Resistant Pest Management Newsletter

A Biannual Newsletter of the **Center for Integrated Plant Systems (CIPS)** in Cooperation with the **Insecticide Resistance Action Committee (IRAC)** and the **Western Regional Coordinating Committee (WRCC-60)**

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Letter from the Editors

This edition of the Resistance Pest Management Newsletter is a little bit different than our normal publication. We are featuring an extensive "*How To*" section about the **Arthropod Pesticide Resistance Database**. This section is at the end of the newsletter and contains articles about how to work the database. If you have never been to the APRD, we encourage you to visit and explore the database. It contains information which we believe will be valuable to our readers. Please take the time to visit the database at http://www.pesticideresistance.com/. You can find more extensive information about the database, how it works, and its contents at the end of this edition of the newsletter. Our hope in publishing these articles is for you to be introduced to this great resource, discover what can be found inside the database, and to become more at ease using the database. We hope that these goals are accomplished through the provided articles. Please feel free to respond with any feedback to RPMNews@msu.edu.

Resistance Management Reviews

Need for Resistance Risk Assessment in Major Pests of Jute to Insecticides

Introduction

The United Nations' resolution proclaims 2009 as the International Year of Natural Fibres in order to increase awareness about natural fibres and to promote sustainability. Jute is one of the most important natural fibre crops of India. During 2005-06, the country earned Rs. 488.80 crores through the export of jute goods (http://agricoop.nic.in/Agristatistics.htm). Though the theoretical potentiality of jute has been estimated at 81.6 q of fibre ha^{-1} (Palit, 1993), the average productivity in 2005 - 2006 was just 21.73 qha^{-1} (http://agricoop.nic.in/Agristatistics.htm). There are several factors responsible for this lower productivity, of which the loss due to insect pests is of major concern.

Major pests

With the intensive cultivation of high yielding, fertilizer responsive cultivars of jute in West Bengal, frequent outbreaks of major pests are very common nowadays. Semilooper *Anomis sabulifera* Guenee, stem-weevil *Apion corchori* Marshall, yellow mite *Polyphagotarsonemus latus* (Banks), hairy caterpillar *Spilosoma obliqua* Walker and the leaf eating caterpillar *Spodoptera exigua* Hübner are the major pests of jute (Das *et al*., 1999). Though few parasitoids were reported as natural bio control agents on jute pests, the mass culture and release technology are yet to be perfected and hence insecticidal interference is inevitable and forms an important component in the IPM programme.

Nature of damage and yield loss

Tossa jute, *Corchorus olitorius* occupies 80 per cent of the jute growing area as opposed to 20 per cent by the white jute, *Corchorus capsularis* (Saha, 2000), but unfortunately the incidence of major pests except *A. corchori* is more on *C. olitorius* than on *C. capsularis.* The jute-semilooper has been reported to occur in all the jute-growing tracts and is the most important foliage pest of the crop (Tripathi and Ghose, 1964; Das and Singh, 1976; Singh and Das, 1979; Das *et al*., 1995). Top leaves with leaf-buds and shoot apex are liable to damage. Damage starts in all cases from unopened leaves or buds, which represent the most susceptible portion. It was observed that 81 per cent of the damage was limited to seven fully opened leaves from the top, and up to 95 per cent down to the 9th leaf (Dutt, 1958). Repeated damage by this pest checks crop growth and induces profuse branching, resulting in ultimate reduction in fibre yield (Tripathi and Bhattacharya, 1963). The pods and unripe seeds are also being damaged by *A. sabulifera,* and the extent of damage varied between 30.50 and 37.50 per cent on important ruling varieties of *C. olitorius* (Singh and Das, 1979). Three waves of infestation by *A. sabulifera* occur in a jute season and the second one is the most destructive (Singh and Das, 1979). Pre-monsoon rains followed by drought conditions favour the outbreaks of

the pest in epidemic forms and the crop loss due to this pest was estimated at up to 50 per cent (Dutt, 1958). Although the damage by *A. sabulifera* to the jute crop was recognized as early as 1954 in Hooghly district of West Bengal (Dutt, 1958), cultivators are realizing the magnitude of loss due to such ravages only recently. Hence, prevention of huge crop loss as often occurs due to semilooper attack deserves the utmost consideration.

S. exigua, which was once considered as a minor pest on jute seedlings, with a maximum observed yield loss of 20 per cent (Dutt, 1958), has now become an important pest responsible for entire crop failure. Resowing is hence warranted very often to maintain the desired population in the field. The *C. capsularis* jute grown early is more liable to damage by this pest than the late sown *C. olitorius*. Bihar hairy caterpillar *S. obliqua* that was once considered as a sporadic pest on jute (Dutt, 1958) is now a major threat to jute crop during the later part of the season every year. The stem weevil, *A. corchori* on the other hand, affects more the quality of fibre than it does the yield. *C. capsularis* is more susceptible to stem-weevil infestation starting from seedling stage to harvest. The adults feed very little on the apical leaves and the rest of the life stages are passed well hidden inside the stem. The pest hence always escapes from the contact of the insecticides (Das and Singh, 1986). Yellow mite, *P. latus* is also one of the important destructive pests of jute (Das and Roychaudhuri, 1979; Das and Singh, 1985a; 1985b; Nair, 1986; Pradhan and Saha, 1997). It sucks the sap from younger leaves and therefore the leaves curl ventrally, and the colour turns from green to brown. The vertical vegetative growth of the crop is arrested and significant yield loss occurs regularly.

Need for resistance risk assessment

Endosulfan was reported to be the most effective against all the major pests of jute (Das and Singh, 1986). It is being used extensively in the jute growing tracts of West Bengal. The possibility of development of resistance to endosulfan due to several decades of exposure cannot be ruled out. A detailed investigation on the status of susceptibility of the jute pests to endosulfan from different parts of India is required to prevent or to delay the development of resistance, if any, to endosulfan. Moreover, most of the farmers under the influence of pesticide dealers apply an array of insecticides as is in vogue for the management of pests affecting other field crops. To combat the unprecedented pressure from insect pests, cypermethrin, endosulfan, quinalphos and dicofol are being used as a matter of routine. It is anticipated that there might be development of resistance due to several decades of exposure to the same groups of insecticides and therefore nowadays the recommended concentration is not very effective and warrants frequent spraying. Though several new chemicals with novel modes of action are available in the market, they may not be recommended unless the baseline toxicity data and field efficacy of these insecticides are generated in the research institute. Moreover, the exposure of the jute pests to the new chemicals in jute growing belts of West Bengal, as well as other parts of the Indian subcontinent, is nil or at least minimal. Hence it is the ideal time to get the susceptible population for generating baseline susceptibility to insecticides with novel modes of action. For effective pest management, detection and continuous monitoring of insecticide resistance are essential. This will also help to phase out the insecticides for which resistance has been built-up and hence the expenditure on ineffective insecticides by the resource poor jute farmers may be minimized. Since no work has been initiated so far in the field of insecticide resistance in jute pests, emphasis needs to be given to monitor the susceptibility to conventional insecticides, to identify the mechanism of resistance if any, and to generate the baseline toxicity for newer insecticides for which the jute pests had never been exposed previously.

Options for bioassay methods and stage of the pest

Topical bioassay using Hamilton repeating dispenser may be employed to dose the insecticides for *A. sabulifera* and *S. exigua*. The relative toxicities of insecticides to *A. sabulifera* (Tripathy, 1967; Chatterji *et al*., 1979; Chatterji and Das, 1983; Das, 1985), *A. corchori* (Chatterji and Das, 1979) and *S. obliqua* (Tripathi, 1966) were determined by directly spraying the insecticides over the larvae or adults using the Potter Tower. The Potter Spray Tower may be considered as a best option to determine the median lethal dose for the hairy caterpillar *S. obliqua* because of the profuse hairiness along the larval body. Moreover, it is easy to handle the larvae in groups as no cannibalism has been reported among the larvae of lepidopteran pests on jute. The Potter Spray Tower may also be employed to impart selection pressure over the generations for establishing the resistant population in the laboratory. Since all the lepidopteran pests on jute are defoliators, the leaf disc bioassay may be adopted with lesser investment in the poorly equipped laboratories in the developing countries. In case of *A. corchori*, the entire life cycle is being spent inside the stem and the adults cause defoliation to the apical leaves for a relatively shorter period of time. Hence it is suggested to utilize the adult weevil for determining the toxicity to insecticides. It is practically feasible and there is no need of destructive sampling in order to collect the grubs.

Need to standardize the artificial diet for the pests on jute

Mass rearing of lepidopteran pests as well as the adults of the stem weevil over several generations is a prerequisite for the studies related to insecticide toxicology. Since the field populations are heterogeneous in nature, the F_1 generation is usually preferred for the bioassy of insecticides. Moreover, the field population of *A. sabulifera, S. exigua* and *S. obliqua* might be naturally infected with entomopathogens and hence the rearing of lepidopteran pests on a natural host may lead to the complete wipe out of the population in the middle of the experiments. Hence it is inevitable to standardize the artificial diet for *A. sabulifera, S. exigua* and *S. obliqua* as far as jute entomology is concerned. It is essential to establish the resistant as well as susceptible populations in the laboratory for cross-resistance studies and to assess the relative fitness of resistant population. Mass culturing of *A. sabulifera* on artificial diet had already been attempted by the earlier workers at the Division of Crop Protection, CRIJAF (Pandit, 2001). However it is yet to be perfected for continuous culturing of the population over several generations. The artificial diet developed at CRIJAF needs to be standardized for culturing over several generations.

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Need for Acaricide Resistance Monitoring in Mites Affecting Tea in South India

The beverage crop tea, *Camellia sinensis* (L) O. Kuntze occupies an important position among the agricultural commodities produced in India, due to their contribution to the nation's economy. In South India, tea is grown mostly in the Western Ghats (between Koppa in the Chickmagalur district in Karnataka and the Kanyakumari district of Tamil Nadu) in contrast to majority of tea areas on flat land in North India. South India, with an area of 115,211 ha, produced 230,781,000 kg of tea in 2004. Tea cultivation started in 1885 with an area of 2,578 ha producing 426,000 kg and has increased enormously in area and productivity to the present level (Muralidharan and Hudson, 2007). The tea growing

regions are *viz*. Anamallais (Coimbatore district), Nilgiris, Nilgiri-Wayanad (Nilgiris District) in Tamil Nadu, Central Travancore, High Range (Idukki District), Wayanad (Wayanad District) in Kerala and Chickmagalur district in Karnataka. The weather conditions show remarkable diversity among different planting districts.

The perennial crop of tea, grown as a monoculture over large contiguous areas, provides a comparatively stable microclimate and steady supply of food for the pest species. About 1,034 arthropod species are found to feed on tea (Chen and Chen, 1989).

Tea cultivation has undergone changes from time to time with new technologies culminating in the highest productivity in the world. For higher productivity, heavy doses of fertilizers and other agrochemicals including pesticides are applied. Though a high input farming system has increased the yield, it has also made the tea plants more vulnerable to various stresses like infestation by pests and drought. More than two dozen species, including mite complex, thrips, tea mosquito bug, shot hole borer, white grub, aphid, mealy bugs, scale etc, have been reported to occur in pest form (Regupathy *et. al.,* 2003). The distribution and abundance of pests in this agroecosystem are greatly influenced by an array of factors such as plucking, pruning, manuring, regulation of shade, use of agrochemicals, jat of tea, biocontrol agents, weed flora, weather and altitude.

Mites are serious pests especially during dry weather. Tea in South India is attacked by six species of mites (Muraleedharan, 1991). The mite complex includes red spider mite, *Oligonychus coffeae* Nietner (Tetranychidae: Acari); scarlet mite, *Brevipalpus californicus* (=*australis*) *Banks* (Tenuipalpidae: Acari); purple mite, *Calacarus carinatus* Green (Eriophyidae: Acari); pink mite; or orange mite, *Acaphylla theae* (Walt.) (Eriophyidae: Acari). Damage by mites may not lead to reduction in yield but reduces the briskness and flavor of processed tea (Muraleedharan, 1997). Earlier the eriophyid purple mite *Calcarus carinatus* (Green) was of common occurrence in South India, and the red spider mite was common in Assam. Recently the red spider mite is the most predominant perhaps due to transport of plants from infested areas of Assam to South India (Personal communication).

At present the tea cultivation is carried out under the following broad categories:

1. Conventional intensive system of cultivation using chemical fertilizers, fungicides, insecticides, and herbicides as per the recommendations of UPASI (United Planters Association of South India) Tea Research Institute, Valparai, Tamil Nadu.
2. Organic system of cultivation using organic fertilizers, fungicides and micronutrients as suggested by organic certification body IMO (Institut für Markökologie, Switzerland).
3. Biodynamic system of farming using biodynamic formulations , calendar and others as suggested by Biodynamic Association of India (BDAI) (Radhakrishnan, *et.al*., 2007) and
4. The last is akin to organic cultivation by default. As many as 22 tea estates have been closed in Kerala, unable to match the income with the expenses (Nambi, 2005). Thousands of the workers thrown out of a job eke out their livelihood on the meager income from whatever available tea is plucked from the abandoned plantations without any inputs like fertilizers, micronutrients and pesticides.

To cater to the demand for organic tea in Germany, France and UK some plantations undertake organic tea cultivation in a limited scale for export purpose. However, the large area of tea cultivation is under the first category due to high cost of inputs, low productivity (Radhakrishnan, *et.al*., 2007), non-compensating price and limited demand for organic/biodynamic tea. Pesticides have been considered to be one of the most essential inputs for increasing tea production. Over the years, the pattern of pesticide usage on tea in India has followed the world trend. At present, the most commonly used insecticides are propargite 57 EC (Omite or Allmite or Simbaa), fenpropathrin30 EC (Meothrin), fenpropathrin 10 EC (Danitol), dicofol 18.5 EC (Kelthane or Colonel- S), wettable sulphur 80% (Thiovit 80% or Ultrasulf 80%), limesulphur, deleltamethrin and endosulfan 30 EC (Muralidharan and Radhakrishnan, 2007). Since a sizable quantity of tea in India is exported to various global destinations, the use of pesticides on them must adhere to the maximum residue limits (MRL) prescribed by the EPA, EEC and other agencies.

The Indian Government and the Indian tea industry are taking various measures to reduce the pesticide residues in tea. Information of pesticide residues has been generated by conducting supervised trials and monitoring studies by UPASI Tea Research Institute, Valparai, Tamil Nadu, Tea Research Association, Tocklai, Assam and Council of Scientific & Industrial Research-Tea Experimental Station, Palampur, Himachal Pradesh.

Based on the dissipation studies, recommendations are being made to wait a period of 10 days (Muralidharan and Radhakrishnan, 2007) between the last pesticide treatment and the harvest so that pesticide residues in crops are within the prescribed MRLs.

The pesticide residues are minimized due to:

- Loss due to various factors such as rainfall, dew, evaporation, photolysis and biodegradation.

- Degradation of pesticides in green leaves through evaporation and thermal decomposition during the manufacturing process. (In South India, tea is manufactured at a temperature above 100^{o}C, and at this temperature, thermal decomposition is high. Thirty to sixty per cent of the pesticide residue is lost during this process.)
- Loss in storing and with the passage of time between the manufacture of tea and use of the same.
- Loss of pesticide residue while making black tea in an infusion of hot water before consumption.
- Avoiding pesticide application just before plucking. This provides time-lag for dissipation and for growth dilution.

Information gathered through supervised trials indicated that residues dissipated to below maximum residue limit (MRL)/detectable limit (BDL) within the available harvest interval. Field surveys of tea samples at periodic intervals undertaken to evaluate the residue level revealed that the residues of fenpropathrin, propargite, dicofol, ethion and endosulfan in the tea samples from various estates in South India were below the MRLs stipulated by Germany.

To avoid cumulative residue and development of resistance, rotation of the chemicals is advocated; under practical situations, planters are likely to apply chemicals more than once for the reasons given below.

1. Fenpropathrin is very effective against red spider mites. Some farmers are so fascinated with the high efficacy that they may be tempted to use it repeatedly.
2. In Vandiperiyar area, repeated application caused the resurgence of thrips.
3. Lime sulphur is effective. Due to dislodgable residues which cause irritation to the tea pluckers, worker reentry problems are observed.
4. MRLs have been fixed only for dicofol, ethion and quinalphos under the Prevention of Food Adulteration Act 1954 (PFA act) in India, and notification is yet to be issued for propargite, fenpropathrin and hexaconazole and data submitted for endosulafan, deltamethrin, chlorpyriphos, profenofos and propiconazole.
5. Generally systemic insecticides (like methyl demeton, dimethoate and monocrotophos) are not preferred due to phytotoxicity observed during hot sunny days.
6. Chemicals such as imidacloprid, lamda cyhalothrin, and bifenthrin have yet to get a label claim for tea.

Recently, it has been observed that dicofol, once commonly used, is not effective against red spider mite. In the case of field control failures, the farmers are likely to enhance the dose leading to a higher residue level. This indicates the onset of acaricide resistance problems in mites and the need for initiating studies on acaricide resistance in mites, especially the red spider mite. The early detection of resistance is of considerable importance to organized pest control campaigns as well as to individual growers since it allows for the timely adjustments of supplies and equipment and for the training of personnel; it also helps growers to avoid the chemicals for which resistance is detected.

For estimating resistance levels in a population, the initial base-line level of susceptibility is essential so that comparisons can be made in the future (Hopkins *et al.*, 1984). For a successful monitoring programme, the base-line susceptibility to different insecticides must be estimated separately for each species in the complex. The laboratory population which has not undergone any exposure to insecticides provides the advantage of a totally susceptible bench mark for calculating resistance ratios.

Resistance in insect pests can be detected by the use of discriminating dose tests. Such detection should be done at the earliest stage of effective pest management. Hence, a suitable monitoring technique needs to be developed not only to detect the presence of resistance, but also to monitor the changes in resistance frequency to determine whether a programme is effective or not.

Bioassay techniques

Out of a number of bioassay techniques available (*viz*., topical assay, leaf residue/leaf disc, foliar application bioassay, thin layer exposure bioassay/surface residue vial bioassay, sticky card technique, slide dip bioassay and glass vial technique), the topical assay, leaf disc and oral feeding methods are more commonly employed (Regupathy, 2001).

A. Topical application: It was earlier considered that topical application on smaller organisms such as mites was difficult. However, Tabata and Saito (1970) made topical application by means of a newly devised application. It consists of a screw micrometer and an ultra micro-syringe that is used for injecting the samples in GC analysis. This device enables the easy application of 2 nl of a solution in furfuryl alcohol on the idosoma of the female adult mite under a binocular microscope. Twenty mites are placed on a round cover glass (22 m diameter) on water–flooded filter paper. After orientation, each mite is topically treated with insecticide/acaricide and held at 25°C, 74% RH.

B. Leaf disc method (Yamada et al., 1986): A detached leaf is placed on a Petri dish. A piece of wet cotton swab is placed on the tip of the leaf to provide moisture for the leaf. The surface of the leaf is surrounded with tangle foot to prevent the mites from escaping. In the adulticidal test, 30 females are inoculated on a

detached leaf, and in the ovicidal test, 100 eggs (0-3 days old) oviposited on the leaf are sprayed with 3 ml of chemical solution by a rotary spray tower and then kept in a room at 25°C.

C. Oral feeding (Yamada et al. 1986): A polyethylene tube 0.017 mm thick is filled with chemical solution. The surface of the tube is surrounded with tangle foot. Thirty adult females are inoculated and allowed to suck the toxicant through the tube. The tube is in a desicator and is regulated at 95% RH with sodium phosphate and is kept in a room at 25°C. Mortality is assessed 48 hours after treatment.

D. IRAC method No. 4: This 'Whole leaf residual contact assay' was developed by Dr. T. Dennehy of Cornell University. This is similar to leaf disc method with a slight modification. A layer of cotton wool is placed over the base of a Petri dish of 9 cm in diameter. Water is added to the point of saturation avoiding build-up of standing water. Individual leaves are treated by dipping for 5 seconds in test liquids. Surface liquid is allowed to dry from leaves before placing them in Petri dishes. Treated leaves are placed, top surface uppermost, on the wet cotton wool base. For *Tetranychus* spp. which live mainly on the lower leaf surface, the leaves may need to be placed with the lower surface uppermost. Cotton wool strips 1 cm in width are soaked in tap water and laid around the perimeter of each treated leaf, half over the leaf and half over the cotton wool bed. A small piece of damp cotton wool is placed around the petiole of each leaf. A population of at least 10 adult mites per leaf is released. With the help of a binocular microscope or hand lens, it is necessary to ensure that there are no gaps between the leaves and cotton wool strips. A minimum of five replicates per treatment needs to be run. The Petri dishes must be left open. Mortality is recorded after 72 or 96 hours depending on speed of action of the test compound. This method was used by Subaharan and Regupathy (2006) for toxicity studies for *Calacarus carinatus*. The IRAC method will be more suitable for *O. coffeae.*

Collection of test insects

Mite populations may be collected from different tea growing regions, especially from fields not exposed to any pesticides (i.e. abandoned plantations). The mite population is maintained continuously for several generations without exposure to pesticides to calibrate discriminating doses.

Mass culturing of mites

The leaves/discs are placed on a water saturated cotton swab in a Petri dish of 10 cm diameter. Field collected adult mites are transferred to leaf discs in Petri dish using an eye brow hair disc fixed to a coconut mid rib. The leaves/discs are changed when necessary, but the water in the Petri dish is changed regularly. The development of the mites is observed under a stereo binocular microscope. (Subaharan and Regupathy, 2006)

Acute toxicity

Adult mites are transferred from stock culture with an eye brow hair disc of 2 cm diameter. Five discs were maintained per replicate. These tea discs were treated by spraying with selected concentrations using a hand atomizer or by Potter's tower, and they were then shade dried for 10 minutes. The discs were then transferred to a Petri dish containing water saturated cotton. Mortality of the mites was recorded 24, 48 and 72 hours after imposing the treatment and percent mortality values were subjected to arcsine transformation prior to analysis. Each treatment was replicated five times.

Discriminating Dose

Preliminary range-finding tests are made to fix the appropriate dosage range for each chemistry. The log-dose/concentration-response curves are fitted. The DD can be arrived at as detailed by Roush and Miller (1986).

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Resistance Management from around the Globe

RELATIVE TOXICITY OF SOME COMMONLY USED INSECTICIDES AGAINST THE ADULTS OF *HELOPELTIS THEIVORA* WATERHOUSE (MIRIDAE : HEMIPTERA) COLLECTED FROM JORHAT AREA TEA PLANTATIONS, SOUTH ASSAM, INDIA

ABSTRACT. Relative toxicity of five commonly used insecticides in tea plantations of Jorhat (South Assam) against male and female *Helopeltis theivora* was determined using the foliage exposure method. The order of susceptibility for the male *H. theivora* was cypermethrin > deltamethrin > dimethoate > imidacloprid > endosulfan, whereas the order was deltamethrin > cypermethrin > imidacloprid > endosulfan > dimethoate for the females. The effective field dosages of these insecticides were computed based on LC_{50} values, and when compared with the recommended dosages, it suggested about 1.54 – 82.85 folds decrease in the susceptibility of the test population. The decrease in susceptibility of *H. theivora* to deltamethrin and imidacloprid was the largest. Three colour variants were identified within males and females of *H. theivora* within a single season presumably due to pesticide selection pressure.

Key words: Relative toxicity, *Helopeltis theivora*, Tea, Susceptibility, Colour variants

INTRODUCTION. Among various biotic stresses that tea plants face, insect attack (especially the tea mosquito bug, *Helopeltis theivora,*) has been a major challenge in recent years. Out of a total of 4.36 lakh hectares, 3.488 lakh hectares (80%) of tea plantations have been suffering from *H. theivora* infestation, causing crop losses of 10-50%. The peak season of *H. theivora* infestation (May-July) and the rainy period (June-July) coincide with the second flush, which has more quality and quantity. Due to year-round infestation, the consumption of insecticides reached up to 8.20-16.94 l/ha and costs increased by Rs.2500 – Rs.6000/-. The dimension of *H. theivora* infestation has been increasing alarmingly, and the consumption of toxic chemical pesticides has doubled (Gurusubramanian *et al*., 2005). The European Union (EU), after analyzing teas (783 samples out of 6217 tea samples all over the world) imported for residue content, has classified the Indian tea in the "higher incidence of pesticide residues" group. The Maximum Residue Limit (MRL) for most of the chemicals in EU has been fixed at 0.1 and below, which has been a major constraint to tea exporting countries (Anonymous, 2004). Following increasing reports from planters that insecticides are becoming less effective against the tea mosquito bug in Northeast India, the present study was conducted to monitor the level of susceptibility of the male and female population of *H. theivora* collected from Jorhat tea gardens to five commonly used insecticides.

MATERIALS AND METHODS. *Helopeltis theivora* adults were collected from the tea gardens in the Jorhat area and placed in rearing jars (20cm x 15 cm) for preconditioning of the tea mosquito bug under laboratory conditions at the temperature of 27± 2^{o} C, 70-80% RH and a 16:10 LD photoperiod for a period of seven days. Insecticides used in the studies were endosulfan 35 EC (Thiodan), dimethoate 30 EC (Rogor), deltamethrin 2.8 EC (Decis), cypermethrin 10 EC (Gem) and imidacloprid 17.8 SL (Confidor). Graded concentrations of insecticides were prepared in distilled water from commercial formulations of the insecticides. TV 1 clone two and bud healthy shoots were collected from the Tocklai experimental garden plots and brought to the laboratory. The leaves were washed thoroughly with distilled water and air-dried. Fifteen tea shoots for each treatment were sprayed with each of the chosen insecticides separately at the respective dilutions using a glass atomizer, and then they were kept in a glass tube containing water and wrapped with cotton. The sprayed tea shoots were kept

under ceiling fans for 15 minutes to evaporate the emulsion. The glass tubes containing tea shoots were placed in glass chimneys. Muslin cloth was tied with the help of rubber bands on top of the glass chimneys, and the tubes were kept at 27 ± 2°C in culture room. Thirty field collected and preconditioned males and females of *H. theivora* were released separately into each glass chimney containing tea shoots. Observations of adult mortality were recorded in all the five replications of each concentration 24 hours after the treatment. Moribund insects were counted as dead (Rahman *et al*., 2006). Five to seven concentrations of each insecticide were tested to obtain a concentration – probit mortality curve. The mortality data was converted to percent mortality and subjected to probit analysis (Finney, 1971; Busvine, 1971) to obtain LC_{50} values, LC_{95} values and a regression equation, from which the relative toxicity values were calculated by taking LC_{50} and LC_{95} values of endosulfan for males and dimethoate for females as unity. The data on the dosage-mortality response of male and female population of *H. theivora* collected from Jorhat area revealed that chi-square values indicated a good fit of probit response in all the bioassays showing that there was no heterogeneity between observed and expected responses. Relative resistance and relative susceptibility were computed using the formula given by FAO (1979).

$$\text{Relative susceptibility} = \frac{LC_{50} \text{ value of insecticide taken as unity}}{LC_{50} \text{ value of other insecticide}}$$

$$\text{Relative resistance} = \frac{LC_{50} \text{ value of other insecticide}}{LC_{50} \text{ value of insecticide taken as unity}}$$

The expected effective concentration of each insecticide was calculated by doubling the LC_{50} value to attain a LC_{100} value, and then effective field dosages of the five insecticides were computed based on the following formula and compared with recommended dosages as per the standard method of Misra (1989).

Expected effective concentration (LC_{100}) (%) = 2 X LC_{50} %
Expected effective dose (g a.i./ ha) = ED /100 X EC X 20 fold
ED = % concentration / EC X 1000 X 400 litres of spray fluid/ha

Colour variation in pronotal area of males and females was observed by collecting the adults from the field and killing them using cyanide jar. The pronotal area of male and female specimens was observed under an advanced research microscope. One hundred specimens each of males and females were subjected to observation of the pronotal colour for colour variation. The sex ratio of *H. theivora* was recorded based on the weekly observations of field populations at different tea gardens of Jorhat for a period of one year.

RESULTS AND DISCUSSION. Comparison of the LC_{50} values of five different insecticides for the male (Table 1) and female (Table 2) populations of H. theivora collected from Jorhat (South Assam) showed the least susceptibility to endosulfan (79.983 ppm) by males and to dimethoate (49.888 ppm) by females. Males were highly susceptible to cypermethrin (2.99 ppm) (Table 1) and females to deltamethrin (10 ppm) (Table 2). The present findings are similar to those of Misra (1989) and Kapoor et al (2002), who reported synthetic pyrethroids as the most toxic of all the tested insecticides against Helicoverpa armigera. The order of susceptibility for male H. theivora was cypermethrin > deltamethrin > dimethoate > imidacloprid > endosulfan (Table 1), whereas it was deltamethrin > cypermethrin > imidacloprid > endosulfan > dimethoate for females (Table 2). Among the pesticides tested, cypermethrin appeared to be 26.75 times more toxic (followed by deltamethrin (2.667 times), dimethoate (2.667 times) and imidacloprid (2.009 times)) than endosulfan in the case of the males (Table 1), but in the females, deltamethrin, cypermethrin, imidacloprid and endosulfan were observed to be 4.989, 3.531, 1.253 and 1.169 times more toxic than dimethoate (Table 2). The difference in relative toxicity among insecticides may be possible because the toxicity of insecticides differ even in between the males and females of H. theivora.

Table 1. Log dose probit response of field strains of *Helopeltis theivora* (Male) to different insecticides

Insecticide	χ^2	Regression equation	LC_{50} (ppm)	LC_{95} (ppm)	S.E.	Fiducial limits (95%)	Relative toxicity	
							LC_{50}	LC_{95}
Endosulfan 35 EC	4.051	Y=6.050x-6.500	79.983	295.12	3.89	86.690–72.440	1.000	2.820
Dimethoate 30 EC	1.443	Y=1.320+2.590x	29.992	831.76	4.71	36.643–24.431	2.667	1.000
Deltamethrin 2.8 EC	1.343	Y=1.649+2.590x	29.992	363.08	4.71	48.750–18.408	2.667	2.290
Cypermethrin 10 EC	9.250	Y=3.800+2.780x	2.990	79.43	4.71	3.540 – 2.500	26.750	10.470
Imidacloprid 17.8 SL	6.689	Y=1.628+2.120x	39.811	630.96	3.61	49.545–31.989	2.009	1.320

Mean of five observations.

Table 2. Log dose probit response of field strains of *Helopeltis theivora* (Female) to different insecticides

Insecticide	χ^2	Regression equation	LC_{50} (ppm)	LC_{95} (ppm)	S.E.	Fiducial limits (95%)	Relative toxicity	
							LC_{50}	LC_{95}
Endosulfan 35 EC	0.290	Y=0.789+2.560x	42.658	954.99	3.40	64.564 – 27.574	1.169	1.950
Dimethoate 30 EC	1.377	Y=5.480x-4.310	49.888	275.42	4.71	60.256 – 45.499	1.000	6.760
Deltamethrin 2.8 EC	2.629	Y=3.037+1.770x	10.000	1862.09	4.71	13.709 – 7.295	4.989	1.000
Cypermethrin 10 EC	1.290	Y=1.190+3.250x	14.125	75.86	4.71	16.453 – 12.238	3.531	24.550
Imidacloprid 17.8 SL	12.761	Y=0.885+2.570x	39.811	562.34	4.71	48.290 – 32.817	1.253	3.310

Mean of five observations.

A comparative study was made to determine the toxicity of different insecticides in terms of relative susceptibility and relative resistance to males (Table 3) and females (Table 4) of *H. theivora*. Regarding the relative susceptibility of five insecticides against males, deltamethrin, dimethoate, imidacloprid and endosulfan were 0.10, 0.10, 0.08, and 0.04 times less toxic than cypermethrin. Imidacloprid and endosulfan were 0.75 and 0.37 times less toxic than deltamethrin, however cypermethrin was 10.03 times more toxic than deltamethrin. Dimethoate toxicity was at par with deltamethrin. The toxicity of cypermethrin was 10.03 times higher than deltamethrin and dimethoate, while imidacloprid and endosulfan were 0.75 and 0.37 times respectively less toxic than deltamethrin and dimethoate. Dimethoate, deltamethrin and cypermethrin were 1.33, 1.33 and 13.31 times more toxic than imidacloprid, and endosulfan was 0.5 times less toxic than imidacloprid. Imidacloprid, dimethoate, deltamethrin and cypermethrin were found to be 2.01, 2.67, 2.67 and 26.75 times more toxic than endosulfan (Table 3). The relative resistance of deltamethrin, dimethoate, imidacloprid and endosulfan with cypermethrin was 10.03, 10.03, 13.31 and 26.75 times more toxic in the case of male *H. theivora*. Imidacloprid and endosulfan were 1.33 and 2.67 times more resistant when deltamethrin and dimethoate were taken as a unity. Endosulfan was 2.01 times more tolerant than imidacloprid (Table 3).

Table 3. Relative susceptibility and resistance of male *Helopeltis theivora* to different insecticides

Insecticide → RR ↓ RS	Cypermethrin 10 EC	Deltamethrin 2.8 EC	Dimethoate 30 EC	Imidacloprid 17.8 SL	Endosulfan 35 EC
Cypermethrin 10 EC	1.00	10.03	10.03	13.31	26.75
Deltamethrin 2.8 EC	0.10	1.00	1.00	1.33	2.67
Dimethoate 30 EC	0.10	1.00	1.00	1.33	2.67
Imidacloprid 17.8 SL	0.08	0.75	0.75	1.00	2.01
Endosulfan 35 EC	0.04	0.37	0.37	0.50	1.00

RR – relative resistance; RS – relative susceptibility

Observations regarding the relative susceptibility and resistance of the five chosen insecticides to female *H. theivora* are summarized in Table 4. The order of the insecticide's relative susceptibility and resistance was different between male and female *H. theivora*. Considering the relative susceptibility of insecticides to the female population, deltamethrin, cypermethrin, imidacloprid and endosulfan were 4.90, 3.53, 1.25, and 1.17 times more toxic as compared to dimethoate. Cypermethrin, imidacloprid, endosulfan and dimethoate were 0.71, 0.25, 0.23 and 0.20 times less toxic than deltamethrin (Table 4). The data on the relative toxicity of endosulfan when taken as unity indicated that deltamethrin, cypermethrin and imidacloprid were found to be 4.20, 3.02 and 1.07 times more toxic, while dimethoate was 0.86 times less toxic than endosulfan. A comparison with toxicity of imidacloprid pointed out that endosulfan and dimethoate were 0.93 and 0.80 times less toxic than imidacloprid, whereas deltamethrin and cypermethrin were 3.98 and 2.82 times more toxic to imidacloprid. Imidacloprid (0.35 times), endosulfan (0.33 times) and dimethoate (0.28 times) were less toxic than cypermethrin; in contrast, deltamethrin was more toxic (1.41 times) (Table 4). The results of the relative resistance to females revealed that cypermethrin, imidacloprid, endosulfan and dimethoate were 1.41, 3.98, 4.20 and 4.90 times more tolerant than deltamethrin. In the case of cypermethrin, the resistance of imidacloprid, endosulfan and dimethoate was 2.82, 3.02 and 3.53 times more toxic. The relative resistance of endosulfan and dimethoate with imidacloprid was 1.07 and 1.25 times more toxic. Dimethoate was 1.17 times more tolerant than endosulfan (Table 4).

Table 4. Relative susceptibility and resistance of female *Helopeltis theivora* to different insecticides

Insecticide → RR ↓ RS	Deltamethrin 2.8 EC	Cypermethrin 10 EC	Imidacloprid 17.8 SL	Endosulfan 35 EC	Dimethoate 30 EC
Deltamethrin 2.8 EC	1.00	1.41	3.98	4.20	4.90
Cypermethrin 10 EC	0.71	1.00	2.82	3.02	3.53
Imidacloprid 17.8 SL	0.25	0.35	1.00	1.07	1.25
Endosulfan 35 EC	0.23	0.33	0.93	1.00	1.17
Dimethoate 30 EC	0.20	0.28	0.80	0.86	1.00

RR – relative resistance; RS – relative susceptibility

Generally, it is accepted that field application rates of insecticides should at least be 20 fold or more of the LC_{50} value (determined through bioassay methods) to achieve satisfactory control of the pest in agriculture (Misra, 1989). By this simple logic, the expected effective dosages of various insecticides were worked out and are presented in Table 5. When these computed dosages were compared with the recommended dosages of the insecticides, it was observed that 1.54 – 82.85 times more of the recommended dosage of different insecticides might be required to achieve desirable control of the pest. The change in susceptibility of *H. theivora* was found to be on the order of 1.54, 3.61, 4.64, 26.56 and 82.85 fold for dimethoate, endosulfan, cypermethrin, imidacloprid and deltamethrin respectively for males and 1.92, 2.61, 2.85, 6.56 and 26.56 fold for endosulfan, dimethoate, cypermethrin, deltamethrin, and imidacloprid for females. A comparison of expected effective dosages of five insecticides based on their LC_{50} values with the recommended dosage revealed a pronounced shift in the level of susceptibility of *H. theivora* to all the

chosen insecticides. The usual recommended dose of synthetic pyrethroids (deltamethrin and cypermethrin), neonicotinoids (imidacloprid), organophosphates (dimethoate) and organochlorines (endosulfan), however, was practically ineffective against this pest. The synthetic pyrethroids are being used widely in tea plantations, and their consumption is about 3-5 litres/ha (Gurusubramanian *et al.* 2005). The reasons are i) per hectare requirement was less (100 ml/ha), ii) having knockdown effect and iii) cost effectiveness. Against the tea mosquito bug, planters using insecticides as prophylactic, due to it being a wet season pest and their peak season (May-July) coinciding with the rainy season (June-July), caused the consumption of pesticides to increase, with about 8-16 applications per year of synthetic pyrethroids on top of other chemical applications. Armes *et al* (1996) reported no marked change in the resistance of endosulfan in *Helicoverpa armigera* since 1987. However, even low levels of endosulfan resistance appeared to be associated with unreliable control of *H. armigera*. This was reported from Australia, where 20-30 fold increases in resistance caused control problems on cotton crops (Gunning and Easton, 1994), and from Andhra Pradesh, where a 13 fold increase in resistance rendered this chemical ineffective for pest control (Mc Caffery *et al*., 1989). Hence, irrespective of the group to which insecticides belong, evidence of the development of resistance to synthetic pyrethroids, organophosphates, organochlorines and neonicotinoids has been experimentally obtained.

Table 5. Expected effective concentration and effective dose of different insecticides for male and female *Helopeltis theivora* in comparison with recommended dose

Insecticide	LC_{50} (%)		Expected effective concentration (%)		Expected effective dose g a.i./ha		Recommended dose g a.i./ha	Times increase	
	Male	Female	Male	Female	Male	Female		Male	Female
Endosulfan 35 EC	0.0079	0.0042	0.0158	0.0084	1264	672	350	3.61	1.92
Dimethoate 30 EC	0.0029	0.0049	0.0058	0.0098	464	784	300	1.54	2.61
Deltamethrin 2.8 EC	0.0029	0.0001	0.0058	0.0002	464	16.00	5.6	82.85	2.85
Cypermethrin 10 EC	0.00029	0.00041	0.00058	0.00082	46.4	65.60	10	4.64	6.56
Imidacloprid 17.8 SL	0.0039	0.0039	0.0078	0.0078	624	624	23.49	26.56	26.56

From our field observations, it was evident that males outnumbered the females 2.12:1 (male: female) and that colour variants were identified within both male and female *H. theivora* (Table 6). The description of the collected colour variants and their incidence in males and females are summarized in Table 6. In males, there were three colour variants: CVM – I was common in Jorhat tea plantations and was found in 72.65 % of the total population; CVM – II occupied the second position in occurrence (20.23 %); and CVM – III incidence was low among male variants in Jorhat area (7.32 %). As in the males, three colour variants (CVF – I, II, and III) were observed in the female population also, and their incidence was 66.12, 11.43 and 22.00 % respectively (Table 6). As per the findings of Eastop (1973) and Russel (1978), the biotypes usually differ due to diurnal or seasonal activity patterns, size, shape, colour, insecticide resistance, migration and dispersal tendencies, pheromone differences or disease vector capacities. In our observations within the population of male and female *H. theivora*, three colour variants were found in Jorhat tea plantations which substantiate the possibility of developing changes in the susceptibility to insecticides as stated by Eastop (1973) and Russel (1978). However, Mann (1907) strongly correlated the colour variation in *H. theivora* with the season in which he found them; males from summer/autumn (July-October) were on the average much darker than those from winter/spring brood (November-June) with the converse being true for females. Furthermore, this observation was validated by a study done by Stonedahl (1991) where populations collected from Vietnam, South India and Assam showed variation in colour pattern of head and pronotum. Mann and Stonedahl found variation in colour pattern in different seasons, but in our observations, colour variation was found both in males and females within a single season presumably due to pesticide selection pressure. This was further confirmed by the elevation of LC_{50} values of different insecticides to male and female *H. theivora*.

Table 6. Incidence of colour variants and sex ratio between male and female of *Helopeltis theivora* collected from Jorhat tea plantations

Colour variants of *Helopeltis theivora*	Description	Per cent incidence of colour variant in the field (Mean ± SE)	Sex ratio Female : Male
CVM I	Pronotal area completely black	72.65 ± 1.14	
CVM II	Pronotal area black with one trench having brown colouration and two brownish dots at lateral side just below the trench	20.23 ± 1.32	
CVM III	Pronotal area black with two trenchs having brown colouration one in top and other in middle	7.32 ± 2.10	1: 2.12
CVF I	Centre area of pronotal area brownish orange	66.12 ± 1.81	
CVF II	Centre area of pronotal area reddish orange	11.43 ± 0.99	
CVF III	Centre area of pronotal area brownish orange with two spots at the distal end	22.00 ± 0.41	

CVM – colour variant male; CVF – colour variant female

Since a shift in the level of susceptibility to insecticides was noticed in *H. theivora*, certain measures can be initiated for combating and delaying the problem of resistance so that it does not assume unmanageable proportions. The measures may be a) since the expected effective dose of deltamethrin, imidacloprid, cypermethrin, dimethoate and endosulfan was higher than the recommended dose, its use should be restricted in *H. theivora* prone areas, b) insecticides should be used judiciously and only if their use is essential, c) no prophylactic spraying of chemicals, d) timing and frequency of applications should be such that it does

not create selection pressure, and e) the insecticides should be altered in such a way that their modes of action are different. Further in-depth studies are needed in other tea growing areas to explore the possibility of determining the resistance level by using resistance enzyme studies and biotype identification through molecular techniques besides the log dose probit assay.

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Susceptibility of the African bollworm, Helicoverpa armigera (Hub.) to endosulfan, chlorpyrifos, carbosulfan and fenpropathrin using different bioassay methods

ABSTRACT. Susceptibility of the 4th instar larvae of the African bollworm, *Helicoverpa armigera* (Hubner), to four insecticides [chlorpyrifos (Dursban®), carbosulfan (Marshal®), endosulfan (Endosulfan®) and fenpropathrin (Danitol®)] from organophosphate, carbamate, cyclodiene and pyrethroid chemical groups was carried out under the laboratory conditions in ARTC, Gezira, Sudan. Three methods were applied (surface treatment, dipping of larvae and dipping of larval food (okra seeds)) in different concentrations from each chemical. LC_{50} and LC_{90} were calculated using probit analysis. Results obtained showed that Dursban® (Chlorpyrifos) was the most effective in controlling ABW. Marshal® (Carbosulfan) and Endosulfan® (Endosulfan) exerted medium effecacy. Danitol® (Pyrethroid group) was the least effective in controlling ABW, compared to the other chemicals used.

Danitol® presented heterogeneity towards the tested insect population, as it recorded the lowest LC_{50} and maximum LC_{90} in surface and larval dipping treatments at 24 and 48 hours, compared to other chemicals used. However, Dursban® exerted high levels of homogeneity towards the tested insects, as it recorded the lowest LC_{90} and a narrow range between the LC_{50} and LC_{90} in almost every treatment. Marshal® and Endosulfan® showed medium resistance levels, falling between Dursban® and Danitol®, but Marshal® was more effective than Endosulfan® in the case of controlling the ABW by larval dipping or food treatments.

All insecticides used exerted a contact rather than an oral effect. However, surface treatment proved to be the most effective method in controlling the ABW, followed by larval dipping and then food dipping.

INTRODUCTION. The African bollworm (ABW) is a very important insect which attacks a wide range of host plants, causing serious damage. *H. armigera* has the widest distribution of any agricultural pests, occurring throughout Africa, the Middle East, southern Europe, India, central and south-East Asia, eastern and northern Australia, New Zealand, and many eastern Pacific Islands (Common, 1953; Anon, 1969; and Zalucki *et al.*, 1986). ABW is listed as a quarantine pest in Africa, Asia, Australia and much of main land of Europe (Anon, 2000). It is recorded as a major pest in the Gezira Scheme since the 1960's, due to the

expanded cotton cultivated area, condensed crop rotation (intensification and diversification), and the injudicious use of insecticides (Balla, 1978, and 1982).

This insect pest causes great economic loss through direct yield reduction, as well as the cost of chemical application and the considerable scouting required to control *H. armigera*. The annual estimates of the cost of damage by *H. armigera* on legumes reached over $300 million in India (Reed and Pawar, 1982), and $25 million due to *H. armigera* and *Heliothis punctigera* Wallengren on many crops in Australia (Wilson, 1982).

The use of broad-spectrum insecticides early in the season drastically affects the natural enemy fauna, which is just beginning to get established in the cotton field (Abdelrahman, 1986). The use of insecticides created many problems, including: development of insecticide resistance, increase in the cost of production, contamination of the environment, and the toxic side effects of these insecticides on non-target organisms including natural enemies, thus the resurgence of the secondary pests (Xiao, 1994). Resistance to insecticides is difficult to assess, but loss of pesticide effectiveness almost invariably entails increased application frequencies and dosages, and finally, more expensive replacement compounds (Roush and Mckenzie, 1987) as new insecticides become increasingly more difficult to discover, develop, register and manufacture (Metcalf, 1980). Therefore, it is essential to develop strategies to delay or minimize the probability of resistance evolution.

The study is designed to study the susceptibility of the 4th larval instar of ABW to some insecticides from different chemical groups, *viz.* the cyclodiene endosulfan (Endosulfan®), the phosphorothioate chlorpyrifos (Dursban®), the carbamate carbosulfan (Marshal®), and the pyrethroid fenpropathrin (Danitol®), and to investigate the effects of the different routes of administration, so as to standardize the method for bioassay.

MATERIALS and METHODS. Three methods were tested and evaluated: surface exposure (Petri dish treatment), larval dipping, and dipping of larval food (okra seeds) in different concentrations. Larvae of the ABW were collected from their host plants grown in Agricultural Research and Technology Corporation (ARC), Gezira Research Station, experimental fields during the 2003-2004 season. The collected larvae were brought to the bioassay laboratory of the Entomology Section, Gezira Research Station. The collected larvae were reared at room temperature. The 4th instar larvae of the first generation reared in the laboratory were subjected to insecticide treatments.

INSECTICIDES TESTED. Four insecticides from different groups were used: Endosulfan® 50 EC (endosulfan; cyclodiene), Dursban® 48 EC (chlorpyrifos; organophosphate), Marshal® 25 EC (carbosulfan; carbamate) and Danitol® 20 EC (fenpropathrin; pyrethroid).

SURFACE TREATMENT. The floor of each glass Petri dish was covered by one of the concentrations (5 Petri-dishes (reps)/conc.). The treated Petri dishes were left to dry for 10-15 min before introducing the larvae (1 larva/Petri-dish) (plate 15). Fresh okra seeds were provided to each larva daily. The experiment was replicated three times.

DIPPING OF LARVAE. Each larva was picked with forceps and dipped in the solution for a second, then allowed to dry for a minute before being transferred to a Petri dish. Five larvae were dipped in each concentration. Okra seeds were provided immediately as food. New pieces of okra fruits were provided daily to each larva. The experiment was replicated three times.

DIPPINGOF LARVAE FOOD. Plastic sieves containing okra seeds (larval food) were dipped for 10 sec in one of the prepared concentrations. Treated sieves and their contents of treated seeds were left to dry for 10-15 min under room conditions (plate 14); the treated seeds in each sieve were distributed to five Petri-dishes, each contained one L4, which was starved for 24 hours. An okra fruit piece was daily provided to the larvae in each Petri dish. The experiment was repeated three times.

DATA COLLECTION and STATISTICAL ANALYSIS.

Data was taken at 24 and 48 hours after treatment. Data was subjected to probit analysis. The LC_{50} and LC_{90} values were obtained graphically and from the equation obtained by probit analysis. Slopes of the LD-P lines were also obtained graphically and mathematically, and the RR' (LC_{90}/LC_{50}) was calculated.

RESULTS and DISCUSSION. The susceptibility of *H. armigera* larvae to different insecticides groups using different bioassay methods at different times was investigated. The results showed that percent mortality of the 4th instar larvae of the African bollworm, *H. armigera*, varied among periods (i.e. 24 and 48 hours).

Endosulfan® When treating the surface of the Petri dish with Endosulfan® at the concentrations specified in Tables 1 and 2, and exposing the larvae to this surface for 24 and 48 hours, the results at 24 hours showed that the LC_{50} was 22.39±0.78 ppm, whereas the LC_{90} was 524.8±1.76 ppm. The population proved to be heterogeneous to this insecticide (slope=0.94) and the range between the two values was wide (RR'=23.44). With regard to the 48 hour treatment, the same

concentrations were tested and the obtained LC_{50} and LC_{90} values were 12.3±0.56 and 109.65±0.96 ppm respectively (Table 2). The slope was greater than at 24 hours (1.36). These results indicated that Endosulfan® might require at least two days to exert its effects on this pest homogeneously. The range between the LC_{50} and LC_{90} was by far narrower than that of 24 hours (RR'=8.9 for 48 hours compared to 23.44 for 24 hours).

Table 1. The Susceptibility of *Helicoverpa armigera* fourth instar to different insecticide groups at 24 hr exposure.

Insecticides	Bioassay Methods	LC 50 (ppm)	LC 90 (ppm)	RR ratio (LC90/LC50)	Slope	Graphic slope
Endosulfan	Surface	22.39 ± 0.78	524.81 ± 1.76	23.44	0.94	0.93
	Dipping	40.74 ± 0.78	794.33 ± 1.56	19.5	0.98	0.96
	Seeds	120.23 ± 5.54	794.33 ± 0.88	6.61	1.53	1.48
Danitol	Surface	12.59 ± 1.81	812.83 ± 3.24	64.57	0.7	0.7
	Dipping	9.55 ± 1.7	1479.1 ± 4.24	154.88	0.58	0.57
	Seeds	117.49 ± 1.04	6309.5 ± 3.28	53.7	0.75	0.75
Dursban	Surface	28.84 ± 0.56	131.83 ± 1.36	4.57	1.91	1.93
	Dipping	40.74 ± 0.78	323.59 ± 1.88	7.94	1.43	1.41
	Seeds	39.81 ± 0.96	512.86 ± 2.52	12.88	1.14	1.15
Marshal	Surface	72.44 ± 0.56	363.08 ± 1.24	5.01	1.83	1.85
	Dipping	14.79 ± 1.1	630.96 ± 3.16	42.66	0.79	0.8
	Seeds	102.33 ± 0.56	467.74 ± 1.46	4.57	1.91	1.9

RR: Resistance Ratio {LC 90/50}

When treating the larvae by dipping into Endosulfan® at the concentrations specified in Table 1, the results at 24 hours showed that the LC_{50} was 40.74±0.78, whereas the LC_{90} was 794.33±1.56 ppm. These results indicate that the population was heterogeneous to this insecticide (slope=0.98). The difference between the LC_{50} and LC_{90} values was high (RR'=19.5). The same concentrations were tested for 48 hours, and the obtained LC_{50} and LC_{90} values were 32.36±0.68 and 398.11±0.96 ppm respectively (Table 2). The slope was higher than that at 24 hours (1.17). These results agreed with that of the surface treatment (i.e. Endosulfan® requires at least two days to exert its effect on this pest homogeneously). These results also confirmed that Endosulfan® exerts its effect by contact, rather than any other way with low action, which in this test appears to be after 48 hours. The range between LC_{50} and LC_{90} proved to be narrower than that at 24 hours (RR'=12.3 for 48 hours compared to 19.5 for 24 hours).

When treating the larval food (Okra seeds) by dipping into Endosulfan® at the concentrations specified in Table 1, and exposing the larvae to the treated seeds for 24 hours, the results indicated that the LC_{50} amounted to 120.23±5.54 ppm, whereas the LC_{90} was 794.33±0.88 ppm. The population proved to be more homogenous to this treatment (slope=1.53). The range between the LC_{50} and LC_{90} values was narrow (RR'=6.61). When these concentrations were tested for 48 hours, the same LC_{90} (794.33±1.24 ppm), and lower LC_{50} (66.07±0.56 ppm) values were obtained (Table 2). The slope was even smaller than that at 24 hours (1.23). These results reflected that Endosulfan® in this treatment exerted its effect on this pest homogeneously at 24 hours, which proved that Endosulfan®'s effect is through stomach poison and contact with the treated seeds. Variation in the slope of Ldp lines indicated the widespread occurrence of a heterozygous population of *H. armigera* in this locality. The difference between the LC_{50} and LC_{90} was higher than that at 24 hours (RR'=12.02 for 48 hours compared to 6.61 for 24 hours).

The mean percent mortality of larvae of African bollworm, *H. armigera*, due to Endosulfan® treatment varied significantly between treatment methods and among the different times. Accordingly, Endosulfan® surfaces treated at 24 hours proved to be effective on this pest when compared with the dipping methods, as it recorded the lowest LC_{50} and LC_{90} values (22.39±0.78 and 524.81±0.76 ppm respectively (Table 1)). The results indicated that Endosulfan® might require at least two days to homogeneously exert its effect on this pest. Khurana (1999) reported that Endosulfan® was found to be most effective against early instars (2^{nd} and 4^{th}), but it was not effective against fully grown larvae. The present findings showed that Endosulfan® surface treatment is best when followed by treated seeds and larval dipping, which recorded the same LC_{90} (794.33±0.88 and 794.33±1.56 ppm) but different LC_{50} values (120.23±5.54 and 40.74±0.78 ppm respectively (Tables 1 and 2)). The results reflected that Endosulfan® treated seeds exerted their effect on this pest homogeneously at 24 hours, which proved that Endosulfan® mode of action is through stomach poisoning in addition to the contact effect.

The results also showed a significant effectiveness of Endosulfan® against *Helicoverpa sp*. It is clear from the results that Endosulfan® has varying degrees of effectiveness due to its various modes of action, but it is an active insecticide for the control of *H. armigera* through all its modes of action. Although its activity is more effective through topical application than stomach or contact actions, it can be concluded that if Endosulfan® is applied at the proper time and concentration and with suitable equipment, it would check the pest below ETL. However, Khalid *et. al.* (2001) stated that care should be taken for the management of resistance development in the pest (like the consecutive usage of a similar group of poison for a long period of time). n China, the resistance has

developed slower and the control efficacy was maintained longer when a mixture of three insecticides (Cyfluthrin, Endosulfan and Quinalphos) was used compared to a two insecticides mixture (Cyfluthrin and Endosulfan or Endosulfan and Quinalphos). Resistance develops rapidly when a single insecticide (Endosulfan) was used (Jiang and Liu, 1995).

Table 2. The Susceptibility of *Helicoverpa armigera* larvae fourth instar to four insecticides representing different chemical groups at 48 hr.

Insecticides	Bioassay methods	LC 50 (ppm)	LC 90 (ppm)	RR ratio (LC90/LC50)	Slope	Graphic slope
Endosufan	Surface	12.30 ± 0.56	109.65 ± 0.96	8.9	1.36	1.33
	Dipping	32.36 ± 0.68	398.11 ± 0.96	12.3	1.17	1.16
	Seeds	66.07 ± 0.56	794.33 ± 1.24	12.02	1.23	1.23
Danitol	Surface	2.82 ± 1.18	457.09 ± 3.42	162.18	0.58	0.57
	Dipping	3.11 ± 0.22	331.13 ± 2.78	106.64	0.63	0.62
	Seeds	102.33 ± 0.88	977.24 ± 0.78	9.55	1.31	1.3
Dursban	Surface	11.75 ± 0.56	89.13 ± 1.18	7.59	1.47	1.5
	Dipping	14.13 ± 0.62	151.36 ± 1.56	10.72	1.25	1.26
	Seeds	11.75 ± 0.68	151.36 ± 1.66	12.88	1.15	1.15
Marshal	Surface	37.15 ± 0.56	169.82 ± 0.78	4.57	1.91	1.9
	Dipping	9.55 ± 0.78	131.83 ± 1.56	13.8	1.13	1.14
	Seeds	91.20 ± 0.56	489.78 ± 1.52	5.37	1.75	1.75

Danitol® Larvae exposed to Danitol® treated surfaces of the Petri dish for 24 hours only, with the concentrations specified in Table 1, indicated that the LC_{50} was 12.59±1.18 ppm, whereas the LC_{90} was 812.83±3.24 ppm, which reflected the heterogeneity of the population to Danitol® (slope=0.7). The difference between the LC_{50} and LC_{90} values was big (RR'=64.57). The same concentrations were tested for 48 hours, and the LC_{50} and LC_{90} values obtained were 2.82±1.18 and 457.09±3.42 ppm respectively (Table 2). The slope was smaller than that at 24 hours (0.58). The difference between the LC_{50} and LC_{90} proved to be bigger than that at 24 hours (RR'=162.18 for 48 hours compared to 64.57 for 24 hours). The overall results concluded that Danitol® more effective after 48 hours than 24 hours of its application. The variation in the slopes indicate the widespread occurrence of a heterozygous population of the *H. armigera* strain in this locality. Therefore, diagnosis of the precise nature of the pyrethroid resistance mechanism is necessary.

When treating the larvae by dipping into the concentrations specified in Table 1, the results showed that at 24 hours the LC_{50} proved to be 9.55±1.7 ppm, whereas the LC_{90} was 1479.11±4.24 ppm. The results also indicated that the population was heterogeneous to Danitol® (slope=0.58). These results reflected that the range between the LC_{50} and LC_{90} values was wide (RR'=154.88). The same concentrations were tested for 48 hours and the obtained results showed that the LC_{50} and LC_{90} values were 3.11±0.22 and 331.13±2.78 ppm respectively (Table 2). The slope was higher than at 24 hours (0.63). These results could indicate that Danitol® shows high efficacy at 48 hours, as reflected in the values of RR' at 48 and 24 hours (106.64 compared to 154.88 respectively).

The present investigation clearly showed that the population of *H. armigera* was heterogeneous to Danitol® (slope=0.75) when treating the larvae food (Okra seeds) by dipping it into the concentrations specified in Table 1, and exposing the larvae to treated seeds. At 24 hours, the data indicated that the LC_{50} was 117.49±1.04 ppm, whereas the LC_{90} was 6309.57±3.28 ppm and an accordingly low level of pyrethroid resistance should be noted (RR'=53.7). The same concentrations were tested for 48 hours, and the LC_{50} and LC_{90} values obtained were 102.33±0.88 and 977.24±0.78 ppm respectively (Table 2). The slope was higher than that at 24 hours (1.31). Variation in the slopes indicated the widespread occurrence of a heterozygous population of *H. armigera*. Danitol® exhibited comparatively low mortality at 24 hours (LC_{50} 117.49±1.04 ppm). Much of the mortality occurred after 48 hours of their treatment (LC_{50} 102.33±0.88 ppm). Afterwards, there was only a marginal increase in the mortality. The results could indicate that Danitol® requires at least two days to exert its effect on this pest homogeneously. The difference between the LC_{50} and LC_{90} proved to be greater than that at 24 hours (RR'=9.55 for 48 hours compared to 53.7 for 24 hours).

Based on LC_{50} and LC_{90} values of Danitol® insecticide against the 4th instar larvae of *H. armigera*, the population was found to be most susceptible to the Danitol® treated surface in the first 24 hours, as it recorded the lowest LC_{90} (812.83±3.24 ppm), followed by Danitol® larvae dipping (1479.11±4.24 ppm (Table 1)), then Danitol® treated seeds, which recorded the highest LC_{90} (6309.57±3.28 ppm). Danitol® treated seeds recorded the highest slope and a lower RR' (0.75 and 53.7 respectively), followed by Danitol® surface treatment (0.7 and 64.57) and Danitol® larvae dipping (0.58 and 154.88 respectively). The 48 hour Danitol® larvae dipping recorded the lowest LC_{90} (331.13±2.78 ppm) followed by the Danitol® treated surface (457.09±3.42 ppm (Table 2)). The results obtained indicated that all Danitol® insecticide treatments exhibited the highest level of mortality within 48 hours. However, the 4th instar larvae of *H. armigera* were highly susceptible to Danitorl® treated surfaces and dipping treatments, possibly due to their continued mobility on the treated surface (the dose taken up was increased) or the direct exposure to the chemical

(dipping treatments). Danitol® treated seeds were less effective since Danitol® is a contact poison, rather than an oral poison.

Dursban® Tests were carried out by treating Dursban® insecticide on the surface of the Petri dish with the concentrations specified in Table 1, and exposing the 4th instar larvae to this surface for 24 hours. The results showed that the LC_{50} was 28.84±0.56 ppm, whereas the LC_{90} was 131.83±1.36 ppm. These results proved homogeneity of the population to Dursban® insecticide (slope=1.91). The difference between the LC_{50} and LC_{90} values was small (RR'=4.57). These results agreed with the work done by Zahid and Hamed (2003) who reported that Lorsban 40 EC showed maximum efficacy: 92% in terms of mortality after 24 hours. The same concentrations were tested for 48 hours, and the obtained results showed that the LC_{50} and LC_{90} were 11.75±0.56 and 89.13±1.18 ppm respectively (Table 2). The slope was smaller than that at 24 hours (1.47) and the RR' was bigger than that at 24 hours (7.59 compared to 4.57). The results indicated that at 24 hours, the LC_{90} is greater (131.83±1.36 ppm) than at 48 hours (89.13±1.18 ppm (Table 2)). It is concluded from the results obtained that Dursban® is more effective at 24 hours.

Regarding larval dipping in the Dursban® concentrations specified in Table 1, the results showed that at 24 hours, the LC_{50} was 40.74±0.78 ppm, whereas the LC_{90} was 323.59±1.88 ppm. The results proved that the population was heterogeneous to Dursban® insecticide (slope=1.43). The difference between the LC_{50} and LC_{90} values was big (RR'=7.94). The same concentrations were tested for 48 hours, and the obtained LC_{50} and LC_{90} values were 14.13±0.62 and 151.36±1.56 ppm respectively (Table 2). In spite of this, the slope was smaller than that at 24 hours (1.25) and the difference between the LC_{50} and LC_{90} proved to be bigger than at 24 hours (RR'=10.72 for 48 hours compared to 7.94 for 24 hours). However, Dursban® requires at least two days to exert its effect on this pest with this mode of treatment.

Dose-mortality regressions were estimated for Dursban® by dipping okra seeds into the concentrations specified in Table 1 and exposing the larvae to these treated seeds for 24 and 48 hours. The LC_{50} obtained at 24 hours was 39.82±0.96 ppm, whereas the LC_{90} was 512.86±2.52 ppm. The results indicated that the population was heterogeneous to Dursban® (slope=1.14 and RR'=12.88). Regarding 48 hours, the same concentrations were tested and the obtained LC_{50} and LC_{90} values were 11.75±0.68 and 151.36±1.66 ppm respectively (Table 2). The slope was approximately equal to that at 24 hours (1.15). The resistance ratio proved to be the same as that at 24 hours (RR'=12.88). However, the 4th instar larvae were more susceptible to Dursban® if exposed to the treated seeds for 48 hours (lower LC_{50} and LC_{90}). Therefore, Dursban® insecticide requires at least two days to exert its effects.

Comparative effectiveness of Dursban® on *H. armigera* collected from the fields and reared in the laboratory showed that treating the surface proved to be the most effective method at both 24 and 48 hours, and 4th instar larvae were found to be more susceptible compared with the dipping methods (Tables 1 and 2). Surface treatment at 24 hours recorded the lowest LC_{50} and LC_{90}, the highest slope, and a lower RR', followed by larvae dipping and then seed treatment. At 48 hours the tested population exerted high heterogeneity to the seed treatment. The same LC_{50} was obtained as that in surface treatment, 11.75±0.68 and 11.75±0.56 ppm respectively (Table 2). Also, seed treatment recorded the same LC_{90} as the dipping of the larvae, 151.36±1.66 and 151.36±1.56 ppm respectively (Table 2). The treated surface at 48 hours recorded the highest slope and a lower RR' (1.47 and 7.59 respectively). That means the 4th instar larvae were highly susceptible to surface and dipping treatments, possibly due to the continued mobility on treated surfaces (the dose taken up was increased) or the direct exposure to the chemical. The seed treatment was less effective since Dursban® is a contact chemical rather than oral. The results indicated that the organophosphorus insecticide (Dursban®) was less tolerated by 4th instar larvae of *H. armigera*. This agreed with the result obtained by Indira (2004), who reported that insecticide resistance to Chlorpyriphos (Dursban®) was low to moderate in the majority of *H. armigera* strains tested in India. Therefore, diagnosis of the precise nature of organophosphate resistance is necessary, along with regular monitoring, for future help in understanding the status of *H. armigera* in Sudan.

Marshal® The differential susceptibility of the 4th instar larvae of *H. armigera* populations by treating the surface of the Petri dish with Marshal® insecticide at concentrations specified in Tables 1 and 2 and exposing the larvae to this surface for 24 and 48 hours was investigated. Based on percent mortality the LC_{50} at 24 hours was 72.44±0.56 ppm, whereas the LC_{90} was 363.08±1.24 ppm. The population was found to be heterogeneous to Marshal® (slope=1.83 and RR'=5.01). The results indicated that the 4th instar larvae were found to be less susceptible compared to the same concentrations when tested for 48 hours, since the obtained LC_{50} and LC_{90} values were 37.15±0.56 and 169.82±0.78 ppm respectively (Tables 1 and 2). The slope is greater than that at 24 hours (1.91), while the RR' proved to be smaller than that of 24 hours (4.57). The results indicated that Marshal® requires at least two days to exert its effect homogeneously on this pest. The excessive use of insecticides had led to problems of insecticide resistance in field population of *H. armigera* due to the absence of baseline susceptibility

data and continued use of insecticides even after their effectiveness has declined considerably. The findings of the present investigations clearly showed that regular monitoring will help in understanding the status of *H. armigera* in Sudan.

When treating the larva with Marshal® by dipping into the concentrations specified in Tables 1 and 2 at 24 and 48 hours, the results at 24 hours showed that the LC_{50} proved to be 14.79±1.1 ppm, whereas the LC_{90} was 630.96±3.16 ppm. The results indicated that the population was heterogeneous to Marshal® (slope=0.79). The difference between the LC_{50} and LC_{90} was big (RR'=42.66). The same concentrations were tested for 48 hours and the obtained results showed that the LC_{50} and LC_{90} were 9.55±0.78 and 131.83±1.56 ppm respectively (Table 2). The slope was higher than that at 24 hours (1.13). Variations in the slopes indicated the widespread occurrence of a heterozygous population of *H. armigera* (Gezira strain). This might indicate that Marshal® requires at least two days to exert its effect on this pest. The difference between the LC_{50} and LC_{90} proved to be smaller than that at 24 hours (RR'=13.8 for 48 hours compared to 42.66 for 24 hours). The findings indicated that the susceptibility of the African bollworm to Marshal® was not appreciably altered from 24 to 48 hours, although fluctuations during the two days were observed.

When okra seeds were treated with Marshal® by dipping into the concentrations specified in Table 1 and 2, and the larvae were allowed to feed on these treated seeds for 24 and 48 hours, the obtained results at 24 hours showed that the LC_{50} was 102.33±0.56 ppm, whereas the LC_{90} was 467.74±1.46 ppm. The findings indicated that the population was homogeneous to this insecticide (slope=1.91). The range between the LC_{50} and LC_{90} values was narrow (RR'=4.57). The same concentrations were tested for 48 hours and the LC_{50} and LC_{90} were 91.20±0.56 and 489.78±1.52 ppm respectively (Table 2). The LC_{90} at 48 hours was greater than LC_{90} at 24 hours. The slope was also smaller than that at 24 hours (1.75). This might indicate that Marshal® exerted more effect at 24 hours on this pest than at 48 hours. However, there were no substantial changes in susceptibility until 48 hours. The range between the LC_{50} and LC_{90} proved to be almost the same (RR'=5.37 for 48 hours compared to 4.57 for 24 hours).

Mean percent mortality of 4th instar larvae of *H. armigera* varied significantly in all the Marshal® treatment methods between the different times. The findings indicated that dipping the larvae proved to be the most effective method in controlling this pest, at both 24 and 48 hours, as it recorded the lowest LC_{50} values (14.79±1.1 and 9.55±0.78 ppm respectively (Tables 1 and 2)) compared to surface treatment (72.44±0.56 and 37.15±0.56 ppm) and seed treatment (102.33±0.56 and 91.20±0.56 ppm respectively). The findings agreed with Khalid *et. al.* (2001) who stated that under field conditions insecticides with contact action are largely chosen for the management of lepidoptrous insects as they are well exposed to the spray particles. These results indicated an increase in the homogeneity of the 4th instar larvae of *H. armigera* towards the larvae dipping and surface treatment methods through time. That means, the larvae were highly susceptible to these treatments due to the continued mobility on the contaminated surface (the dose taken up was increased) or the direct exposure to the chemical. Seed treatment was less effective, as the chemical was a contact rather than an oral poison. Our present study also proved the topical effectiveness of Marshal®.

Various insecticides belonging to different classes are used for management of this pest all over the world. Mean percent mortality of 4th instar larvae of African bollworm, *H. armigera*, varied between all the insecticides tested (*viz.* Endosulfan®, Marshal®, Danitol® and Dursban®), among the different bioassay methods and time intervals. The results indicated the susceptibility of the African bollworm to these insecticides based on the LC_{50} values obtained in the present investigation at 24 hours. The LC_{50} values of the tested insecticides using surface (contact) treatment varied from 12.59±1.18 to 72.44±0.56 ppm (Table 1), with the lowest value for Danitol® and the highest value for Marshal®. The LC_{50} values at 48 hours of the tested insecticides using surface (contact) treatment varied from 2.82±1.18 ppm to 37.15±0.56 ppm (Table 2), with the lowest value for Danitol® and the highest value for Marshal®, as at 24 hours. The overall results concluded that insecticides concerning Danitol®, Endosulfan®, and Dursban® in particular proved better at 24 hours, and the same trend continued at 48 hours of their application. Comparatively Marshal® shows low efficacy in killing the insect. Although the results indicated that the surface treatment by Marshal® proved the homogeneity of the population tested, the Danitol® treatment showed high heterogeneity to the tested population. However, findings of most researchers (Singh *et. al.* 1987; Sherma *et. al.* 1989 and Lohar and Jumo 1995) who tested different pyrethroids against the *H. armigera* in the field found it as an effective insecticide. Similar findings were obtained in the present study, confirming that the pyrethroid group chemicals are very effective, as compared to Endosulfan® and other insecticides tested.

The LC_{50} values of the tested insecticides using the larval dipping (topical application) at 24 hours varied from 9.55±1.17 to 40.74±0.78 ppm (Table 1), with the lowest value for Danitol® and the highest value for Endosulfan® and Dursban®. At 48 hours the LC_{50} values of the tested insecticides varied from 3.11±0.22 to 32.36±0.68 ppm (Table 2), with the lowest value for

Danitol® and the highest value for Endosulfan®. The results indicated that susceptibility of the 4th instar larvae to these insecticides was not appreciably altered from 24 to 48 hours, although fluctuations were observed (Tables 1 and 2). Moreover, there were no substantial changes in susceptibility to Danitol®. Among the different insecticides tested, the susceptibility was high for Danitol®, followed by Marshal®, Dursban®, and then Endosulfan®.

Results obtained after 24 and 48 hours by giving treated seeds to 4th instar larvae of *H. armigera* in the laboratory showed differences in the LC_{50} values between the insecticides used (*viz.* Endosulfan®, Marshal®, Danitol® and Dursban®). At 24 hours the results indicated that susceptibility of the 4th instar larvae to these insecticides based on the LC_{50} values ranged from 39.81±0.96 to 120.23±5.54 ppm (Table 1), with the lowest value for Dursban® and the highest value for Endosulfan®. At 48 hours, the LC_{50} values of the tested insecticides varied from 11.75±0.68 to 102.33±0.88 ppm (Table 2), with the lowest value for Dursban® and the highest value for Danitol®. The results reflect that Dursban® in seed treatment exerts its effect at 24 and 48 hours on this pest much better compared to the other insecticides tested. This proved that Dursban® exerts its effect by stomach poison, in addition to its contact effect.

Based on the obtained LC_{90} values of the tested insecticides, Danitol® was the least effective chemical as it recorded maximum LC_{90} values in surface treatment at 24 and 48 hours (812.83±3.24 and 457.09±3.42 ppm respectively). In contrast, Dursban® recorded the lowest LC_{90} values (131.83±1.36 and 89.13±1.18 ppm respectively (Tables 1 and 2)). Regarding the dipping bioassay method of the 4th instar larvae, the results obtained at 24 hours showed that LC_{90} values varied from 323.59±1.88 to 1479.11±4.24 ppm respectively (Table 1), with the lowest value for Dursban® and the highest for Danitol®. The results obtained at 48 hours indicated that the lowest LC_{90} values were obtained in Marshal® and Dursban® treatments (131.83±1.56 and 151.36±1.56 ppm respectively (Table 2)), and the highest LC_{90} values were obtained in Danitol® and Endosulfan® treatments (331.13±2.78 and 398.11±0.96 ppm respectively (Table 2)). These results indicate that Dursban® was the most effective chemical when the 4th instar larvae of the African bollworm were directly exposed to the chemical, or came into contact with the treated surface at 24 and 48 hours. The susceptibility of the 4th instar larvae of *H. armigera* to insecticides used by giving treated seed to larvae, based on the LC_{90} values at 24 and 48 hours, demonstrated that Marshal® was the most effective as it recorded the lowest LC_{90} at 24 hours (467.74±1.46 ppm (Table 1)). Danitol® appeared less effective, because of its high LC_{90} value (6309.57±3.28 ppm (Table 1)). Results at 48 hours supported the results at 24 hours as Danitol® remained the least effective chemical, having the highest LC_{90} value recorded (977.24±0.78 ppm (Table 2)). The lowest LC_{90} value recorded was Dursban® (151.36±1.66 ppm (Tables 1 and 2)).

CONCLUSIONS and RECOMMENDATIONS.

Chlorpyrifos (Dursban®) performance was the best among the chemicals tested, compared with carbosulfan (Marshal®), endosulfan (Endosulfan®) and fenpropathrin (Danitol®) (lowest LC_{90} in surface and seed). Consequently, a spraying technique that guarantees good and homogeneous plant coverage is recommended.

The cyclodiene endosulfan exerted the second best kill, in the case of surface treatments. Therefore, good and homogeneous coverage of the treated crop must be applied. Carbosulfan obtained the best results when the larval dipping technique and larval food (okra seed) treatment at 48 hours were adopted, compared to other bioassays chemicals. It should therefore be applied as an EC, so as to deposit more spray volume on the larval cuticle and the larval food (the crop). Fenpropathrin exerted its contact effect via the surface exposure and larval dipping routes of administration only (the lowest LC_{50} in surface and dipping). A high level of hydrolases, *viz.* esterases, might be the reason pyrethroids must be potentiated by AchE- inhibitors, e.g. Ops or carbamates.

Furthermore, research on the monitoring and management of insecticides resistance in *H. armigera* in Sudan needs to get started. Diagnosis of the precise nature of organophosphate, carbamate, and pyrethroid resistance mechanisms is necessary. This, as well as regular monitoring, will help to understand the status of *H. armigera* in the Sudan in the future.

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Resistance to acetolactate synthase (ALS) inhibiting herbicides in UK populations of *Alopecurus myosuroides* (black-grass)

This is a summary of a PhD project (Marshall, 2007); further information is available from the author.

INTRODUCTION. The objective of this project was to characterise resistance to sulfonylurea herbicides in UK populations of the grass-weed *Alopecurus myosuroides* Huds. Investigations included field studies, glasshouse bioassays, biochemical and molecular analyses. Responses to the selective sulfonylurea herbicides mesosulfuron-methyl + iodosulfuron-methyl sodium mixture and flupyrsulfuron-methyl were investigated, while extensive use was made of the non-selective sulfonylurea herbicide sulfometuron-methyl at a rate of 100g a.i. ha^{-1} as a screening test for possible ALS target site resistance in *A. myosuroides* based on work done with *Lolium rigidum* in Australia.

MATERIALS and METHODS. Four biotypes of *A. myosuroides* were used for the initial whole plant and enzyme work: (a) Roth03, a susceptible standard (b) Pel96, an enhanced metabolism standard population from Peldon in Essex which shows cross resistance to a range of different herbicides (c) Pel02, a population from Peldon collected in 1992 which shows around 15% highly resistant plants following treatment with 100g a.i. ha^{-1} sulfometuron-methyl and (d) PelRES, a sulfometuron selected line from Peldon containing around 40% highly resistant plants in sulfometuron screening tests.

The four standard populations were subjected to dose response tests and ALS enzyme assays with the herbicides flupyrsulfuron-methyl and sulfometuron-methyl. Dose response assays included 16 reps (plants) per dose and eight doses of each herbicide. Plants were sprayed at the three leaf stage and foliage weights were recorded four weeks after spraying. Plant material for ALS assay was grown to a 6 tiller stage and harvested in 50g batches then immediately extracted and assayed (Singh et al., 1988). A screening test was developed from dose response data and used 100g a.i. ha^{-1} of sulfometuron-methyl to discriminate between highly resistant plants likely to possess ALS target site changes and susceptible or partially resistant plants.

Sulfometuron screening was performed for 9 populations collected from field sites around the UK where resistance to mesosulfuron-methyl + iodosulfuron-methyl sodium was observed in the field. These were as follows: Pel02, Cock06 (Essex); Chal05, Thame05 (Oxfordshire); East06 (Lincolnshire); Key06, R30-06 (Cambridgeshire); Maid05, Wilts04 (Berkshire). Between 42 and 200 plants from each population were grown to the 3 leaf stage. Leaf samples were taken from each individual and stored at -80°C. All plants were then sprayed with sulfometuron at 100g a.i. ha^{-1} and assessed for damage after four weeks. Genomic DNA from leaf material of highly resistant and completely susceptible plants was extracted using a Qiagen kit and primers were designed to span the five conserved domains of the ALS gene in two parts; the F10 (AAGGGCGC(G/C)GACATCCT), R1 (ATCTGCTG(C/T)TGGATGTCCTT) combination for PCR amplification of Doms C, A and D and the F3 (TGGTAGCTTCCTCATGAACATT), R10 ((A/G)TCCTGCCATCACC(T/A)TCCA) primer combination for Doms B and E. PCR reactions were carried out according to a standard method (Prado et al., 2004). Direct sequencing of PCR products was performed using a BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, USA) and an ABI Prism 3100 Genetic Analyser. In addition to the direct sequencing work, PCR products from two highly resistant Pel02 plants were cloned and sequenced using a TOPO TA Cloning Kit (Invitrogen)

RESULTS and DISCUSSION. Initial glasshouse tests identified that resistance to flupyrsulfuron was widespread in UK populations of *A. myosuroides*, while resistance to both mesosulfuron-methyl + iodosulfuron-methyl sodium mixture and sulfometuron-methyl was identified in only 9 populations. All 9 populations contained a proportion of plants which survived treatment with sulfometuron-methyl at the screening dose of 100g a.i. ha^{-1}. DNA sequencing of the ALS gene confirmed that a single amino acid substitution at position Pro197 was found to segregate with high level resistance to sulfometuron-methyl in 7 of the 9 populations tested. These were Pel02, Chal05, Cock06, East06, Key06, Maid05 and Thame05. In total 17 polymorphic sites were identified in the F10/R1 sequenced region, with 14 synonymous changes and 3 non-synonymous, at Ala135, Pro197 and Pro232, respectively. No segregation with sulfometuron resistance was observed for the Ala135 or Pro232 polymorphisms with most plants appearing heterozygous regardless of phenotype. All highly resistant individuals from the 7 populations appeared heterozygous for a Pro-197-Thr substitution, while susceptible individuals appeared homozygous for Pro. Cloning of resistant Pel02 individuals showed two alleles were present, with each plant containing one copy of both Pro and Thr alleles. No amino acid substitutions were found to segregate with resistance in the 330 bp sequenced region spanning Domains B and E in any of the populations tested. Two populations, Wilts04 and R30-06, showed very high levels of sulfometuron resistance but no evidence of any polymorphism segregating with resistance. Further work is required to determine the mechanism of resistance in these biotypes. Enzyme inhibition assays with sulfometuron confirmed that resistance was due to an altered form of the ALS enzyme less susceptible to inhibition by sulfonylurea herbicides in the Peldon population. Comparison of I_{50} values showed a 16-fold difference in enzyme sensitivity between the most resistant sulfometuron selected Peldon line and a susceptible standard. This is one of the first cases of ALS target site resistance in a European grass-weed which has been characterised at both molecular and enzyme levels. Results from segregation of sulfonylurea resistant and susceptible phenotypes in crossing experiments indicated that ALS target site resistance in *A. myosuroides* is conferred by a single, dominant nuclear allele but that additional effects are also present. Currently resistance to mesosulfuron-methyl + iodosulfuron-methyl sodium had been confirmed in *A. myosuroides* from 81 farms in the UK, based on glasshouse pot assays of seed samples sent to Rothamsted from sites where reduced control was observed in the field. The precise mechanism of resistance in the majority of these samples is not known. Further work is currently underway investigating the molecular basis of resistance in these populations in order to gain a better understanding of the frequency of ALS target site resistance compared to other mechanisms. ALS target site resistant *A. myosuroides* is predicted to increase with more widespread use of mesosulfuron-methyl + iodosulfuron-methyl sodium in coming years.

SUMMARY

- A single point mutation conferring a predicted Pro-197-Thr ALS enzyme target site substitution was found to segregate with resistance to the non-selective SU herbicide sulfometuron-methyl in 7 out of 9 mesosulfuron + iodosulfuron-methyl sodium resistant *A. myosuroides* populations. The Pro-197-Thr substitution was present in all resistant individuals but was not present in any susceptible individuals. No other mutations segregating with resistance were found across all five conserved domains of the ALS gene.
- The same Pro-197-Thr mutation was found in highly resistant plants from 7 separate geographical locations in the UK. All biotypes showed varying proportions of plants highly resistant to sulfometuron at the screening dose of

100g a.i. ha^{-1} and the high level resistant trait was associated with Pro197 substitution in all cases.

- These resistant *A. myosuroides* biotypes represent the first examples of confirmed ALS target site resistance in a European grass weed where the molecular basis of the resistance has been characterised, and only the fourth confirmed case in grass weeds world wide (Park & Mallory-Smith, 2004, Kaundun et al., 2006, Laplante, 2006).
- Based on crossing experiments the genetic basis of ALS target site resistance in the Peldon biotype seems to be as a single gene dominant trait and this is consistent with results from other species.
- Work on the evolution of target site resistance at Peldon shows that the trait can build up relatively quickly with continuing flupyrsulfuron treatment, even though enhanced metabolism provides a resistance mechanism which allows plants not possessing ALS target site resistance to survive. Pre-selection with the ALS inhibitor chlorsulfuron is a risk factor in the development of ALS target site resistance. Given the relative efficacy of mesosulfuron-methyl + iodosulfuron-methyl sodium on enhanced metabolism populations compared to flupyrsulfuron, it is expected that the build up of ALS target site resistance will not be slowed by the widespread adoption of this herbicide.
- Sulfometuron at 100g a.i. ha^{-1} has potential as an indicator of target site resistance in *A. myosuroides* populations. Sulfometuron has demonstrated the ability to overcome enhanced metabolism type resistance in the Peldon biotype.
- The presence of the Pro197 substitution in sulfonylurea resistant biotypes is consistent with data from other weed species and is the most commonly found mutation conferring resistance to sulfonylurea herbicides. Mutations at this position are known to confer high levels of resistance to sulfonylureas but not to imidazolinones in most cases.

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Insecticide susceptibility of field-collected *Plutella xylostella* from Virginia

INTRODUCTION. The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a major insect pest of crucifer crops worldwide (Talekar and Shelton 1993). Some populations of the pest have developed resistance to almost every class of insecticide used against it (Diaz-Gomez et al. 2000, Shelton et al. 2000). For instance, Georghiou (1981) reported *P. xylostella* resistance to 36 insecticides in 14 countries. In North America, some populations of *P. xylostella* were reported to be more than 100 fold resistant to the pyrethroid, permethrin, the carbamates, and methomyl, and more than 400 fold to *Bacillus thuringiensis* subsp. *kurstaki* (Shelton and Wyman 1990, Shelton et al. 1993a). Insecticide resistance patterns in *P. xylostella* populations appear to be localized geographically (Tabashnik et al. 1987, Shelton and Wyman 1990, Shelton et al. 2000).

The levels and types of insecticide resistance in *P. xylostella* populations from Virginia are not currently known. Historically, control measures for *P. xylostella* and other pests in commercial cabbage, broccoli, and collards in Virginia have involved multiple applications of insecticides with little regard for pest population level (Lasota and Kok 1986). In the 1980's, the frequently-used insecticides on crucifer crops included the organophosphate, methamidophos, the carbamates, methomyl, the pyrethroids, fenvalerate and permethrin, and *B. thuringiensis* (Lasota and Kok 1986). Since the mid-1990's, crucifer growers have incorporated several newer lepidopteran-targeted insecticides into their spray programs including spinosad, emamectin benzoate, indoxacarb, and methoxyfenozide (Kuhar et al. 2006). Resistance to each of these compounds has been reported in isolated populations of *P. xylostella* from around the world (Iqbal and Wright 1997, Sayyed and Wright 2004, Zhao et al. 2002, 2006). The purpose of this study was to assess the current susceptibility of a *P. xylostella* field population from Virginia to some of the traditional insecticides as well as some of the newer

insecticide chemistries and compare these susceptibility levels with those of a known susceptible strain of *P. xylostella*.

MATERIALS AND METHODS

***Plutella xylostella* field population.** Several hundred larvae and pupae of *P. xylostella* were collected weekly during July and August of 2003 and 2004 from collards (*Brassica oleracea* L. acephala group, variety 'Vates') located on the Eastern Shore of Virginia, the major commercial vegetable region in the state. Larvae and pupae were initially quarantined in separate containers to remove any parasitized individuals, and then all unparasitized *P. xylostella* pupae were used to initiate a laboratory colony. Insects were maintained on potted collard plants inside of screen cages (24 x 24 x 24 cm) in a rearing room at 27 ± 3°C, 40 to 70% RH, and a photoperiod of 14:10 (L:D). *Plutella xylostella* adults were fed with 10% sugar solution in distilled water. Bioassays were conducted using 2nd instars of the first laboratory generation of *P. xylostella*.

***Plutella xylostella* susceptible population.** An insecticide-susceptible *P. xylostella* colony (>80 generations) was acquired from Benzon Research® (Carlisle, PA) and reared on artificial diet number F9441B (Bioserv Inc., Frenchtown, NJ) at 27 ± 2°C, 40 to 70% RH, and a photoperiod of 14:10 (L:D). As with the field-collected population of *P. xylostella,* all bioassays were conducted using 2nd instars.

Table 1. Insecticides tested in laboratory toxicity assays conducted on a field-collected population of *Plutella xylostella* from the Eastern Shore of Virginia and a susceptible strain of *P. xylostella*, 2003 and 2004.

Insecticide (ai)	Product name (manufacturer)	Insecticide Class	Recommended field application rate (kg [ai]/ha)
Acephate	Orthene 97 (Valent BioScience Corp. Libertyville, IL)	Organophosphate	1.087
Acetamiprid	Assail 70WP (Cerexagri, Inc., King of Prusia, PA)	Neonicotinoid	0.084
Azadirachtin	Neemix 4.5EC (Certis USA L.L.C., Columbia, MD)	Botanical – Neem extract	0.011
Bacillus thuringiensis subsp. *kurstaki* strain HD-1	DiPel DF (Valent BioScience Corp. Libertyville, IL)	Microbial	0.605
Emamectin benzoate	Proclaim 5WDG (Syngenta Crop Protection Inc., Greensboro, NC)	Avermectin	0.008
Esfenvalerate	Asana XL (E. I. du Pont de Nemours and Co., Wilmington, DE)	Pyrethroid	0.032
Indoxacarb	Avaunt 30WG (E. I. du Pont de Nemours and Co., Wilmington, DE)	Pyrazoline	0.072
Methomyl	Lannate LV (E. I. du Pont de Nemours and Co., Wilmington, DE)	Carbamate	0.504
Methoxyfenozide	Intrepid 2F (Dow AgroSciences LLC, Indianapolis, IN)	Insect growth regulator	0.112
Novaluron	Rimon 0.83EC (Crompton Corporation, Middlebury, CT)	Insect growth regulator	0.087
Spinosad	SpinTor 2SC (Dow AgroSciences LLC, Indianapolis, IN)	Spinosyn	0.026

Insecticides. Eleven different commercial insecticides were assayed using serial dilutions of the lowest recommended field application rate on crucifer crops (Table 1). Insecticides were diluted in a volume of distilled water proportional to a typical field spray volume of 355 liters per ha. Four to eight concentrations of each insecticide were prepared in serial dilutions including a control of distilled water. To improve uniform coverage over the leaf surface, a spreader-sticker, Latron B-1956® (Loveland Industries Inc., Greeley, CO), was added to each insecticide solution and the control at a concentration of 0.25% vol:vol.

Toxicity bioassays. For each insecticide, bioassay experiments were replicated a minimum of four times and a maximum of seven times depending upon the number of *P. xylostella* larvae available at the time of assaying. The toxicity bioassay utilized a leaf dip method similar to that used by Shelton et al. (1993a, 1993b, 2000). Leaf disks of 8.5 cm diameter were cut from the outer layers of cabbage heads (not including wrapper leaves). Leaf disks were dipped for 10 seconds in each concentration, held vertically to allow excess solution to drip off, and placed in a drying rack in a fume exhaust hood to air dry for 2 hours. Leaf disks were then placed individually into 9 cm diameter plastic Petri dishes along with approximately 10 *P. xylostella* 2nd instars. Petri dishes were maintained at 27 ± 2°C, 40 to 70% RH, and a photoperiod of 14:10 (L:D). Mortality of the 2nd instars was determined after 48 hours of exposure for esfenvalerate, indoxacarb, spinosad, methomyl, acephate, and emamectin benzoate; 72 hours for acetamiprid, *B. thuringiensis*, novaluron, and methoxyfenozide; and 96 hours for azadirachtin. Larvae were considered dead if they did not move when prodded.

Statistical Analyses. The dose-mortality for each insecticide concentration was estimated using probit analysis in Polo Plus (LeOra Software 2002). Control mortality, which averaged 9.2% (range 0 - 14%), was corrected for by using Abbott's formula (Abbott 1925) for each probit analysis. The LC_{50} and the corresponding 95% fiducial limits (FL) were estimated for each insecticide tested on *P. xylostella* populations. The LC_{50} and the corresponding 95% FL are the criteria used to compare insecticide susceptibilities between *P. xylostella* populations (Tabashnik et al. 1990, Kobayashi et al. 1992, Shelton et al. 1993a, Zhao et al. 2002, Liu et al. 2003). The response curves of two populations to a particular insecticide were considered different if their corresponding 95% FL did not overlap (Shelton et al. 1993b). When the response to one insecticide was different, the tolerance ratio (TR) was calculated by dividing the LC_{50} of the field population by the corresponding LC_{50} of the

susceptible population. The more neutral term 'TR' was used rather than 'RR' (Resistance Ratio) because of the latter's potentially unfounded implications (Shelton et al. 2000).

RESULTS AND DISCUSSION

Based on non-overlap of the 95% FL of the $LC_{50,}$ significant differences in toxicity response to esfenvalerate, acetamiprid, indoxacarb, methoxyfenozide, methomyl, and acephate were found between the Eastern Shore of Virginia population and the susceptible population of *P. xylostella* (Table 2). The largest difference in toxicity occurred with esfenvalerate, where the LC_{50} of the field population was 15.01 mg AI/liter compared with 0.008 mg AI/liter for the susceptible population, representing a tolerance ratio of 1876. High tolerance ratios to pyrethroids have also been found in many other populations of *P. xylostella* in the U.S. (Shelton et al. 1993a, Liu et al. 2003), and it has been suggested that past selection by older insecticides such as DDT, cyclodienes, and organophosphates have caused some cross-resistance to pyrethroids (Tabashnik et al. 1987).

For acetamiprid, the LC_{50} of our *P. xylostella* field population was 131.54 mg AI/liter compared with 0.94 mg AI/liter for the susceptible population, a tolerance ratio of 139. This was surprising because acetamiprid has not yet been registered on crucifer crops in the U.S. Also, selection pressure to the neonicotinoid class of insecticides should be extremely low in *P. xylostella* because they are a relatively new class of pesticide, are typically not applied for control of lepidopteran pests, and have not been used often on crucifer crops in Virginia. However, Tomizawa and Casida (2003) suggest that cross-resistance between neonicotinoids and nicotinoids may be associated with evolutionary selection for nicotine tolerance, over expression of *CYP450* oxidative enzymes, and other mechanisms.

The tolerance ratios for methomyl, methoxyfenozide, indoxacarb, and acephate in our field population were 32, 26, 19, and 8, respectively. Shelton et al. (1993a) suggest that because no standards define problematic resistance levels for diamondback moth, resistance ratios close to 10 cannot be attributed to the insecticide toxicity itself, but may be the impact of other factors such as variability in field population response to insecticides or experimental procedures. Resistance to carbamates and organophosphates is very common in *P. xylostella* populations around the world (Shelton et al. 1993a). The levels of tolerance to methomyl (32x) and acephate (8x) exhibited by the Eastern Shore population of *P. xylostella,* suggest a conservative use of these insecticides to avoid increase of resistance by *P. xylostella* in the future.

No signs of tolerance in *P. xylostella* were found to *B. thuringiensis,* novaluron, azadirachtin, emamectin benzoate, and spinosad. In Virginia, these insecticides currently are highly efficacious in the field (Cordero et al. 2006), appear to be excellent insecticide resistance management tools for *P. xylostella*, and are all IPM-compatible products with reduced impact on natural enemies (Cordero et al. 2007). However, *P. xylostella* has developed resistance to some of these (or similar) insecticides in other regions. For instance, toxicity ratios of 1641 and 1125 for *B. thuringiensis* products were reported by Shelton et al. (1993b) and Perez and Shelton (1996). Iqbal and Wright (1997) reported toxicity ratios of 357 for teflubenzuron and 190 for abamectin. Sayyed and Wright (2004) reported toxicity ratios of 1170 to spinosad and 2840 to *B. thuringiensis*. A toxicity ratio of 6422 for spinosad in Hawaii after ~2.5 years of use of this insecticide was reported by Zhao et al. (2002). Because *P. xylostella* has already shown the ability to develop resistance to some of these novel insecticides, continued monitoring of *P. xylostella* field populations to insecticide susceptibility should be done, particularly in those instances when field failures have been reported.

Table 2. Laboratory susceptibility to insecticides of 2nd instar *Plutella xylostella* collected from a field population at the Eastern Shore, Virginia and a known susceptible population.

Population	Insecticide	n	LC_{50}[a]	95% FL[a]	Slope ± SE	χ^2 (df)	TR[b]
Painter	esfenvalerate	250	15.009	2.203-44.211	1.18 ± 0.17	6.99 (3)	1876
Susceptible	esfenvalerate	200	0.008	0.001-0.020	0.76 ± 0.15	0.63 (3)	
Painter	acetamiprid	320	131.53	70.346-191.394	1.45 ± 0.31	2.16 (4)	139
Susceptible	acetamiprid	200	0.944	0.131-7.828	0.30 ± 0.09	0.91 (3)	
Painter	methomyl	250	621.14	464.536-770.36	3.05 ± 0.63	2.07 (4)	32
Susceptible	methomyl	250	19.159	5.631-111.227	0.43 ± 0.10	0.88 (3)	
Painter	methoxyfenozide	300	144.12	75.212-230.805	1.28 ± 0.23	1.21 (4)	26
Susceptible	methoxyfenozide	200	5.414	1.976-20.565	0.58 ± 0.11	1.01 (3)	
Painter	indoxacarb	390	4.563	1.354-10.572	1.32 ± 0.13	21.46 (6)	19
Susceptible	indoxacarb	240	0.235	0.074-0.660	0.51 ± 0.08	0.15 (3)	
Painter	acephate	300	133.94	67.417-201.815	1.91 ± 0.27	6.22 (5)	8
Susceptible	acephate	200	16.708	8.583-34.526	0.73 ± 0.11	1.61 (3)	
Painter	*B. thuringiensis*	720	1.976	0.330-7.499	0.98 ± 0.08	13.84 (3)	
Susceptible	*B. thuringiensis*	380	0.613	0.137-1.629	0.43 ± 0.07	0.39 (3)	
Painter	novaluron	200	2.098	0.581-10.934	0.41 ± 0.10	1.92 (3)	
Susceptible	novaluron	800	1.142	0.003-6.614	0.57 ± 0.06	30.71 (6)	
Painter	azadirachtin	220	22.353	12.410-30.545	2.57 ± 0.88	0.42 (3)	
Susceptible	azadirachtin	360	9.826	1.836-26.497	0.98 ± 0.19	8.26 (5)	
Painter	emamectin benzoate	680	0.018	0.003-0.050	0.86 ± 0.08	19.94 (5)	
Susceptible	emamectin benzoate	200	0.009	0.002-0.018	1.08 ± 0.23	1.25 (3)	
Painter	spinosad	510	0.213	0.079-0.429	1.13 ± 0.13	8.11 (5)	
Susceptible	spinosad	240	0.022	0.001-0.112	0.73 ± 0.11	4.85 (3)	

[a] mg AI/l

[b] TR, tolerance ratio = LC_{50} of field population-Painter, VA/ LC_{50} of susceptible population

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Research in Resistance Management

Transgenic peas (*Pisum sativum* L.) to control the pea weevil (*Bruchus pisorum* L.)

INTRODUCTION

Transgenic plants now provide a new alternative to the use of chemical pesticides for controlling insect pests, and one approach is to introduce protein inhibitors into crop species (Gatehouse et al. 1992, Gatehouse and Gatehouse 1997, Gatehouse 1999).

The α-amylase inhibitor (α-AI), a seed protein that is toxic to insect larvae, is a naturally occurring defence mechanism present in some leguminous seeds (Fory et al. 1996). Shade et al. (1994) showed that seeds of common bean (*Phaseolus vulgaris* L.) are resistant to some bruchid beetles, mainly because of the presence of α-AI. Their larvae develop inside the seed, eat the cotyledons and reduce the yield and quality of the seeds (Janzen 1981, Clement et al. 1999). Most bruchids are highly specialised and their larvae may have the ability to detoxify secondary metabolites in the seeds (Ishimito and Chrispeels 1996, Giri et al. 1998).

In Australia, the major environmental factors influencing the field pea are drought and high temperatures during the spring flowering period (Ali *et al.*, 1994). Significant production losses in field pea can also result from damage by the seed-feeding beetle

Bruchus pisorum L. (pea weevil) (Smith 1990b). α-AI peas have been developed in three common pea cultivars, Greenfeast, Laura and Dundale by transferring the inhibitor–producing genes from the common bean. In transgenic pea seeds that express the *α-Ai-1* gene, larval development of the pea weevil is apparently blocked at an early stage of larval development (Schroeder et al. 1995, Morton et al. 2000). In transgenic pea seeds expressing the *α-Ai-2* gene, the development of larvae is delayed by at least one month compared with development in non-transgenic pea seeds (Morton et al. 2000).

Through our glasshouse and field experiments, it was found that transgenic peas with the α-AI-1 inhibitor expressed in their seeds effectively controlled the pea weevil. The present study focused on whether the level of the α-amylase inhibitor (α-AI-1) varied when transgenic peas were grown under water and heat stress and, if the inhibitor level was affected, whether this had any influence on the ability of the transgenic plants to control the development of the pea weevil. The field trial experiment was conducted during two consecutive pea growing seasons (1999-2000).

RESULTS and DISCUSSION

This glasshouse study utilized an improved larval measurement procedure to evaluate the impact of transgenic pea plants expressing the α-amylase inhibitor 1 or 2 (α-AI-1 or α-AI-2) proteins in their seeds on the pea weevil (*Bruchus pisorum* L.). Seeds of the transgenic pea cvs. Laura expressing the α-AI-1 inhibitor reduced pea weevil survival by 93–98%. Larval mortality occurred at an early instar stage. Conversely, in non-transgenic cultivars approximately 98–99% of the insects emerged as adults (Fig. 1). Similar results were found with transgenic Greenfeast peas seeds expressing the α-AI-1 protein.

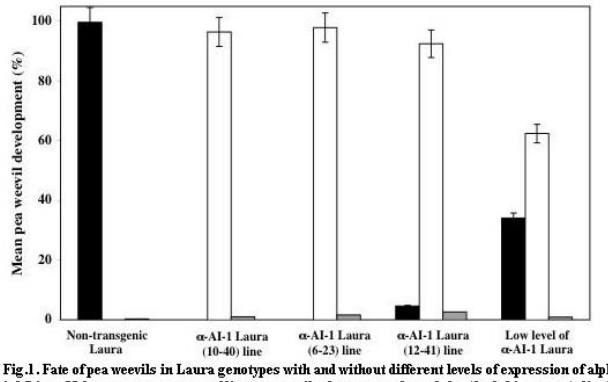

Fig.1. Fate of pea weevils in Laura genotypes with and without different levels of expression of alpha-AI-1 inhibitor. Values are percentage of live pea weevils that emerged as adults (dark histogram), live larvae and pupae (striped histogram), and dead larvae (white histogram) in pea seeds harvested at maturity from non-transgenic Laura (n = 75 pots), three alpha-AI-1 Laura lines (n = 40 pots) and a transgenic line of Laura with low level of alpha-AI-1 (n = 10 pots), presented as means +/- standard error (when this is larger than the line thickness), (from Sousa-Majer et al. 2007).

By measuring the head capsule size it was possible to determine that larvae died at the first to early–third instar stage in α-AI-1 transgenic peas, indicating that this inhibitor is highly effective in controlling the insect. By contrast, transgenic pea cvs. Laura and Dundale expressing the α-AI-2 inhibitor did not affect pea weevil survival, but did delay larval development. After 77 days of development, the head capsule size indicated that the larvae were still at the third instar stage in the transgenic (α-AI-2) peas, while adult bruchids had developed in the non-transgenic peas (Fig.2) (Sousa- Majer et al. 2007).

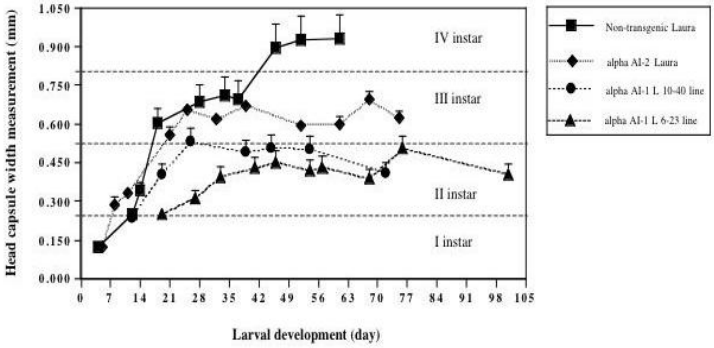

Fig. 2. Change with time in the mean maximum width + standard error (when this is larger than the symbol) for the head capsules of pea weevil larvae developing in seeds of non-transgenic and transgenic peas that express the alpha-amylase inhibitors 1 and 2. Dashed lines dividing instars are based on Smith's (1990a) data where range (mm): 1st instar (0.100 – 0.230), 2nd instar (0.260 – 0.460), 3rd Instar (0.490 – 0.770) and 4th instar (0.800 – 1.108), (from Sousa-Majer et al. 2007).

Transgenic and non-transgenic plants were subjected to heat stress treatments under controlled conditions in a growth cabinet. Transgenic and non-transgenic Greenfeast pods from plants grown at 27/22°C and 32/27°C were inoculated with pea weevil eggs to evaluate whether the reduction in level of α-AI-1 in the transgenic pea seeds reduced the efficacy of the inhibitor. The level of α-AI-1 was reduced on average by 36.3% in transgenic peas at 32/27°C compared to those grown at 27/22°C. At the higher temperatures, 39% of adult pea weevil emerged, compared with 1.2% in the transgenic peas grown at the lower temperatures, indicating that heat stress reduced the protective capacity of the transgenic peas (Fig 3) (Sousa-Majer et al. 2004).

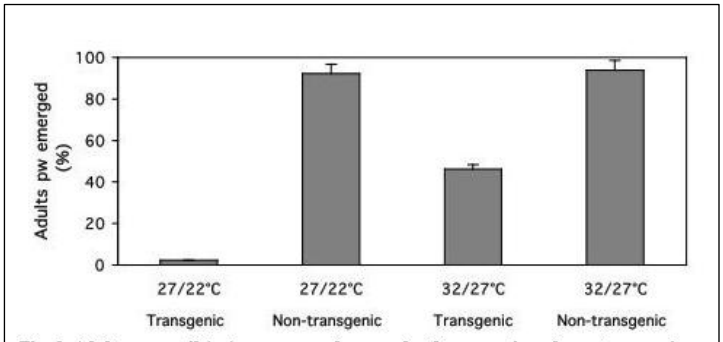

Fig. 3. Adult pea weevil (pw) emergence from seeds of transgenic and non-transgenic peas in two temperature treatments, 27/22 C and 32/27 C, (from Sousa-Majer et al. 2004).

Field trials of 0%, 80%, 90% and 100% transgenic peas were conducted in 1999 and 2000, at six farms located near Northam and York in the Western Australian wheat belt region. The results indicated that, relative to the non-transgenic plots, population growth rates of weevils were reduced by 87–89% in the 80% transgenic plots, by 88–96% in the 90% transgenic plots and from 97–98% in the 100% transgenic plots**.** Variation between years in population growth of the pea weevil is apparently common, perhaps depending on weather and cropping conditions for peas. Our results showed that in transgenic pea plots (α-AI-1 Laura line12–41), relative to the non-transgenic plots population growth rates of weevils were reduced by 97–98%. Also, germination tests of post-harvested field pea seeds from 1999 and 2000 showed 90-99% germination for transgenic but 28-66% for non-transgenic seeds.

Based on this study, we conclude that although highly effective under water and cooler conditions, it is possible that the transgenic peas may confer less effective protection in warmer regions. Release of transgenic peas into hotter environments needs to be undertaken with caution. The temperatures used in the current experiments were consistent with the maximum temperature observed in the field, but the peas were exposed to a constant high temperature of 32°C for 12 h per day, whereas maximum temperatures in the field only occur for a short period around midday. If mean daily temperatures are at, or over, 27°C during flowering, growers may need to use insecticidal sprays to control pea weevils. In the field studies reported by Sousa-Majer (2002), while day temperatures above 27°C occurred for several days, there was no significant increase in pea weevil survival under field conditions. These results will help us to devise better guidelines for risk-assessment of transgenic crops

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The resistance forming dynamics to different insecticides in the housefly

It was determined the resistance forming dynamics of 6 strains of housefly selected of insecticides from 3 chemical classes: organophosphate insecticides, pyrethroids, chitin synthesis inhibitors. Resistance forms quickly to the more toxic insecticides deltamethrin and fenvalerat.

INTRODUCTION

Development of resistance to insecticides in insect populations is defined by the interaction of three groups of factors (Malinovsky, 2001). The first group is genetic factors (presence and number of resistant dominant genes, interaction of resistant alleles, and selection under the influence of previous insecticides). The second group is biological and ecological factors (duration of the development of the first generation and its quantity, manner of breeding, migration, and manner of feeding). The third group is operating factors (the chemical composition of the insecticide, its resemblance to previously applied pesticides, duration of the action, etc.). The first two factors can not be influenced by humans, but their cognition is required for risk assessment of the displayed resistance. Knowledge of the third factor and its influence upon insect resistance allows humans to restrain its impact.

The number of generations of insects subjected to systematic influence insecticides before the appearance of a significant degree of resistance varies in a relatively small range, usually from 5 to 50 generations (May, Dobson, 1986). Documented resistance, caused by a single dominant mutation, has been shown to occur in as few as 10-15 generations, but for a recessive mutation, resistance was observed to occur in 18-25 generations (Zilberminc, 1991).

There are three periods in the development of resistance to insecticides: choice within the limits of a standard reaction, defining nonspecific polyfactorial tolerance; uneven growth of general resistance at the expense of the accumulation of resistant mutants; elimination of the sensitive individual and choice within the limits of a standard reaction of the mutants (i.e. stabilization of resistance on the maximum level (Zilberminc, Smirnova, 1979)).

In this study the resistance forming dynamics in the housefly were studied to insecticides from three different classes: organophosphates (OP), pyrethroids and derivates of benzoylphenylurea (chitin synthesis inhibitors, CSI).

MATERIALS and METHODS

The objects of this study were imago and 3rd instar housefly larvae. In the laboratory, imago houseflies were kept in a kapron warren cage with metallic framework (25x25x25 cm) and fed on dry milk and water. The larvae of the flies were maintained in 800 cm^3 glasses on moist wheaten bran. The warren and glasses were in an insectarium with a temperature of 24-28°C and moisture of 40-50%.

For studying the resistance forming dynamics in houseflies, imago flies of a sensitive strain were divided into groups and each group was selected by a corresponding insecticide. The selection was conducted at a rate of LK_{50-80} by the spill method (Sawicki, Farnham, 1964) using the following insecticides: phosmet (phtalaphos, 20% e.c., strain R-ft) and phocsim (volaton, 50% e.c., strain R-v) from the OP class; and deltamethrin (decis, 2.5% e.c., strain R-d), fenvalerat (sumicidin, 20% e.c., strain R-fv) and ethophenprox (trebon, 30% e.c., strain R-tr) from the pyrethroids class. The chitin synthesis inhibitor chlorfluazuron (eim, 12% e.c) was added in the fodder given to the larvae.

For determining the level of resistance to the OP and pyrethroids, 3-4th dayly imago of the house flies were used. The acetone solutions of insecticides were marked topically on the fly's mid-back, 1 µl per individual by means of a micro syringe. Six to seven different concentrations of the insecticide in 3-th repeating on each concentration, an 20 flies per repetition were used.. The control flies were processed with an equivalent amount of acetone. The processed flies were contained in Petri dishes at room temperature (24±1°C), and the death-rate was taken into account within 24 hours after treatment. Anestheitzed by CO_2, flies were weighed before topical treatment.

For determining the level of resistance to the derivatives of benzoylphenylurea, 3rd instar larvae were placed in 50 ml glasses with 10 g of fodder (wheat bran) treated with 30 ml of emulsified preparation solution. In the control group, the fodder was moistened only with water. The glasses were stored at room temperature, and the efficiency of the preparation was defined by quantity of flown out imago. The determination level of the resistance was conducted through each of 6 generations.

The criterion of sensitivity of imago flies to the preparations was determined by the lethal concentration which kills 50% of the individuals (LC_{50}, %), which was defined by the method of probit-analysis. The LD_{50} values (in mcg/g alive mass) were calculated by the following formula using the mass of the imago flies processed from the OP and pyrethroid treatments:

$$LD_{50} = LC_{50}\ 1000\ V/\ P$$

where *V* is the volume of the insecticide, *P* is the weight of the insect. The degree of gained resistance by the housefly was characterized by the resistance index (RI), which is calculated by dividing the LC_{50} value of the resistant strain by the LC_{50} value of the

sensitive strain. The statistical analysis of data was conducted using the Student t-Test (Lakin, 1990).

RESULTS and DISCUSSION

The LD_{50} of each preparation for the sensitive strain (the strain S) was determined before beginning the study of resistance forming dynamics. The results are provided in Table 1.

From the results of the preparation for contact action, deltamethrin possesses the most toxicity, following are fenvalerat, ethophenprox, and the organophosphates.

It is seen from this table that resistance to both organophosphate preparations developed slowly: to the 30th generation, RI= 4,8 for the strain selected with phocsim (R-v) and RI= 2,07 for the strain selected with

Table 1. Initial sensitivity of the strain S housefly to insecticides of different classes

Class	Organophosphates		Pyrethroids			Derivate of benzoylphenyl-urea
Insecti-cide	Phocsim	Phosmet	Ethophenprox	Delta-methrin	Fenvalerat	Chlorfluazuron
LD_{50}, mcg/g	11,64± 0,57	17,23± 0,94	5,44± 0,31	0,057± 0,006	0,25± 0,03	0,000031± 0,0000017

Note: For chlorfluazuron meaning EK_{50} (%) is given

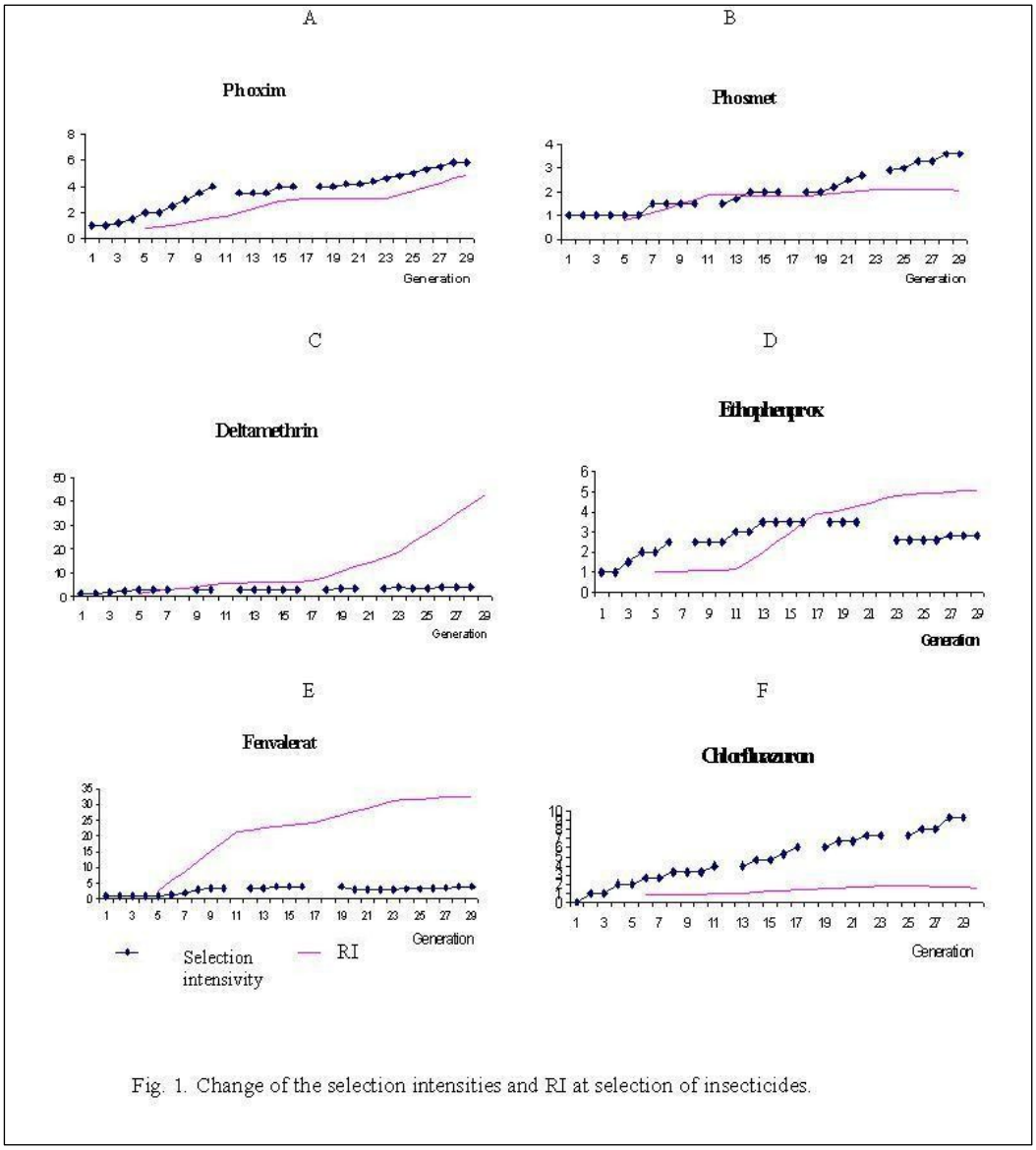

Fig. 1. Change of the selection intensities and RI at selection of insecticides.

phosmet (R-pht). Thereby, these strains may only be conditionally resistant. During the same time period, resistance to volaton formed quicker. Apparently, both selected strains during the first 30 generations pass only stage I of resistance formation: selection within the standard of the reaction, and the meaning of the resistance index grows in proportion to the growth of the intensity of the selection in both strains, which presents itself as a ratio of the select concentration in a given generation to its concentrations for the F_1 generation, and, like a resistance index, is a non-dimensional value (fig. 1, A, B).

Similar results were received during the selection of the potato beetle by these preparations (Berim, Byhovec, 1980); for 8 generations, the RI increased 2,25 times during selection by phtalophos and 2,4 times during selection by volaton. Slow-forming resistance was noted during selection of the houseflies by etaphos (Roslavceva et al., 1982). In spite of increasing the selected concentration 20 times (from 0,002 to 0,04%) during 30 generations the RI only varied from 1,2-4,4. In the 30th generation, it was equal 2,6. But during selection of the houseflies by gardone, resistance formation was very quick (Roslavceva, 1979). Already through 5 generations, the flies became resistant to the preparation (RI=17-30). In the 11th generation, RI reached 12; in the 15th, it unevenly increased to 1000 and, during the following 30 generations, was maintained at this level.

Resistance to deltamethrin developed slowly in the initial stages. Up to the 6th generation, the RI almost did not change (RI=1,58); in the 12th generation, a small leap occurred (RI=5,61), and then resistance grew slowly; in the 18th generation the RI=6,67. In the 24th and 30th generations also, it showed an uneven formation of resistance in spite of the fact that select concentrations changed insignificantly (Fig. 1, C).

In the trebon-selected strain (R-tr), resistance formation occurred considerably slowly, even in the 30th generation where the RI=5 (Fig. 1, D). In the strain R-fv, unlike the two previous strains, resistance on the initial stage developed quickly. Already in the 12th generation, the uneven formation of resistance occurred (RI=21,44), but then resistance increased slowly (i.e., came out of a plateau (Fig. 1, E)).

Data on resistance formation to fenvalerat conformed, in general, to the data of H. Malinovski (1986; 1989), who studied the development of resistance in houseflies to deltamethrin, cypermethrin and fenvalerat. During the first 10 generations, resistance formation occurred slowly, but then quick resistance development occurred. Through 20 generations, resistance to fenvalerat increased 23 times (in 18th generation RI=24). Flies in our laboratory developed resistance to deltamethrin slowly. The resistance index in the 30th generation was 42, but in the Malinovski study it was so in the 20th generation. Our data on resistance formation to deltamethrin is closer to the studies of N. Sales et al (1988), which conducted the selection of the flies *Lucilia cuprina* by deltamethrin during 20 generations. The resistance index increased to 25, and then under further selection it did not change. Significant levels of resistance to deltamethrin existed in the mosquito *Culex pipiens pallens*. Using selection by this preparation during 53 generations, the RI was increased 136 times (Chen, 1990). For a laboratory strain of beetles (*Callosabruchus chinensis*) treated with deltamethrin during 7 generations, the RI was increased 3,45 times (Jain, Yadav, 1989). Data about resistance formation to ethophenprox was not found published in literature.

Thereby resistance to pyrethroids in our study formed considerably quicker than to OP. Moreover, correlation between toxicity of the insecticide and resistance index existed. The highest level of RI in flies was selected by the most toxic insecticide – deltamethrin. The lowest level of RI in flies was selected by the least toxic ethophenprox.

At the beginning of selection by chlorfluazuron, a small increase in sensitivity of the houseflies to the selectant existed (RI=0,74 in the 6th generation). A similar phenomenon existed in selection by diflubenzuron in caterpillars *Spodoptera littoralis* (Radwan et al., 1978) and in the larvae of the potato beetle and codling moth (Byhovec and al., 1980). This increase in sensitivity to the selectant is conditioned, probably by reduction of the defensive quality of the organism. At further selection, the resistance index increased minimally. As can be seen from Fig. 1, F, resistance to chlorfluazuron is practically not formed. In spite of increases in the concentrations of the selectant (9,3 times for 30 generations), the RI was increased only to 1,55 (i.e. remained within the standard reactions of the insect organism).

According to literary data, resistance formation to chitin synthesis inhibitors is possible and may be faster. For instance, resistance to diflubenzuron developed quicker in caterpillars *S. littoralis*. For selection of the caterpillars of the 1st age field population, the resistance index to the selectant increased from 0,6 to 2,3 for four generations (Watson, Geurguis, 1988). For selection of the caterpillars of the 2nd age during 5 generations, there was a fivefold increase in resistance to the preparation (Ahmed et al., 1987). Also, the resistance index increased 5 times for selection of mosquitoes *C. pipiens* by diflubenzuron during 11 generations (Brown et al., 1978).

Therefore, significant differences exist in the rate of resistance formation depending on the insecticide-selectant used, insect species, and initial population (laboratory or natural).

From the six preparations studied concerning three different chemical insecticide classes, resistance in the houseflies is formed quicker to pyrethroids

(deltamethrin and fenvalerat), and slower to chitin synthesis inhibitor (chlorfluazuron). The intermediate position is occupied by the pyrethroid ethophenprox and organophosphates phocsim and phosmet.

On the grounds of previously mentioned material and analysis of literary data, it is possible to determine that the revealed resistance formation dynamics have a general nature for many insect species. The rate and degree of the resistance development to insecticides of the contact action depends on their toxicity to insect: if the insecticide is more toxic, the degree of the resistance development is higher, and the rate of formation is quicker.

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Resistance Management News

Establishment of IRAC South East Asia

Brief outline of IRAC SEA

The recenly formed IRAC South East Asia is a regional group of IRAC with following objectives:

- Communication & Education of external and internal stakeholders
- Sharing of baseline & other resistance management options
- Issue management for all existent and new AI
- Interaction with regulatory bodies and research providers on key resistance related issues

IRAC SE Asia's role is related to insecticide resistance management - proactive and reactive - in SE Asia.

The committee members of IRAC SE Asia are:

Chair - Siddharth Jain, Syngenta Asia Pacific
Vice Chairman - Kai-Uwe Brueggen, Bayer Crop Sciences

Secretary - Samsudin Amit, Dow Agrochemicals asamsudin@dow.com
Treasurer - Cecille Ebuenga, BASF cecille.ebuenga@basf.com
Enquiries about IRAC SEA including interest in joining IRAC SEA can be sent to any of the committee members listed below:
siddharth.jain@syngenta.com
kai-uwe.brueggen@bayercropscience

~Special Feature~

Arthropod Pesticide Resistance Database

What is the APRD?

Resistance is a widespread phenomenon where arthropod populations develop the ability to avoid the lethal effects of normally fatal concentrations of pesticides and biopesticides. Resistance frequently leads to the increased use, overuse, and even misuse of pesticides, which poses a risk to the environment, phytosanitation, market access, global trade, and public health. There is a worldwide need for accurate and easily accessible pest resistance information – information to be used by numerous stakeholders in agriculture, human health, animal protection, and other pest management arenas.

The Arthropod Pesticide Resistance Database (APRD) is a web-based resistance case entry system that serves as a tool to access arthropod resistance information, and provides a forum for real-time and scale appropriate reporting of the current status of arthropod resistance across the globe. The APRD is a gateway search engine (found on-line at **www.pesticideresistance.com**) that is the only comprehensive arthropod resistance resource worldwide. The Database details thousands of resistance cases since 1914 and is recognized as the world's largest repository of resistance information. Currently, the database receives over 540,000 visits annually that last ten minutes or longer.

The APRD also allows for on-line submission of resistance cases by registered users. Anyone can register and submit cases, but only cases approved by a peer review panel of resistance experts will be accepted into the database. A person does not have to be registered to search the database for resistance cases; however, searching without registration will only reveal basic information such as the insect, pesticide, year of report, location, and reference. Registered users can view the entire case summary, including resistance ratios, bioassay information, and other critical information when available.

How to Contact the Database

The Arthropod Pesticide Resistance Database (APRD) can be found at **www.pesticideresistance.com**, and the following contact information can be found under the "Contact Us" link at the top of the page.

- Lisa Losievsky, System Administrator, lisalos2000@yahoo.com
- Mark E. Whalon, Project Director, whalon@msu.edu
- David Mota-Sanchez, Resistance Specialist, motasanc@msu.edu
- Robert M. Hollingworth, Project Co-Director, rmholl@msu.edu
- Lee Duynslager, Webmaster, duynslag@msu.edu

Please feel free to contact any of these people with questions about the Database, or about a submission to the Database.

For questions regarding case submissions, the best contact method is email, but you can also call the Whalon Lab at 1-517-355-1768. (Reaching us by phone may be difficult, so please email if possible.) Also, you can track your case submission online at www.pesticideresistance.com. For online tracking, see "How to Submit a Case to the Database."

The Resistant Pest Management (RPM) Newsletter was developed to spread knowledge of resistance around the world. The goal of the RPM Newsletter is to inform researchers, industry workers, pesticide policy bureaucrats and field personnel of ongoing changes and advances in pesticide resistance management, provide an archival resource to national and international policy leaders, and enhance communication of ideas among resistance managers worldwide. The bi-annual publication has over 1000 electronic subscribers (mostly in government, industry and academia), and hard copies are now part of 61 libraries' serial listings

worldwide. Example countries with serial listings include the United States, Germany, Italy, the United Kingdom, India, Japan, Taiwan, Egypt, Kenya, Costa Rica, Australia, Malaysia, and New Zealand.

To contact the Resistance Pesticide Newsletter (in which your submission will be published if accepted) please email RPMNews@msu.edu or call 1-517-355-1768. We will respond to your email as soon as possible.

How to Submit a Case to the Database

The submission process is completely electronic. It is a simple, peer-reviewed procedure that usually takes less than two weeks. However, only registered members can submit a new case for publication to the database. Therefore, the first step in submitting a resistance case is to become a registered member.

To become a registered member:
Go to **www.pesticideresistance.com** and click on the "Sign Up" link near the top of the page. This will take you to an electronic form, which must be filled out. (Anything with a red asterisk is required.) Once the form is submitted, a verification email will be sent to the email address that you provided. Click on the link in the email to verify the registration request. In approximately 24 hours, a confirmation email will be sent to your account stating that you are now registered with the database. If you ever lose your password, you can email David Mota-Sanchez to receive a new one. His email is motasanc@msu.edu, and can be found on the "Login" page.

To submit a resistance case:
Once you are registered, go to **www.pesticideresistance.com** and log on. Once you are logged on, click on the "Submit" link and fill out the necessary information. (Anything with a red asterisk is required.) A Chief Editor will contact you with a password that you will need for submitting your resistance case to the Editorial Board.

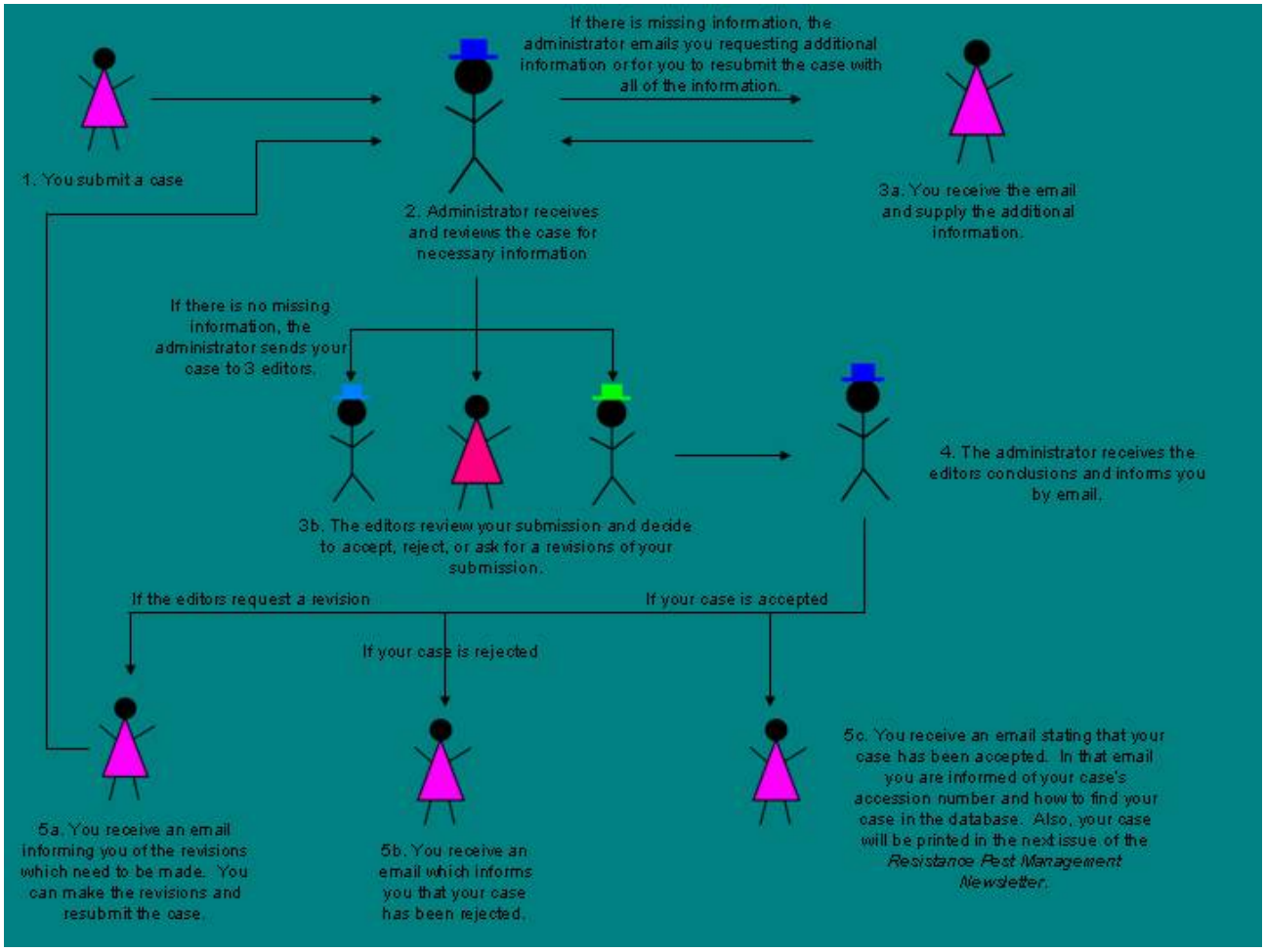
When your case is submitted it will be reviewed for all necessary information. If your case contains the necessary information, it will be assigned to 3 editors. If information is missing, the administrator will email you requesting either the missing information, or a resubmission of your case with the necessary revisions.

When the editors review the case, they can approve, reject, or request revision of the case. If the case is rejected, you will receive an email informing you of your case's rejection. If the editors ask for a revision of the case, then you will receive an email regarding the editors' decision, including necessary revisions for acceptance. You can revise the case submission and resubmit it. If the case is accepted, then you are emailed with their decision of acceptance. Once accepted, the case is submitted into the Database, where it receives an accession number (for citation purposes) and information about where to find the case in the Database. The case is also included in the next issue of the Resistance Pest Management (RPM) Newsletter, a biyearly publication.

To track your resistance case:
During the submission process, you can track your submission to see its submission status. On your "My Account" page, you can see a list of your submissions by genus. This page will tell you the status of your submission, and whether it has been accepted, rejected, or needs revision.

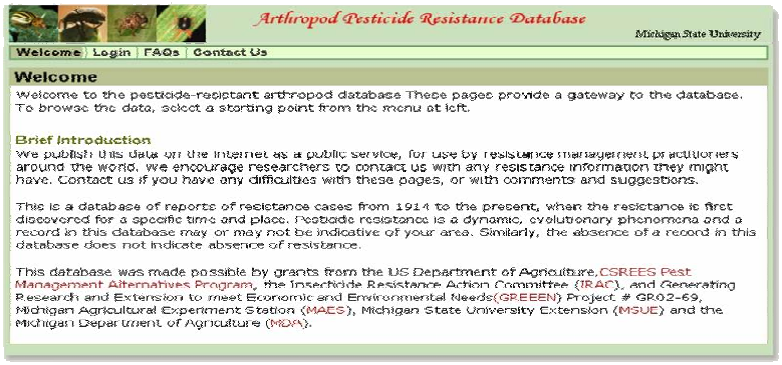
To use the cloning function:
The cloning function is used when you have more than one resistance case of the same type, and most of the information for each case remains the same. If the resistance case that you are submitting is already published, you can submit your case as a clone. To do this, log on to your account and go to the "My Account" page. You will see a record of the resistance cases that you have submitted and where they are in the submission process. Next to each submission, there is a "Clone" tab that you can click on to clone your case. The cloned case will appear at the end of the submitted cases on your "My Account" page.

An example of the cloning feature: You have found resistance in the German cockroach from several populations in Georgia, USA, to fipronil. Each population was tested using the same bioassay method but each population has a different resistance ratio. For this example, after you submit the first case of resistance you can "clone" it. Go to "My Account," where you will find the case you submitted and an exact replica of that case - the clone - which will be listed last. You can click on the cloned case and you only have to change the resistance ratio, since insect, pesticide, location, collection dates, bioassay type, and reference are already entered from the previous case.


If there is missing information, the administrator emails you requesting additional information or for you to resubmit the case with all of the information.
1. You submit a case
2. Administrator receives and reviews the case for necessary information
3a. You receive the email and supply the additional information.
If there is no missing information, the administrator sends your case to 3 editors.
4. The administrator receives the editors conclusions and informs you by email.
3b. The editors review your submission and decide to accept, reject, or ask for a revisions of your submission.
If the editors request a revision
If your case is accepted
If your case is rejected
5a. You receive an email informing you of the revisions which need to be made. You can make the revisions and resubmit the case.
5b. You receive an email which informs you that your case has been rejected.
5c. You receive an email stating that your case has been accepted. In that email you are informed of your case's accession number and how to find your case in the database. Also, your case will be printed in the next issue of the Resistance Pest Management Newsletter.

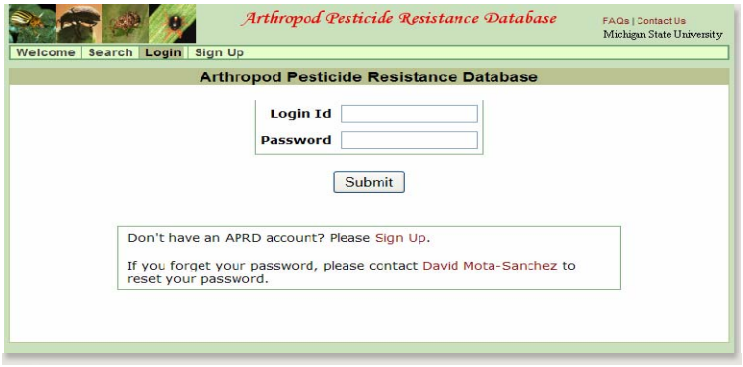
How the Database Works

Welcome



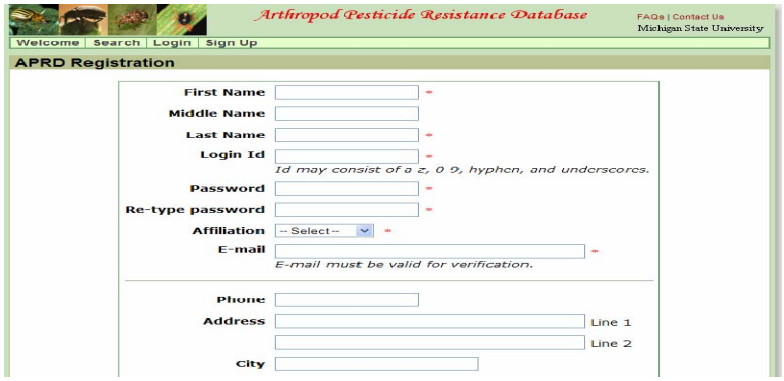
The home page of the database, found at www.pesticideresistance.com, gives a brief introduction and has tabs to other parts of the database.

Login



By clicking on the "Login" tab, you are taken to this page where you can either sign into your account (by entering your username and password into the correct fields), or register with the database by clicking "Sign Up."

Sign Up



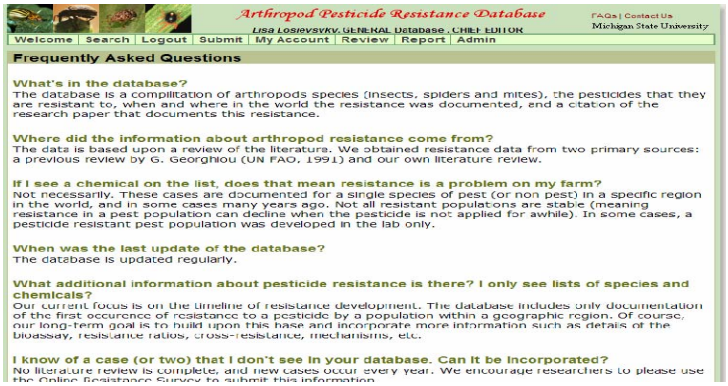
If you click "Sign Up," you are taken to this page. Please see the subsection "To become a registered user" under "How to Submit a Case to the Database" for further information.

My Account

Once you sign into your account, click on the "My Account" tab, which will take you to a page similar to the one shown above. This page lists all of the cases which you have submitted to the database, and their submission status. Please see "How to Submit a Case to the Database" for further information.
You can change your password by clicking on the "Change Password" tab.

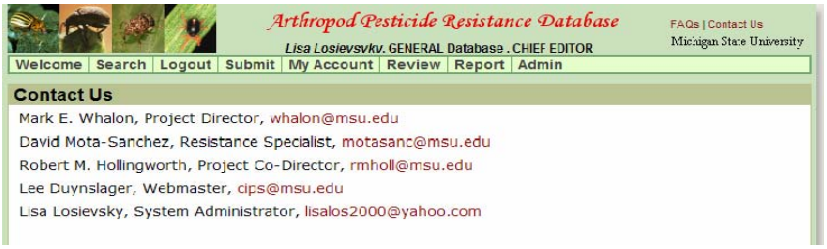
By clicking on the "Update Profile" tab, you can change the information that you submitted when you created your account

FAQs



By clicking on "FAQs" at the top right-hand corner of the webpage, you will be directed to this page. The FAQs page is designed to answer common questions about the Database. If you have a question, please check this page first. If your question is not answered here, please contact us.

Contact Us

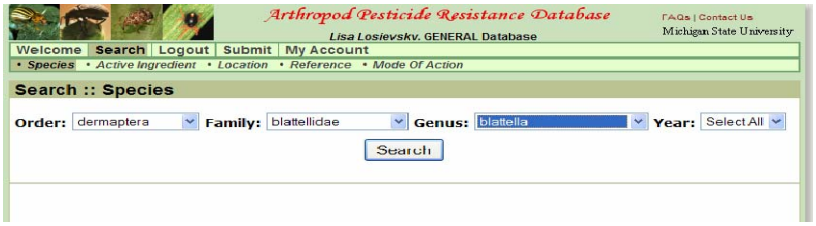


By clicking "Contact Us" at the top right-hand corner of the webpage, you will be directed to this page. If you have a question or problem that is not addressed on the FAQs page, please email anyone listed on this page. For more contact information, please see "How to Contact the Database."

Searching the Database

Searching by Species

Search



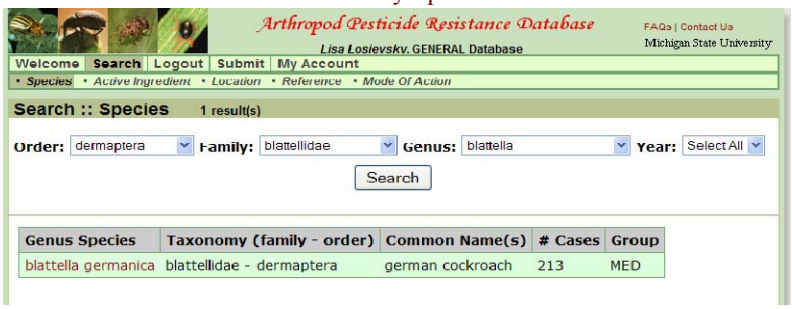
By clicking the "Search" tab, you are taken to this search page. From here you can search for any resistance case in the Database. You can search based on species, active ingredient, location, reference (author, title or publication year), or mode of action.

To illustrate how to use each search function, an example of German cockroach (*Blattella germanica*) resistance to fipronil reported in 2003 in North Carolina, USA will be used. (This is the same example that was used to explain cloning in "How to Submit a Case to the Database.")

The species search page displays drop down boxes for Order, Family, Genus, and Year. If you are looking for a specific species you can go directly to the Genus drop down menu and make your selection. This action will automatically fill in the Order and Family boxes for you. If you are interested in finding broader resistance within an Order or Family, begin by making your selection within one of those categories and click search. This action will display all of the insects within that Order or Family that have been reported as resistant.

In our example, we begin my selecting *Blattella* from the Genus drop down menu (shown above) and clicking search. The year was left at "Select All" although 2003 could have been entered for it. When APRD administration submits cases, the year is usually the year in which the resistance was discovered, which is not necessarily the same year it was published. Keep in mind that if you are looking for a specific case and you select the year it was published, you may not find the case you are looking for.

Search by Species



Clicking Search displays a list of all the species within the Order, Family, or Genus you selected. In our example there is only one species within the Genus *Blattella*. For each species in the list, the common name, number of cases, and importance group are shown. The importance groups are medical, agricultural, parasite, pollinator, and other.

List of Active Ingredients

Clicking on the name of the species you are interested in shows all of the active ingredients (pesticides) to which the species is resistant. To find the case that you are looking for simply scroll down the list of active ingredients and click on the one you want. If you do not see the active ingredient you are looking for, no resistance case has been submitted for it. For our search, we will click on "fipronil" to see the resistance cases associated with it. (Note: the page has been cropped and does not show all active ingredients to which *B. germanica* is resistant.)

Resistance Cases

This page lists all of the resistance cases of the German cockroach to fipronil. As you can see above, there are two cases of resistance in *B. germanica* to fipronil listed under the heading "Resistance Case(s)." On this page you can also click on the word fipronil, which will take you to a list of all resistance cases reported to fipronil. This page can be reached alternatively by an active ingredient search (covered in the next section). Clicking on "blatella germanica" will take you back to the page listing all the active ingredients.

The case we are searching for is from North Carolina, and we can access that by clicking on the case id "7084." This opens a pop-up window which gives all of the information about this resistance case, including the reference.

Since this case was added prior to updating the database, some categories in the summary are blank. Newer cases will have information entered for these categories including field or lab selected, bioassay method, and resistance ratios. We are slowly working to update these older cases to include all available information, but this will undoubtedly take several years.

Pop-Up Window: Case Information

Case 7084 (GENERAL)

Arthropod Classification

Species or Subspecies	germanica
Other Known Name(s)	german cockroach
Genus	blattella
Order	dermaptera
Family	blattellidae
Class	insecta

Active Ingredient (AI)

AI 1	fipronil GABA-gated chloride channel antagonists; Fipronil or Phenylpyrazoles

Origin of the Resistance Population

The population was resistant when collected in the field.
No

The population was created solely by selection and/or genetic manipulation in the laboratory.
No

The population was selected further in the laboratory after collection.
No

Population's Mechanism(s) of Resistance

Geographic Location

Country	USA
State or Province	North Carolina
County, Prefecture, or Nearest City	Cental, southeaster, northeastern

Collection Site

Date of Collection	

Bioassay

Date	
Life Stage	
Sex	
Method	

Discriminating Dosage: No

Multidose Bioassay Results: No

Impact of the Resistance: No

Reference

Type	Published
Title	Origin and Extent of Resistance to Fipronel in the German Cockroach, Blattella germanica (L.) (Dictyoptera: Blattellidae)
Author	Holbrook, G.L, J. Roebuck, C.B. Moore, M.G. Waldvogel, and C. Schal
Journal	Journal of Economic Entomology
Volume	96(5)
Pages	1548-1558
Year	2003

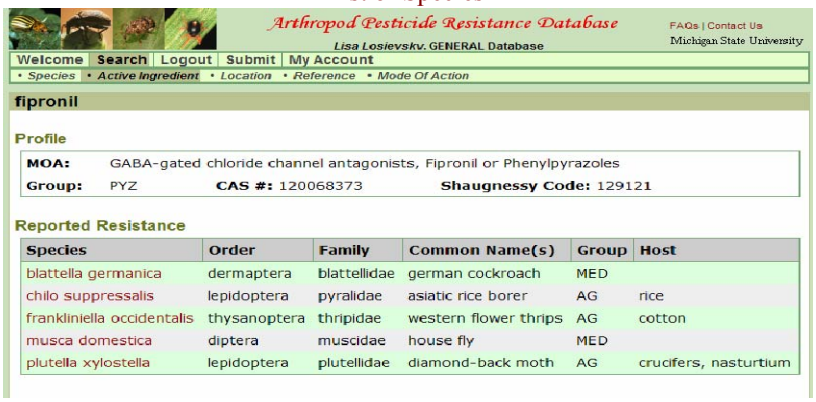
Cross Resistance: No

Searching by Active Ingredient

Search by Active Ingredient

The active ingredient search page is accessed by clicking the "Active Ingredient" tab. This takes you to a page in which the active ingredients are listed in alphabetical order. To scroll to the next page click the word "next." Keep clicking the "next" button until you see the active ingredient you are looking for. Once you see the active ingredient you want, access that page by clicking on the ingredient (in this case, fipronil).

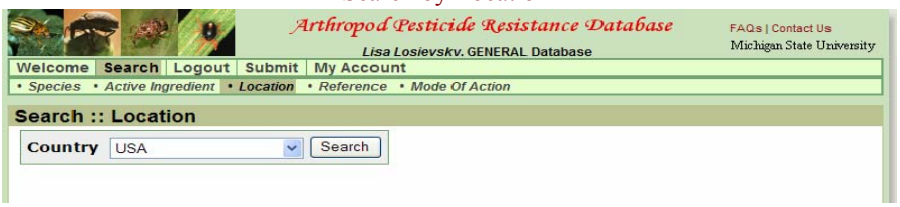
List of Species



This page will list every species with a reported resistance to the active ingredient that you are searching for. Click on the species that you want (in this case, *Blattella germanica*). Doing so opens the webpage which lists every reported resistance case of the German cockroach to fipronil.

Searching by Location

Search by Location



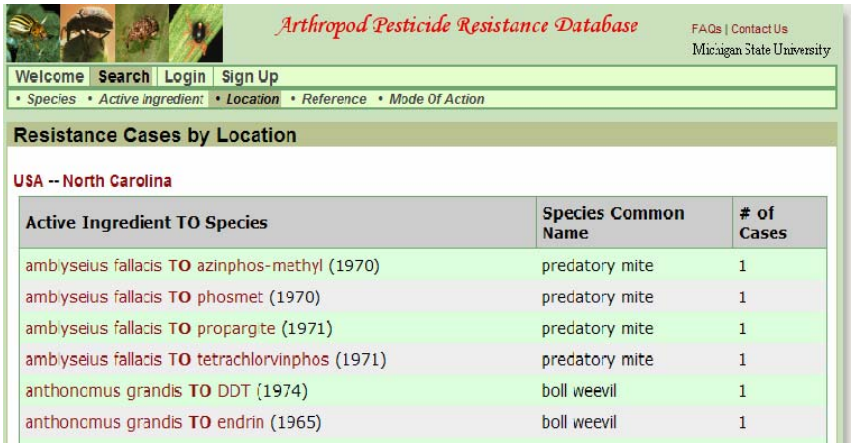
You can search by location by clicking the "Location" tab. This brings up a page with a drop down box to search by country. Select the country for which you are searching and click "Search."

List of Regions

Clicking "Search" displays a page listing regions of the country where resistance has been reported.

Scroll down the page to find the region that you wish to search for, and click on the name of the region (in this case, North Carolina). This displays a page listing every case of resistance in that region, listed alphabetically by genus, species and then active ingredient. Scroll down the list to the species and active ingredient for which you are searching. If the species or active ingredient is missing, then no case of resistance has yet been reported.

Resistance Cases

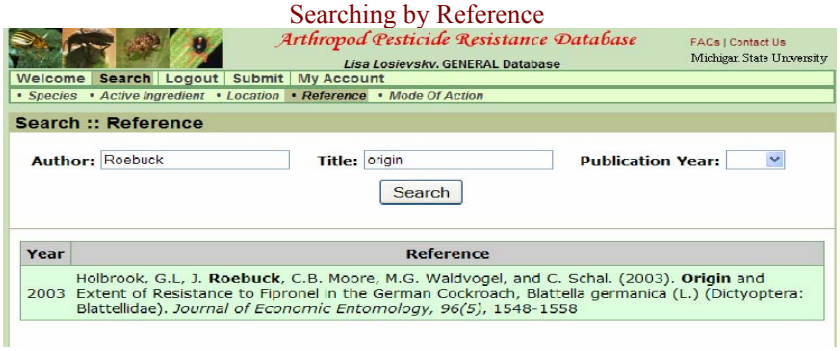


Clicking on the genus, species, and active ingredient for which you are searching will again take you to the page listing all cases of resistance, which was shown before.

Scroll down the list to the desired species and active ingredient. If the species or active ingredient is missing, then no case of resistance has yet been reported.

Searching by Reference

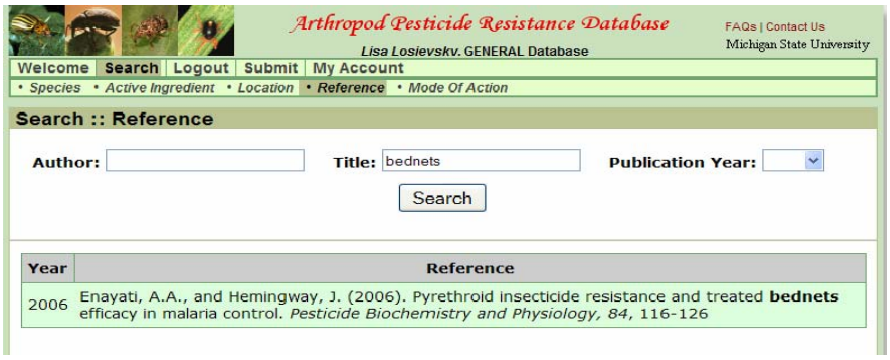
Searching by Reference



You can search by reference by clicking the "Reference" tab. This brings up a page in which you can type author or title information, or select a year from the drop-down box. Entering any of the above information will give you the citation information for any resistance case meeting your criteria. When using searching by author, you do not have to use the primary author (the first author listed in the reference); you can use any author cited in the reference.

When searching by title, you do not have to use the complete title, but if you do it must be spelled correctly and be exactly the same as it was entered into the system. It is easy to make a mistake when searching for whole titles. If you use a whole title and cannot find what you are looking for, try using a partial title or key word.

Title Search

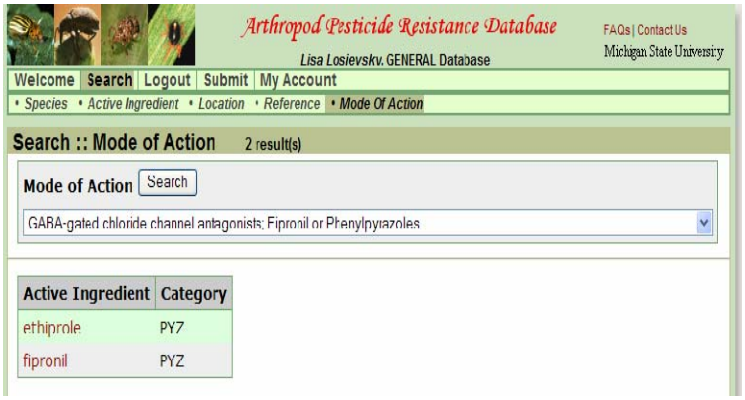


When you are searching for a specific topic, it is helpful to search using a key word in the title search. For example, if you are looking for resistance cases involving bednets, you can type "bednets" into the title box and it will list any reference that used that word in the title.

Searching by Mode of Action

To access the mode of action search, click the "Mode of Action" tab. Select the mode of action you are interested in from the drop down list and click search.

Searching by Mode of Action



Clicking search displays a table listing all of the active ingredients which act by this mode of action. Click on the active ingredient (in this case, fipronil) for which you are searching. This will bring up the page listing all of the species with known resistance to the ingredient. From here, it is exactly like an active ingredient search, so please see that section for more information.

Editor List

We would like to acknowledge and thank our editors of the APRD.

Reviewer	**Class of Pesticide**
Tom Anderson	Flonicamid
Nigel Armes	Phenylpyrazoles (Fiproles)
	Organotin miticides
	Uncouplers of oxidative phosphorylation via disruption of proton gradient
	Inhibitors of chitin biosynthesis, type 0, Lepidopteran
	Hydramethylnon
	Mitochondiral complex electron transport inhibitors
	Metaflumizone
Andrea Bassi	Indoxacarb
Carlos A. Granadino	Pyriproxyfen
	Etoxazole
	Und Pyridalyl
Alasdair Haley	Propargite Tetradifon
	Neuronal inhibitors (unknown mode of action)
Graham Head	Microbial disruptors of insect midgut membranes (includes transgenic crops expressing Bacillus thuringiensis toxins)
Alan McCaffery	Nicotine
	Chloride channel activators
	Fenoxycarb
	Pyrnetrozine
	Inhibitors of chitin biosynthesis, type 0, Lepidopteran
	Moulting disruptor, Dipteran
Ralf Nauen	Neonicotinoids
	Nicotine
	Inhibitors of lipid synthesis

Venkat Pedibhotla	Phenylpyrazoles (Fiproles) Uncouplers of oxidative phosphorylation via disruption of proton gradient Mitochondrial complex electron transport inhibitors Metaflumizone
Suresh Prabhakaran	Sulfuryl fluoride
Phil Robinson	Cryolite Organotin miticides
David Rogers	Neonicotinoids Inhibitors of lipid synthesis
Caydee Savinelli	Chloride channel activators Pyrnetrozine
Robin Slatter	Und Pyridalyl
Tom Sparks	Organophosphates Nicotinic Acetylcholine receptor agonists (allosteric)(not group 4) Juvenile hormone analogues Fenoxycarb Pyriproxyfen Diacylhydrazines Octopaminergic agonists
Bruce Stanley	Carbamates Triazemate Indoxacarb
Nick Storer	Microbial disruptors of insect midgut membranes (includes transgenic crops expressing Bacillus thuringiensis toxins)
Gary Thompson Bob Hollingworth David Mota-Sanchez Mark Whalon	Nicotinic Acetylcholine receptor agonists (allosteric)(not group 4)

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We would like to acknowledge all of the people who have a part in updating and maintaining the Arthropod Pesticide Resistance Database and the *Resistance Pest Management Newsletter*.

Mark E. Whalon	Project Director,
David Mota-Sanchez	Resistance Specialist
Robert M. Hollingworth	Project Co-Director
Lee Duynslager	Webmaster
Lisa Losievsky	System Administrator
Saunté Sutton	System Administrator
Jeanette Wilson	RPM Newsletter Coordinator
Abbra Puvalowski	RPM Newsletter Coordinator

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Announcements and Submission Deadlines

Thank you to those who contributed to this issue - you have really made the newsletter a worthwhile reading experience! Our contributors truely increase the newsletter's success at sharing resistance information worldwide.

We encourage all of our readers to submit articles, abstracts, opinions, etc (see the newsletter online at http://whalonlab.msu.edu/rpmnews/general/rpm_submission.htm for submission information).

The Newsletter is a resource to many around the globe. It is also a wonderful and effective way to enhance the flow of ideas and stimulate communication among global colleagues.

We appreciate your efforts to support the newsletter and we look forward to your continued contributions.

The next two **submission deadlines** are:

Monday, March 17, 2008
Monday, September 15, 2008

We hope you continue to consider the newsletter as a forum for displaying your ideas and research

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