of exposure time. In all the larval stage experiments, by increasing ozone concentration and exposure time, the percentage of mortality was increased.

Pupae

Mortality of pupae was increased with increasing exposure time and ozone concentration. Pupal mortality differed among different treatments, and these differences were significant ($F = 2.72$; $df = 9, 95$; $p = 0.0080$). The highest mortality for this life stage was 80.00±6.83% which was observed at 5 ppm of ozone concentration for 2 h of exposure time.

Discussion

Not surprisingly, ozone had less effect on *P. interpunctella* egg mortality. At the highest ozone concentration × exposure time treatment, the mortality ratio of eggs (56.66±4.94%) was lower than those of 5-(90.00±3.65%), 12-(95.00±3.41%), 17-day-old larvae (85.00±9.21%), and of pupae (80.00±6.83%). The results presented by others show that eggs cannot be completely eradicated. For example, Leesch (2003) reported that high concentrations of ozone at 10.000 ppm for a 4-hour exposure time did not kill 100% of the eggs of *P. interpunctella*. Wood (2008) found eggs of the greater wax moth, *Galleria mellonella* L., to be more tolerant to ozone compared with larvae and adults. Niakousari *et al*. (2010) reported that a concentration of 4.000 ppm of ozone for 2 h resulted in only 80% mortality of *P. interpunctella* eggs on date fruits. Bonjour *et al*. (2011) found that ozone at a concentration of 70 ppm for a period of 4 days did not have a significant effect on the eggs of *P. interpunctella*. These results show that eggs are more resistant to ozone. An explanation is that ozone has an initial problem penetrating through the egg (Niakousari *et al*. 2010).

Generally, it is expected that sensitivity to ozone increases as larvae get larger in size and have a larger contact surface to ozone. However, our findings on the efficacy of ozone as a fumigant against immature stages of *P. interpuctella* may be compared with several studies on the efficacy of ozone to control insect pests of stored products. The present results support those of Kells *et al*.

(2001), who found a higher mortality rate for the larval stage compared with other stages of *P. interpunctella* exposed to 50 ppm of ozone for 3 days. Leesch (2003) tested the toxicity of gaseous ozone on different stages of *P. interpunctella.* Through the testing, it was found that, except the egg stage, all life stages of *P. interpunctella* were more or less susceptible to laboratory treatment with ozone at 300 ppm for a 4-h exposure period. Similarly, Isikber and Oztekin (2009) found that larval stage of *Tribolium confusum* Duv. with 86.3% mortality, was more susceptible to ozone than other life stages of the tested insect.

Some studies showed results which were similar to our present study results. In a study on the potential of ozone as a fumigant to control pests in honey bee hives, James (2011) reported that pupae of *G. mellonella* were more resistant to ozone than larvae. Also, Isikber and Oztekin (2009) found that pupae of *T. confusum* were more resistant to ozone than larvae. In contrast to the present results, Bonjour *et al*. (2011) evaluating the efficacy of ozone fumigation against the major grain pests in stored wheat, reported that pupae of *P. interpunctella* were more susceptible than eggs and larvae.

Overall, the present study showed that there are some differences in the susceptibility of different immature stages of *P. interpunctella* to ozonation. Based on the present results and those of others, it seems that ozone toxicity for insects varies depending on both the life stages and species of test insects. For example, larval and pupal stages of *Tribolium castaneum* Hbst. are ozone sensitive with sensitivity decreasing with age (Erdman 1980). Also, Bonjour *et al*. (2011) showed that ozone treatment on pupae were more effective than on eggs and larvae of *P. interpunctella*. The mortality rate of *Ephestia kuehniella* Z. and *T. confusum* was studied by Isikber and Oztekin (2009). In their study, they observed that insect mortality during ozonation was not only dependent upon the life stages specific for both the species but also was insect specific. They observed a higher susceptibility and higher mortality for larvae, pupae, and adult stages of *E. kuehniella* (90–100%) compared to *T. confusum* (1.3–22.7%) under similar experimental conditions. Also, Leesch (2003) reported a higher susceptibility rate for *P. interpunctella* compared to *T. confusum*.

Lethal concentrations and exposure times for ozone have been reported to range between 5 ppm for 5 days (Mason *et al*. 1997) to 135 ppm of ozone for 8 days (Hansen *et al*. 2012) for insects living within stored products. The present study showed that a much lower concentration and time period (5 ppm for 120 min) can result in a substantial mortality of 12-day-old larvae of *P. interpunctella* when they were freely exposed to ozone. There is variation of the effective levels of ozone in different studies evaluating the efficacy of ozone to control stored product insects. Such variation is considered to be acceptable considering that the majority of studies have used insect specimens placed inside the grain masses or inside the kernels of grain or other products. In such cases, it is obvious that gaseous ozone cannot easily reach the target pest and insects are, to some extent, protected from exposure to ozone. A much higher concentration and a longer exposure time is needed to obtain full control of the treated insects. Moreover, the efficacy of ozone to control insect pests in deeper layers of products is less than of those insects on the surface of the product. Hansen *et al*. (2012) showed that full mortality of those stored product pests feeding within kernels needed 135 ppm of ozone for 8 days compared with 35 ppm for 6 days, for freely exposed stages of main stored pests.

Our study showed that ozone in gas form at a low concentration of 5 ppm for 2 h of exposure has the potential to control, immature stages (eggs, larvae and pupae) of *P. interpunctella*. This same procedure was effective against micro flora on date fruits (Habibi Najafi and Hadad Khodaparast 2009). Further experiments may show that higher concentrations of ozone, different exposure durations, or a combination of both, will increase effectiveness against both *P. interpunctella* and micro flora on stored date fruits or pistachio nuts.

Acknowledgements

The authors are grateful to Mehdi Eftekhari for reviewing an earlier draft of this manuscript and to Dr. Mojtaba Hosseini in the Plant Protection Department, Ferdowsi University of Mashhad, for providing statistical tips. The Department of Food Science and Technology of Ferdowsi University of Mashhad provided ozone facilities for the experiments. We wish to thank the Ferdowsi University of Mashhad, Iran, where this study was carried out.

References

Bonjour E.L., Opit G.P., Hardin J., Jones C.L., Payton M.E., Beeby R.L. 2011. Efficacy of ozone fumigation against the major grain pests in stored wheat. J. Econ. Entomol. 104 (1): 308–316.

Cline L.D. 1978. Penetration of seven common flexible packing materials by larvae and adults of eleven species of stored-product insects. J. Econ. Entomol. 71 (5): 726–729.

EPA 1999. Alternative disinfectants and oxidants guidance manual. United States Environmental Protection Agency (US EPA), Publication 815 R 99014, 30 pp.

Erdman H.E. 1980. Ozone toxicity during ontogeny of two species of flour beetles, *Tribolium confusum* and *T. castaneum*. J. Environ. Entomol. 9 (1): 16–17.

Habibi Najafi M.B., Hadad Khodaparast M.H. 2009. Efficacy of ozone to reduce microbial populations in date fruits. J. Food Control 20 (1): 27–30.

Hansen L.S., Hansen P., Jensen K.M.V. 2012. Lethal doses of ozone for control of all stages of internal and external feeders in stored products. Pest Manage. Sci. 68 (9): 1311–1316.

Isikber A.A., Oztekin S. 2009. Comparison of susceptibility of two stored-product insects, *Ephestia kuehniella* Zeller and *Tribolium confusum* to gaseous ozone. J. Stored Prod. Res. 45 (3): 159–164.

James R.R. 2011. Potential of ozone as a fumigant to control pests in honey bee (Hymenoptera: Apidae) Hives. J. Econ. Entomol. 104 (2): 353–359.

Johnson J.A., Gill R.F., Valero K.A., May S.A. 1996. Survival of navel orangeworm (Lepidoptera: Pyralidae) during pistachio processing. J. Econ. Entomol. 89 (1): 197–203.

Kells S.A., Mason L.J., Maier D.E., Woloshuk C.P. 2001. Efficacy and fumigation characteristics of ozone in stored maize. J. Stored Prod. Res. 37 (4): 371–382.

Kim J.G., Yousef A.E., Dave S. 1999. Application of ozone for enhancing the microbiological safety and quality of foods: a review. J. Food Prot. 62 (9): 1071–1087.

Leesch J.G. 2003. The mortality of stored-product insects following exposure to gaseous ozone at high concentrations. p. 827–831. In: "Advances in Stored Product Protection" (P.F. Credland, D.M. Armitage, C.H. Bell, P.M. Cogan, E. Highley, eds.). Proc. 8th Int. Working Conference on Stored-Product Protection, York, UK, 22–26 July 2002, 965 pp.

Mason L.J., Woloshuk C.P., Maier D.E. 1997. Efficacy of ozone to control insects, molds, and mycotoxins. p. 665–670. In: Proc. Int. Conference on Controlled Atmosphere and Fumigation in Stored Products (E.J. Donahaye, S. Navarro, A. Varnava, eds.). PrintCo Ltd., Nicosia, Cyprus, 780 pp.

Mullen M.A., Arbogast R.T. 1979. Time-temperature-mortality relationships for various stored product insect eggs and chilling times for selected commodities. J. Econ. Entomol. 72 (4): 476–478.

Na L., Ryoo M. 2000. The influence of temperature on development of *Plodia interpunctella* (Lepidoptera: Pyralidae) on dried vegetable commodities. J. Stored Prod. Res. 36 (2): 125–129.

Niakousari M., Erjaee Z., Javadian S. 2010. Fumigation characteristics of ozone in postharvest treatment of Kabkab dates (*Phoenix dactylifera* L.) against selected insect Infestation. J. Food Prot. 73 (4): 763–768.

Pimentel M.A.G. 1991. World resources and food losses to pests. p. 5–11. In: "Ecology and Management of Food Industry Pests" FDA Technical Bulletin 4 (J.R. Gorham, ed.). Assoc. Off. Anal. Chemists, Arlington, Virginia, USA, 595 pp.

Sait S.M., Begon M., Thompson D.J., Harvey J.A., Hails R.S. 1997. Factors affecting host selection in an insect host-parasitoid interaction. Ecol. Entomol. 22 (2): 225–223.

Sauer J.A., Shelton M.D. 2002. High temperature controlled atmosphere for post-harvest control of Indian meal moth (Lepidoptera: Pyralidae) on preserved flowers. J. Stored Prod. Res. 95 (5): 1074–1078.

Shadia E. Abd El-Aziz 2011. Control strategies of stored product pests. J. Entomol. 8 (2): 101–122.

Shojaaddini M., Moharramipour S., Sahaf B.Z. 2005. Effects of different photoperiods on some biological parameters of the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) on fried and unfired pistachio cultivars. Turkish J. Entomol. 29: 279–287.

Shojaaddini M., Moharramipour S., Sahaf B.Z. 2008. Fumigant toxicity of essential oil from *Carum copticum* against Indian meal moth, *Plodia interpunctella*. J. Plant Prot. Res. 48 (4): 411–419.

Sousa A.H., Faroni L.R.D.A., Guedes R.N.C., Totola M.R., Urruchi W.I. 2008. Ozone as a management alternative against phosphine-resistant insect pests of stored products. J. Stored Prod. Res. 44 (4): 379–385.

Wood M. 2008. Ozone might help make bee hives cleaner and safer. New York Berry News 7 (5): 8–9.

Zettler J.L., Halliday W.R., Arthur F.H. 1989. Phosphine resistance in insects infesting stored peanuts in the Southeastern USA. J. Econ. Entomol. 82 (6): 1508–1511.

Zhanggui Q., Xiaoping Y., Xia W. 2003. Trials of ozone reducing pesticide residues in grain. Grain Storage 32 (3): 10–13.