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Multiple resistance mechanisms to abamectin in the two-spotted spider mite

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ABSTRACT

The two-spotted spider mite, *Tetranychus urticae*, is one of the most destructive pests of various orchard trees and garden plants. Biochemical mechanisms of abamectin resistance in two *T. urticae* strains (PTF, 239-fold resistance; AbaR, ca. 4753-fold resistance) were investigated. The involvement of both esterase (Est) and mixed function oxidase (MFO) in abamectin resistance was suggested by synergistic bioassays, in which median lethal time (LT_{50}) values were significantly reduced by pretreatment with triphenyl phosphate (TPP) and piperonyl butoxide, respectively. Detoxification enzyme assays confirmed that Est and MFO were related to abamectin resistance as metabolic factors. Moreover, some Est bands on a native isoelectric focusing gel were specifically inhibited by TPP, implying their association with resistance. Pretreatment with verapamil in synergistic bioassays did not reduce the LT_{50} to abamectin, suggesting that the ABC transporter is not likely involved in resistance. However, enhanced MFO and Est activities in the AbaR strain were not enough to account for the extremely high level of abamectin resistance, which suggests the involvement of additional resistance mechanisms, such as target site insensitivity.

Introduction

Abamectin (Avermectin B_1, MK-936) is a macrocyclic lactone compound. It belongs to the avermectins, which are fermented from *Streptomyces avermitilis*, a soil actinomycete microorganism. Abamectin has been used widely as an insecticidal, nematicidal and acaricidal agent (Clark et al., 1995). Abamectin is most active against the two-spotted spider mite, *Tetranychus urticae* Koch, which is one of the most destructive pests on ornamental, horticultural, and agronomic crops worldwide (Nauen et al., 2001).

T. urticae has a high potential of resistance development to almost all kinds of acaricides, including abamectin (Whalon et al., 2004), due to its short life cycle, high biotic potential and parthenogenic reproduction (Saito et al., 1983). Abamectin-resistant populations of *T. urticae* were found in several countries including the United States (California, Florida and Washington), the Netherlands, Brazil and Columbia (Campos et al., 1995, 1996; Beers et al., 1998; Stumpf and Nauen, 2002; Sato et al., 2005). In South Korea, abamectin resistance has been reported in some populations collected from glasshouses and apple trees in 1995, 2003 and 2009 (Cho et al., 1995; Lee et al., 2003; Koh et al., 2009).

Enhanced activities of mixed function oxidases (MFO) and glutathione-*S*-transferase (GST) were determined to be involved in *T. urticae* abamectin resistance through biochemical analyses and synergism studies using piperonyl butoxide (PBO) and diethyl maleate (DEM) (Stumpf and Nauen, 2002). In a genetics study of abamectin resistance in *T. cinnabarinus*, a sibling species of *T. urticae*, the inheritance of abamectin resistance was incompletely recessive and was suggested to be controlled by more than one gene (He et al., 2009).

In this study, abamectin resistance levels in two different resistant strains of *T. urticae* were determined using the residual contact vial (RCV) bioassay method, and biochemical mechanisms of abamectin resistance were investigated.

Materials and methods

T. urticae strains

An abamectin-susceptible UD strain was collected from the Ulleung islands in June 2004 and maintained without any acaricides. A susceptible laboratory strain (PyriF) was obtained from the National Academy of Agricultural Science (Suwon, Korea). Abamectin-resistant PTF and AbaR strains were collected from rose greenhouses in Pyeongtaek and Suweon, respectively, and had been selected by abamectin three times. *T. urticae* were maintained in the laboratory

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under of 25 °C and 50–60% humidity with 16 L: 8D photoperiod on kidney bean plants.

Phylogenetic analysis based on the mitochondrial cytochrome oxidase I subunit and internal transcribed sequence 2 markers suggested that both the PyriF and PTF strains belong to the major green-type *T. urticae* but that the UD and AbaR strains belong to two independent clades within the *T. urticae* complex (Kwon and Lee, unpublished data).

Determination of median lethal concentration (LC_{50}) and median lethal time (LT_{50})

RCV was conducted for the determination of LC_{50} and LT_{50}. Abamectin (>99% pure, Chem Service, West Chester, PA) was dissolved in acetone to various concentrations (0.06–480 ppm). A 100 µl aliquot of abamectin solution was transferred to a 5-ml glass vial, and the inside wall of the vial was coated with the abamectin solution using a rolling wave rotator (Eberbach, Ann Arbor, MI) under a fume hood for 1 h. Fifteen female mites were transferred into each abamectin-coated vial and mortality was determined at 8 h post-treatment. For LT_{50} determination, 15 females were transferred into a 15 ppm abamectin-coated vial and the knockdown response was observed at 15–30 min intervals during an 8-h period. If mites showed uncoordinated movement or/and trembling legs, they were considered as knocked down. Three replicates of the LC_{50} and LT_{50} bioassays were conducted. Both LC_{50} and LT_{50} values were calculated by PoloPlus (LeOra software Company, Petaluma, CA).

Determination of synergistic effects by triphenyl phosphate (TPP), PBO and verapamil

For the evaluation of synergistic effects, 5-ml glass vials were coated with 100 µl of 100 ppm TPP (an esterase inhibitor, Chem Service) or 100 ppm PBO (an MFO inhibitor, Chem Service) together with 100 µl of 15 ppm abamectin solution. Bioassays were then conducted by RCV method, as described above. For the verapamil synergistic bioassay, verapamil [an ATP-binding cassette (ABC) transporter inhibitor, Sigma] was orally administered by feeding mites kidney bean leaf disks (5.0 cm in diameter) that were soaked for 24 h in verapamil solution (200 ppm in distilled water). The verapamil-pretreated mites were then subjected to either 15 ppm (PTF strain) or 240 ppm (AbaR strain) abamectin in RCV bioassays, as described above.

Enzyme preparation

Adult mites (ca. 30 mg) were homogenized with 300 µl of 0.1 M Tris–HCl (pH 7.8) buffer using glass–glass tissue grinder (Radnoti, Monorovia, CA) and the homogenate was centrifuged at 10,000 × *g* for 15 min. The supernatant was filtered through a glass wool filter and the flow-throw was used as the enzyme source for the measurement of esterase (Est), GST and MFO. Protein concentration was determined using a bicinchonic acid kit (Sigma, St Louis, MO) following the manufacturer's instructions, with bovine serum albumin as a standard protein.

Enzyme assay

Est

Enzyme samples (5 µg protein) were incubated with 0.2 mM 1-naphthyl acetate (1-NA) or 1-naphthyl butyrate (1-NB) for 10 min at 25 °C (Van Asperen, 1962). The reaction was terminated by adding 50 µl stop solution (0.25% fast blue RR with 0.5% SDS). The optical density was measured at 595 nm using a microplate reader (Victor3™ plate reader, PerkinElmer, MA). An equal amount of enzyme solution was also incubated with 0.125 mM 4-nitrophenyl acetate (4-NPA) or 4-nitrophenyl butyrate (4-NPB) for 10 min 25 °C and the optical density was measured at 405 nm using the microplate reader.

GST

GST assays were conducted following the method of Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. Enzyme samples (5 µg protein) were incubated with 20 mM CDNB and 10 mM GSH for 10 min at 25 °C and their activities were measured at 355 nm by Victor3™ plate reader (PerkinElmer).

MFO

Enzyme samples (20 µg protein) were incubated with 0.4 mM 7-ethoxycoumarin (7-EC) and 1 mM NADPH for 30 min at 30 °C in the 96-well microtiter plate. Fluorescence of NADPH was removed as described by Chauret et al. (1999) by adding 10 µl oxidized glutathione (100 mM in water) and 20 µl glutathione reductase (1 U). After 10 min of incubation at room temperature, the reaction was stopped with 125 µl 50% acetonitrile in 50 mM Tris buffer (pH 10). The amount of 7-HC was quantified with the SPECTRAmax GEMINI XS spectrofluorometer (Molecular Device Inc. Sunnyvale, CA) with 390 nm excitation and 465 nm emission wavelengths.

Est band screening after treatment of TPP

One hundred fifty female mites (PTF or AbaR) were transferred to a 5-ml glass vial pre-coated with 100 µl of 100 ppm TPP and incubated for 2 h. Enzyme extraction and protein quantification were conducted as described above. The enzyme samples (ca. 25 µg protein) with 10 µl IEF marker (Sigma) were loaded into each well of a Novex pH 3–7 precast gel (Invitrogen, Carlsbad, CA). The gel was focused for 1 h at 100 V, 1 h at 200 V and 30 min at 500 V. After focusing, the IEF marker lane was cut out and stained with Coomassie Brilliant Blue G250 (Sigma). The remaining lanes were stained for Est activity with 1-NA as a substrate. The gel was soaked in 10 mM 1-NA solution for 5 min and then transferred to 0.5% fast blue RR salt solution to visualize Est bands.

Results

Abamectin resistance levels determined by bioassay

Abamectin resistance levels were determined by comparing the LC_{50} values among the UD, PyriF, PTF and AbaR strains. The mortality of the AbaR strain, even at the highest concentration (480 ppm) tested, was ca. 37% (Table 1). This suggested that its LC_{50} was higher than 480 ppm. Based on the LC_{50} values, the PTF strain showed ca. 238.7- and 56.8-fold resistance compared to the UD and PyriF strains, respectively (Table 1). The AbaR strain showed an extremely high level of resistance (>4753- and >1132-folds) compared to the UD and PyriF strains, respectively (Table 1).

Synergistic effects of metabolic inhibitors on abamectin resistance

The LT_{50} to abamectin was determined by the RCV method. The PTF strain exhibited ca. 5.2- and 5.3-folds more delayed responses

Table 1
Resistance levels in abamectin-susceptible and -resistant strains of *T. urticae* as determined by RCV bioassay.

Strains	*N*	Slope	χ^2	LC_{50} (95% CL), ppm	Resistance ratio	
					To UD	To PyriF
UD	179	2.94	3.3	0.10 (0.08–0.12)	1.0	–
PyriF	219	1.30	13.5	0.43 (0.26–2.46)	4.2	1.0
PTF	222	1.90	3.7	24.1 (18.1–39.3)	238.7	56.8
AbaR	220	1.36	10.3	>480	4753	1132