Journal of Asia-Pacific Entomology 15 (2012) 101–105

Contents lists available at SciVerse ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Effects of azadirachtin on the development and gene expression of fifth instar larvae of Indianmeal moth, *Plodia interpunctella*

Ohn Mar Lynn [a,b], Jang-Eok Kim [a], Kyeong-Yeoll Lee [a,*]

[a] School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 702-701, Republic of Korea
[b] Department of Entomology, Yezin Agricultural University, Yezin, Myanmar, Myanmar

ARTICLE INFO

Article history:
Received 9 June 2011
Revised 20 August 2011
Accepted 23 August 2011

Keywords:
Azadirachtin
Development
Gene expression
Heat shock proteins
Indianmeal moth
Plodia interpunctella

ABSTRACT

The effects of azadirachtin on the rates of pupation, adult eclosion, and the expression of nine selected genes were evaluated in *Plodia interpunctella*. The newly molted fifth instar larvae (V0) were fed a pollen diet containing different amounts of azadirachtin. When larvae ingested azadirachtin at 1 and 10 ppm, pupation was 85 and 75.8%, respectively, and adult eclosion was 81.7 and 55.3%, respectively. The effects of azadirachtin on gene expression were analyzed using real-time RT-PCR analysis. When V0 larvae ingested azadirachtin for 2 days, expression of some genes related to development, stress, and immunity was affected by azadirachtin. mRNA levels of *shsp, hsp90, ecdysone receptor, ultraspiracle, prophenoloxidase* and *hemolin* were upregulated, although both *ultraspiracle* and *hemolin* were downregulated at higher doses. Levels of *hsc70, hsp70* and *β-1,3-glucan recognition protein* were not changed. Overall, azadirachtin significantly inhibited post-embryonic development and differentially modulated gene expression patterns of *P. interpunctella*.

Introduction

Azadirachtin, a triterpenoid in the limonoid class, is present mostly in the seeds of neem tree (*Azadirachta indica*). It is one of the most biologically active natural inhibitors of insect growth and development (Schmutterer, 1990; Ascher, 1993; Mordue (Luntz) and Blackwell, 1993; Morgan, 2009). Since it was first found to be a feeding deterrent to the swarming desert locust, *Schistocerca gregaria*, in India, it has been shown to be effective in more than 500 insect species (Butterworth and Morgan, 1968; Schmutterer and Singh, 2002). It has a broad spectrum and is effective against many other groups in arthropods, as well as nematodes and annelids. However, it has low or no toxicity to vertebrates (Mordue (Luntz) et al., 2005).

Azadirachtin affects three aspects of insect biology: behavior, growth and development (Mordue (Luntz) et al., 2005). First, azadirachtin has a strong antifeedant affect in various insects. Second, it acts as an insect growth regulator by inhibiting growth and development by blocking the biosynthesis of insect hormones such as ecdysteroids. Third, when insects ingest azadirachtin, the development of reproductive organs and vitellogenesis are inhibited which reduce fertility and fecundity of the adults.

Molecular techniques have been used to determine its mode of action and molecular interactions at cellular and molecular levels. Azadirachtin is particularly active on mitotic cells by blocking polymerization of microtubules (Salehzadeh et al., 2003). It has antiproliferative effects by arresting the cell cycle and inducing apoptosis in the *Spodoptera litura* SI-1 cell line (Huang et al., 2011). In addition, azadirachtin damages nuclear DNA directly and binds to a large complex of proteins in extracts of *Drosophila* Kc167 cells. A heat shock protein 60 (hsp60) is identified as one of putative azadirachtin-binding proteins (Robertson et al., 2007). It also interacts with retinoic acid receptors and exerts anti-inflammatory and anti-metastatic responses in human cell lines (Thoh et al., 2011). Therefore, current evidence suggests that azadirachtin is highly reactive with various cellular molecules in the cytoplasm and nucleus. Azadirachtin may also alter activities of specific genes and proteins (Mordue (Luntz) et al., 2005).

In this study, the effects of azadirachtin on the rates of pupation and adult eclosion, and on the transcription rate of 9 selected genes which are important in development, stress, and immunity of insects were evaluated in the Indianmeal moth, *Plodia interpunctella*, a serious pest of postharvest and stored agricultural products.

Materials and methods

Insects

A *P. interpunctella* colony was maintained in a rearing room at 27 ± 2 °C, 70 ± 5% relative humidity, and a 16-hour light and 8-hour dark (16L:8D) photoperiodic cycle. Larvae were reared in a plastic box ($15 \times 15 \times 10\ cm^3$) on a diet consisting of wheat bran, pollen, honey, glycerin, and water (1:1:0.3:0.3:0.15, v/v) (Aye et al., 2004). Except where noted, fifth instar larvae were used in all experiments. The day

* Corresponding author. Tel.: +82 53 950 5759; fax: +82 53 950 6758.
E-mail address: leeky@knu.ac.kr (K.-Y. Lee).

1226-8615/$ – see front matter © Korean Society of Applied Entomology, Taiwan Entomological Society and Malaysian Plant Protection Society, 2011. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.aspen.2011.08.008

of ecdysis to the fifth larval instar was designated as day 0 (V0). Wandering fifth instar larvae were allowed to pupate in corrugated cardboard rolls. Pupae were collected and placed in a new plastic box where they were allowed to eclose to adults and then mate. Eggs were collected and transferred to a new box containing diet.

Ingestion of azadirachtin

Azadirachtin (>95% purity; Sigma-Aldrich, St Louis, USA) was dissolved in 10% isopropanol and diluted with distilled water. Newly molted fifth instar larvae (0-day-old) were reared individually in each of the 24 wells (diameter 16 mm) of a plastic plate (SPL, Seoul, Korea). They were fed 10 mg of pollen grains (*Actinidia arguta* Planch) mixed with 10 μl of different concentrations of azadirachtin solution. The final doses of azadirachtin of each diet were 0, 1, 10, and 100 ppm. The rates of pupation and adult eclosion were determined until 23 days after treatment. Each experiment used 60 individuals and was replicated three times.

Real-time RT-PCR analysis

Total RNA was extracted from whole bodies of two-day-old fifth instar larvae (V2) using RNeasy mini kit (QIAGEN, USA). To eliminate DNA contamination, RNase-free DNase I was used according to the manufacture's protocols. The cDNA synthesis reactions for each total RNA (2 μg) were prepared using a Reverse Transcriptase System Kit (Applied Biosystems, USA) and performed in PTC-200 thermal cycler (MJ Research, Watertown, MA, USA). Using nucleotide sequences from the NCBI database, the gene-specific primers of *shsp (piac25)* (Shirk et al., 1998), *hsc70* and *hsp90* (Shim et al., 2009), *hsp70* (NCBI database), *ecdysone receptor* (Siaussat et al., 2004), *ultraspiracle* (Siaussat et al., 2005), *prophenoloxidase* (Hartzer et al., 2005), *β-1,3-glucan recognition protein* (Fabrick et al., 2003), and *hemolin* (Aye et al., 2004) were designed as shown in Table 1. cDNA samples (0.2 μl) were run in 7300 Sequence Detection System (Applied Biosystems, USA) using Power SYBR green PCR Master Mix (Applied Biosystems, USA) for 1 cycle (95 °C for 10 min), followed by 40 cycles (95 °C for 15 s; 55 °C for 20 s; 72 °C for 35 s), followed by 1 cycle for dissociation stage (95 °C for 15 s; 60 °C for 30 s; 95 °C for 15 s).

The Ct (Threshold cycles) values were used to calculate the relative quantities of each gene. Data were analyzed using the formula, $2^{-\Delta\Delta Ct} = 2^{-[\Delta Ct\ \text{treatment} - \Delta Ct\ \text{control}]}$. The partial nucleotide sequence of the ribosomal protein S7 gene (*rpS7*) of *P. interpunctella* (EB828957) was identified from the *Plodia* EST database in NCBI Genbank using the *rpS7* sequence of *Manduca sexta* (L20096). The mRNA level of *rpS7* was used as a reference to normalize expression levels between samples. All data were relatively calculated and expressed against *rpS7* levels to compensate for any differences in reverse transcriptase efficiency.

Table 1
Sequences of the gene-specific primers.

Target genes	Primer	Sequence (5′→3′)	Product lengths (bp)	Accession numbers
shsp (piac25)	F	CGGGACATCGGCTCGA	71	U94328
	R	GAGAAATGCTGCACGTCCAA		
hsc70	F	TTGGGTGGCGGTACCTTTG	81	EF202591
	R	AGTGTCACCATTGGTAGCAACC		
hsp70	F	CTGAACGTCCTACGCATCATCA	71	EU556149
	R	TTTAAGTTCTTGTCGAGGCCG		
hsp90	F	GTACGCTGACCATCATCGACA	76	DQ988682
	R	TTAGCAATCGTACCCAAGTTGTTC		
Ecdysone receptor	F	CCTGGCTTCTCCAAAATCTCA	81	AY489269
	R	CACTCGCAACATCATCACCTC		
Ultraspiracle	F	CGTGTCGCGAAGAGAGGAATT	81	AY619987
	R	CGAGGCACTTTTGGTACCGA		
Prophenoloxidase	F	GATACAACGTGGAGCGCATGT	72	AY665397
	R	CTATGGGCTGCCTGAAGTCG		
β-1,3-glucan recognition protein	F	GATGAAGGAGAAGATCGGCATT	71	AF532603
	R	CTTGCTTCCAAATCAGCGTATAA		
Hemolin	F	CGGCAGCCCAAACACAAT	81	AY771598
	R	CAGGTAAATCCTCTCCCCGAC		
rps7	F	TATGTGCCGATGCCCAAAC	71	EB828957
	R	GCCGCTGAACTTCTTTTCCA		

Data analysis

Transcript levels of each gene were subject to analysis of variance (ANOVA) using a SAS program (2000). The separation of treatment means was conducted by using Duncan's multiple range tests at the 95% confidence level.

Results

Effect of azadirachtin on the development of Indianmeal moth larvae

To determine the effect of azadirachtin on the rate of pupation of *P. interpunctella*, newly molted V0 fifth instar larvae were fed a pollen diet containing different amounts of azadirachtin. Control larvae, which were not fed azadirachtin, initiated pupation at day 10 and pupation was 100% completed by day 18. Pupation was reduced to 85% and 75.8% when larvae ingested azadirachtin at 1 and 10 ppm, respectively. However, pupation was completely inhibited when the larvae ingested a diet containing 100 ppm azadirachtin (Fig. 1). Adult eclosion of pupated individuals in the control group started at day 17 and 98.3% eclosed by 23 days after treatment (Fig. 1B). At day23, the eclosion rates of larvae fed 1 and 10 ppm azadirachtin diet were 81.7% and 55.3%, respectively (Fig. 1B).

Effect of azadirachtin on the gene expression of fifth instar larvae

mRNA levels of 9 different genes relative to *rpS7* levels were determined in V2 fifth instar larvae (Table 2). *Hsc70* levels were highest at 20% of the control. Both *hsp90* and *prophenoloxidase* were approximately 4–5%, but levels of *β-1,3-glucan recognition protein*, *hemolin,* and *shsp* were lowest at 0.1% or less of the control (Table 2).

To determine the effect of azadirachtin on gene expression of fifth instar larvae, V0 larvae were fed different doses of azadirachtin for 48 h. Among the 9 tested genes, the levels of 7 changed in a dose-dependent manner (Fig. 2). Upregulation of mRNA levels was detected in *shsp, hsp90, ecdysone receptor, ultraspiracle, prophenoloxidase,* and *hemolin.* Those rates increased 2 to 3 times of the control. In addition, both *ultraspiracle* was downregulated at 1 ppm and *hemolin* was downregulated at 10 ppm. However, *hsc70, hsp70* and *β-1,3-glucan recognition protein* levels were not changed.

Discussion

Ingestion of azadirachtin inhibits growth and development of many insects. Pupation and eclosion rates of *P. interpunctella* declined in a dose-dependent manner when V0 fifth instar larvae ingested a pollen diet containing azadirachtin. Generally, azadirachtin is most active in the 1 to 10 ppm range in most insect species (Govindachari and Gopalakrishnan, 1998; Lynn et al., 2010). Similarly, our results showed that pupation was reduced when larvae were fed 1 and 10 ppm but completely failed when larvae were fed 100 ppm. Further, adult eclosion rates were reduced in larvae fed 1 and 10 ppm. Similar observations were reported in fourth instar larvae of *Plodia* who had lower weight and higher larval and pupal mortality when they ingested azadirachtin (Rharrabe et al., 2008).

Azadirachtin modulates the biosynthesis of proteins involved in development. Ingestion of azadirachtin significantly changed the rates and

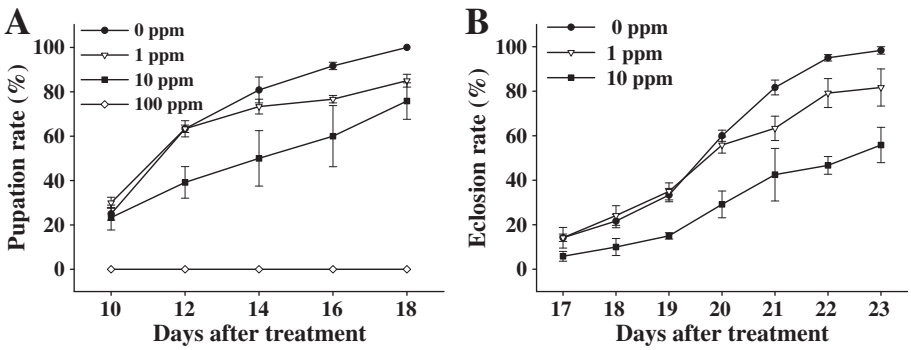

Fig. 1. Effect of azadirachtin on the development of fifth instar *Plodia interpunctella* larvae. Neonate fifth instar larvae (V0) were fed pollen grains containing different doses of azadirachtin. The rates of pupation (A) and adult eclosion (B) were determined until 23 days after treatment. Each point represents the mean ± SE (n = 60).

patterns of protein synthesis in midgut and fat body cells and in the cell line in a dose-dependent manner (Timmins and Reynolds, 1992; Huang et al., 2004). Azadirachtin inhibited the synthesis of most proteins in Sf9 cells but some proteins were upregulated in their synthesis (Robertson et al., 2007). Azadirachtin also reduced activities of various gut enzymes in *S. litura* (Senthil et al., 2005a), *Cnaphalocrocis medinalis* (Senthil et al., 2005b), and *P. interpunctella* (Rharrabe et al., 2008), which may be caused by either inhibition of protein synthesis or toxicity of azadirachtin. Therefore, we expected that azadirachtin may regulate various genes at the transcription level. Genes associated with feeding and development may be inhibited in their transcription rates, but other genes related with stress, immunity, and detoxification may be increased in their transcription rates by azadirachtin uptake.

We tested 9 genes associated with stress, development, and immunity of insects. Our results showed that azadirachtin differentially modulated the expression rates of some genes we tested in a dose-dependent manner. Heat shock proteins (HSPs) are a large group of cellular proteins which act as molecular chaperones during the processes of protein synthesis and assembly within the cell (Feder and Hofmann, 1999; Hartl and Hayar-Hartl, 2002). They are divided into at least three groups based on their size and sequence similarity: small heat shock proteins (sHSP), heat shock proteins 70 (HSP70), and heat shock proteins 90 (HSP90) (Lindquist and Craig, 1988). Each *hsp* gene can respond differentially to various extracellular stimuli, such as temperature extremes, desiccation, toxic substances, and pathogens (Denlinger et al., 2001; Sørensen et al., 2003). Our results showed that *shsp* and *hsp90*, but not *hsp70*, were upregulated by azadirachtin ingestion in a dose-dependent manner. Although there is no previous information on the role of HSPs and azadirachtin ingestion, this increased expression of *shsp* and *hsp90* may be associated with the inhibition of cellular growth and proliferation in larvae which ingested azadirachtin. Azadirachtin inhibits cell division in actively reproducing cells, such as those in fat bodies, ovaries, and testes (Mordue (Luntz) et al., 2005). One of the major modes of action is the blockage of microtubule formation in the inhibition of cell division (Salehzadeh et al., 2003). It is possible that the upregulation of both *shsp* and *hsp90* may increase proteins that can act as cellular chaperones to protect from them from damage during synthesis, folding, assembly, and localization of proteins in the cells.

The HSP70 family contains two sub-groups based on their expression patterns although they share many common primary structures (Kiang and Tsokos, 1998). Namely, the *hsp70* is induced rapidly under various stresses, but is expressed at a very low level under normal conditions. But the heat shock cognate 70 (*hsc70*), which is similar in nucleotide sequence and molecular structure to *hsp70*, is constitutively expressed in normal cells and changes relatively little in stress conditions. Our results indicated that *hsc70* levels did not change when larvae consumed azadirachtin although it occurs at the highest level of the 9 tested transcripts of *Plodia*. In addition, *hsp70* levels were not significantly changed by azadirachtin although it gradually increased dose-dependently.

Azadirachtin modulates ecdysteroid hormone action. This natural compound inhibits the release of prothoracicotropic hormone (PTTH) from the corpous cardiacum, a neurohemal organ that lies posterior to the brain (Rembold et al., 1989). Azadirachtin also inhibits the activity of ecdysone-20-monooxygenase, an enzyme that converts ecdysone to 20-hydroxyecdysone (20E), the active form of molting hormone in the hymolymph (Mitchell et al., 1997). Thus, azadirachtin-treated insects have a reduced level of 20E in the hymolymph which results in the delay or termination of further development. Unexpectedly, however, our results showed that *Plodia* larvae which consumed azadirachtin upregulated both nuclear ecdysteroid receptor genes, *ecdysone receptor* (*EcR*) and *ultraspiracle (USP)*, a heterodimer of EcR. The complex of 20E and receptors binds to DNA and leads transcriptional activation of many other genes involved in molting process (Riddiford et al., 2000). It is possible that the increased mRNA level of both hormone receptor genes may facilitate the action of ecdysteroid hormone. However, as mentioned previously, growth and molting rates were significantly inhibited by azadirachtin. Huang et al (2004) also reported that an EcR is one of proteins downregulated by the ingestion of azadirachtin in *S. litura* pupae. Thus, it is unlikely that the transcriptional upregulation of ecdysteroid receptor genes by azadirachtin is associated with those protein levels or with further hormonal regulation of development.

Our results showed that some components of the defense system in *Plodia* larvae were activated by azadirachtin ingestion. Hemolin and β-1,3-glucan recognition protein are pattern recognition receptors that can bind to pathogen-associated surface molecules during the immune process (Yu et al., 2002; Aye et al., 2008). They recognize pathogens and foreign components and transfer signals to induce immune reactions including the synthesis of antibacterial proteins. Phenoloxidase kills bacteria and synthesizes melanin for wound healing and pathogen encapsulation (Hartzer et al., 2005). Among three immune-related genes of *Plodia* larvae, *prophenoloxidase* and *hemolin* were upregulated by azadirachtin in a dose-dependent manner, but *β-1,3-glucan recognition protein* was not changed. However, *hemolin* was downregulated again by 100 ppm of azadirachtin. Thus, genes associated with immune and detoxification systems were selectively

Table 2
Relative mRNA values of each gene to rps7 at 2-days-old fifth instar larvae.

Target genes	Relative values (×100)
shsp (piac25)	0.07 ± 0.01
hsc70	19.89 ± 6.18
hsp70	0.54 ± 0.19
hsp90	5.74 ± 3.43
Ecdysone receptor	0.37 ± 0.29
Ultraspiracle	0.62 ± 0.34
Prophenoloxidase	4.26 ± 2.12
β-1,3-glucan recognition protein	0.11 ± 0.04
Hemolin	0.09 ± 0.02

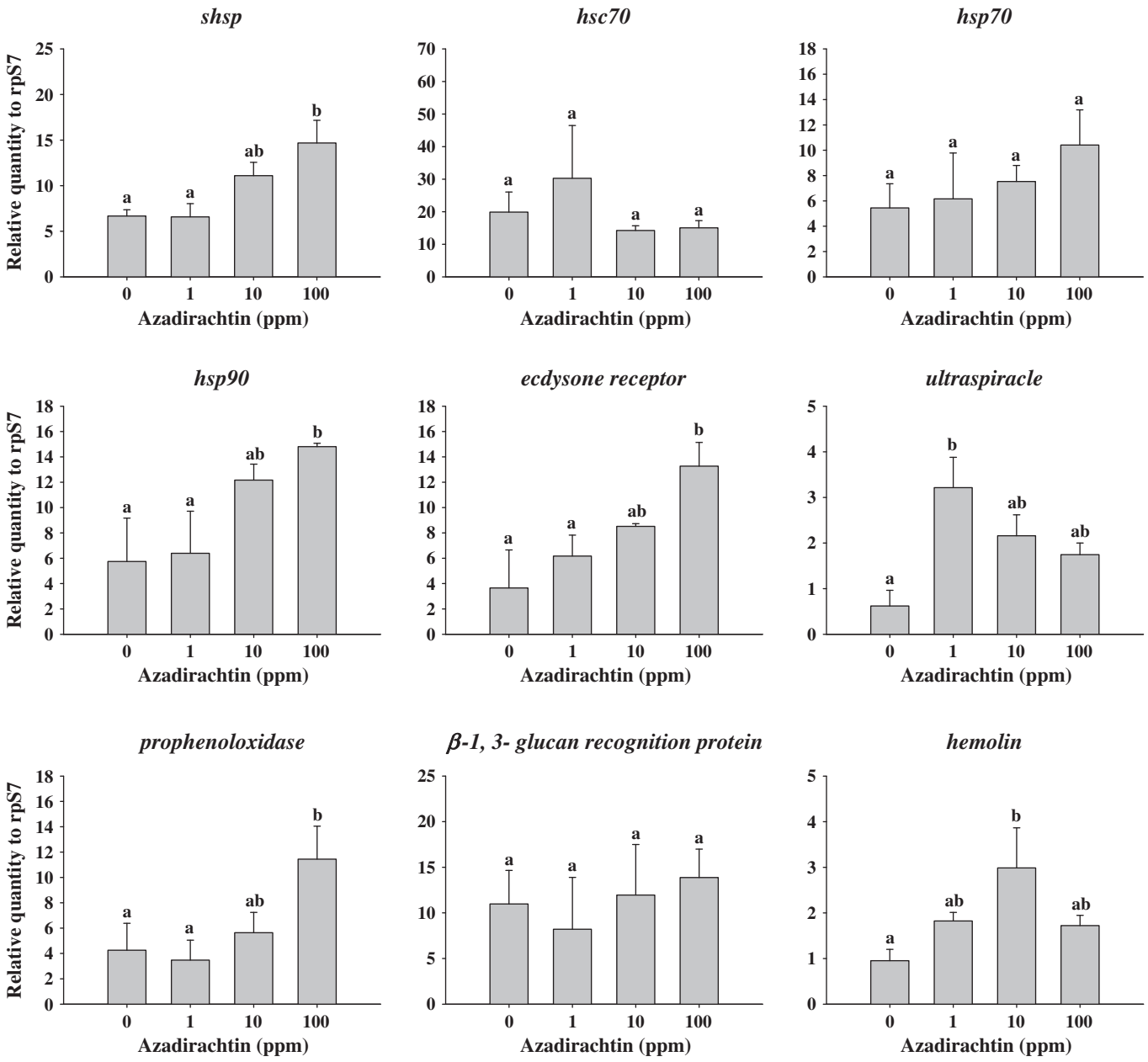

Fig. 2. Effect of azadirachtin on the gene expression of fifth instar *Plodia interpunctella* larvae. Neonate fifth instar larvae (V0) were fed pollen grains containing different doses of azadirachtin for 48 h. Total RNA samples were extracted from the whole bodies of 2-day-old fifth instar larvae. Real-time PCR analysis was performed with 0.2 μl of each cDNA sample. The *rpS7* level was used as a reference to normalize the expression levels between samples. Bars with different superscripts are significantly different ($P<0.05$). Each point represents the mean ± SE of 3 replicates.

modulated at the transcription level by azadirachtin ingestion in *Plodia* larvae.

Overall, ingestion of azadirachtin significantly inhibited post-embryonic development of *P. interpunctella*. In addition, azadirachtin differentially regulated expression patterns of specific genes associated with development, stress, and immunity in fifth instar larvae. Our study helps us to understand the physiological response of insects to azadirachtin at the molecular level.

Acknowledgments

We sincerely thank reviewers for critical reading and invaluable comments on this manuscript. We also thank Jong-Hee Kim, Jung-Yeok Lee, and Jung-Eun Jung for technical assistance in Indianmeal moth rearing. This work was supported in part by a grant of the Agricultural Research and Development Promotion Center, Korea and a grant (no. PJ006325) from the Agenda Program of the National Academy of Agricultural Science in Rural Development of Agriculture of Korea.

References

Ascher, K.R.S., 1993. Nonconventional insecticidal effects of pesticides available from the neem tree, *Azadirachta indica*. Arch. Insect Biochem. Physiol. 22, 433–449.

Aye, T.T., Lee, K.-Y., Kwon, Y.-J., 2004. Cloning of immune protein hemolin cDNA from the Indian meal moth, *Plodia interpunctella*, and its high induction during development and by bacterial challenge. Entomol. Res. 34, 269–275.

Aye, T.T., Shim, J.-K., Ha, D.-M., Kwon, Y.-J., Kwon, J.-H., Lee, K.-Y., 2008. Effects of gamma irradiation on the development and reproduction of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). J. Stored Prod. Res. 44, 77–81.

Butterworth, J., Morgan, E., 1968. Isolation of a substance that suppresses feeding in locusts. Chem. Commun. 1, 23–24.

Denlinger, D.L., Rinehart, J.P., Yocum, G.D., 2001. Stress proteins: a role in insect diapause? In: Denlinger, D.L., Giebultowicz, J.M., Saunders, D.S. (Eds.), Insect Timing: Circadian Rhythmicity to Seasonality. Elsevier, Amsterdam, pp. 155–171.
Fabrick, J.A., Baker, J.E., Kanost, M.R., 2003. cDNA cloning, purification, properties, and function of a beta-1,3-glucan recognition protein from a pyralid moth, *Plodia interpunctella*. Insect Biochem. Mol. Biol. 33, 579–594.
Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 61, 243–282.
Govindachari, T.R., Gopalakrishnan, G., 1998. Supermolecules for insect control. J. Indian Chem. Soc. 75, 655–661.
Hartl, F.U., Hayar-Hartl, M., 2002. Molecular chaperones in the cytosol: from nascent chain to folded protein. Science 295, 1852–1858.
Hartzer, K.L., Zhu, K.Y., Baker, J.E., 2005. Phenoloxidase in larvae of *Plodia interpunctella* (Lepidoptera: Pyralidae): molecular cloning of the proenzyme cDNA and enzyme activity in larvae paralyzed and parasitized by *Habrobracon hebetor* (Hymenoptera: Braconidae). Arch. Insect Biochem. Physiol. 59, 67–79.
Huang, Z., Shi, P., Dai, J., Du, J., 2004. Protein metabolism in *Spodoptera litura* (F) is influenced by the botanical insecticide azadirachtin. Pest. Biochem. Physiol. 80, 85–93.
Huang, J.-F., Shui, K.-J., Li, H.-Y., Hu, M.-Y., Zhong, G.-H., 2011. Antiproliferative effect of azadirachtin A on *Spodoptera litura* Sl-1 cell line through cell cycle arrest and apoptosis induced by up-regulation of p53. Pest. Biochem. Physiol. 99, 16–24.
Kiang, J., Tsokos, G., 1998. Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. Pharmacol. Ther. 80, 183–201.
Lindquist, S., Craig, E.A., 1988. The heat-shock proteins. Annu. Rev. Genet. 22, 631–677.
Lynn, O.M., Song, W.-G., Shim, J.-K., Kim, J.-E., Lee, K.-Y., 2010. Effects of azadirachtin and neem-based formulations for the control of sweet potato whitefly and root-knot nematode. J. Korean Soc. Appl. Biol. Chem. 53, 598–604.
Mitchell, M.J., Smith, S.L., Johnson, S., Morgan, E.D., 1997. Effects of the neem tree compounds azadirachtin, salannin, nimbin, and 6-desacetylnimbin on ecdysone 20-monooxygenase activity. Arch. Insect Biochem. Physiol. 35, 199–209.
Mordue (Luntz), A.J., Morgan, E.D., Nisbet, A.J., 2005. Azadirachtin, a natural product in insect control. In: Gilbert, L.I., Iatrou, K., Gill, S.S. (Eds.), Comprehensive Molecular Science, vol. 6. Elsevier, Oxford, pp. 117–135.
Mordue, A.J., Blackwell, A., 1993. Azadirachtin: an update. J. Insect Physiol. 39, 903–924.
Morgan, E.D., 2009. Azadirachtin, a scientific gold mine. Bioorg. Med. Chem. 17, 4096–4105.
Rembold, H., Subrahmanyam, B., Muller, T., 1989. Corpus cardiacum: a target for azadirachtin. Experientia 45, 361–363.
Rharrabe, K., Amri, H., Bouayad, N., Sayah, F., 2008. Effects of azadirachtin on post-embryonic development, energy reserves and α-amylase activity of *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae). J. Stored Prod. Res. 44, 290–294.
Riddiford, L.M., Cherbas, P., Truman, J.W., 2000. Ecdysone receptors and their biological actions. Vitam. Horm. 60, 1–73.
Robertson, S.L., Ni, W., Dhadialla, T.S., Nisbet, A.J., McCusker, C., Ley, S.V., Mordue, W., Mordue (Luntz), A.J., 2007. Identification of a putative azadirachtin-binding complex from Drosophila Kc167 cells. Arch. Insect Biochem. Physiol. 64, 200–208.
Salehzadeh, A., Akhkha, A., Cushley, W., Adams, R.L.P., Kusel, J.R., Strang, R.H.C., 2003. The antimitotic effect of the neem terpenoid azadirachtin on cultured insect cells. Insect Biochem. Mol. Biol. 33, 681–689.
Schmutterer, H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu. Rev. Entomol. 35, 271–297.
Schmutterer, H., Singh, R.P., 2002. List of insects pests susceptible to neem products, In: Schmutterer, H. (Ed.), The Neem Tree *Azadirachta indica* A. Juss. and Other Meliaceous Plants, Second ed. Neem Foundation, Mumbai, pp. 411–456.
Senthil, N.S., Kalaivani, K., Chung, P.G., 2005a. The effects of azadirachtin and nucleopolyhedrovirus on midgut enzymatic profile of *Spodptera litura* Fab. (Lepidoptera: Noctuidae). Pest. Biochem. Physiol. 83, 46–57.
Senthil, N.S., Kalaivani, K., Murugan, K., Chung, P.G., 2005b. The toxicity and physiological effect of neem limonoids on *Cnaphalocrocis medinalis* (Guenee) the rice leaffolder. Pest. Biochem. Physiol. 81, 113–122.
Shim, J.K., Aye, T.T., Kim, D.W., Kwon, Y.J., Kwon, J.H., Lee, K.Y., 2009. Gamma irradiation effects on the induction of three heat shock protein genes (piac25, hsc70 and hsp90) in the Indian meal moth, *Plodia interpunctella*. J. Stored Prod. Res. 45, 75–81.
Shirk, P.D., Broza, R., Hemphill, M., Perera, O.P., 1998. α-crystallin protein cognates in eggs of the moth, *Plodia interpunctella*: possible chaperones for the follicular epithelium yolk protein. Insect Biochem. Mol. Physiol. 28, 151–161.
Siaussat, D., Bozzolan, F., Queguiner, I., Porcheron, P., Debernard, S., 2004. Effects of juvenile hormone on 20-hydroxyecdysone-inducible EcR, HR3, E75 gene expression in imaginal wing cells of *Plodia interpunctella* lepidoptera. Eur. J. Biochem. 271, 3017–3027.
Siaussat, D., Bozzolan, F., Queguiner, I., Porcheron, P., Debernard, S., 2005. Cell cycle profiles of EcR, USP, HR3 and B cyclin mRNAs associated to 20E-induced G2 arrest of *Plodia interpunctella* imaginal wing cells. Insect Mol. Biol. 14, 151–161.
Sørensen, J.G., Kristensen, T.N., Loeschcke, V., 2003. The evolutionary and ecological role of heat shock proteins. Ecol. Lett. 6, 1025–1037.
Thoh, M., Babajan, B., Raghavendra, P.B., Sureshkumar, C., Manna, S.K., 2011. Azadirachtin interacts with retinoic acid receptors and inhibits retinoic acid-mediated biological responses. J. Biol. Chem. 286, 4690–4702.
Timmins, W.A., Reynolds, S.E., 1992. Azadirachtin inhibits secretion of trypsin in midgut of *Manduca sexta* caterpillars: reduced growth due to impaired protein digestion. Entomol. Exp. Appl. 63, 47–54.
Yu, X.Q., Zhu, Y.F., Ma, C., Fabrick, J.A., Kanost, M.R., 2002. Pattern recognition proteins in *Manduca sexta* plasma. Insect Biochem. Mol. Biol. 32, 1287–1293.