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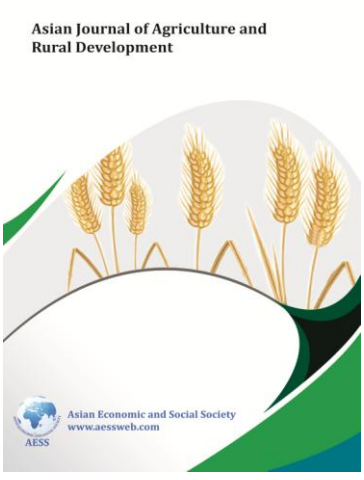

The Response of the Red Morph of the Tobacco Aphid, *Myzus Persicae Nicotianae*, to Insecticides Applied under Laboratory and Field Conditions

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Abstract

The tobacco aphid, *Myzus persicae nicotianae* is an economically important pest of tobacco, causing extensive yield losses especially as it is an important host of numerous viruses and the sudden shift from the green morph to the red morph is of concern as reports of insecticide resistance are common in this morph. The efficacy of several insecticides was studied, both in the laboratory and in the field, to establish the pest status of the red coloured morph of the tobacco aphid with respect to resistance build up. The laboratory tests confirmed that resistance could be of concern in Monocrotophos only and the field work appeared to show some build up of resistance additionally in Aldicarb. The results point at a need for continued monitoring as well as use of rotations in chemical use in order to reduce the chance of resistance build up.

Keywords: Green morph, Monocrotophos, Red morph, Aldicarb, Laboratory tests

Introduction

The cosmopolitan peach-potato aphid, M. persicae, is an economically important insect. It is a vector of many plant viruses and has developed resistance to a wide range of insecticides (Devonshire *et al*., 1998). The tobacco (Nicotiana tabacum L. Solanaceae) adapted form is considered to be a distinct subspecies and is known as the tobacco aphid, M. persicae nicotianae (Blackman), (Margaritopoulos *et al*., 2003). The tobacco aphid causes significant losses in tobacco directly by feeding and honeydew deposition and indirectly by contamination and as a vector of a wide range of viruses. Colour differences may be correlated with insecticide resistance (Abdel-Aal, 1992; Lampert and Dennis, 1987), reproductive performance (Araya *et al*., 1996) and resistance to aphid parasitoids (Tomiuk and Wöhrmann, 1980). Before 2002, the main colour form of the tobacco aphid in Zimbabwe was green. However, during the 2003/04 tobacco season, red forms of the aphid were observed to be more prevalent than the green one at Kutsaga Research Station. During the 2004/05 season, green forms could not be found in some areas and where they existed, only a few constituted this form.

Elsewhere in the world, the red form of the tobacco aphid was observed as far back as 1985 in North Carolina, USA (Harlow and Lampert, 1990) and appeared to be a more serious pest than the green form. In the United States, where the tobacco aphid Myzus p. nicotinae was previously described as a tobacco form of the green peach aphid (Blackman, 1987), the red-coloured form was found to consistently express resistance to malathion and acephate. On the other hand, the resistance in the green form was inconsistent (Harlow and Lampert, 1990).

In North America, red morphs present on tobacco plants were deemed to be more resistant to organophosphorus insecticides than the green form (Harlow and Lampert, 1990) and reports of control failure by some tobacco growers during the 2004-05 season in Zimbabwe brought about awareness of the ever-present threat of insecticide resistance caused by unilateral reliance on pesticides. Pesticide resistance is even more threatening where the tobacco aphid is concerned because

resistance, should it occur, is permanent. This is because there is no mechanism for crossing of gene pools as a result of the anholocyclic (devoid of sexual reproduction) nature of reproduction exhibited by this aphid in Zimbabwe (Blair, 1990).

The occurrence and subsequent dominance of the red form of M. p. nicotianae brought about the need for a study of its biology as this is an important tool for the development of effective control strategies. Results from a study on the biology of these red and green morphs of the tobacco aphid have shown that the red morph of the tobacco aphid has a greater reproductive potential and rate of population increase than the green morph (unpublished Tobacco Research Board of Zimbabwe Annual report, 2011).

The objective of this study was to determine the response of the red morph of the tobacco aphid Myzus p. nicotianae, on flue-cured tobacco to insecticides already registered for the green morph in Zimbabwe.

Materials and Methods

Laboratory Studies

Foliar insecticides were evaluated using the plant dip method (Kerns *et al*., 1996) in three trials. Tobacco plants were washed in distilled water and foliar portions of the tobacco plant dipped for five seconds into insecticide solutions (Imidacloprid, Thiamethoxam, Aldicarb, Monocrotophos, Acephate, Dimethoate and Pirimicarb at the Tobacco Research Board's recommended rate of each insecticide (TRB Handbook, 2002). Plants were removed and allowed to dry at room temperature for 30 minutes, and were then transferred to plastic pots. Pots were prepared by drilling 1 cm diameter holes into the lids of the clear plastic small cup shaped containers (75 mm x 105 mm diameter). Plants were placed into these holes with roots protruding from the holes into the pot and secured into place using sticky tape. Approximately 400 ml of distilled water were added into each pot forming a hydroponic reservoir.

Twenty adult red tobacco aphids, from greenhouse tobacco plant cultures originally obtained from Kutsaga fields, and reared in the Entomology Laboratory at Kutsaga, were placed on each plant and the cups were put in a growth room at temperature of 24º C and 16 hours photoperiod. For systemic aphicides, plant roots were dipped in 500 ml hydroponic reservoirs treated with the respective insecticide rates at ambient temperature for 48 hours. This allowed the systemic movement of the insecticide from the roots to the leaves before aphids were introduced.

After 24 hours, mortality was determined by gently probing the aphids with a fine brush. If the aphid moved or walked it was considered alive. If no movement was observed or if the aphid could not walk, it was considered dead. The experiment was a randomised complete block design with 8 treatments in 3 blocks and was repeated in 3 different years, 2007-2009.

Field experiments

Three experiments were carried out under field conditions at Kutsaga Research Station in Zimbabwe (17º 55' S, 31º 08', altitude 1480 m above sea level, average annual rainfall 882 mm), from 2008-2009. Seedlings were sown in float beds as described by Mazarura and Asher (2011), in August and transplanted into the lands in November. Planting-hole aphicides (Imidacloprid, Thiamethoxam, Aldicarb and Thiflu) were applied as per manufacturer's recommendation (Table 1) soon after planting, and artificial aphid infestation with 4 aphids plant-1 were done at 3 weeks after planting. Curative sprays (Dimethoate, Acephate, Monocrotophos and Methamidophos) and aphid assessments were done at 6 weeks after planting, when infestation levels had reached a score of at least 1(1-10 aphids) in the untreated control plots. There-after, this was done on a weekly basis until topping at 9 weeks after planting.

Aphid infestation was assessed and scores from 0 to 4 assigned for each treatment. The scores were: 0 for no aphids, 1for 1-10 aphids, 2 for 11-100 aphids, 3 for 101-1000 aphids, and 4 for 1000 or more aphids.

Analysis of Variance (with Genstat 9th edition) was used to determine the response of the aphid to the insecticides and Abott's formula (Abott, 1987) was used to correct for mortality in the untreated plots. The

experiment was a randomised complete block design with 9 treatments in 3 blocks and was repeated in 3 different years (2007 to 2009).

Results

Laboratory experiments

There were no significant differences ($P > 0.05$) in aphid mortality among the systemics, namely Imidacloprid, Thiamethoxam, Aldicarb and Pirimicarb in 2008 and 2009 (Table 1); although in 2007 Thiamethoxam gave significantly ($P < 0.001$) the least control. Significant differences in aphid mortality among the contact aphicides though, were apparent with aphid mortality with Monocrotophos being significantly ($P < 0.001$) lower than for Acephate and Dimethoate; and the systemic aphicides from the three years (2007, 2008, 2009) of the study.

A comparison of the insecticides in the field is given in Tables 2 - 4. The field studies were done when Pirimicarb had been banned for use due to its toxicity properties and it was replaced with Thiflu which was a new product accepted to be good against aphids.

Table 1: Percent Mortality in Currently Registered Aphicides of the Tobacco Aphid *Myzus p. nicotianae*

Treatments	Active ingredients	Mode of action	Rate 100 L^{-1} water	Year of trial		
				2007	**2008**	**2009**
Pirimicarb 50 dg	Pirimicarb	systemic	125g	82.1	96.3	97.5
Monocrotophos 40 ec	Monocrotophos	contact	175g	33	71.3	69.1
Dimethoate 40 ec	Dimethoate	contact	375g	87.5	98.8	97.4
Temik 15 g	Aldicarb	systemic	0.7g plant-1	97.4	97.5	96.3
Confidor 200 sl	Imidacloprid	systemic	220 ml	76.3	93.8	98.8
Actara	Thiamethoxam	systemic	125 g	59.5	91.3	96.2
F-Probability						
LSD (0.05)				17.28	7.76	4.39

*** = P<0.001.

dg =dispersible granule, ec = emulsifiable concentrate, sl =soluble liquid

Field experiments

For the 2007 season, artificial aphid infestation was carried out at 5 weeks after planting (WAP) and curative contact sprays were done after assessment at 6 weeks after planting. Planting hole treatments with the neonicotinoid aphicides, Imidacloprid, Thiamethoxam and Thiamethoxam + gave significantly better aphid control from planting, as compared to the untreated control ($p < 0.001$) (Table 2), till 8 weeks after planting, a time when aphids ceased to become a problem. Aldicarb gave similar control up to 6 weeks after planting. Curative sprays of Acephate, Dimethoate, Methamidophos and Monocrotophos gave better control than the untreated control (see weeks 7 and 8). Although better than the control, Monocrotophos performed worse than all other aphicides (Table 2).

Table 2: Aphid Scores to Currently Registered Aphicides of the Tobacco Aphid *Myzus p. nicotianae* for 2007

Treatments	Active ingredients	Mode of action	Weeks after planting			
			5	6	7	8
Untreated control	Nil	Nil	0.6	1	3.83	0.49
Thiflu 1.25 gr	Thiamethoxam+	systemic	0	1.29	0.16	0.07
Confidor 200 sl	Imidacloprid	systemic	0.02	0.01	0.26	0.18
Lancer 75 wp	Acephate	contact	0.79	1.24	0.75	0.13
Temik 15 g	Aldicarb	systemic	0.02	0.05	1.49	0.41
Dimethoate 40 ec	Dimethoate	systemic	0.35	0.97	1.07	0.27
Monocrotophos 40 ec	Monocrotophos	contact	0.47	0.99	1.87	0.18
Tamaron 60 sl	Methamidophos	contact	0.81	1.12	0.46	0.2
Actara 25 wg	Thiamethoxam	systemic	0	0	0.65	0.16
F-Probability						
LSD			0.21	0.29	0.47	0.23

*** = P <0.001

dg =dispersible granule, ec = emulsifiable concentrate, sl =soluble liquid

Table 3: Aphid Scores to Currently Registered Aphicides of the Tobacco Aphid *Myzus p. nicotianae* for 2008

Treatments	Active ingredients	Mode of action	Weeks after planting			
			6	7	8	9
Untreated control	Nil	Nil	1.77	3.29	3.28	3.48
Thiflu 1.25 gr	Thiamethoxam+	systemic	0.37	0.09	0.29	0.35
Confidor 200 sl	Imidacloprid	systemic	0.57	0.13	0.58	1.26
Lancer 75 wp	Acephate	contact	1.92	2.92	1.84	0.65
Temik 15 g	Aldicarb	systemic	0.35	0.23	0.51	0.91
Dimethoate 40 ec	Dimethoate	systemic	1.61	2.8	1.03	0.67
Monocrotophos 40 ec	Monocrotophos	contact	1.66	2.94	1.66	1.5
Tamaron 60 sl	Methamidophos	contact	1.57	3.04	0.84	0.28
Actara 25 wg	Thiamethoxam	systemic	0.05	0.16	0.32	0.51
F-Probability						
LSD			0.8	0.72	0.78	0.86

*** P<0.001

dg =dispersible granule, ec = emulsifiable concentrate, sl =soluble liquid

Unlike in 2007 when aphid pressure was lower, in 2008 natural aphid infestation was very high but despite this, artificial aphid infestation was carried out at 6 WAP, and curative contact sprays assessments at 7 WAP. As was the case in 2007, preventative planting hole aphicides applied at plating, Imidacloprid, Thiamethoxam, Thiamethoxam and Aldicarb were significantly ($P < 0.001$) better than the control throughout the season ($p < 0.05$) (Fig.1). The contact aphicides Acephate, Methami-dophos and Dimethoate were also very effective and were significantly different from the control (Table 3). Most of the aphicides showed as good or better control than the standard aphicides, Dimethoate.

Curative contact aphicide sprays were done soon after assessments at 6 weeks after planting. Preventative planting hole aphicides Imidacloprid, Thiamethoxam and Thiflu were again the most effective throughout the season showing better control throughout the season($p < 0.0001$) (Table 2) than the control and comparable control relative to the standard aphicides, Dimethoate. Aldicarb, however, was effective only up to 7 WAP. Contact aphicides such as Acephate, Methamidophos and Dimethoate were also very effective and were significantly better than the control ($p < 0.001$) (Table 4). The control by Monocrotophos was just better than the untreated control.

Table 4: Aphid Scores to Currently Registered Aphicides of the Tobacco Aphid *Myzus p. nicotianae* for 2009

Treatments	Active ingredients	Mode of action	Weeks after planting			
			6	7	8	9
Untreated control	Nil	Nil	1.98	1.85	2.56	2.56
Thiflu 1.25 gr	Thiamethoxam+	systemic	0.41	0.48	1.52	1.52
Confidor 200 sl	Imidacloprid	systemic	0.09	0.01	0.18	0.18
Lancer 75 wp	Acephate	contact	1.02	0.27	0.18	0.18
Temik 15 g	Aldicarb	systemic	0.69	1.23	2.54	2.54
Dimethoate 40 ec	Dimethoate	systemic	1.51	0.42	1.26	1.26
Monocrotophos 40 ec	Monocrotophos	contact	1.64	1.17	1.74	1.74
Tamaron 60 sl	Methamidophos	contact	1.67	0.37	0.29	0.29
Actara 25 wg	Thiamethoxam	systemic	0.17	0.33	1.24	1.24
F-Probability						
LSD			0.43	0.29	0.31	0.31

dg =dispersible granule, ec = emulsifiable concentrate, sl =soluble liquid

Discussion

Based on our data, we cannot be certain of insecticide resistance in the red morph infesting tobacco. However, it is certain that significant differences in insecticide response existed. Tobacco growers and pest control advisors should avoid using Monocrotophos as a curative treatment where red-coloured morphs are present and under high aphid pressure such as in late planted tobacco it is advisable for growers to use planting hole aphicides. These will give good aphid control and result in reduced levels of sooty-mould and virus diseases. Where growers use curative aphicide sprays, it is advised that they rotate the aphicides used to minimise the development of pesticide resistance. The relationship between red colour and resistance to insecticides is common among several aphid species, including potato aphids. Our results, however, failed to detect any consistent reduced efficacy relative to the control. Insecticide resistance to organophosphates such as dimethoate, monocrotophos and methamidophos has already been documented for *M. persicae* in Chile (Unruh *et al.*, 1996; Fuentes-Contreras *et al.*, 2004).

Resistance to organophosphates, carbamates and pyrethroid insecticides has been attributed to increased levels of a single carboxylesterase (E4) which express activity towards a broad range of insecticides (Devonshire and Swicki, 1979). Harlow and Lampert (1990) classified tobacco aphids from North Carolina into three resistant categories based on their colour and response to malathion. They reported that red-coloured tobacco aphids were consistently 3.3 to 4.3-fold more resistant than a susceptible population. In our case, however, the red morph was controlled effectively by most of the insecticides used in the study. Studies done in Yuma, Arizona, with red and green-yellow colour forms showed that the red-coloured aphids were consistently more resistant to dimethoate and lambdacyhalothrin than the green-yellow ones (Kerns *et al.*, 1996), something we did not observe perhaps because the red morph is still a new pest in the country.

Elsewhere in the world, the red form of the tobacco aphid had been observed as far back as 1985 in North Carolina, USA and appeared to be a more serious pest than the green morph as it was is more tolerant of higher temperatures and is also more prone to develop resistance to insecticides (Harlow and Lampert, 1990). Results therefore are consistent with those from North America where red morphs present on tobacco plants were deemed to be more resistant to organophosphorus insecticides than the green form (Harlow *et al.*, 1991). Our data found out that, for the time being, the red morph of the tobacco aphid *Myzus p. nicotianae*, has not yet developed resistance to insecticides already registered for the green morph in Zimbabwe. However, these studies were done on a population from one location and cannot represent the response of other populations from various parts of the country. Of interest, nevertheless, is that Monocrotophos and Aldicarb which are known to be very

effective in controlling the green morph of the aphid, appeared to have reduced efficacy with regards to the red morph.

Conclusion and Recommendations

The results of this study showed that current aphicides were still effective in controlling the red morph of the tobacco aphid. The study also highlighted the need for further work to elucidate the nature of resistance to insecticides of both the red and green morphs using molecular methods and also to determine the spatial distribution of this red morph in Zimbabwe. Although these results showed that insecticide resistance in the red morph may not be prevalent in the population we studied, caution must be taken in the use of Monocrotophos and Aldicarb as there appears to be some reduced efficacy. Aphicide rotations employing insecticides with different modes of action must, therefore, be encouraged in order to reduce the risk of insecticide resistance build up by varying the selection pressure. Further work must be carried out to monitor for resistance build up in populations collected from other parts of the country.

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