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RESISTANCE TO MONOCROTOPHOS IN THE COCONUT BLACK-HEADED CATERPILLAR : OUTLINING DEVELOPMENT OF RESISTANCE IN ASYNCHRONOUS POPULATIONS

K. MURALIMOHAN, V.T. SANNAVEERAPPANAVAR and Y.B. SRINIVASA[1,*]

Department of Entomology, University of Agricultural Sciences, GKVK, Bangalore 560 065 India
[1]Institute of Wood Science and Technology, P.O. Malleswaram, Bangalore 560 003, India
*E-mail : ybsrinivasa@gmail.com

ABSTRACT : Monocrotophos has been the most frequently used insecticide in the management of coconut black-headed caterpillar, *Opisina arenosella*. Seven spatially segregated populations from Southern Karnataka, India with different records of insecticide applications were selected for the study. The population from Nittur was being continuously exposed to monocrotophos, while infested orchards from Arasikere, Kadur, Channapatna, Mandya and K R Pet taluks had only a past history of exposure to monocrotophos. The population from Mangalore was never exposed to the chemical. We discovered that Nittur population had 134 folds greater LC_{50} value than the most susceptible one (Mangalore population). The results showed that *Opisina* can potentially develop populations resistant to monocrotophos. Considering that *Opisina* has discrete spatially segregated populations that are also asynchronous (which is uncommon among tropical insects), we make an attempt to provide a general framework to trace the development of resistance in such populations.

Key Words : *Opisina arenosella*, insecticide resistance

INTRODUCTION

Coconut gardens plagued by the black-headed caterpillar, *Opisina arenosella* (Walker) (Lepidoptera: Oecophoridae) (hereafter *Opisina*) causes substantial loss to growers (Rao *et al.*, 1948; Joy and Joseph, 1972). Caterpillars of this species feed on leaflets of the coconut palms. Insecticides, especially monocrotophos, are administered for managing this menace (Kanagaratnam, 1976; Nadarajan and ChannaBasavanna, 1981; Rao *et al.*, 1981; Sundaramurthy and Jayaraj, 1985; and Kanagaratnam and Pinto, 1985). However, the frequency of application does not usually exceed three per year. Monocrotophos is not only effective against *Opisina* but also exhibits several characteristics that make its usage to coconut palms very convenient (Pushpalatha, 1986). Its systemic nature (favouring distribution throughout the palm), ability to move upwards when injected into the stem or fed through the roots, and negligible phytotoxicity even when undiluted commercial formulations are used has led to its general popularity in managing populations of *Opisina*. The recommendation by the state agricultural universities and supplies made at subsidized rates has increased the usage

of this chemical. Since ~30 years monocrotophos has been used in managing the pest in Karnataka. The widespread usage of this insecticide led us to investigate the variation in susceptibility of *Opisina* to monocrotophos in gardens with varying histories of its usage.

Opisina occurs in spatially segregated populations with each following discrete generation cycles temporally asynchronous with generation cycles of others (Ramkumar *et al.*, 2006), which suggests that there is an almost 'zero' probability of immigration/emigration between populations (Muralimohan, 2006). Evidences strongly point to the existence of long-term periodicity in populations of *Opisina* (Parera *et al.*, 1988), which means that there are periods when there are a large number of discrete breeding populations and periods when populations crash to only a few. Therefore, the consequences of insecticide resistance in *Opisina* could be different from a species where there is constant intermixing of populations, the latter being common among tropical insect pests.

The present study, carried out in 2005, records concentration-mortality response to monocrotophos among selected geographically isolated populations of *Opisina* in Karnataka and briefly discusses the possible implications of insecticide resistance in a species with practically non-interbreeding populations.

METHODOLOGY

Larvae and pupae of *Opisina* were collected from different regions of Karnataka (fig. 1). Of the seven populations selected, Nittur population had a continuous history of monocrotophos application, including the population sampled. Arasikere, Kadur, Channapatna, Mandya and K R Pet had a history of application of monocrotophos in the region, but the population currently sampled was not

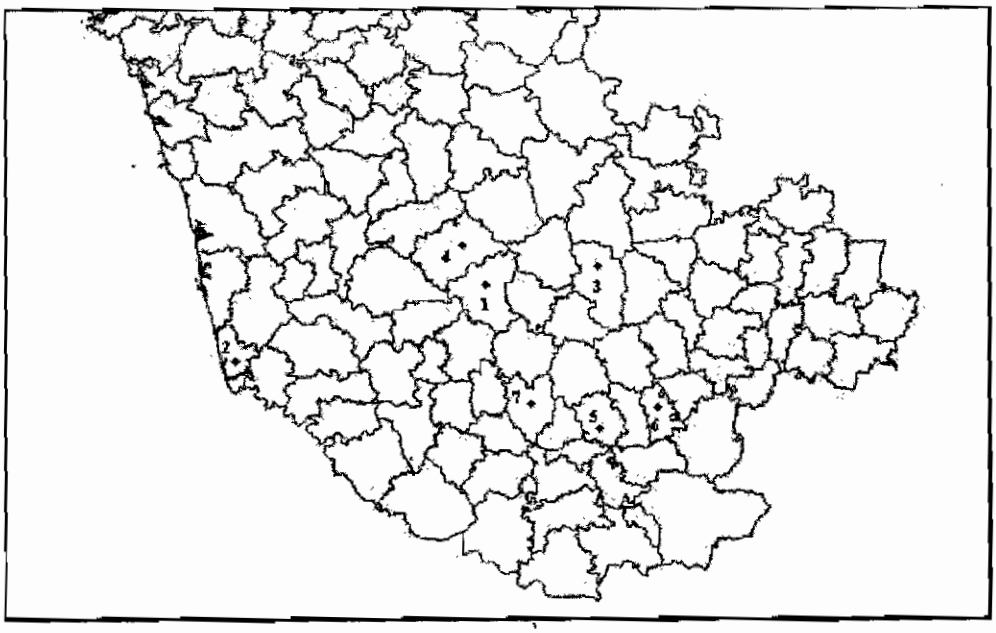

Fig. 1. Locations in southern Karnataka from where populations of *Opisina arenosella* were drawn for testing their susceptibility to monocrotophos – 1-Arasikere; 2-Mangalore; 3-Nittur; 4-Kadur, 5-Mandya; 6-Channapatna; 7-K. R. Pet

exposed to any chemical; the populations sampled in these gardens have been present for at least 18 months during which time 7 or 8 generations may have been completed. Mangalore region has no history of insecticide application against *Opisina*, especially around the areas where samples were drawn.

Larvae were reared on bits of coconut leaflets in the laboratory till pupation. Adults that emerged from these pupae were allowed to mate and eggs were collected (Ramkumar *et al.*, 2001). Eggs were incubated at 25°±1°C in a BOD incubator. On hatching, neonates were transferred to plastic vials containing fresh bits coconut leaflets and reared till they reached third instar. Bioassays were conducted on the third instar larvae using monocrotophos 36 SL (Luphos® 36SL). Leaf dip method was employed in the bioassays. A preliminary bracketing experiment was carried out for each of the populations to set limits on concentrations encompassing larval mortality from 10 to 90 per cent (refer table 1 for details on the number of dilutions and concentration limits for each population). Coconut leaf bits approximately two inches long were dipped in a selected concentration for 20 seconds. Such leaf bits were dried under shade for an hour before placing them in plastic vials @ one leaf bit/vial. Fifteen third instar larvae of uniform size were released into each vial and three replications were maintained per concentration. A control in three replications, where only distilled water was used to treat the leaf bits, was maintained for each population. The experimental units were placed in a BOD incubator set at 25°±1°C. Larval mortality was recorded at an interval of 24 h until there was no further mortality for three successive days in any of the test concentrations in each population. Data were subjected to probit analysis (Finney, 1964) for determining the LC_{50}. χ^2 values were generated to test homogeneity within populations and resistance ratio, which is the ratio of the LC_{50} value of a population to the LC_{50} value of the most susceptible population, was used to compare the levels of susceptibility across the test populations. The population with the lowest LC_{50} value was considered to be the susceptible population as there was no susceptible laboratory culture available.

RESULTS AND DISCUSSION

Among the test populations, the LC_{50} values varied from $2.0x10^{-5}$ per cent for the Mangalore population to $2.7x10^{-3}$ per cent for Nittur population (table 1). The remaining populations had LC_{50} values between $2.0x10^{-5}$ and $8.0x10^{-5}$ per cent. In the absence of baseline susceptibility data for *Opisina* against monocrotophos, LC_{50} value of the Mangalore population, which was the lowest among the populations tested, was considered for calculating resistance ratio for all the remaining populations. Nittur population recorded the highest resistance ratio of 134.5, which indicated that the Nittur population had 134.5 folds of resistance over the most susceptible population, which was found to be resistant to monocrotophos as the fiducial limits ($1.9x10^{-3} - 3.8x10^{-3}$) did not overlap with that of the susceptible population ($1.0x10^{-5} - 5.0x10^{-5}$). The fiducial limits of the remaining populations overlapped with that of the Mangalore population.

Results of this investigation show the possibility of development of insecticide resistance in *Opisina*. Although LC_{50} values for Nittur population was 134 folds more than that of the susceptible Mangalore population, the situation may not be alarming. The LC_{99} of the Mangalore population was $1.3x10^{-2}$, which may be used as the diagnostic dose to detect development of resistance in *Opisina* populations.

Development and management of insecticide resistance in a species whose populations are spatially and temporally isolated from each other is expected to be different from that of a species with intermixing populations. In

Table 1 Probit analysis of concentration-mortality responses of third instar larvae of *Opisina arenosella* of spatially segregated populations to monocrotophos

Location	Concentration limits (%)	No. of dilutions	Regression equation	LC50 (%)	Fiducial limit (%)		χ^{2*}	Resistance factor**
					Lower	Upper		
Arasikere	$5.4x10^{-4} - 4.22x10^{-6}$	8	y = 1.29 +5.73x	$3.0x10^{-5}$	$3.0x10^{-5}$	$5.0x10^{-5}$	7.72	1.5
Mangalore	$2.7x10^{-4} - 1.05x10^{-6}$	9	y = 0.84 + 3.91x	$2.0x10^{-5}$	$1.0x10^{-5}$	$5.0x10^{-5}$	13.07	1.0
Nittur	$5.0x10^{-2} - 2.0x10^{-4}$	9	y = 0.89 + 2.28x	$2.7x10^{-3}$	$1.9x10^{-3}$	$3.8x10^{-3}$	3.21	134.5
Kadur	$1.08x10^{-3} - 4.22x10^{-6}$	9	y = 0.45 + 4.92x	$4.0x10^{-5}$	$3.0x10^{-5}$	$5.0x10^{-5}$	9.96	2.0
Mandya	$1.08x10^{-3} - 2.11x10^{-6}$	10	y = 1.01 + 4.31x	$5.0x10^{-5}$	$4.0x10^{-5}$	$8.0x10^{-5}$	4.93	2.5
Channapatna	$1.08x10^{-3} - 1.35x10^{-6}$	11	y = 0.30 + 2.75x	$8.0x10^{-5}$	$4.0x10^{-5}$	$2.0x10^{-4}$	18.26	4.0
K. R. Pet	$1.08x10^{-3} - 2.11x10^{-6}$	10	y = 0.94 + 4.10x	$4.0x10^{-5}$	$3.0x10^{-5}$	$6.0x10^{-5}$	4.06	2.0

* calculated χ^2 values were less than the critical values for all the test populations at $\alpha = 0.05$

** resistance factor was calculated considering LC_{50} of the most susceptible population (Mangalore) as the base-line value

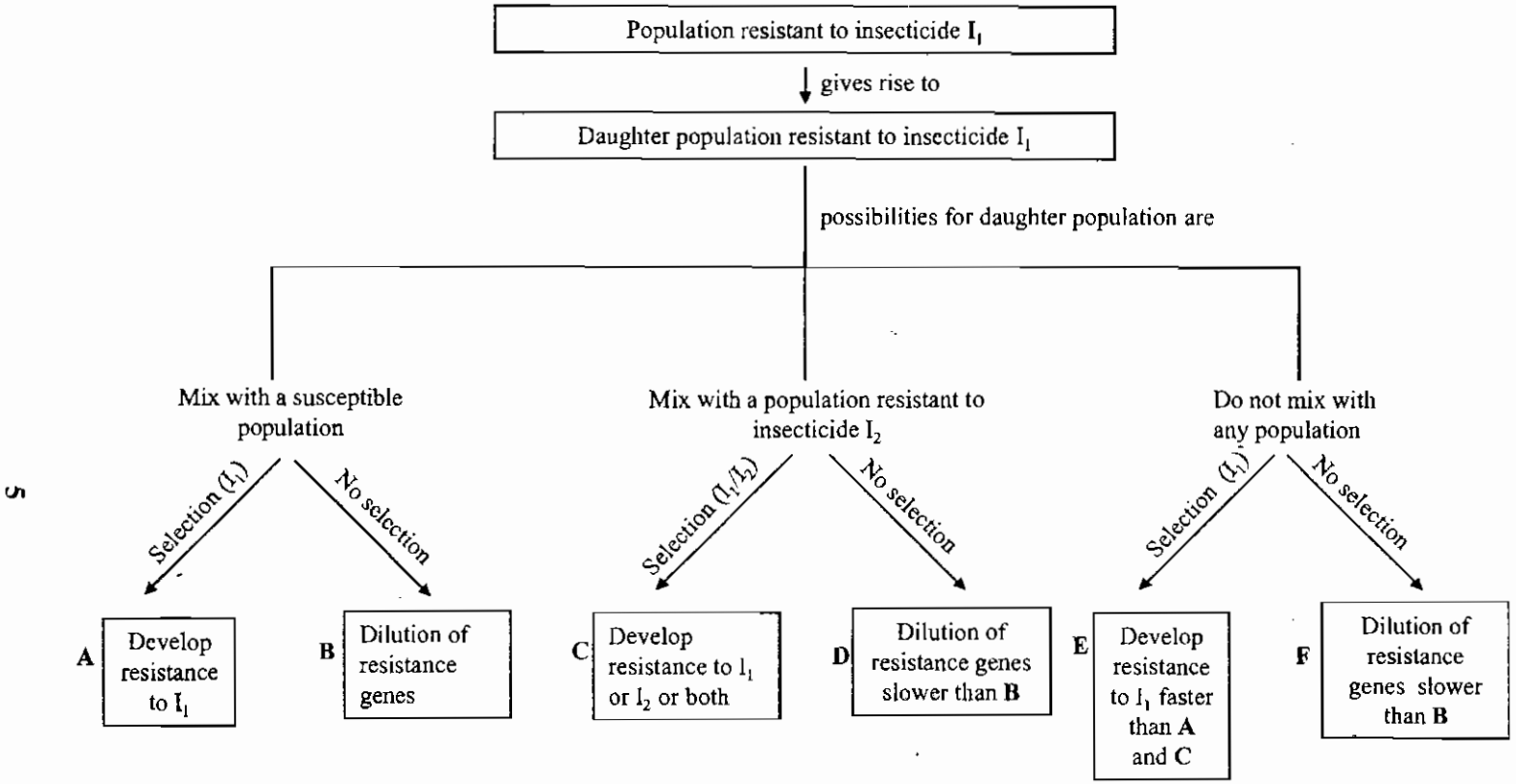

Fig. 2. Comparison of development of insecticide resistance across populations that are discrete and asynchronous (such populations do not generally mix with each other) as against populations that continuously mix.

the former, each population acts as an independent breeding unit with 'zero' emigration/ immigration (Muralimohan, 2006), which is highly uncommon among tropical insect species where intermixing populations are almost a rule. Therefore, spread of *Opisina* to newer areas should involve splitting of a parent population into several daughter populations, and, that each of the daughter populations remains isolated in space and time from other populations. Mixing is possible only when two dispersing populations converge on the same coconut garden. It is also necessary to note that each population may complete at least 15 discrete generations in a given location (Muralimohan and Srinivasa, manuscript under review).

Results obtained in the present study – increased LC_{50} values for the population continuously exposed to the chemical, and comparable LC_{50} values among populations with and without any history of application of monocrotophos should be interpreted with care due to the following reasons. It must be noted that development of resistance in populations that do not mix may be different from populations that mix (Fig. 2 provides a diagrammatic explanation of the same). Any population resistant to an insecticide (I_1) gives rise to daughter populations each of which may 1) mix with a susceptible population, or 2) mix with a population resistant to an insecticide I_2, or 3) remain distinct without mixing with any population (or mix with another population resistant to I_1). The first two situations are applicable to most tropical insect pests while the last is applicable to *Opisina*. Based on the mode of spread in *Opisina* one can predict that several resistant daughter populations could arise from a resistant parent population. As each daughter population remains distinct without interbreeding with other populations, dilution of the resistant genes would be slow even under a situation where selection is withdrawn (Fig. 2; outcome F), which is often the case because it takes several generations for a daughter population to reach pestiferous levels to warrant insecticide application. Due to the slow dilution of resistant genes in the population, a farmer may very quickly face a resistant population (assuming that he is continuing with the insecticide to which the population is resistant; monocrotophos, in the present case). Here, the rate of development of resistance can be much faster (Fig. 2; outcome E) than where populations mix continuously (Fig. 2; outcome A). Therefore, it is possible that farmers at Arasikere, Kadur, Channapatna, Mandya and K R Pet may face resistant populations more quickly than farmers at Mangalore if they administer monocrotophos (although the LC_{50} values are presently comparable).

When populations mix it is always possible that the resultant may rapidly develop resistance to different insecticides (Fig. 2; outcome C). Based on the current knowledge on the spatial distribution and generation cycles of populations of *Opisina*, it appears improbable that such a situation would arise in this species. Fortunately, thus far, the species has been exposed only to monocrotophos, which makes it the correct time to chalk out resistance management strategies. It must also be admitted that although monocrotophos has been the only recommended chemical (some of the recent recommendations include the usage of neem-based formulations), its usage has not been rampant.

Developing resistance management plans for tropical insect pests has been largely plagued, among other things, by the difficulty of assessing the potential resistance of various intermixing populations. The unusual population ecology of *Opisina* although might lead to faster rate of resistance development, lack of intermixing populations allows development of an easier population-by-population resistance management strategy.

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