RESISTANCE TO PHOSPHINE IN STORED GRAIN INSECTS IN BRAZIL. I.

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ABSTRACT

The objectives of this study were (1) to evaluate the present status of resistance to phosphine in field populations of stored grain coleoptera in Brazil and (2) to verify the possible relation between climate and grain storage management, particularly phosphine application techniques, and the development of resistance.

Populations of Sitophilus oryzae, S. zeamais, Rhyzopertha dominica, Tribolium castaneum and Cryptolestes spp. were collected from storage grain facilities located in the South (States of Rio Grande do Sul and Santa Catarina), Center (State of Goiás) and Northwest (States of Acre and Rondônia) and submitted to doses of up to ten times the discriminating concentration and/or to exposure periods of up to 168 hours.

Resistance to phosphine was detected in populations of stored grain insects from all the states sampled.

All the populations of R. dominica and Cryptolestes spp., 86% of S. oryzae and 77% of T. castaneum showed resistance. No resistance was shown by S. zeamais.

Resistant populations of S. oryzae, R. dominica, T. castaneum and Cryptolestes spp. from the cooler Southern area, where grains are generally stored in bulk, were controlled by ten times the discriminating concentration and exposure periods of 80 hours. With the same concentration of phosphine, R. dominica from the Central and Northwestern areas, where grain is generally stored in bags, was not controlled. Warm climates, the storage method used, inadequate management, and poor application techniques have probably contributed to enhance the selection pressure for resistance in these areas.

INTRODUCTION

Phosphine (PH_3) has been, in the last 15 years, pratically the only fumigant used in Brazil for the fumigation of stored grain and by

products. The ease and safety of application, and its great capacity of penetration certainly contributed to its wide acceptance, with the consequent decrease in the use of other fumigants.

It is known, however, that the incorrent use of this fumigant, mainly with respect to sealing and exposure time, can lead to a selection of insect populations tolerant to the fumigant, and with repeated usage, to the development of resistant populations.

According to WINKS (1986) the transmission of resistance by hereditary means occurs in an efficient way. Also BOND (1983) states that when successive generations of insects are selected by means of a toxic substance, allowing some individuals to survive and reproduce, an increase in tolerance to the toxic substance is observed. According to this author, this resistance results from a selection of the most tolerant individuals, resulting in a resistant population. He also indicates that, the resistance to fumigants by stored grain insects can develop in the same way as the resistance to insecticides. Laboratory experiments with several species have indicated that under appropriate selection pressure, considerable resistance to fumigants can be developed.

In Brazil the labels on the commercial products do not include information on the exposure period. The period of 72 hours for temperatures above 20°C was probably sufficient for the control of adult insects before the development of resistance. Nowadays, the recommended period is at least 120 hours (GASCA, 1986). PRICE (1986), determined that the most probable mechanism of resistance to phosphine by insects was exclusion, meaning that the resistant insects apparently developed the capacity to actively exclude the gas from their tissues only accumulating the toxin very slowly. This could explain why the time factors is more important than concentration in the case of resistant insects. According to WINKS (1986), the fact that phosphine is more efficient when applied for longer exposure periods is probably less due to a deviation of Haber's rule (ct = k), than to the fact that phosphine is more efficient against first instar larvae and adults. Thus if an adequate concentration were applied for a period of time long enough for the eggs in the last stage of maturation to hatch, or for the pupae to reach the last pupal stage or become adults, a high degree of control should be expected.

Observations performed in storage units located in the State of São Paulo indicated the presence of large populations of insects a short time after fumigation. It was verified that phosphine treatments were being conducted under unsatisfactory conditions of sealing with consequent underdosage and inadequate exposure periods. These conditions were the same as those that resulted in the development of resistant storage pests in Bangladesh (CONWAY, 1981 as cited by TAYLOR & HALLIDAY, 1986; MILLS, 1983, TYLLER *et al.*, 1983), in the United Kingdom (MILLS, 1986) and in Paquistan, Butão and Botswana (TAYLOR, 1986). This fact led to the suspicion that insects were developing resistance to phosphine in Brazil. A few populations of insects collected in those units were then taken to the NRI - Natural Resources Institute, England, where tests indicated the presence of resistance to the fumigant (TAYLOR & HALLIDAY, 1986, TAYLOR, 1989). The detection of resistance was the motive for carrying out a survey in the State of São Paulo. The results of this survey showed that the 12 populations of *S. oryzae*, 2 of *S. zeamais*, 10 of *R. dominica*, 10 of *T. castaneum* and 8 of *Cryptolestes* tested,, 12, 0, 9, 9 and 8 respectively were resistant to the fumigant (PACHECO *et alii*, in press). These results motivated the current research which aimed at verifying the

distribution of resistant populations of the main species of stored grain insects in Brazil and also at drawing attention to the importance of the adequate use of this fumigant.

MATERIAL AND METHODS

Populations of stored grain insects were collected in storage units located in different states and taken to the laboratories of the Grain Storage Section of ITAL. Collected adult insects of the species S. oryzae, S. zeamais, T. castaneum and Cryptolestes spp. have been multiplied in the laboratory, and were tested for resistance. During sampling, observations were made with respect to the storage structures and insect control management, also considering the technique used for phosphine application.

Samples were taken from storage units in different parts of the States of Rio Grande do Sul, Santa Catarina, Goiás, Acre and Rondônia.

Tests were conducted according to FAO Method nº 16 (FAO, 1975), which is based on the exposure of adult insects for 20 hours using the discriminating concentration of the gas for the species under test. Populations that showed resistant individuals were then exposed to greater concentrations and/or exposure times with the objective of obtaining total mortality. Discriminating concentrations for each species are as follows:

Species	Discriminating Concentration (mg PH_3/l)
Sitophilus spp	0.04
T. castaneum	0.04
R. dominica	0.03
Cryptolestes spp.	0.06

Phosphine was obtained in a generator, according to FAO methodology (FAO, 1975) and the concentration of the gas was determined by the conductimetric method of Harris, based on the reaction of phosphine with mercurium chloride (HARRIS, 1986). The volume of gas necessary for the obtention of the desired concentration was calculated, and applied to the dessicators with a gas tight syringe. After the exposure period, the insects were transferred to flasks containing a small quantity of wheat flour and closed with a screened top, returning them to the chamber at 26 ± 2°C and 70 ± 5% R.H., where they were kept for 14 days. After this period the live, dead and knocked-out insects were counted, considering the knocked out ones as dead. The percent mortality obtained was corrected according to Abbott's formula (ABBOTT, 1925).

RESULTS

The average mortalities of populations of S. zeamais, S. oryzae, R. dominica, T. castaneum and Cryptolestes spp collected in the States of Rio Grande do Sul, Santa Catarina, Goiás, Acre and Rondônia are presented in Tables 1 to 3.

TABLE 1. Resistance to phosphine (PH_3) in stored grain insects from five States in Brazil.

Location collected	Species	Number of populations tested	Number of resistant populations
• Rio Grande do Sul	S. zeamais	1	0
	S. oryzae	5	4
	R. dominica	3	3
	T. castaneum	1	1
	Cryptolestes spp.	3	3
• Santa Catarina	S. zeamais	5	0
	S. oryzae	1	1
	T. castaneum	1	0
• Goiás	S. zeamais	2	0
	R. dominica	5	5
	T. castaneum	8	8
	Cryptolestes spp.	3	3
• Acre	R. dominica	5	5
	T. castaneum	1	0
• Rondônia	S. zeamais	3	0
	R. dominica	3	3
	T. castaneum	1	1

TABLE 2. Response of populations of stored grain insects resistant to phosphine to increased exposure periods.

Location collected	Species	Average mortality at disc. dosage (%)	Exp.periods with the disc. conc. (hours)	Average mortality (%)
• Rio Grande do Sul	S. oryzae	28	40	50
			80	72
			120	89
	R. dominica	27	40	57
			80	73
			120	86
	T. castaneum	88	40	90
			80	100
	Cryptolestes spp.	23	40	44
			80	54
			120	84
• Santa Catarina	S. oryzae	84	40	93
			80	90
			120	100
• Goiás	R. dominica	32	40	35
			80	46
			120	61
• Acre	R. dominica	26	40	36
			80	38
			120	39
• Rondônia	R. dominica	28	40	33
			80	35
			120	46
	T. castaneum	38	40	48
			80	62
			120	95

TABLE 3. Response of resistance populations to ten times the discriminating concentration of phosphine and increased exposures periods.

Location collected	Species	Average mortality at dis.dose (%)	Conc. PH_3 (mg/l)	Exp.period (hours)	Average mortality (%)
• Rio Grande do Sul	S. oryzae	28	0.4	20	86
			0.4	40	99
			0.4	80	100
	R. dominica	27	0.3	20	80
			0.3	40	90
			0.3	80	100
	Cryptolestes spp.	23	0.6	20	69
			0.6	40	90
			0.6	80	100
• Goiás	R. dominica	32	0.3	20	48
			0.3	40	64
			0.3	80	87
			0.3	120	100
• Acre	R. dominica	26	0.3	20	32
			0.3	40	49
			0.3	80	66
			0.3	120	82
			0.3	168	99
• Rondônia	R. dominica	28	0.3	20	46
			0.3	40	58
			0.3	80	92
			0.3	120	96
			0.3	168	--
	T. castaneum	38	0.4	20	97
			0.4	40	100

• Rio Grande do Sul

The climate is cool and almost the totality of the grain is stored in bulk.

Wheat is fumigated every three or four months with 1.4g PH_3/t. The only non-resistant population of S. oryzae from this State was collected in wheat that was stored in an improvised above ground "pool" and had not yet been fumigated. Two of the three populations of R. dominica were collected from paddy stored in bulk that had been fumigated almost montly with 2.4g PH_3/t.

• Santa Catarina

The only resistant population of S. oryzae collected in this State was

collected in wheat that had been stored in bags for 14 months and had been submitted to fumigation with 1g PH_3/t every 100 days.

• Goiás

The climate is warm and dry. About 85% of the product sampled was stored in bags. Fumigation had been carried out using 1g PH_3/t for 72 hours, frequently with inadequate sealing, using plastic sheets in bad condition, poorly set, and with occasional lack of sand snakes. In a vertical metal silo in Maurilândia, fumigation was conducted by placing the fumigant in the aeration ducts, and turning on the aeration fans, without subsequent sealing of the silo.

• Acre

Situated in the Amazon Basin, the climate is warm and humid. All the grains are stored in bags in conventional warehouses. A few also house processing and packaging operations and operate as deposits for other products. As a result it is almost impossible to carry out adequate cleaning and sanitation procedures, aggravating the problem of resistance by the presence of residual infestations. Fumigation using 1g PH_3/t for 72 hours is frequently conducted at one month intervals.

• Rondônia

Situated next to Acre in the Amazon Basin, the climate is also warm and humid. All the grains are stored in bags in conventional warehouses. Fumigation is conducted using 2g PH_3/t for 72 hours.

GENERAL DISCUSSION AND RECOMMENDATIONS

Although with different intensities, resistance to phosphine was detected in populations of stored grain insects from the five states sampled. All the populations of *R. dominica* and *Cryptolestes* spp., 86% of *S. oryzae* and 77% of *T. castaneum* showed resistance. No resistance was shown by *S. zeamais*. Resistant populations of *S. oryzae*, *R. dominica*, *T. castaneum* and *Cryptolestes* spp. from the cooler southern area, where grain are generally stored in bulk, were controlled using ten times the discriminating concentration and exposure periods of 80 hours. *R. dominica* was the most difficult species to control in the central and northwestern areas, where the climate is warmer and the grain is generally stored in bags: 60% of the populations collected in Goiás required exposure periods of 120 hours and 65% from Acre and Rondônia required 168 hours, 35% not being completely controlled even with these dosages.

In the worldwide survey of pesticide susceptibility carried out by FAO in the period 1972-1973 (CHAMP & DYTE, 1976) no resistance to phosphine was detected in stored grain insects from Brazil. Sampling included 2 populations of *S. oryzae* (from Goiás and São Paulo), 13 populations of *S. zeamais* (from Amazon, Rio Grande do Sul and São Paulo), 2 of *R. dominica* (Goiás and São Paulo) and 5 of *T. castaneum* (Goiás, Rio de Janeiro, Rio Grande do Sul and São Paulo). The same survey indicated the occurrence of phosphine

resistance in 33 of the 86 countries sampled, involving 82 of a total 849 populations tested. No resistance was detected in S. zeamais. Resistance was detected in 50% of the populations of S. oryzae and T. castaneum and in 23% of R. dominica.

WINKS (1986) also observed higher levels of resistance in R. dominica before and after selection in the laboratory as compared to T. castaneum, T. confusum and Sitophilus spp. MILLS (1986) indicated that in Bangladesh resistant populations of R. dominica survived after being exposed to 0.8mg PH_3/l for 168 hours at 25°C.

In the central and nortwestern areas the warm climate, the frequent inadequate storage management added to poor fumigant application techniques, have probably enhanced the selection pressure for resistance. Corrective measures to limit the further development of resistant populations and to control the existing ones are badly needed. Well trained operators, conscious of the importance of the correct use of the fumigant, wide distribution of folders with clear and precise instructions for the apllication and a modification of the instructions included in the labels of commercial products, adapting concentrations and exposure times to the state of tolerance of the insect populations to phosphine, are measures that cannot be postponed.

Operational methods used should minimize the problem of residual infestations and lower the selection pressure. For the control of resistant populations it is essential to achieve a degree of sealing which allows for the maintenance of the determined concentration of gas for an adequate period. A minimum exposure period of 5 days and a final concentration of phosphine of approximately 0.2mg/l is recommended as a miminum standard for efficient fumigation (GASCA, 1986).

In regions where the atmospheric temperature can be relatively low, forced aeration of the grain bulk may cause a lowering of the temperature, sufficient to halt or retard the multiplication of the insects, lowering the selection pressure.

Finally, it is necessary to remember that no method of control is efficient when used alone. It is necessary to adopt an integrated method of control involving obligatory good housekeeping, sanitation and periodic inspections of the storage unit and its surroundings.

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RESISTANCE A LA PHOSPHINE DES INSECTES DES GRAINS STOCKES AU BRESIL

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RESUME

Les objectifs de cette publication sont : 1) d'évaluer l'état actuel de la résistance des insectes à la phosphine en milieu naturel chez les populations de coléoptères des grains stockés au Brésil ; 2) de vérifier les techniques de fumigation employées ainsi que leur possible rapport avec le développement de la résistance.

Des populations de *S. oryzae, S. zeamais, Rhyzopertha dominica, Tribolium castaneum* et *Cryptolestes spp.* ont été récoltées sur des installations de stockage de grains situées dans de nombreuses régions du pays et ont été soumises au test de résistance recommandé par les normes de la FAO. Les populations d'insectes semblant présenter des signes de résistance ont alors été soumises à des doses allant jusqu'à 10 fois la concentration discriminante et/ou à une durée d'exposition allant jusqu'à 20 heures.

Jusqu'à présent, toutes les populations de *R. dominica* et de *Cryptolestes spp.*, 82 % de *T. castaneum* et 86 % de *S. oryzae* ont présenté des signes de résistance. Aucun signe n'a été remarqué chez *S. zeamais*. Il a fallu augmenter à la fois la concentration et la durée d'exposition pour éliminer les populations résistantes. Cette résistance peut probablement être attribuée à une mauvaise étanchéité ainsi qu'à des durées d'exposition trop courtes.