Final Project Report to the NYS IPM Program, Agricultural IPM 2003-2004

TITLE: A NEW APPROACH FOR DETECTING INSECTICIDE RESISTANCE IN ONION MAGGOT POPULATIONS

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TYPE OF GRANT: Monitoring, forecasting, and economic thresholds

PROJECT LOCATIONS: Research was conducted in most of the major onion growing regions in NY. The survey information is applicable only to NY onion growers, but the technique developed is applicable worldwide.

ABSTRACT:

The primary goal of this research was to develop a rapid assay for identifying insecticide resistance in onion maggot populations and then to use it for predicting controllability of onion maggot infestations in the field. In 2002 and 2003, chlorpyrifos (Lorsban® 4E) was evaluated for managing onion maggot infestations in a total of 23 commercial onion fields. Populations from 12 of these fields were established and LC_{50}s for them were determined using the larval assay. The LC_{50} value for the chlorpyrifos-susceptible population (ST) was 14 ppm, whereas LC_{50} values for populations collected from commercial onion fields ranged from 63 ppm to 4343 ppm. These results indicate that resistance to chlorpyrifos occurs in some commercial onion fields and that the levels of resistance can vary dramatically. Control using chlorpyrifos in the field also differed considerably among fields. In both years, the percentage of onion plants killed by onion maggot in untreated and chlorpyrifos -treated plots were positively correlated, indicating that chlorpyrifos lost effectiveness as onion maggot pressure increased. To determine if the lack of onion maggot control in chlorpyrifos-treated plots was primarily due to resistance, a negative correlation should occur between an increase in the resistance ratios and the percentage reduction of onion plants killed by onion maggot using chlorpyrifos in the field. However, this was not the case. Therefore, based on the absence of this negative correlation, the larval assay will not be a reliable tool for predicting the controllability of an onion maggot infestation using chlorpyrifos in the field. However, the larval assay is an effective tool for identifying levels of resistance. It is likely that the efficacy of chlorpyrifos for controlling onion maggot in the field is largely affected by factors in addition to resistance, such as environmental conditions and the level of pest pressure.

BACKGROUND AND JUSTIFICATION:

Onion maggot, *Delia antiqua*, continues to be a major pest of onion in New York. Onion maggot overwinters within onion fields and completes three generations in New York. Rotation of onion fields far from the previous season's onion fields significantly reduces onion maggot infestations, but logistical constraints and economics preclude its use in most situations. As a consequence, onion fields are rarely rotated and onion maggot infestations can become severe. Onion maggot infestations are controlled primarily using insecticides applied at planting. Because there are only two products labeled for onion maggot control in New York, they are often used continuously in the same fields. Resistance to one of these products, chlorpyrifos (Lorsban®), has been documented in many fields leaving the other, cyromazine (Trigard®), as the only alternative. The loss of either product to resistance makes long-term management of this pest increasingly difficult. Monitoring the susceptibility of onion maggot populations to insecticides each year using a laboratory bioassay could be used to predict whether or not resistance to an insecticide is developing and if the insecticide will be effective for controlling next season's infestation. Because onion maggot populations are likely to be relatively localized in onion fields, this strategy appears feasible.

In the early 1990s, a laboratory assay was developed in Canada and later modified to detect chlorpyrifos resistance in onion maggot adults. This assay requires female flies to be treated with a dose of chlorpyrifos that will kill most susceptible individuals while allowing most resistant ones to survive. Although this assay works, there are operational constraints. The procedure is quite labor-intensive, time consuming and requires a high level of expertise to perform the experiments. The assay is designed for use only with adults; however, the larval stage is the stage that is targeted in the field. Perhaps most importantly, it is not known how accurately the results from this assay can be used to predict control of onion maggot infestations in the field. Thus, the primary goal of this project was to develop an assay in which larvae rather than adults can be tested for resistance to chlorpyrifos and that the assay can be used to predict field-level control.

OBJECTIVES:

1. To identify insecticide dose-larval mortality relationships for chlorpyrifos and onion maggot populations using a laboratory assay.

2. To determine field-level control of these onion maggot populations.

3. To relate larval mortality in the laboratory assay to mortality in the field to predict field-level control using the laboratory assay.

4. To evaluate this project, the predictability of resistance in onion maggot populations will be evaluated in commercial onion fields.

PROCEDURES:

The resistance assay will be developed initially for detecting chlorpyrifos resistance in onion maggot populations. Currently we are maintaining seven colonies of onion maggot, which were collected from NY onion fields in 2003. Additionally, we have a colony that is known to be susceptible to chlorpyrifos (originated from an organic onion farm in Michigan).

Objective 1: Log dose-larval mortality relationships will be determined for each of the seven onion maggot colonies. To do this, 7 d- old larvae (2nd instars) will be subjected to a range of doses of chlorpyrifos. Larvae will be submerged in a solution containing chlorpyrifos for one minute and then placed in a plastic diet cup containing a small piece of wheat germ-based artificial diet. Survival of larvae will be recorded after 24 hr. Preliminary experiments have shown this technique to work. Data will be analyzed using Polo to describe the log dose – mortality relationships. From these results, a discriminating dose of chlorpyrifos that kills 99% of the susceptible insects will be identified.

Objective 2: Evaluating levels of onion maggot control using chlorpyrifos in the field will be conducted in small plot experiments in at least 12 commercial fields. Fields with varying levels of onion maggot pressure or known resistance to chlorpyrifos will be selected. Fipronil (Regent® 6.2TS) will be included in these tests as a positive control.

Objective 3: In order to use results from the assay to predict field-level control of onion maggot infestations using chlorpyrifos, the relationship between level of mortality in the lab bioassay and control in the field must be described. This will be achieved using linear and/or non-linear regression analysis.

Objective 4: The predictability of resistance in onion maggot populations to chlorpyrifos using the assay will be evaluated. Because maggot populations are localized, maggots will be tested in 2003 to predict controllability in 2004.

RESULTS AND DISCUSSION

Larval assays were conducted for populations in which a sufficient number of larvae were collected to establish a colony. In 2002, five populations were established and seven have been established for collections in 2003. The larval assays were completed for onion maggot populations sampled in 2002 and are currently underway for populations sampled in 2003 **(Table 1)**. The LC_{50} value for the chlorpyrifos-susceptible population (ST) was 14 ppm, whereas LC_{50} values for populations collected from commercial onion fields ranged from 63 ppm to 4343.3 ppm. These results indicate that resistance to chlorpyrifos occurs in some commercial onion fields and that the levels of resistance can vary dramatically.

TABLE 1. Susceptibility of *Delia antiqua* larvae to chlorpyrifos in New York in 2002 and 2003.

Year	Population	N	Slope	LC_{50} (ppm)	95% CL	X^2(df)	RR
2002	ST[1]	395	4.68 (0.56)	14.4	-	6.11 (1)	1.0
	J. Frilito	210	-	>400[2]	-	-	-
	Gianetto	838	2.27 (0.15)	70.7	53.3-92.1	9.02 (4)	4.9
	Mortellaro	700	1.24 (0.12)	91.0	61.9-136.3	5.85 (4)	6.2
	Sacheli	491	1.62 (0.15)	84.4	38.6-185.9	20.27 (4)	5.9
	Yurchuck	720	2.31 (0.17)	63.1	22.1-117.1	23.27 (3)	4.4
2003	Datthyn	300	3.69 (0.41)	4343.3	3817.2-4993.1	0.80 (2)	301.2

[1]Population maintained continuously in the laboratory after collecting larvae from an organic onion farm in Michigan during the late 1970's.

[2]43.3% mortality at 400 ppm.

In 2003, the percentage of onion plants killed by onion maggot in commercial fields varied tremendously **(Table 2)**. Within each region, especially in the central and western regions, there was a wide range in percentage of untreated plants killed by onion maggot **(Table 2).** These results suggest that onion maggot pressure can vary among fields, even within the same growing region. Fipronil provided better control of onion maggots than chlorpyrifos in most fields. Mean percentage of plants killed by onion maggot in fipronil-treated plots was not correlated with the percentage of those killed in untreated plots (F= 2.7; df= 1, 8; P=0.1375), indicating that fipronil provides excellent control of onion maggot even when pressure is very high. In contrast, mean percentage of plants killed by onion maggot in chlorpyrifos-treated plots was correlated positively with percentage of plants killed in untreated plots (y= 1.4 + 0.8 x; R^2 = 0.67; F= 16.1; df= 1, 8; P=0.0039), indicating that control using chlorpyrifos fails under high pressure. Based simply on these field results, it can not be determined if the reduction in control using chlorpyrifos in some fields was due to resistance, loss of product due to leaching or high onion maggot pressure.

TABLE 2. Mean cumulative percentage of onion seedlings killed by onion maggot, *Delia antiqua*, in commercial fields in three general growing regions in New York in 2003.

EAST- Orange County

	Mean cumulative % seedlings killed by onion maggot[1]		
Treatment	Ruskiewicz	Myruski	Yurchuck
Untreated	24.3 a	14.9 a	11.5 a
Lorsban® 4E	13.2 b	3.6 b	8.3 ab
Regent® 6.2TS	2.7 c	2.2 b	1.2 c

CENTRAL – Yates, Wayne and Oswego Counties

	Mean cumulative % seedlings killed by onion maggot[1]				
Treatment	Sacheli	Datthyn	D. Frilito	Dunsmoor	J. Frilito
Untreated	31.2 a	84.9 a	52.1 a	35.9 a	5.1 a
Lorsban® 4E	5.3 b	42.2 b	30.9 b	6.5 b	1.3 b
Regent® 6.2TS	0.7 c	11.2 c	4.4 c	0.3 c	0.9 b

WEST - Orleans County

	Mean cumulative % seedlings killed by onion maggot[1]		
Treatment	Mortellaro	Triple-G	Kasmer
Untreated	70.3 a	81.7 a	34.3 a
Lorsban® 4E	17.4 bc	10.4 bc	11.5 bc
Regent® 6.2TS	2.3 c	1.7 c	1.3 c

[1] Means within a column followed by the same letter are not significantly different ($P > 0.05$; LSMEANS). Data were transformed using square root ($x + 0.001$) function before analysis, but untransformed means are presented.

To determine if the lack of onion maggot control in chlorpyrifos-treated plots was primarily due to resistance, a negative correlation should occur between an increase in the resistance ratios (see Table 1) and the percentage reduction of onion plants killed by onion maggot using chlorpyrifos in the field. This was not the case (F=0.67; df= 1, 3; P=0.4731). Therefore, based on the absence of this negative correlation, the larval assay will not be a reliable tool for predicting the controllability of an onion maggot infestation using chlorpyrifos in the field. However, the larval assay is an effective tool for identifying levels of resistance. It is likely that the efficacy of chlorpyrifos for controlling onion maggot in the field is largely affected by factors in addition to resistance, such as environmental conditions (e.g., high soil moisture that causes leaching of chemical) and the level of pest pressure.