AGRISCIENTIA, 2003, VOL. XX : 99 - 110

REVISIÓN

Monitoring for insecticide resistance in major stored product pests in Argentina: a review

Stadler, T; B. Subramanjam and A. A. Ferrero

SUMMARY

Insecticide resistance monitoring is not a simple recording of the lack of susceptibility in an insect population. It represents a useful tool for the assessment of the effectiveness of control measures as well as a forecast to predict pest outbreaks. This review focuses on the research-work that has been carried out in this field in Argentina; it encompasses both the study of the occurrence of resistance in stored-cereal insects and the role that ecological and physiological factors play in the evolution of resistance, since these factors directly affect the magnitude and speed of the process. The aim of the Insecticide Resistance Project in Argentina is to develop feasible and available monitoring tools for Insecticide Resistance Management, in agreement with the IORPM standards. However, this goal can only be achieved through readily available reference insect strains, through the harmonization of accurate laboratory testing and rearing methodologies and through the development of field-testing methods for an immediate approach to the problem.

Key words: insecticide resistance monitoring, *Sitophilus oryzae*, *Tribolium castaneum,* stored product pests.

Stadler, T; Subramanjam, B. and Ferrero, A. A., 2003. Monitoreo de la resistencia a insecticidas en las plagas principales del grano almacenado de la Argentina: una revisión. Agriscientia XX: 99 - 110

RESUMEN

Esta revisión sobre investigaciones en resistencia a insecticidas en plagas de los granos almacenados en Argentina, abarca el estudio del fenómeno y del papel que los factores eco-fisiológicos juegan en la evolución de la resistencia, considerando que éstos afectan en forma directa la magnitud y la velocidad del proceso. El

---

Fecha de recepción: 17/10/03

monitoreo de resistencia es un registro de cambios en la susceptibilidad de una población de insectos a un producto determinado, pero también una herramienta para evaluar la eficacia de las medidas de control y un método de predicción para la aparición de plagas.El proyecto sobre Resistencia a Insecticidas en Argentina tiene como objetivo el desarrollo de herramientas para el monitoreo de la resistencia en coincidencia con los estándares de la IORPM, que incluyen las cepas de referencia, la armonización de las técnicas de ensayo y cría, y técnicas de evaluación a campo para una aproximación rápida al problema.

Palabras clave: insecticidas, monitoreo de resistencia, *Sitophilus oryzae*, *Tribolium castaneum,* plagas de granos almacenados.

T. Stadler, Laboratorio de Parasitología y Ecotoxicología, U.N. del Comahue (UNCo-CONICET), Buenos Aires 1400 Q8300 BCX Neuquén-Argentina; E-mail : lpe@uncoma.edu.ar; B. Subramanjam, University Minnesota, College of Agricultural, Food and Environmental Sciences, Department of Entomology, MN55108-6125 EEUU; E-mail: subra001@maroon.tc.umn.edu; A. A. Ferrero, Departamento de Biología, Bioquímica y Farmacia. U.N. del Sur, San Juan 670 (8000) Bahía Blanca-Argentina; E-mail: aferrero@uns.edu.ar

INTRODUCTION

Insecticide resistance has emerged as one of the key constraints to continued successful crop protection, while introduction of new insecticide compounds is slowing down. Unfortunately most pesticide use does not incorporate Insect Pest Management (IPM) strategies, which could prolong the development of insect resistance to insecticides. As an aftermath, Insect Resistance Management (IRM) programs were introduced as a response to field failures, as a curative and not as a preventive way to manage resistance. However, the principles of Insect Resistance Management have been clearly understood for a long time and it is mandatory that all pest control strategies be based on them. It should also be realized that pest resistance to pesticides is an international problem, given the free movement of commodities in international trade (Champ & Highley, 1983)

Major stored grain insect pests in Argentina are: *Sitophilus oryzae*, *Rhizopertha dominica*, *Tribolium castaneum*, *Sitotroga cerealella* and *Tenebrio molitor*. Damage of grain in storage attacked by these insect-pests was customarily recorded until 1991 by the National Agrifood Service (SENASA ex IASCAV). At that time, corn and maize damaged by insect attack was 1.22% and 1.38% respectively over whole yield of 10,891,300 Ton corn and 7,770,400 Ton maize in 1990/91 (IASCAV files). Yield did not vary substantially in the last 12 years and damage by insect attack sets below 8% in corn and maize (Ing.Adriana Digiulio, SENASA; pers. comm.) Unfortunately, there are no historical records available about distribution of stored product pests, pesticide treatments or resistance cases in stored grain in Argentina.

Since 1985 some attempts have been made in Argentina to study the occurrence of resistance in stored cereal insects and the output is a very useful package of basic biological and toxicological information on *S. oryzae* and *T. castaneum* (Picollo *et al.*,1985; Stadler, 1988; Stadler *et al.*, 1990; Ferrero *et al.*, 1991; Picollo *et al.*, 1992). However, there were no local or international findings available to support the existing research work or to encourage the development of the science and data that would permit more intelligent deployment of pest controls so as to minimize the selection of resistance.

In the late 80`s, Malathion resistance was widespread in stored grain insect populations from grain manufactories in Argentina; thus, multiple outbreaks of resistant pests and high chemical bills were routine. However, this information was never formally recorded or published.

In order to provide a basis for the future development of an effective resistance monitoring program in stored grain insects, which should involve conservation of susceptibility to all classes of insecticides, several research groups have arisen in Argentina out of which only three are still working on resistance cases. The Centro de Investigación de Plagas e Insecticidas (CIPEIN, CITEFA-CONICET), principally fosters research programs on hematophagus insect vectors of human diseases and household pests. The Laboratorio de Parasitología y Ecotoxicología (UNCo-CONICET) is involved in research projects on insecticides in the framework of IPM programs on several crops and pesticide resistance monitoring in stored products, cotton and Soya bean pests. Finally, the Cátedra de Zoología de Invertebrados II of the Universidad Nacional del Sur carries out standard laboratory tests on insecticide susceptibility of Chagas disease vectors and monitor insecticide resistance in stored grain, wheat and apple insect pests.

These three research groups have linked objectives, focusing their research on ways to revert or mitigate the phenomenon of insecticide resistance and its unfortunate consequences. To achieve such goals in this area, the first basic issue addressed is the study of mechanisms leading to resistance buildup, the biochemical processes related to the degradation of a xenobiotic within the insect. The second is the role that ecological and physiological factors play in resistance buildup, because they directly affect the magnitude and speed of resistance development and when combined in different ways, they can cause, promote or delay the process.

The actions undertaken in "monitoring of resistance" in insect populations living on stored grain lead to:

- The detection of cases of control failure
- The selection of an appropriate type of bioassay
- Laboratory rearing of susceptible reference strains of the main insect pests species
- The establishment of baseline response data from susceptible strains
- The adequate measurement of the level of resistance
- Regional resistance mapping
- The identification of the most active compounds among different insecticides
- Focus on the influence of exogenous and endogenous variables that influences the susceptibility of insects to insecticides
- The development of basic research in order to devise, for each pest, a sufficiently sensitive method to measure susceptibility and discriminate resistance from stage- dependent or age-dependent tolerance
- The recording of the resistance status for regulatory agencies

The aim of the IRM Project is to develop a suitable methodology for monitoring the resistance in stored grain insect pest populations, and the output should be a feasible, easy and handy monitoring tool for Insecticide Resistance Management within the local conditions and in agreement with the international IORPM standards.

Thus, the analysis and standardization of those factors upon whose interaction the variability in a pest population depends is mandatory. Rearing methods need to be defined by fixing all possible variables in order to obtain laboratory strains with a low coefficient of variation for individual size, nutritional state and all the other characteristics that may affect pesticide bioassays in one way or another. The analysis of these variables will be based on those indicators normally used in experimental insect physiology such as mortality, fecundity, individual size and overall evolution of the culture, focusing on the influence of exogenous (ecological and environmental) and endogenous (physiological and populational) variables on insecticide susceptibility and development of resistance (Cichy, 1971; Georghiou & Taylor, 1977; Gomez *et al.*, 1983; Stadler, 1988; Stadler *et al.*, 1990; Rosenheim & Tabashnik, 1990). In order to accomplish these goals, the basic premise is that bioassays must be comparable, in other words, reproducible.

S. oryzae has been extensively studied in Argentina (Picollo *et al.*, 1985, Stadler, 1988; Stadler *et al.*, 1990) following FAO (1974) guidelines. The information available on *S. oryzae* is sufficient to develop an accurate method for resistance monitoring in populations of this species and should be used at the same time as a model for the development of basic research on other stored grain insect pests.

INSECT- PEST REFERENCE STRAINS FROM LOCAL STORED PRODUCTS USED IN RESEARCH WORK IN ARGENTINA

S. oryzae; PARTOX-S Strain: "Malathion-susceptible strain". Obtained in 1980 from a population reared on wheat and lacking any previous chemical control, from the Cátedra de Terapéutica Vegetal, Facultad de Agronomía, Universidad Nacional de La Plata, Buenos Aires-Argentina, transferred to CIPEIN and further, in 1984, to our rearing facilities.

S. oryzae; PARTOX-RM Strain: "Malathion-resistant strain". The initial population that gave rise to it was obtained at the milling company "Leticia" (Buenos Aires, Argentina), transferred to CIPEIN and further, in 1984, to our rearing facilities. This strain starts with an early Resistance Factor of 6.7 (Picollo *et al.*, 1985), reared on wheat, selected with Malathion (in vitro) and maintained around RF = 4.0.

T. castaneum, PARTOX -S Strain: "Malathion-susceptible strain". Obtained in 1994 from CIPEIN.

T. castaneum, PARTOX -RM Strain: "Malathion-resistant strain". Obtained in 1994 from CIPEIN. The early Resistance Factor of this population reared on wheat was RF = 5, selected and kept up in vitro by recurrent exposures to Malathion.

Rhyzopertha dominica, PARTOX-S Strain: "Malathion-susceptible strain". Obtained in 1994 from CIPEIN.

All reference strains are reared under standard conditions (Stadler, 1988) at 27°C ± 1°C and RH was kept constant at 77% ± 2% by means of a NaCl solution (Winston & Bates, 1960).

BASELINE TOXICITY DATA FROM LOCAL STRAINS OF *Sitophilus oryzae* and *Tribolium castaneum*

Relative toxicity of different insecticides was assessed in laboratory trials using different strains of *S. oryzae* and *T. castaneum*. This information was acquired from local strains and may be taken as baseline data for local populations (Tables. 1, 2 & 3).

Table 2: Susceptibility of *S. oryzae* to four different insecticides in the filter paper test (FAO, 1974) (Stadler,T. unpublished).

Insecticide	LD$_{50}$ µg/cm^2 PARTOX-S STRAIN (Fiducial Limit 95%)	Slope
Cypermethrin	3.07 (2.32 - 5.21)	1.85
β-cypermethrin	1.40 (0.98 - 2.18)	2.67
Deltamethrin	0.63 (0.41 - 0.85)	2.46
Permethrin	3.80 (0.42 - 0.54)	1.91

Table 1: Susceptibility of two *S. oryzae* strains to three different insecticides in the topical application test (Stadler *et al.*,1990)

Insecticide	LD$_{50}$ µg/Insect CIPEIN-S STRAIN (Fiducial Limit 95%)	LD50 µg/Insect CIPEIN-RM STRAIN (Fiducial Limit 95%)	Resistance Factor (RF)
Malathion	0.026 (0.025-0,028)	0.168 (0.164-0.171)	6.46
Deltamethrin	0.002 (0.001-0.002)	0.002 (0.001-0.003)	0.00
Lindane	0.480 (0.424-0.543)	0.14 (0.107-0.182)	0.29

Table 3: Susceptibility of two *T. castaneum* strains to five different insecticides in the filter paper test (Picollo *et al.*, 1992)

Insecticide	LD$_{50}$ µg/cm^2 ULP Suscept. STRAIN (Fiducial Limit 95%)	LD$_{50}$ µg/cm^2 ML Resistant STRAIN (Fiducial Limit 95%)	Resistance Factor (RF)
Malathion	21.28 (19.8-23.0)	271.41 (218.0-336.0)	12.75
Fenitrothion	0.38 (0.10-1.46)	2.68 (1.57-4.63)	7.00
Pirimiphos methyl	1.43 (1.40-1.46)	1.25 (1.21-1.27)	0.87
Fenothrin	>1300	>1300	------
Deltamethrin	385.29 (198.3-748.4)	22.95 (20.55-25.64)	0.006

TECHNOLOGY FOR THE MONITORING OF INSECTICIDE RESISTANCE IN STORED CEREAL INSECTS: *Sitophilus oryzae* A CASE STUDY

S. oryzae is one of the most important pests of stored grain in the world and also a valuable model for toxicological research. Virtually all aspects of its biology have been studied. Rearing the weevil in laboratory is relatively easy, and the succession of generations is quite fast. However, the major constraint for the use of this species in bioassays aiming at the elucidation of ecological, physical or toxicological matters, is its "variability", so widely mentioned in the results of those assays. This high variability appears when different strains, different generations of a strain, or different substrates are used. References to "variability" in *S. oryzae* are abundant and mainly refer to insect size, body weight, tolerance to toxicants, response to different kinds of food (Phadke & Bhatia,1974; Sharma, 1985), life cycle and reproductive potential (Kiritani, 1965; Evans, 1982; Thaung & Collins, 1986).

In populations of *S. oryzae* under selection pressure with DDT, 15-fold higher factors of resistance to this insecticide were obtained when the population was grown on rice than when reared on barley (Cichy, 1971). Besides, if the diet is modified for one generation, the development of resistance is different, and different diets have dissimilar effects depending on the insecticide used. Generalizing, the diet plays an important role since it affects the susceptibility of a population to a certain insecticide in the short term, and the speed at which resistance develops, in the long term.

Considering only exogenous factors, the variability in susceptibility of *S. oryzae* to different pesticides is mainly due to temperature, relative humidity and diet (Stadler *et al.*, 1990). By means of the standardization of the rearing method for *S. oryzae*, successive generations with similar characteristics are obtained (Stadler, 1990). These populations have low coefficients of variation in size and overall state of the individuals. Moreover, the stability of these two elements contributes to the verisimilitude of toxicological assays.

Physiological, environmental and population factors affecting the susceptibility to pesticides and the selection of resistance in *Sitophilus oryzae* and other insect pests.

The response of a living organism to a xenobiotic is a characteristic of each combination of species and toxicant. It also differs between populations of the same insect, or between phases, stages, age intervals within a stage, or simply between individuals with a different physiological state.

DIET AND REARING CONDITIONS : The life cycle of *S. oryzae* is influenced, mainly, by humidity and temperature, and their effect is especially evident during embryonic and larval development. Food and environmental humidity sensibly affect the development of larvae and, to a lesser extent, that of eggs and pupae (Easthman & Segrove, 1947). In the non-feeding phases (egg and pupa), water balance is guaranteed, since the insect's metabolism is mainly catabolic. During the feeding and fast-developing phase (larval), anabolic processes prevail, and water demand increases (Singh & Sinha, 1977). Mortality of embryos of the weevil is minimum at 29°C an 90% RH, and the limits within which embryos achieve full development are 15°C and 34.5°C. Embryonic mortality in *S. oryzae* increases dramatically near the upper limit, being 20% at 34.0°C and 100% at 34.5°C (Birch, 1944). As regards changes in temperature and relative humidity, the most sensitive phase is the larval one, and its first instar shows the highest mortality indexes. In favorable enough conditions for the development of L1, mortality in the following instars is practically null (Birch, 1945).

Fecundity also deserves an analysis of two variables such as temperature and humidity combined, and female age. At an even temperature (27°C) and varying the humidity content in food, different fecundity values are obtained per female, in a period of 90 days: 11% RH = 91.8; 12.5%RH = 148.7; 14%RH = 192.6 (Stadler, 1988). According to Longstaff & Evans, 1983, fecundity is highest at 24°C with a grain humidity of 14%. In these conditions, the whole life cycle lasts six weeks, approximately. With high fecundity values -similar to those obtained at 24°C- the life cycle takes place in only four weeks when incubation temperature is 27°C to 30°C and grain humidity ranges from 12.5% to 14%. Within this range, the peak of fecundity in females occurs in the age interval between 1 and 4 weeks (Stadler, 1988).

The reproductive performance of *S. oryzae*, related to its population density has lead to a great number of studies. Early research in this area belongs to MacLagan, 1932, who defines population density and number of eggs laid per female as an inversely proportional relation that can be described by a hyperbolic function (MacLagan & Dunn, 1935). Subsequent studies show that fecundity values fall at very low population densities, showing a much more complex function. Cuff & Hardman's (1980) model shows the evolution of a population of *S. oryzae* taking into account the interaction between initial population density and two variables, namely temperature and atmosphere inside the incubation chamber.

According to different authors (Richards, 1947; Evans, 1977; and Holloway, 1985, Stadler, 1988), sex ratio in a natural population of *S. oryzae* is 1:1. Crossbreeding among individuals of different strains sometimes gives rise to an F1 with a higher proportion of females and, therefore, an unbalanced sex ratio (Shazali, 1982). This bias can be observed along several generations and reciprocal crossings. In spite of the fact that the sex ratio (1:1) is genetically determined, fecundity reaches optimal levels when the male: female relation is 2:5 ratio (Stadler, 1988). This ratio does not address a high copulation frequency, it just guarantees a maximum of mating with at least ones per female. Thus, to start the rearing units, only a short number of individuals will be necessary, avoiding intraspecific competition, which seriously affects fecundity and survival. However, the 2:5 ratio must be considered as related to a certain place/space, or otherwise be defined as a function of population density.

The nature of the relation between population density and fecundity in *S. oryzae* is similar to that established for *S. granarius* by MacLagan, 1932. The appropriate description for this relation is Longstaff's model (1981):

$$f = a.(\log_e N)^b.N^{-c}$$

where N is the number of females per a fixed number of wheat grains, f is the number of eggs per female, and the constants are: a = 6.8211; b = 2.3221; and c = 0.3993. This function is the reflection of two opposite processes determining fecundity. The term $(\log_e N)^b$ represents the positive feedback, or social density-increasing effect, while N^{-c} refers to the phenomenon of overpopulation or negative feedback. When referring to large volumes of cereal, these effects nullify each other for a population density of one individual per 2000 wheat grains. In this situation, fecundity is highest. At higher densities than those suggested by Longstaff (1981), survival of immature phases greatly diminishes. However, on low volumes (150g) exposed to the pest during short intervals, the maximum fecundity and survival is reached with densities of $7x10^4$ weevils per 10^6 grains. If the initial population density and sex ratio in a *S. oryzae* culture is determined, its evolution under controlled conditions can be predicted with a certain degree of precision.

Diet is the factor having the greatest incidence on reproduction rate, body size and physiological state of *S. oryzae* (under optimum environmental conditions) both from a qualitative and a quantitative point of view (Stadler, 1988). Early observations concerning this phenomenon were empirical and, by means of them, it was proved that grain size is directly correlated to the injury level caused by *S. oryzae* (Dogget, 1957).

Shazali (1986) experimentally determined the reproduction rate of *S. oryzae* on big and small grains, correlating it with grain weight loss and calculating the frequency distribution for the number of ovipositions per grain. In other rearing experiments, using two different grain sizes plus a mixture of both, the difference between number of ovipositions on big grains and the mixture was not significant, but 76% of the eggs laid on the mixture were concentrated on the big grains and 24% on the small ones. The preference of *S. oryzae* towards ovipositing on bigger grains was initially noted (Richards, 1947; and Russel, 1966) and discussed by different authors; however, no explanation beyond mere speculation has been found so far. Different authors have demonstrated, in different ways, that food type and kernel-size are directly responsible for individual size of the weevils (Surtees, 1965; Lavadinho, 1975) and this, in turn, for fecundity in the population. Differences in reproduction rate between two populations from the same strain of *S. oryzae* reared on different wheat varieties may sometimes be more significant than those obtained on different types of grain, like corn and wheat (Phadke & Bhatia, 1974; Khare & Agraval, 1963). Not only reproduction rate, but also mortality rate (Russel, 1966; Koura *et al.*, 1971), as well as imago (F1) size and weight (Ungsunantwiwat & Mills, 1985) are altered by the use of different grain varieties when rearing *S. oryzae*. Factors that are responsible for these differences are, mainly, physical and chemical, and are generally related to characteristics of the grain pericarp. The well-known variations among different generations of the same strain of *S. oryzae* are mainly due to the lack of a "standard" food, with which the effect of variables such as pericarp toughness, texture and structure; attractive or repellent substances contained in the pericarp, and grain size, can be minimized or homogenized. These characteristics are subject to inter- or intra-hybrid variations (Russel, 1966; Stevens & Mills, 1973; Gomez *et al.*, 1983), but also suffer modifications during grain storage (Peng *et al.*, 1983).

The use of an artificial diet for rearing of reference strains of *S. oryzae*: On the basis of issues already discussed concerning nutrition and metabolism, a balanced diet was formulated due to rear *S. oryzae* by using a standard interlaboratory method (Stadler, 1988). The artificial diet fulfills the weevil's needs of carbohydrates, proteins, vitamins and other essential components. The mixture was fractionated into 500-mg tablets (double grooved) by means of an automatic pastille maker, due to the fact that *S. oryzae* oviposites only on compact substrates. Tablets can be conserved at +2°C to +5°C for over six months, since they are waterless.

Assays concerning artificial food acceptance showed that 100% of the individuals feed on the tablets during the first 48 hours, after which 20% preferred wheat (Stadler, 1988). Besides, it would be interesting to determine whether those individuals found on wheat remain there, or if some of them alternatively visit both types of food during the assay.

The number of descendants (F1) obtained from PARTOX-S and PARTOX -RM strains on wheat and on artificial diet does not vary significantly between both diets, the differences being mainly qualitative, in coincidence with Bansode & Bhatia's (1981) results. The interval needed for the development of a generation has a smaller standard deviation on artificial diet than on wheat.

The diet determines the size and overall physiological state of an insect population, those populations that fed on substrates of a lower food value (incomplete diet) show a higher susceptibility to Malathion in toxicological trials (Table 4). This phenomenon can partially be due to the homogeneous distribution of all nutrients within the tablet, whereas in wheat grain these nutriments are compartmentalized, forming a heterogeneous system, and not always within reach of the larvae, especially during early growth stages. The difference in susceptibility between populations fed on wheat and artificial diet is also conspicuous.

Table 4: Susceptibility to Malathion of three populations of *S. oryzae* reared on different diets in the topical application test (Stadler *et al.*, 1990)

Diet	LD_{50} (μg/insect)	Fiducial Limit 95%	LD_{95} (μg/insect)
incomplete	0.022	0.020 - 0.031	0.094
artificial	0.037	0.035 - 0.039	0.073
wheat	0.027	0.025 - 0.028	0.061

In spite of the good performance of rearing procedures and bioassays obtained by using artificial diet in our trials, the food value of wheat grain is undeniable. However, by using the artificial diet, there is no influence of variables such as texture, physicochemical characteristics of the pericarp and grain size, which depend on hybridization and storage (Peng *et al.*, 1983). Consequently, the heterogeneity of the natural diet negatively influences the chance to repeat a bioassay.

TEMPERATURE: Among external factors, temperature is certainly the one that most significantly affects the response of these insects to a toxicant. This factor acts at different levels:

- Temperature at which the strain evolved. This is especially important for resistant strains.
- Incubation temperature during rearing.
- Temperature at which insects were kept before, during and after the bioassay.

The last item is particularly important, due to the close interrelation between temperature and insect activity regarding phenomena like intoxication, detoxification, concentration of the toxicant in the target, and eventual recovery from intoxication.

Champ & Chambell-Brown,1970, showed that a temperature shift from 25°C to 30°C causes an increase of nearly x1.5 in Malathion, Diazinon, and Phenytrothion toxicity to *S. oryzae*. Besides, Thaung & Collins (1986) showed that phenytrothion toxicity to *S. oryzae* increases with temperature. Toxicological assays at 15°C, 26°C and 30°C show that susceptibility to Malathion is positively correlated with temperature (Table 5), while for deltamethryn, this correlation is negative (Stadler *et al.*, 1990).

According to previous considerations, it was established that 28°C ± 1°C is an acceptable value from both the physiological and the operational

Table 5: Toxicity of Malathion and Deltamethrin at three different temperatures in *S. oryzae* populations reared at standard conditions, in the topical application test (Stadler *et al.*, 1990)

Insecticide	Temperature °C	LD_{50} μg/ Insect	Fiducial Limit 95%
Malathion	15	0.053	0.051-0.062
	26	0.037	0.035-0.039
	30	0.039	0.038-0.040
Deltamethrin	15	0.0010	0.0006-0.0019
	26	0.0019	0.00016-0.0021
	30	0.0022	0.0018-0.0028

points of view, for toxicological trials.

RELATIVE HUMIDITY : Water economy in stored grain pests has peculiar characteristics, since they were adapted to living without any free water, in environments with a very low water activity. In order to make up for water loss, these insects regain water from food or from the atmosphere, or gain it from primary carbohydrate and lipid oxidation (metabolic water).

In *S. oryzae*, most of the water lost by perspiration is replaced by water taken in from the environment, in passive and active ways. Perspiration is, in turn, regulated by the internal water concentration (internal a_v), which is constant and independent from the external one (external a_v). The ability to regulate water intake by modifying food intake, according to their own internal balance, is an important adaptation of this and other species developing on such extreme habitats (Arlian, 1979). Net water loss by perspiration can be compensated via digestion only in atmospheres bearing an a_v between 0.85 and 0.65. Below or over these limits ($0.65 << a_v >> 0.85$), *S. oryzae* significantly reduces food intake. The overall water balance in the weevil can be summarized as follows:

- With vapor activities of 0.225, water loss due to perspiration exceeds its gain via metabolism, absorption, and incorporation with food. The metabolic rate shows no variation to compensate the deficit.
- With vapor activities between 0.65 and 0.85, water intake due to diet, absorption and metabolism compensates perspiration and metabolic losses.
- With vapor activities of 0.99, water incorporation via digestion diminishes, since food intake falls. Absorption, in this case, compensates all losses.

It can be concluded that, for those strains that develop on insecticide-treated grain, and in environments with a low RH, the rapid increase in resistance is an exclusive consequence of the raise in the doses of pesticide which, in turn, is due to an increase in food intake.

In order to standardize the bioassays that keep *S. oryzae* off desiccation or humidity excess stresses, it is convenient to fix RH at 77% ± 2% (both for insect rearing and toxicological assays).

AGE AND DEVELOPMENTAL STAGE : A lower Malathion susceptibility, as well as a higher deviation in the toxicological response, is observed in *S. oryzae* populations during the first five days of life of imagines. In the figure Nº 1, a gradual increase can be noted in Malathion susceptibility during the former three days. This phenomenon gradually reverts from the fifth day on, and remains stable until the fifteenth day, after which it slightly increases. The variation in susceptibility observed during the first five days of the imagos life are, probably, due to a series of physiological events that modify the penetration and metabolism of the toxicant. These phenomena are probably related to cuticle hardening, sexual maturation and important changes in lipid and carbohydrate metabolism that occur at the beginning of the adult stage (Singh & Sinha, 1977).

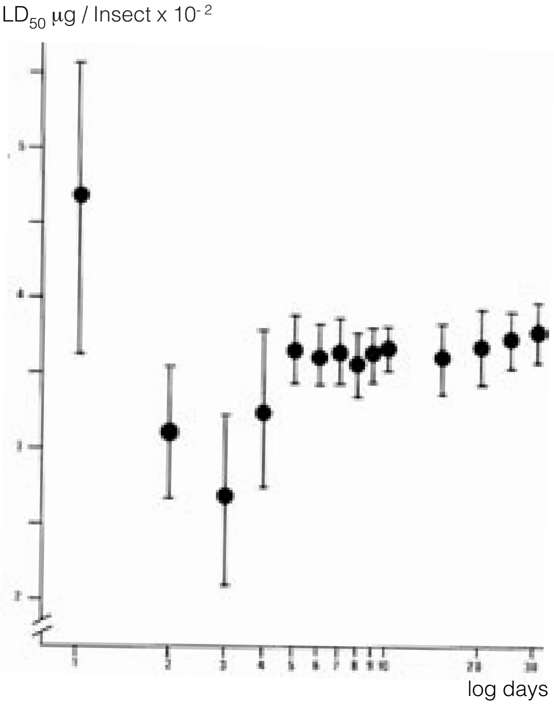

Figure 1 : Age dependent tolerance to Malathion in a susceptible strain of *S. oryzae* in the topical application test (Stadler *et al.*, 1990)

The age and stage dependent tolerance cases studied up to now show that the tolerance is related to certain biochemical mechanisms. In *Blatella germanica* (L.) fifth instar nymphs and adult males show differential tolerance toward Propoxur due to enhanced microsomal oxidation (Valles *et al.*, 1996). In other taxa like Diptera (Brattsten & Metcalf, 1973) and Lepidoptera (Yu, 1983), age-dependent variation of susceptibility to pesticides and differential detoxification capacity was shown.

Therefore, it can be stated that in *S. oryzae* the recommended age interval of individuals to be used in toxicological experiments is 5 to 10 days.

Table 6: Sex dependent tolerance in *S. oryzae* to three insecticides in the topical application test (Stadler *et al.*, 1990

SEX	INSECTICIDE	STRAIN CIPEIN-S LD$_{50}$ µg/Insect	STRAIN CIPEIN-MR LD$_{50}$ µg/Insect
MALE	MALATHION	0,024 (0.022-0.026)	0,168 (0,164-0,171)
	DELTAMETHRIN	0,002 (0,001-0,002)	0,002 (0,001-0,003)
	LINDANE	0,309 (0,260-0,367)	0,092 (0,070-0,120)
FEMALE	MALATHION	0,029 (0,028-0,031)	0,169 (0,165-0,172)
	DELTAMETHRIN	0,002 (0,001-0,002)	0,002 (0,001-0,002)
	LINDANE	0,650 (0,588-0,720)	0,188 (0,145-0,245)

SEX : In *S. oryzae* the mean body weight is nearly 15% higher in females than in males (FEMALES, x=1.52mg, SD=0.314; MALES, x=1.27mg, SD=0.277), the toxicological response is similar for both of them, regarding Malathion and Deltamethryin. Whereas susceptibility for Lindane in males is 100% higher than that in females (Table 6), both in susceptible and resistant strains (Stadler *et al.*, 1990).

CONCLUSIONS

A standardized rearing method for *S. oryzae* offers the possibility to define or characterize a strain, fixing all ecophysiological variables, and to obtain a biological material of qualitatively and quantitatively constant characteristics, both among individuals within a population and among different generations. This is achieved by means of:

The control of the temperature, humidity, parental population density and sex ratio, the removal of parents after a short oviposition period and the elimination of fine particles (frass) will stabilize the heat production, water balance and gas concentration during incubation and will avoid :

* the high mortality indexes, as well as low fertility indexes, caused by the unfavorable situation generated, in uncontrolled conditions, from a non-specific interaction among exogenous variables.
* the difference in life cycle length among different populations.
* the massive development of fungi and invasion of mites in cultures.

- Defined nutritional conditions: The use of artificial food eliminates qualitative and quantitative differences, both intra- and inter-hybrid, as well as the influence of physicochemical factors that alter grains. It is, therefore, possible to obtain more homogeneous populations as regards:
  * overall physiological state.
  * reproduction rate.
  * individual size (related to food size), which also affects, through fecundity, the final population density (F1).

Artificial diet, together with specific incubation conditions helps to characterize a strain, or compare two strains, on the basis of defined ecophysiological variables. The number of descendants (F1) obtained from PARTOX -S AND PARTOX -RM, reared both on wheat and an artificial diet does not vary significantly. The only changes observed were those of the coefficients of variation (CV) that vary proportionally in each strain". For toxicological studies, it is convenient to use individuals reared on the same diet, preferably an artificial one. Besides, the use of artificial diets for laboratory rearing of *S. oryzae* shows other advantages, namely:

- For toxicological bioassays, eggs, larvae of different instars and pupae can be obtained in a short time and in practically unlimited numbers, breaking the tablets to pieces under water stream on a sieve (250m). This method replaces the seed dissection technique (Soderstrom, 1960).
- Imagines (F1) which normally emerge from wheat within 14 days, do so in only 8 days when reared on artificial food. With this procedure, a greater number of individuals of the same age interval can be obtained from smaller cultures.
- The temperature and relative humidity recommended for toxicological assays with *S. oryzae* are: 28°C ± 1°C and 77% ± 2% RH. Under these conditions, individuals keep their normal activity and keep off those stress conditions caused by alterations in the water balance.
- The optimum age interval of imagines to be used for toxicological studies is 5 to 10 days. Within this interval, deviations in toxicological response are lowest.

The use of the proposed rearing methodology for *S. oryzae* and its transfer to other insect pest species will contribute to the unification of bioassay criteria for future standard interlaboratory programs on insecticide resistance monitoring.

PRIORITY AREAS FOR RESEARCH AND TECHNOLOGY DEVELOPMENT FOR MONITORING OF INSECTICIDE RESISTANCE

It has to be acknowledged that the only effective resistance-monitoring program is the one that detects resistance before control failures, so resistance assessment must be a tool of great precision (Roush & Miller, 1986). For this reason, harmonization of criteria for future IRM programs is a priority.

It has to be assumed that there is a lot of information available for stored grain pests from research conduced over the last 60 years all over the world. Likewise, some of this information has to be evaluated regionally and some other needs to be validated for different pest species.

However, there are three levels of information that must be obtained in order to build up a comprehensive resistance-monitoring program in stored product insects. The first, the rearing of reference strains as a material for validation of insecticide screening. The second, the harmonization of accurate laboratory testing and rearing methodology and the third level of information is the development of a field testing method to attain a prompt approach to the problem.

It is here suggested that a field-testing method for the different stored grain pests by using the "glass vial bioassay" (Plapp *et al.*, 1987) should be developed. Routine monitoring by using this simple but efficient methodology will offer an effective and economical choice for a prompt detection of resistance in the field. This notwithstanding, it will be necessary to preserve samples for future confirmatory, comparative and cross-resistance testing in the laboratory.

International cooperation is also essential because it is not reasonable to expect that this work be developed by a single research group. Therefore, it is important that there be close cooperation between agencies, institutes and scientists to address a harmonized resistance monitoring program for stored cereal insects. It is important, as well, that the best international scientific expertise be involved with the project to assist in research, transfer and harmonization. This cooperation should encourage the formation of an International Stored Grain Pest Resistance Monitoring Task Force.

ACKNOWLEDGMENTS

We acknowledge Ing. Agr. A. Luqui and Ing. A. Digiulio (Servicio Nacional de Sanidad y Calidad Agroalimentaria-Argentina; SENASA, ex IASCAV) for the useful historical information on annual yields and grain damage in Argentina.

LITERATURE CITED

Arlian, L.G., 1979. Significance of passive sorption of atmospheric water vapor and feeding in water balance of the rice weevil, *Sitophilus oryzae*. Comp. Biochem. Physiol., 62A:725-733.

Bansode, P. C. and S.K. Bathia,1981. Note on reduced reproductive ability in a Malathion-resistant strain of *Sitophilus oryzae* (L.) (Coleoptera : Curculionidae). Protection Ecology, 3: 63-64.

Birch, L.C., 1944. The effect of temperature and dryness on the survival of the eggs of *Calandra oryzae* L. (small strain) and *Rhizopertha dominica* Fab. (Col.) Australian J. Exp. Biol. Med. Sci., 22 (4): 265-269.

Birch, L.C.,1945. The influence of temperature, humidity and density on the oviposition of the small strain of *Calandra oryzae* L. and *Rhizopertha dominica* Fab. (*Coleoptera*). Aust. J. Exp. Biol. Med. Sci.,23 (3):197-203.

Brattsten, L.B. and R.L. Metcalf, 1973. Age-dependent variation in the response of several species of diptera to insecticidal chemical. Biochem. Pharmacol. 18, 1403.

Cichy, D., 1971. The role of some ecological factors in the development of pesticide resistance in *Sitophilus oryzae* L. and *Tribolium castaneum* Herbst. Ekol. Polska, XIX (36): 567-616.

Cuff, W.R. and J.M. Hardman, 1980. A development of the Leslie Matrix formulation restructuring and extending an ecosystem model : the infestatioln of stored wheat by *Sitophilus oryzae*. Ecological Modelling, 9: 281-305.

Champ, B. R. and M.J. Champbell-Brown,1970. Insecticide resistance in Australian Tribolium castaneum (Herbst). I.- A test method for detecting insecticide resistance. J. Stor. Prod. Res.,6: 53-70.

Champ, B.R. and E. Highley, 1983. Pest resistance to pesticides: 385-397, in Pest Resistance to Pesticides. G. P. Georhiou and T. Saito, eds. NY Plenum.

Dogget, H. 1957. The breeding of sorghum in East Africa. I.- Weevil resistance in sorghum grain. Emp. J. exp. Agric., 25:1-9.

Easthman, L. E. S. and F. Segrove, 1947. The influence of temperature and humidity on instar length in *Calandra granaria* Linn. J. Exp. Biol. 24: 79-94.

Evans, D.E., 1977. The capacity for increase at a low temperature of several Australian populations of *Sitophilus oryzae* (L). Aust. J. Ecol. 2: 55-67.

Evans, D.E., 1982. The influence of temperature and grain moisture content on the intrinsic rate of increase of *Sitophilus oryzae* (L) (Coleoptera : Curculionidae). J. Stor. Prod. Res. 18: 55-66.

FAO, 1974. Recomended methods for the detection and measurement of resistance to agricultural pests to pesticides. FAO Method No 15. Fao Plant Prot. Bull 22:127-137.

Ferrero, A., E. Wood, M.Y. Picollo de Villar, C. Reale y E. Zerba, 1991. Relación entre la actividad de esterasas y la resistencia a Malatión en una cepa de *Tribolium castaneum*. Acta Bioq. Clin. Latinoamericana 25 (4): 435-442.

Georghiou, G.P. and C.E. Taylor, 1977. Genetic and biological influences in the evolution of insecticide resistance. J. Econ. Entomol.,70 (3): 319-323.

Gomez, L.A., J.G. Rodriguez, C.G. Poneleit, D.F. Blake, 1983. Relationship between some characteristics of the corn kernel pericarp and resistance to the rice weevil (Coleoptera : Curculionidae). J. Econ. Entom. 76 (4): 797-800.

Holloway, G.J., 1985. The effect of increase grain moisture content on some life history characters of *Sitophilus oryzae* (L.) after staining egg-plugs with acid fuchsin. J. Stored Prod. Res. vol.21 (4): 165-169.

Khare, B.P. and N.S. Agrawal, 1963. Effect of temperature, relative humidity, food material and density of insect population on the oviposition of *Sitophilus oryzae* (Linn.) and *Rhyzopertha dominica* Fab. Bull. Grain Technol. 1: 48-60.

Kiritani, K., 1965. Biological studies on the *Sitophilus complex* (Coleoptera : Curculionidae) in Japan. J. Stor. Prod. Res. 1: 169-176.

Koura, A., M. El Halfawy, S. Risk and F.D. Aly, 1971. Type of food as a factor influencing the longevity of some stored products insects. Agric. Res. Rev. vol.49: 30-33.

Lavadinho, A.M.P., 1975. Toxicological studies on adult *Sitophilus granarius* (L), influence of individual body weight on the susceptibility to DDT and Malathion. J. Stor. Prod. Res. 12: 215-224.

Longstaff, B.C., 1981. Density dependent fecundity in *Sitophilus oryzae* (L.) (Coleoptera : Curculionidae). J. Stored Prod. Res. 17: 73-76.

Longstaff, B.C. and N. Evans, 1983. The demography of the rice weevil *Sitophilus oryzae* (L) (Coleoptera : Curculionidae) submodels of age-specific survivorship and fecundity. Bull. Ent. Res. 73: 333-344.

MacLagan, D.S., 1932. The effect of population desity upon rate of reproduction with special reference to insects. Proc. R. Soc. London, Ser. B, 111: 437-454.

MacLagan, D.S. and E. Dunn, 1935. The experimental analysis of the growth of an insect population. Proc. R. Soc. Edinburgh, 55: 126-138.

Peng, W. K., S.J Hsu and K.K. Ho, 1983. Studies on the varietal resistance of rice weevil *Sitophilus oryzae* (L.). Phitopathologist & Entomologist NTU (19): 27-38.

Phadke, K. G. and S.K. Bhatia, 1974. Population growth of *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F) in wheat varieties. Ind. J. Entomol.,36: 251-260.

Picollo de Villar, M. I., E. Seccacini y E.N. Zerba, 1985. Resistencia a Malatión en insectos plaga del grano almacenado de la República Argentina. IDIA no 441-444: 59-63

Picollo de Villar, M. I., A. Ferrero, E. Seccacini y E.N. Zerba, 1992. Perfil de toxicidad de insecticidas en cepas susceptibles y resistentes al malatión en *Tribolium castaneum* (Coleoptera : Tenebrionidae). Rev. Soc. Entomol. Argent. 51 (1-4): 71-78.

Richards, O.W., 1947. Observations on grain weevils *Calandra* (Col. , Curculionidae) I. General Biology and Oviposition. Proc. Roy. Zool. Soc. London 117:1-43.

Plapp, F. W., G.M. McWhorter and W.H. Vance, 1987. Monitoring for resistance in the tobacco budworm in Texas in 1986. In Proc. Beltwide Cotton Prod. and Res. Conf. National Cotton Council, Memphis TN: 324-326.

Rosenheim, J.A. and B.E. Tabashnik, 1990. Evolution of pesticide resistance: Interactions between generation time and genetic, ecological, and operational factors. J. Econ. Ent. 83 (4): 1184-1193.

Roush, R.T. and G.L. Miller, 1986. Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol. 79 (2): 293-298.

Russel, M.P., 1966. Effects of four sorghum varieties on the longevity of the lesser rice weevil *Sitophilus oryzae* (L). J. Stor. Prod. Res. 2: 75-79.

Sharma, V.K.,1985. Effect of mated females alone and in association with males on the adult emergence of *Sitophilus oryzae* (L.) infesting different wheat varieties. J. Ent. Res. (New Delhi) 8 (2): 213-214.

Shazali, M.E.H., 1982. The biology and population ecology of four insect pests of stored sorghum with particular references to competition and succession. Ph.D. Thesis, Univ. of Reading, Sudan.

Shazali, M.E.H., 1986. Effect of sorghum grain size on developmental ecology of *Sitophilus oryzae* (L.). Z. Angew. Zool. 73: 293-300.

Singh, N.B. and R.N. Sinha, 1977. Carbohydrate, lipid and protein in the developmental stages of *Sitophilus oryzae* and *S. granarius* (Coleoptera : Curculionidae). Ann. Ent. Soc.Amer. 70 (1): 107-111.

Soderstrom, E.L., 1960. Recognition and duration of larval instars of the rice weevil and the granary weevil. J. Kansas. Ent. Soc. 33 (4): 157-161.

Stadler, T., 1988. Normalización de las variables ecofisiológicas durante el desarrollo de *Sitophilus oryzae* (L.) (Coleoptera : Curculionidae). Bol. San. Veg. Plagas, 14: 541-552.

Stadler, T., M.Y. Picollo y E. Zerba, 1990. Factores ecofisiológicos relacionados con la susceptibilidad a insecticidas y la resistencia a malatión en *Sitophilus oryzae* (L.) (Coleoptera : Curculionidae). Bol. San. Veg. Plagas, 16: 743-754.

Stevens, R.A. and R.B. Mills, 1973. Comparison of techniques for screening sorghum grain varieties for resistance to rice weevil. J. Econom. Entomol. 66 (5): 1222-1223.

Surtees, G., 1965. Effect of grain size on development of the weevil *Sitophilus granarius* (L.) (Coleoptera : Curculionidae). Proc. Roy. ent. Soc. London A (40): 38-40.

Thaung, M. and P.J. Collins, 1986. Joint effects of temperature and insecticides on mortality and fecundity of *Sitophilus oryzae* (Coleoptera : Curculionidae) in wheat and maize. J. Econ. Entomol. 79 (4): 909-914.

Ungsunantwiwat, A and R.B. Mills, 1985. Influence of rearing medium on size and weight of adults of four *Sitophilus* populations and on weight loss of host kernels (Coleoptera : Curculionidae) J. Stored Prod. Res. 21 (2): 89-93.

Valles, S. M., S.J. Yu and P.G. Koehler, 1996. Biochemical mecanisms resposible for stage-dependent propoxur tolerance in the german cockrach. Pest. Biochem. Physiol. 54: 172-180.

Winston, P. W. And D.H. Bates, 1960. Saturated solutions for the control of humidity in biological research. Ecology 41: 232-237.

Yu, S.J., 1983. Age variation in insecticide susceptibility and detoxification capacity of fall army worm (Lepidoptera : Noctuidae) larvae. J. Econ. Entomol. 76: 219.