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Full Length Article

Toxicity and Field Efficacy of Emamectin Benzoate (ARETOR) against Red Palm Weevil, by using Syngenta Tree Micro-Injection Technique

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Abstract

Invasions of the red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) has become a well-known problem for several palm species in date palm-growing states, including Saudi Arabia. Although several control measures have been implemented in efforts against RPW, yet, no approach has proven consistently effective against this invasive pest. Therefore, further efforts are still needed to develop an effective and efficient control method for RPW management and mitigation. The objectives of the present study were to determine emamectin benzoate (ARETOR) efficacy against RPW larval stages, in the laboratory using a diet incorporation bioassay, along with field tests of the Syngenta Tree Micro-Injection Technique. Our laboratory bioassay showed that the median lethal concentration of emamectin benzoate (ARETOR) was 0.30 μg/mL^{-1} against wild populations of RPW larvae. In the field studies, emamectin benzoate (ARETOR) showed promising effects on RPW mortality for all stages. We found 100% mortality for both larval and pupal stages for all exposure times, except after 2 months, where 86% mortality was observed for larvae. Thus, we conclude that emamectin benzoate (ARETOR) is an effective biopesticide against RPW. Our results show that it killed RPW all stages, also prevented further damage to the healthy tissues of date palms, in addition, provided protection against RPW spread to other neighboring healthy date palms.

Keywords: Date palm; Chemical control; Emamectin benzoate (ARETOR); Red palm weevil; Infestation

Introduction

The date palm *Phoenix dactylifera* (Linnaeus) is an oldest important crop cultivated in the Arabian Peninsula and in temperate regions of the world (Chao and Krueger 2007). Many insect pests are known to attack and damage date palms, however the red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) is known as a primary pest. The RPW has emerged as one of the most destructive pests of date palm worldwide and young date palms are more vulnerable to RPW attack (Faleiro 2006). Both larvae and adults of RPW feed inside the tree trunk and complete many generations inside a single palm tree (Faghih 1996; Salama *et al*. 2009). The life cycle of red palm weevil may range from 3 months and above depending upon the rearing medium. In the date palm trunk red palm weevil population stays as long as the entire tissues of the trunk are available to feed.

The RPW infestation of date palm requires intensive investigation for proper management. Several non-chemical approaches have been applied, but still the problem persists, and chemical use cannot be ignored as a curative measure. Many previous studies have investigated the efficacy of different insecticides, such as imidacloprid, deltamethrin, and fipronil, under laboratory and field conditions against RPW (Azam and Razvi 2001; Kaakeh 2006; Dembilio and Jacas Miret 2012). Imidacloprid was found to be an excellent insecticide, at concentrations of 3.5 mL/L and 1000 mL/L of water in the laboratory and field, respectively (Kaakeh 2006). Similarly, imidacloprid applied at a rate of 1 mL/L to 2nd and 4th instar RPW larvae resulted in 84 and 79% mortality, respectively, after 20 days of exposure (Malik *et al*. 2016). Deltamethrin was shown to be highly toxic against 20-day-old RPW larvae and adults after 24 h of exposure, followed by the application of emamectin benzoate and imidacloprid (Shawir *et al*. 2014). Al–Shawaf *et al*. (2010) found that fipronil was the most toxic

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insecticide against both larval and adult stages of the RPW and date palm offshoots treated with fipronil (0.004%) were prevented from attack by RPW (Al-Shawaf *et al*. 2013). Organophosphate insecticides, pirimiphos-methyl and oxydemeton-methyl, also exhibited strong effects against adult and larval stages of RPW, respectively (Ajlan *et al*. 2000). Trunk injection with a mixture of endosulfan and dimethoate resulted in 100% recovery of infested date palms (Azam and Rizvi 2001).

Emamectin benzoate, a biopesticide, has a mode of action involving glutamate-gated chloride channel allosteric modulators (Sparks and Nauen 2015). It is toxic to foliar insects and borers, and has been demonstrated to protect trees from pest infestation (Mashal and Obeidat 2019). For example, a population of armyworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), in a rice field was reduced up to 92% following the application of emamectin benzoate at 0.3 g/L (Mainali *et al*. 2014; Burkhard *et al*. 2015). Similarly, emamectin benzoate injected into date palms and ornamental palms killed 100% of RPW larvae, and cured 95% trees of infestation after one year in Spain and Jordan. Moreover, no residues of emamectin benzoate were detected in the fruit of injected trees (Gomes and Ferry 2019; Mashal and Obeidat 2019).

Emamectin benzoate (ARETOR) is currently in use for the control of RPW in Saudi Arabia. However, there have been no reports on the toxicity of emamectin benzoate (ARETOR) against RPW under laboratory and field conditions in Riyadh, Saudi Arabia. Therefore, we evaluated the toxicity/efficacy of emamectin benzoate (ARETOR) on RPW under laboratory as well as field conditions to determine the effectiveness of this insecticide in Riyadh, Saudi Arabia.

Materials and Methods

Collection and rearing

A sample of 150–200 RPW larvae, pupae, and adults were collected from a date palm field located in Dirab, Riyadh, Saudi Arabia (24.4164°N, 46.5765°E). Insects were collected manually and kept in 1 kg plastic boxes. After collection, insects were transferred on the same day to the laboratory for rearing and were maintained to get the F_1 progeny. The larvae were reared on an artificial diet consisting of ground date palm petioles, corn flour, wheat flour, and distillate water as primary components, with potassium benzoate, sorbic acid, ascorbic acid, and agar added as protectants from contamination. All the ingredients were mixed together with a specific composition as follows: 500 g of ground petiole, 250 g wheat flour, 250 g corn flour, and 2 g ascorbic, 1.6 g potassium benzoate, 1.6 g sorbic acid, 20 g agar and 2 litres distilled water) (Mehmood *et al.* 2018). The population was maintained in a growth chamber (Steridium, Australia) at 27 ± 2°C, 70 ± 5% RH, on a 12:12 h (light: dark) photoperiod. The diet was refreshed every 3 days.

Insecticide and bioassay

Emamectin benzoate (Revive/Aretor 4EC, Syngenta, Switzerland) was used to assess its toxicity in RPW larvae. A bioassay was performed on fourth instar larvae using the diet incorporation method (Abbas *et al*. 2012). Briefly, five serial diluted concentrations of emamectin benzoate were incorporated into a semi-synthetic diet. Each concentration was repeated 3 times. After mixing thoroughly, the treated diet was transferred into 50 cm diameter plastic cups. Four plastic cups were prepared for each replicate, and an individual larva was placed in the plastic cup. Twelve larvae were used for each concentration, for a total of 60 larvae for the bioassay. The bioassay was kept in a growth chamber at 27 ± 2°C, 70 ± 5% RH and 12:12 h (light:dark) photoperiod. Larval mortality was documented at 72 h post-treatment. All larvae that did not make a coordinated movement when touched with a fine hair camel brush were considered dead.

Emamectin benzoate (ARETOR) efficacy under field conditions

To test the field efficacy of Syngenta Tree Micro-Injection Technique, a formulation of Revive/(ARETOR) insecticide containing 4% emamectin benzoate micro-emulsion was injected into date palms naturally infested by RPW. Experiments were carried out using a complete randomized block design at Alraiyanh date palm farm, Al-Kharj, Riyadh region, Saudi Arabia (24.14.84°N, 15.182°E). Each Revive/Aretor insecticide treatment was coupled with a control (water injection) and observed at three time points: after 2, 6 and 12 months. Each treatment contained 10 replications, with one individual palm tree in each replicate. In total, 60 date palms were used in this experiment.

Selection of date palms

Medium-sized date palms of similar age approximately 12–15 years old, with medium RPW infestation levels, were selected randomly based on externally visual symptoms by experienced personnel of the Ministry of Environment, Water, and Agriculture, Riyadh, Saudi Arabia. These external symptoms included tunnels on the trunks of trees and at the bases of date palm fronds, the presence of frass consisting of chewed plant tissue with fermenting odour, the oozing of thick brown liquid material, and the remains of weevil cocoons around the tree.

Insecticide application

Selected date palms were treated with 48 mL Revive/ ARETOR insecticide per tree, dispersed around four directions (12 mL/direction). Four injection points were marked at the lower level of trunk bases (above the roots) in four directions (East, North, West, and South) and holes were made using a drilling machine equipped with a brad

point drill-bit (diameter, 8 mm) at an angle of 15–20 degrees, to a depth of 1/3 trunk diameter. The insecticide was delivered undiluted into the trunk immediately after drilling using the Tree Micro Injector (TMI) device (Fig. 1). After injection, a biodegradable micro-injection plug was inserted into the drilled hole to act as a barrier restricting any backflow of the insecticide.

Data collection

According to the experimental plan, insecticide efficacy data were recorded at three time points as mentioned above after treatment. To record observations, each tree was cut down at the base, and then the trunks were cut longitudinally into one-meter lengths to make the logs. All logs were inspected carefully from top to base, and any sign of RPW infestation was recorded. In addition, each part was split first into two equal halves and then into quarters for detailed interior observations. Each quarter was thoroughly observed (peripheral side, inner side, top, and base) to record any signs of RPW infestation (galleries) and record counts of live and dead RPW individuals (Table 3). The method for cutting the trees is shown in Fig. 2 and the log coding in Table 1. The levels of RPW infestation were classified as either good, moderate, medium, heavy or dead, according to defined RPW infestation index rates (Fig. 3).

Statistical analysis

The data were analyzed and means were compared using Analysis of Variance and Least Significant Difference test ($P \leq 0.05$) using SAS 9.2 (SAS 2008). Bioassay data were analyzed by probit analysis using POLO PLUS software (LeOra Software 2002) to determine the median lethal concentration of emamectin benzoate.

Results

Due to rapid pest-killing action, chemicals are recommended for use against various pests worldwide, including in Saudi Arabia. Knowledge of the toxicity of different synthetic chemicals is crucial for selection of the most potent compounds for the management of insect pests. The biopesticide emamectin benzoate (ARETOR) is a powerful weapon for integrated pest management due to its high selectivity and its comparative safety for the environment and non-targeted fauna. In the present study, the LC_{50} of emamectin benzoate (ARETOR) was 0.30 μg/mL, suggesting higher toxicity of the insecticide against RPW in the laboratory (Table 2).

After cutting of the date palms it was found that some of the date palms in all treatments were heavily infested, but these infestations were old. In contrast, some trees were completely healthy. Mortality rates of all RPW stages after 2, 6 and 12 months of pesticide application were statistically compared (Table 3). The results revealed significant

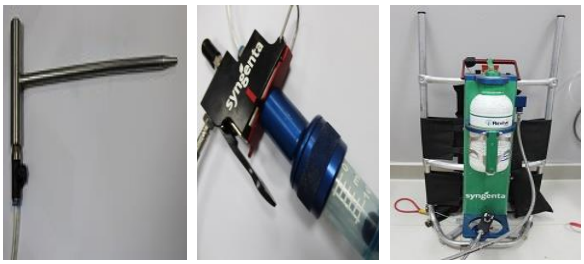

Fig. 1: Tree Micro injector (TMI) Device

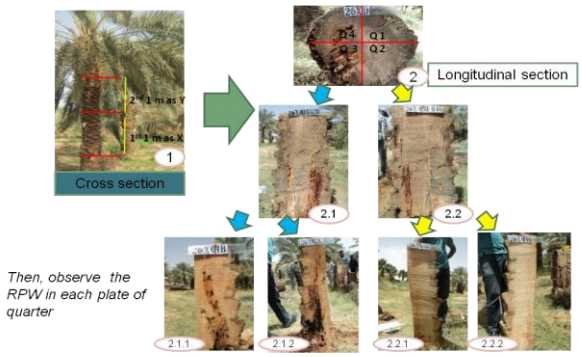

Fig. 2: Dissection of date palm for detailed observation and data collection

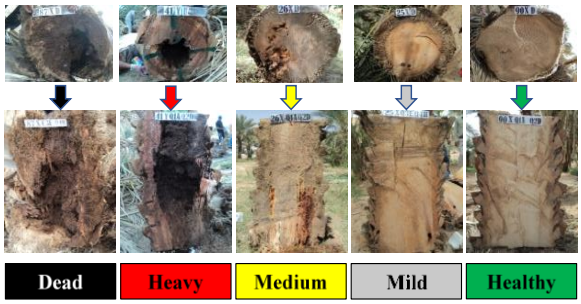

Fig. 3: Established levels of red palm weevil infestation index

difference between the treatment and control for both larval and pupal RPW stages after 2 months of pesticide application according to the found and collected individuals (although surprisingly few RPW individuals (live or dead) were found). Mortality rates were 86 and 100% for larvae and pupae, respectively. Date palms observed after 6 months of pesticide application revealed 100% mortality, with 12 dead RPW larvae. Date palms observed after 12 months of pesticide application revealed 100% larval mortality and revealed significant difference in comparison with the control. Moreover, no live or dead RPW pupae were recorded.

Discussion

In our study, emamectin benzoate (ARETOR) LC_{50} value was 0.30 μg/mL, suggesting higher toxicity against RPW under the laboratory conditions. Previously, toxicities of

Table 1: General coding of experimental logs used in field study

Date palm	Log code	Log quarter designation						Final code
		Quarter	Position	Up/down final code	Direction	Side	Position	
Tree No…	Log X	1	Upper side	1X1U	East	A	left side of log quarter	1X1A
	first meter from bottom		Down side	1X1D		B	right side of log quarter	1X1B
		2	Upper side	1X2U	South	A	left side of log quarter	1X2A
			Down side	1X2D		B	right side of log quarter	1X2B
		3	Upper side	1X3U	North	A	left side of log quarter	1X3A
			Down side	1X3D		B	right side of log quarter	1X3B
		4	Upper side	1X4U	West	A	left side of log quarter	1X4A
			Down side	1X4D		B	right side of log quarter	1X4B
	Log Y	1	Upper side	1Y1U	East	A	left side of log quarter	1Y1A
	Second meter from bottom		Down side	1Y1D		B	right side of log quarter	1Y1B
		2	Upper side	1Y2U	South	A	left side of log quarter	1Y2A
			Down side	1Y2D		B	right side of log quarter	1Y2B
		3	Upper side	1Y3U	North	A	left side of log quarter	1Y3A
			Down side	1Y3D		B	right side of log quarter	1Y3B
		4	Upper side	1Y4U	West	A	left side of log quarter	1Y4A
			Down side	1Y4D		B	right side of log quarter	1Y4B
	Log Z	1	Upper side	1Z1U	East	A	left side of log quarter	1Z1A
	Third meter from bottom		Down side	1Z1D		B	right side of log quarter	1Z1B
		2	Upper side	1Z2U	South	A	left side of log quarter	1Z2A
			Down side	1Z2D		B	right side of log quarter	1Z2B
		3	Upper side	1Z3U	North	A	left side of log quarter	1Z3A
			Down side	1Z3D		B	right side of log quarter	1Z3B
		4	Upper side	1Z4U	West	A	left side of log quarter	1Z4A
			Down side	1Z4D		B	right side of log quarter	1Z4B

Table 2: Toxicity of emamectin benzoate in field population of red palm weevil

Population	Number of larvae	LC_{50} (FL 95%) (μg mL^{-1})	Slope ± SE	df	χ^2	P
Field (G_1)	72	0.30 (0.23-0.38)	4.45 ±1.07	4	7.65	0.10

Table 3: Field efficacy of Syngenta Tree Micro-injection Technique using Revive/ARETOR insecticide against red palm weevil

Stage	Exposure (months)	Treatment	No. of trees	Average alive	Average dead	Total average	Mortality (%)
Larvae	2	Emamectin benzoate	5	0.4	2.4	2.8	86a
	2	Control	3	1.3	0	1.3	0b
Pupae	2	Emamectin benzoate	5	0	1	1	100a
	2	Control	3	0	0	0	0b
Larvae	6	Emamectin benzoate	3	0	12	12	100a
	6	Control	3	0	0	0	0b
Pupae	6	Emamectin benzoate	3	0	0	0	0
	6	Control	3	0	0	0	0
Larvae	12	Emamectin benzoate	2	0	2.5	2.5	100a
	12	Control	2	4	2.5	6.5	38b
Pupae	12	Emamectin benzoate	2	0	0	0	0
	12	Control	2	0	0	0	0

Similar letters within each category are not significantly different, $\alpha = 0.05$

different insecticides have been investigated in RPW (Ajlan *et al.* 2000; Kaakeh 2006; Al–Shawaf *et al*. 2010; Shawir *et al.* 2014; Malik *et al*. 2016). A laboratory evaluation of abamectin at 500 ppm resulted in 100% larval mortality of RPW within 48 h of application (Abo-El-Saad *et al.* 2013). These findings are in line with our results.

To date, very few studies reported on the emamectin efficacy for RPW infestation control in date palms. In lab experiments with RPW larvae, the same effects of emamectin have been observed as when applied with a micro injector to the date palms. Larval mortality rates of 97 and 100% were recorded after 90 days, when 50 or 100 mL/tree emamectin were injected, respectively (Gomes and Ferry 2019). In another recent study, Mashal and Obeidat injected Revive 4 and 9% into date palms infested with RPW for periods of 3, 6 and 12 months, and found all-individual mortality after 6 months and up (Mashal and Obeidat 2019). These results support our findings, and the concept of long-term persistence of emamectin via a sophisticated translocation mechanism in the trees that can kill borers and other insects (Burkhard *et al.* 2015).

Unfortunately, limited numbers of RPW individuals were recorded in the date palms dissected after 2, 6, and 12 months of pesticide application. However, we believe that the 2-month period may have been too long to leave enough individuals to be counted. It is possible that larvae died inside the date palm trunk as a result of the insecticide application and disintegrated by the time we dissected the trees. Supporting this, we observed some slimy material in date palm trunks dissected after 2-months of insecticide

application, likely resulting from larval degradation in the galleries created by larvae. Therefore, dissection of date palms after one and two weeks of insecticide application is recommended in future studies to avoid the disintegration of RPW larvae, and resulting complications in calculating the real efficacy of the insecticide. Our findings showed significant differences in mortality percentage for all RPW stages between the treatments and control.

Conclusion

The Syngenta Tree Micro-Injection Technique using emamectin benzoate (ARETOR) is a promising solution for control of RPW, which in our experiments resulted in 100% mortality of both larval and pupal stages at all exposure times, except for the larvae collected from date palms after 2 months, which showed 86% mortality. Moreover, no live RPW at any stage were recovered from trees treated with Revive insecticide, except for one tree. This reflects that the insecticide killed all RPW stages, while and stopping further damage to the healthy tissues of trees and also providing protection against the spread of RPW to the other neighboring healthy trees. Additional comprehensive studies are recommended using date palms with active infestations before and during treatment to obtain more detailed mortality data and draw further concrete conclusions.

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Author Contributions

KGR, MH, and ASA participated in the planning, design and coordination of the study. SS, KM, KDS participated in the red palm weevil rearing, conducted practical work, KGR and MH, and NA collected and analysed data, and write up. ASA and KGR supervised the work. All authors have read the final version of the manuscript carefully and approved it.

Conflicts of Interest

The authors declare that they have no conflicting interests.

Data Availaility

All the data is present in the manuscript.

Ethics Approval

The work is original, Moreover, no legal permission was required to conduct the experiments in the laboratory as well as in the field.

References

Abo-El-Saad MM, HA Elshafie, IA Bou-Khowh (2013). Toxicity of bio-insecticide, Abamectin, on red palm weevil, *Rhynchophorus ferrugineus* (Olivier). *Intl J Agric Sci Res* 2:107–115

Abbas N, SA Shad, M Razaq (2012). Fitness cost, cross resistance and realized heritability of resistance to imidacloprid in Spodoptera litura (Lepidoptera: Noctuidae). *Pestic Biochem Physiol* 103:181–188

Ajlan AM, MS Shawir, MM Abo-El-Saad, MA Rezk, KS Abdulslam (2000). Laboratory evaluation of certain organophosphorus insecticides against the red palm weevil, *Rhynchophorus ferrugineus* (Olivier). *Sci J King Faisal Univ (Basic Appl Sci)* 1:15–26

Al–Shawaf AA, Al-Shagagh, MM Al-Bakshi, SA Al-Saroj, SM Al-Badr, AM Al-Dandan, AB Abdallah (2010). Toxicity of some insecticides against red palm Weevil, *Rhynchophorus ferrugineus*. *Ind J Plant Prot* 38:13–16

Al-Shawaf AM, A Al-Shagag, M Al-Bagshi, S Al-Saroj, S Al-Bather, AM Al-Dandan, AB Abdallah JR Faleiro (2013). A quarantine protocol against red palm weevil *Rhynchophorus ferrugineus* (Olivier) (Coleptera: Curculiondae) in date palm. *J Plant Prot Res* 53:409–415

Azam KM, SA Razvi (2001). Control of red palm weevil, *Rhynchophorus ferrugineus* Oliver, using prophylactic spraying of date palms and trunk injection. *In: Proceedings of the 2nd Intl Conf on Date Palms*, pp: 216–222. Al-Ain, UAE

Burkhard R, H Binz, CA Roux, M Brunner, O Ruesch, P Wyss (2015). Environmental fate of emamectin benzoate after tree micro injection of horse chestnut trees. *Environ Toxicol Chem* 34:297–302

Chao CT, RR Krueger (2007). The date palm (*Phoenix dactylifera* L.): Overview of biology, uses, and cultivation. *HortScience* 45:1077–1082

Dembilio O, JA Jacas Miret (2012). Bio-ecology and integrated management of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), in the region of Valencia, Spain

Faghih A (1996). The biology of red palm weevil, *Rhynchophorus ferrugineus* Oliv. (Coleoptera, Curculionidae) in Saravan region (Sistan & Balouchistan province, Iran). *Appl Entomol Phytopathol* 63:16–18

Faleiro JR (2006). Insight into the management of red palm weevil: Based on experiences on coconut in India and date palm in Saudi Arabia. *In*: *I Jornada Internacional Sobre el Picudo Rojo de las Palmeras (November, 2005)*, pp: 35–57 Agroalimed F (Ed). Fundacion Agroalimed. Valencia, Spain

Gomes S, M Ferry (2019). A simple and low cost injection technique to protect efficienlty ornamental *Phoenix* against the red palm weevil during one year. *Arab J Plant Prot* 37:124–129

Kaakeh W (2006). Toxicity of imidacloprid to developmental stages of *Rhynchophorus ferrugineus* (Curculionidae: Coleoptera): Laboratory and field tests. *Crop Prot* 25:432–439

Mainali RP, N Dangi, S Aryal, GS Bhandari, ASR Bajracharya (2014). Management of armyworm outbreak in the rice field of Chitwan district. *Asian J Sci Technol* 5:774–776

Malik MA, M Manzoor, H Ali, A Muhammad, S Ul Islam, M Qasim, N Ahmad, A Idrees, A Muhammad, HSA Saqib (2016). Evaluation of imidacloprid and entomopathogenic fungi, *Beauveria bassiana* against the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *J Entomol Zool Stud* 4:262–268

Mashal MM, BF Obeidat (2019). The efficacy assessment of emamectin benzoate using micro injection system to control red palm weevil. *Heliyon* 5:e01833

Mehmood K, M Tufail, M Husain, KG Rasool, A Aldawood (2018). *Molecular characterization, expression profiling of vitellogenin gene and reproductive ability disruption by RNAi in red palm weevil, Rhynchophorus ferrugineus (Olivier)*. King Saud University, Riyadh, Saudi Arabia

LeOra Software (2002). *Polo Plus, a User's Guide to Probit or Logic Analysis*. LeOra Software, Berkeley, California, USA

Salama H, F Zaki, A Abdel-Razek (2009). Ecological and biological studies on the red palm weevil *Rhynchophorus ferrugineus* (Olivier). *Arch Phytopathol Plant Prot* 42:392–399

SAS I (2008). SAS/STAT 9.2 *User's Guide*. SAS Institute, Cary, North Carolina, USA

Shawir MS, MAL Abbassy, YM Salem (2014). Laboratory evaluation of some insecticides against larval and adult stages of red palm weevil's *Rhynchophorus ferrugineus* (Olivier). *Alex Sci Exch* 35:76

Sparks TC, R Nauen (2015). IRAC: Mode of action classification and insecticide resistance management. *Pestic Biochem Physiol* 122–128