

## STATUS OF ACTELIC RESISTANCE IN THREE STORED PRODUCT INSECTS INFESTING SORGHUM AND BEANS IN RWANDA

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### ABSTRACT

Populations of stored grain and bean insects resistant to actellic (pirimiphos methyl) were suspected to be developing in government warehouses in Rwanda after a national survey of beans, sorghum and maize was completed in 1986. Actellic has been used prophylactically (0.1% powder) since 1982 for all beans (Phaseolus vulgaris L.) and sorghum (Sorghum bicolor) bought by the government warehouses. The authors hypothesized that this resistance had begun in the areas of prophylactic use and not at areas, such as the cooperative storages, where actellic was infrequently used. The main loss causing insects in beans, sorghum and maize in Rwanda are Acanthoscelides obtectus Say, Sitophilus oryzae L., and Rhyzopertha dominica F. In 1989, these three species were collected at government warehouses and cooperative storages in Rwanda and reared through one or two generations in the laboratory. In controlled conditions the same as that used for rearing, adult bioassays were conducted with acetone dilutions of liquid actellic. Our probit analyses indicated that populations of A. obtectus were 150 times less sensitive at the Kicukiro warehouse than at the cooperative in Kopakokazo. At one of the newest government warehouses (Bugesera), populations of A. obtectus were only 50 times less sensitive than that of the cooperative. Populations of S. oryzae at the Kicukiro warehouse were 110 to 210 times less sensitive than those at the Codegi (Gishoma) Cooperative. Populations of R. dominica were similarly sensitive at areas where actellic had and had not been used prophylactically. Alternatives to actellic in the management of A. obtectus and S. oryzae are being developed within Rwanda.

### INTRODUCTION

Insect susceptibility to insecticides is a natural resource which has been over-exploited in many developed countries such as the USA. Generally this misuse of the susceptibility resource has occurred when insecticides are used prophylactically. The result is often a population of insects which have evolved a resistance to the routinely

used insecticide. Better management of the susceptibility resource requires more information on ecology and population genetics of insects as well as the political will to make resistance management strategies work (Mallett 1989). In following the model of the US and other developed countries, many developing countries have initiated a prophylactic use of insecticides, particularly with stored grain and other stored products.

Actellic (pirimiphos methyl) is an organophosphate approved in the US for commercial use as a residual insecticide for stored grain in 1986. It is considered low impact to non-target mammals (LD50 = 1180-2050 mg/kg), but actellic is toxic for birds (207-633 mg/kg), fish, and zooplankton (Samylin and Kerbabayev 1982).

Since 1983, Actellic has been used prophylactically for control of the bean bruchid, *Acanthoscelides obtectus* Say, in dry edible beans, *Phaseolus vulgaris* L., in government storages in Rwanda (East Central Africa). In Rwanda, *A. obtectus* is the major cause of postharvest loss (Dunkel et al. 1988a). A similar situation has occurred with the postharvest management of grain sorghum, *Sorghum bicolor*, in Rwanda. The major insect problems in stored sorghum in Rwanda are the rice weevil, *Sitophilus oryzae* L. and the lesser grain borer, *Rhyzopertha dominica* F. For both beans and sorghum, a powder formulation of Actellic at 1% is mixed by hand and by shovel with the bulk commodity within weeks after reception at the government warehouse (Figure 1). This generally occurs one to two months after harvest. After treatment, the commodities are placed in woven polyethylene or jute bags which are then stacked on pallets in a brick, metal, or concrete warehouse (Figure 1).

Prior to the use of Actellic, malathion was used prophylactically in these Rwandan storages. The use of malathion was discontinued because government warehouse managers indicated that it was no longer suppressing the populations of these storage insects.

The necessity of developing a sustainable insect protectant program for the large scale government storages in Rwanda is now urgent. The urgency is dictated by the government decision to begin long-term strategic storage of beans and sorghum (Dunkel et al. 1988b). In this program, which is now underway, beans will be stored a maximum of two years and sorghum a maximum of five years. In 1984, at one of the government warehouses (Nyanza), the density of *S. oryzae* increased significantly from 11.5 to 137.5 adults/kg between five and eleven months postharvest following the typical actellic prophylactic treatment plan. Similarly, in the same warehouse during the same time period, *R. dominica* increased from 0.9 to 22.1 adults/kg (Dunkel et al. 1988a).

We therefore hypothesized that the development of actellic resistant populations of *S. oryzae*, *R. dominica*, and *A. obtectus* was occurring in government warehouses in Rwanda. We further hypothesized that this loss of sensitivity was not occurring in the populations of these insect species located on farm and in the cooperative warehouses where insecticides were not used prophylactically. This research constitutes the first study of the susceptibility levels to actellic in stored product insects in Rwanda.

## MATERIALS AND METHODS

The three insect species were collected from government warehouses of Rwanda

in Nyanza, Bugasera, Kibungo, and Kicukiro and from cooperative storages at Kopakokazo and Codegi (Gishoma) in Rwanda. Collections were made in the spring and summer of 1989. Insects were reared through two to three generations in the research laboratory (OPROVIA-GRENARWA II - Recherches) (OPROVIA = Office National pour le Developpement et la Commerciale des Produits Vivriers et des Productions Animales) (GRENARWA = Grenier National au Rwanda) in Kigali. The A. obtectus were maintained on the Rubono variety of dry beans and the other species on red sorghum. Insects were reared in glass jars (1l capacity) with screen lids ( $27 \pm 1$  degrees C;  $65 \pm 5\%$  relative humidity (RH); 12L:12D photoperiod). Fresh grain or beans was provided for each new generation of ovipositing females.

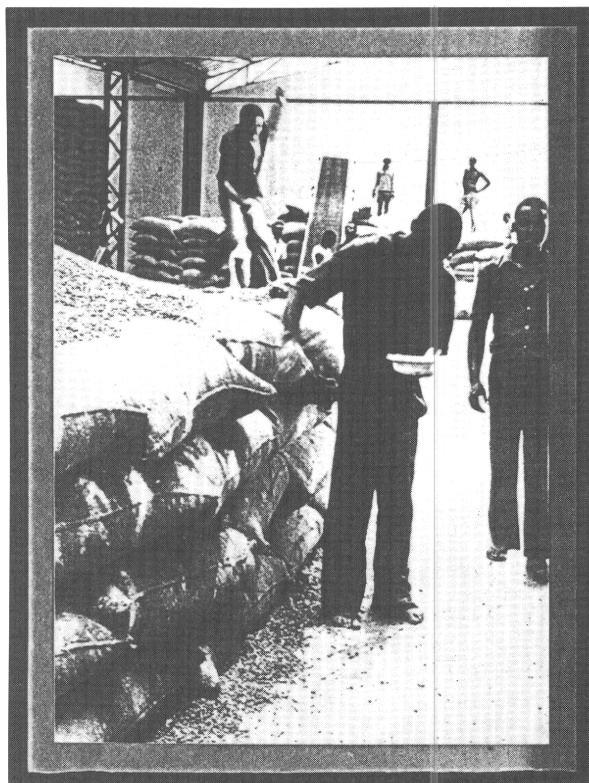
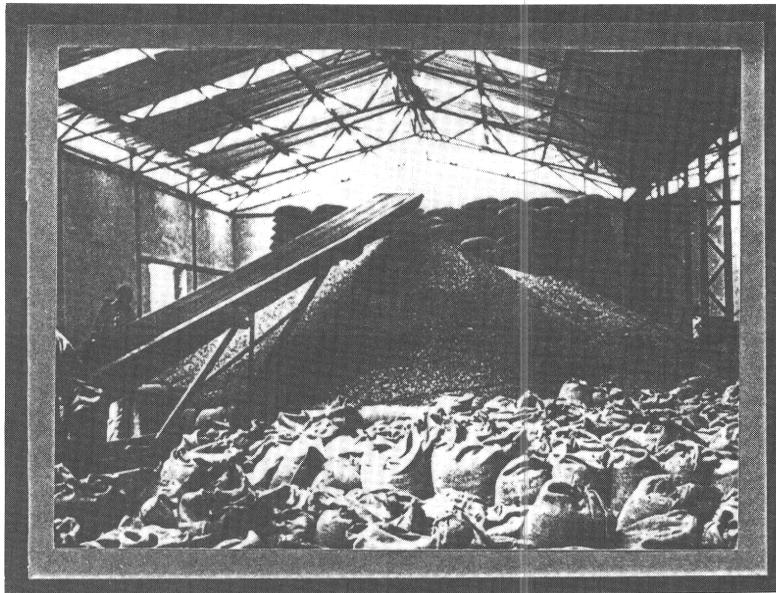
Technical grade actellic (ICI/TWIGA Nairobi, Kenya; liquid 25% a.i. = active ingredient) was diluted with acetone (99.8%) immediately prior to use. The original dilution series was: 4.0, 2.0, 1.0, 0.1, and 0.01 % volume/volume (v/v). If 100% mortality was not achieved with 4% dilution, alternate dilutions (Set A-high series) (1.0, 2.0, 4.0, 8.0, and 10.0%) (Set B-low series) (0.01, 0.05, 0.1, 0.5, and 1.0%) were used.

The bioassay (modified FAO 1974 procedure) was conducted under the same conditions as the rearing. Whatman No. 1 filter papers (9 cm diam) were placed in a pyrex glass petri dish and treated with 0.8 ml of the actellic dilution or with acetone only as the control. Treated papers were dried in the dish for two hours. Ten adult insects of mixed sexes and known ages were inoculated in each of five replicates. Readings were made at four and 24 hours after inoculation. Mortality and morbidity were counted as such if the insect was not able to right itself after gentle stimulation with a fine brush.

Probit regression analyses (Finney 1952) were estimated on the concentration mortality data analyzed using a microcomputer-based probit analysis (Hubert 1988) for the IBM-PC. The median lethal concentration (LC50) of actellic was calculated for each insect strain. Control mortality never exceeded 17%. Abbott's formula (Abbott 1925) was applied to the mortality data prior to performing the regression analyses. The resistance ratio (RR) of government warehouse strains was computed using these LD50 values ( $RR = LC50$  of insect strains collected from suspected resistance site/ $LC50$  of insect strain collected from storages where actellic was not used).

## RESULTS

In 1989, the first actellic bioassays performed on storage insects in Rwanda indicated there was over 100 fold less sensitivity to actellic in stored product insect populations at government warehouses than populations of the same species at cooperative storages. The population of A. obtectus from the Kopakokazo Cooperative where actellic is not used prophylactically had LC50s of 0.0005 % or below (Table I) (Figure 2a). On the other hand, at Kicukiro, the warehouse where actellic has been used regularly for the longest period of time (at least since 1982) than any location in Rwanda, the A. obtectus population had an LC50 of 0.0749% (Figure 2b)(Table I). The government warehouse at Bugesera is one of the newest in Rwanda. The LC50 of its bean bruchid population was intermediate to that of the identified sensitive population and the hypothesized resistant population (Table I)(Figure 2c).



**Figure 1. Prophylactic treatment of beans with actellic (pirimiphos methyl) at a Rwandan national (OPROVIA) warehouse in Kicukiro prior to bagging and building of stacks on pallets (background) a). creation of bulk of newly purchased beans to which powdered actellic is added and mixed, b). adding actellic around edge of bean bulk.**

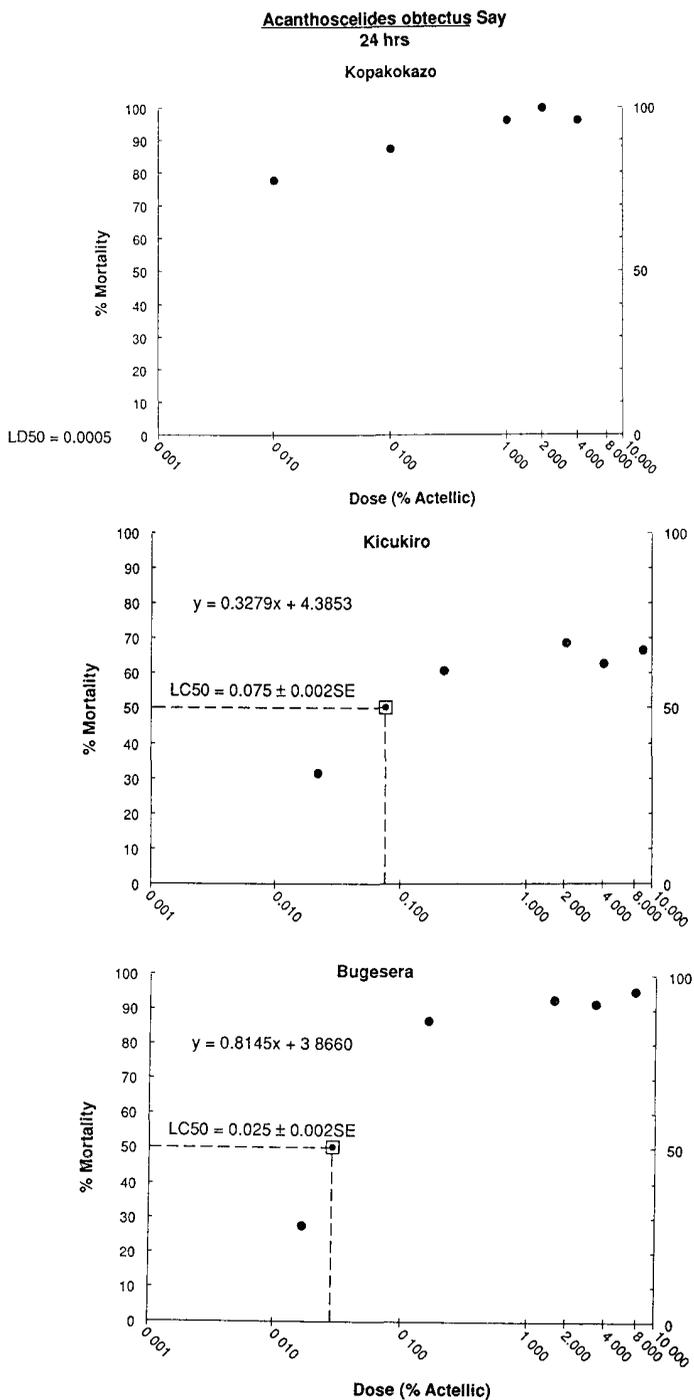


Figure 2. Probit analyses of Rwandan strains of Acanthoscelides obtectus Say exposed to actellic solutions on filter paper in petri dish bioassay chambers, 0 to 3 days after adult emergence from beans a). A. obtectus from Kopakokazo Cooperative, b). A. obtectus from Kicukiro warehouse, c). A. obtectus from national (OPROVIA) warehouse in the Bugesera.

Table I. Responses to the actelic bioassay at 4 and 24 hours of Acanthoscelides obtectus Say, Rhyzopertha dominica F., and Sitophilus oryzae collected at selected locations in Rwanda.

24 hrs A. obtectus

| Location                              | No. of Insects <sup>a</sup> | Slope  | LC50(95% CL) <sup>b</sup> | RR <sup>c</sup> |
|---------------------------------------|-----------------------------|--------|---------------------------|-----------------|
| *Cooperative<br>Kopakokazo<br>14Mar89 | 250                         | 0.5635 | 0.0005(0.0001,<br>0.0024) | *               |
|                                       | 125                         | 0.2034 | 0.0000(0.0000,<br>0.0023) |                 |
| Warehouse<br>Kicukiro<br>23Jun89      | 250                         | 0.3279 | 0.0749(0.0234,<br>0.2400) | 149.8           |
| Warehouse<br>Bugesera<br>7Sept89      | 125                         | 0.8145 | 0.0247(0.0104,<br>0.0583) | 49.4            |

24 hrs S. oryzae

| Location                                  | No. of Insects <sup>a</sup> | Slope  | LC50(95% CL) <sup>b</sup>  | RR <sup>c</sup> |
|---|-----------------------------|--------|----------------------------|-----------------|
| *Cooperative<br>Codegi (Gishoma)<br>7Ap89 | 250                         | 0.5548 | 0.003(0.0001,<br>0.0018)   | *               |
| Warehouse<br>Kicukiro<br>15Sept89         | 250                         | 1.4870 | 0.6305(0.4576,<br>0.08687) | 210.2           |
|   | 250                         | 0.6226 | 0.588(0.0277,<br>0.1246)   | 196.0           |

24 hrs R. dominica values too close to 100% to run probit

<sup>a</sup>number of insects tested excluding controls.

<sup>b</sup> concentrations are reported in % v/v (volume/volume) of insecticide applied to bioassay chamber.

<sup>c</sup> resistance ratio LC50 of strains collected from suspected resistant areas (government warehouses) ÷ LC50 of strains collected from areas where actelic has not been used (cooperative storages).

\* = used as the standard sensitive response for that species

There was a 200 fold lower sensitivity to actellic in the population of S. oryzae collected from the Kicukiro warehouse than in the S. oryzae population obtained at a cooperative storage where actellic had never been used prophylactically. For R. dominica, probit data was only available for the response after four hours and only for one government warehouse. Again this data indicated resistance had developed at a level similar to that of other storage insect species at the other actellic-using warehouses.

## DISCUSSION

Resistance to actellic appears to have occurred in populations of the major species of stored product insects in Rwandan government warehouses where actellic has been used prophylactically for the past eight years. Grain and beans do not generally move from the government warehouses to the cooperative storages. Stored commodities, however, do move frequently between government warehouses. The direction of this movement depends on where the surplus production areas have been in comparison to the needs of the human population. Because the primary stored product insects in Rwanda, A. obtectus, S. oryzae, R. dominica, are all obligate internal feeders of beans and other seeds, they are easily transferred to other locations with the beans or grain. New warehouses that begin a prophylactic regime of actellic usage may receive a few resistant individuals in a grain or bean shipment from an older and longer actellic using warehouse. If the new warehouse is using actellic, these few resistant individuals may rapidly increase in numbers. Eventually, those few insects will develop into a large population that are resistant to actellic.

If actellic and other organophosphate insecticides are to be used with efficacy and confidence, resistance management strategies must be implemented immediately. Viable alternatives to actellic must be readily available in Rwanda which has a low input of foreign exchange. With this in mind, the following alternatives have been in development since the first indication of possible resistant insect populations was observed in 1984 at the government warehouse at Nyanza (Dunkel et al. 1988a). A national survey of local bean varieties of P. vulgaris developed by Rwandan farmers was conducted (Lamb and Hardman 1986). Of the over 300 varieties identified, 8 varieties were determined in laboratory studies to be resistant to bruchids (Lamb and Dunkel 1988). Underground sealed storage of Rwandan beans indicated that, for at least one year, the resultant low oxygen environment prevented development of insect populations, maintained an acceptable sensory quality in the beans, and did not require any insecticide treatment (Hanegreefs et al. in press). Studies presently underway already indicate that local medicinal and insecticidal plants used for stored product protection by Rwandan farmers have potential for large scale protection of beans in government warehouses (Dunkel et al. 1990a). Other insecticidal plants such as neem (Dunkel et al. 1990b) and marigolds are being investigated for their potential for production in Rwanda and utilization in government storages. As suggested by Brattsten et al. (1986), the new insecticides need not be more toxic than current products. New stored product insecticides should, however, have a novel mode of action and have a reasonable (12 to 18 month) stability.

Stored product insect susceptibility to insecticides is an important resource in Rwanda. Without its conservation, it will not be possible to develop long term

strategic storages that hold grain and beans without severe losses due to insects.

## CONCLUSIONS

- The most serious stored product insects in Rwanda have developed resistance to actellic (pirimiphos methyl).
- Alternatives to actellic in government warehouses are being sought.
- Alternatives need to address the issues of novel mode of action and processing which does not require foreign currency.

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ETAT DE RESISTANCE AU PIRIMIPHOS-METHYL CHEZ TROIS INSECTES  
DES DENREES STOCKEES INFESTANT LE SORGHO ET LES HARICOTS AU  
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RESUME

Au Rwanda, le pirimiphos-méthyl est le seul insecticide résiduel actuellement employé en prophylaxie pour lutter contre les insectes des grains et des haricots stockés dans les entrepôts nationaux. Le phénomène de résistance a été observé chez *Rhyzopertha dominica* F. récolté dans les entrepôts gouvernementaux à Nyanza (site de l'observation originelle de 1985) et a conduit à l'hypothèse qu'une résistance au pirimiphos-méthyl était en train de se développer. Les sensibilités des trois principaux insectes des denrées stockées, sensibles et résistants au pirimiphos-méthyl, *R. dominica* provenant de Nyanza, *S. oryzae* L. provenant des entrepôts gouvernementaux de Kicukiro et *Acanthoscelides obtectus* Say provenant de la coopérative de Kopako-Kaso ont été étudiées en 1989. La résistance a été mesurée en utilisant des boîtes de Pétri et du papier buvard imprégné de pirimiphos-méthyl. La mortalité a été mesurée 24 heures après contact. *R. dominica* de Nyanza s'est montré sensible à 0,1, 2,0 et 4,0 % de pirimiphos-méthyl. *S. oryzae* de Kicukiro s'est montré résistant à 0,01 et 1,0 % de pirimiphos-méthyl, modérément résistant à 4 % et sensible à 8 % et 10 % ; tandis que *S. oryzae* de Codegishoma s'est montré résistant à 0,01 et sensible à 1,0 et 2,0 % et 4,0 % de pirimiphos-méthyl. *A. obtectus* provenant de la coopérative de stockage de Kopaza-Kaza s'est montré résistant à 0,01, 1,0 et 2,0 % de pirimiphos-méthyl et sensible à 4 %. En conclusion, des individus résistants au pirimiphos-méthyl existent dans les populations des principaux insectes des denrées stockées au Rwanda. Ces individus existent à la fois dans les populations d'entrepôts ayant subi l'utilisation prophylactique du pirimiphos-méthyl (par ex. : l'entrepôt gouvernemental de Nyanza) mais également dans celles où il n'y a pas eu d'utilisation de l'insecticide (par ex. : Kopako-Kaso).