A Western Australian farm survey for phosphine-resistant grain beetles

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Abstract

A survey of 4547 Western Australian farms for phosphine resistance in rust-red flour beetle, *Tribolium castaneum* (Herbst), confused flour beetle, *Tribolium confusum*, Jacq. du Val, rice weevil, *Sitophilus oryzae* (Linnaeus), granary weevil, *S. granarius* (Linnaeus), lesser grain borer, *Rhyzopertha dominica* (Fabricius) and sawtoothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus) collected separately from handling equipment, sealed and unsealed grain storages during 1991–92. A discriminating dose test detected resistance in 17.1% of *T. castaneum*, 9.4% of *S. oryzae*, 37.5% of *S. granarius*, 15.2% of *R. dominica* and 15.6% of *O. surinamensis* samples. No resistance was found in *T. confusum*. Frequency of phosphine resistance between grain handling equipment, sealed and unsealed storages was similar, although sealed storages were 2.8 times as effective as unsealed storages in minimising the incidence of grain insect infestations. Infrequent treatments with phosphine in grain-handling equipment may have resulted in more rapid development of resistance than in grain storages.

Introduction

Despite having storage conditions that are more suitable to rapid grain insect development than many of its overseas competitors, Australia has, through judicious use of protectants and management of insecticide resistance, established a reputation as an exporter of insect-free grain. The challenge now is to meet increasingly frequent market specifications which require 'residue-free' grain. In order for Western Australia to take advantage of these burgeoning markets for 'residue-free' grain, it must guarantee that its product does not contain contact insecticide residues applied at either the central handling system or on-farm.

Since 1990 all Western Australian grain exports were shipped without the use of contact protectants during storage in the central handling system. This has been brought about by strategic planning and co-operation of industry and Government over the last decade (Dean, these proceedings).

A major strategy to achieve this is through the use of controlled atmosphere (CA) storage. This significant advance in grain storage management was inaugurated in 1980 when Co-operative Bulk Handling Western Australia (CBH) decided that CA storage was the most efficient method of bulk grain storage. To this end CBH embarked on a program of sealing storage facilities which has resulted in the upgrading of over 65% (5 Mt) of its permanent storage capacity. Currently these storages are fumigated with phosphine, although other CA alternatives could be readily implemented.

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Sealed storage with phosphine fumigation has also proved to be the most effective and economic method for the on-farm storage of grain. The Agriculture Protection Board of Western Australia (APB) has had an extension campaign in effect since the early 1980s, which has actively promoted this form of grain storage on-farm. It is no longer possible to purchase an unsealed farm silo in Western Australia, as all manufacturers now produce only sealed units.

Western Australia is becoming more dependent on phosphine for grain insect control both on-farm and in the central handling system. Phosphine (Worthing 1991) has many benefits over other fumigants, including ease of use, penetration and cost. There are few alternatives to phosphine which have the same advantages, particularly with the inevitable withdrawal of methyl bromide owing to its ozone-depleting properties. In order that phosphine remain available as a fumigant to the industry it is essential that any risks that could lead to its removal be identified and eliminated.

One threat to the industry is from the delivery to the central handling system of phosphine-resistant strains of grain insects which may develop in farm storages through the use of phosphine in unsealed and poorly maintained sealed storages. It has been shown that storages in this condition are unable to meet the exposure periods required for effective control. There is much evidence to suggest that the development of phosphine resistance is associated with poor fumigation techniques (Banks 1986; Friendship et al. 1986; Mills 1986; Price 1986; Taylor 1986; Tyler et al. 1983; Webley 1984).

Alternatively, some Australian researchers working with grain insect resistance have expressed concern that inefficient use of sealed silos rather than unsealed silos poses the greater threat through the development of higher levels of resistance (G.G. White, personal communication, 1990).

Entomologists involved in grain storage have been concerned for some time over the potential for grain insects to develop phosphine resistance. The U.K. Tropical Development and Research Institute (TDRI 1985) recognised the value of the FAO worldwide survey of insecticide resistance (Champ and Dyte 1976) and noted that this survey indicated the potential for a phosphine resistance problem. It considered that assessment of the status of phosphine resistance in grain insects be classed as most urgent.

In Western Australia, APB inspectors have routinely collected grain insect samples for phosphine resistance testing since 1986. However, these results do not give an accurate indication of the extent of phosphine resistance in grain insects because farms with a history of resistance are specifically chosen by APB inspectors. The objective of the APB campaign is to detect and eradicate resistant strains, not to survey the extent of resistance.

In order to accurately determine the extent and level of phosphine resistance in grain insects on farms in Western Australia, a random farm survey was implemented during 1991/92.

Western Australia is able to conduct an extensive resistance survey because of the declaration of grain insects under the Agriculture Protection Board Act and the co-operation between this group, the Western Australian Department of Agriculture and other industry bodies.

This farm survey for phosphine resistance in grain insects has determined the extent of phosphine resistance throughout the grain-producing areas of Western Australia and will facilitate the establishment of a resistance-management plan which can be used to limit the development and spread of phosphine-resistant strains of grain insects. This plan will provide a model which could be used by similar industries in other parts of Australia and in other countries. As Brattsten et al. (1986) aptly stated, 'A critical prerequisite to resistance management is anticipation of resistance before control actually fails'.

Materials and Methods

Insect collection

Preliminary monitoring by the APB indicated that approximately 30% of Western Australian farms were infested with grain insects (APB Annual Reports 1981–90) and that approximately 14% of all farms were infested with phosphine-resistant insects (Table 1).These preliminary data were used to calculate that at least 5000 farms should be surveyed to achieve return of sufficient (>200) farms with resistant grain insects.

The APB database has over 12000 registered grain-producing properties throughout Western Australia. To simplify selection, every second grain-producing property in APB zones 4,5,7 and 8 (Fig. 1) was randomly selected by computer. These zones cover the major grain-producing areas of the State. Farms smaller than 500 ha were excluded as these were considered to be too small to be relevant. The total number of farms included in the survey was 5612.

Species collected for resistance testing were *Tribolium castaneum*, *T. confusum*, *Sitophilus oryzae*, *S. granarius*, *Rhyzopertha dominica* and *Oryzaephilus surinamensis*.

APB officers were given 12 months (July 1991–June 1992) to carry out the sampling of grain insects from farms. They were instructed to collect separate samples of grain insects from sealed storages, unsealed storages and grain handling equipment (headers, augers etc.) from each property. The insects were removed from the grain residue and placed in a vial with a 1:1 mixture of chemical-free rolled oats and flour. The infestation level in each of the storage types was recorded, the codes used were:

H—high infestation, large numbers of insects easily found and superficially obvious

M— moderate infestation, many insects present but not superficially obvious

L— low infestation, only a few insects present on close inspection

N— no insects found on close inspection.

Farms which did not have sealed or unsealed storages, or grain handling equipment, were recorded as A (absent) for that equipment type. The entry E (error) was used where access to the property could not be obtained. These farms were deleted from the survey, leaving a total of 4547 farms inspected.

The insect samples were then sent by post to the Department of Agriculture laboratories at South Perth for phosphine-resistance testing.

Resistance testing procedures

Grain insects were sorted by species and tested for phosphine resistance directly from the field if at least 50 live insects were present, or cultured at 26°C, 60% relative humidity (r.h.) until populations were of sufficient numbers to test. Standard procedures recommended by the Food and Agriculture Organisation (FAO) for phosphine resistance testing were used (Anon 1975).As there were over 1000 tests to be conducted, a discriminating dose procedure was used. Phosphine was generated from pellets containing aluminium phosphide, collected over acidified water. Fumigation was carried out in 6.1 L desiccators. Insects were confined during fumigation in 50 mL polystyrene vials fitted with muslin. Phosphine was injected with a gastight syringe through a rubber septum fitted to a socket in the desiccator lid. Phosphine was mixed with air in the desiccator by a magnetic stirrer. Susceptible control insects were included in every test to ensure that there were no protocol failures (e.g. blocked syringe, broken seals).

Test insects were exposed to phosphine in the desiccators for 20 hours at 25°C, 60% r.h. then transferred to an incubator and a mixture of 1:1 flour and broken wheat was added. Mortality assessments were made 14 days after treatment. Insects were classified as dead if incapable of co-ordinated movement. The discriminating dosages shown in Table 2 are taken from the FAO method except for the *T. castaneum dosage* which was increased by 0.008 mg/L in accordance with earlier trials which had shown this dose to be more effective (Moulden 1987).

The FAO method states that the probability of a single insect in a batch of 100 being unaffected due to chance is less than 0.1. For the purpose of this paper strains were classified as resistant if more than 1 insect in 10 tested survived the discriminating dose.

Results

The resistance data in this paper are presented as a proportion of resistant strains or a proportion of farms with resistant grain insects. Many of the farm totals will not equal the sum of the sub-totals (made up of species and storage types) because some farms may have several resistant species or resistant insects in both sealed and unsealed storages. These farms will be counted only once when totals are calculated.

Phosphine resistance monitoring carried out by the APB from 1985 to 1992 is summarised in Table 1. This routine monitoring, which is biased because the APB selects properties with a history of grain insect infestation or phosphine resistance, shows that in 1991–92, 12% of farms inspected were infested with resistant insects. Over the same period this survey found 7% (Table 5).

Infestation levels in sealed storages, unsealed storages and grain handling equipment are outlined in Table 3. Infestations are lowest in grain handling equipment. More importantly, infestations of grain insects in unsealed storages are almost three times those in sealed storages. An overall infestation level of 38.31% (6.91% heavily infested) is similar to that found during routine monitoring by the APB over the same period (Agriculture Protection Board, 1992).

Table 4 summarises phosphine resistance based on strains. Of the 2238 strains tested 16% were phosphine resistant.

The frequency of resistance expressed as a percentage of farms inspected is shown in Table 5. The total percentage of farms infested with resistant insects is 7%, this is considerably lower than the figure for resistant strains shown in Table 4 (16%) because only 38% of farms are actually infested with grain insects (Table 3).

The relationship between storage type and frequency of resistance is summarised in Table 6. Of the 1861 strains collected from unsealed storages, 16.7% were resistant compared with 15.8% from sealed storages.

Fig. 1 . Agriculture Protection Board zones and districts.

Discussion

Infestations

The use of sealed storages on-farm has been extensively promoted in Western Australia as the most effective method of grain insect control (Newman 1987).This survey for phosphine resistance in grain insects provided a unique opportunity to compare infestation levels on sealed and unsealed farm storages (Table 3).This survey indicates that infestations in unsealed storages are 2.8 times more common than in sealed storages (39% v. 14%).Clearly, sealed storages should be the preferred option for grain insect control. Infestation levels of 3% in grain handling equipment are insignificant and recommended control methods, which include treatment with organophosphate insecticides and amorphous silica dust, should be maintained.

The percentage of heavily infested properties shown in Table 3 is considerably higher than previous routine monitoring by the APB (7% Table 3 v. 3% APB Annual Reports 1981–1991), this has been attributed to reduced grain insect inspections and control programs by the APB during the previous year.

Population resistance

Surveys conducted by Attia and Greening (1981) in New South Wales between 1968 and 1980 detected phosphine resistance in 3% of *R. dominica*, 12%of *T. castaneum* and 29% of *T. confusum* samples. Herron (1990) found phosphine

Table 1. Monitoring of phosphine resistance on Western Australian farms 1985 to 1992.

	1985–86	1986–87	1987–88	1988–89	1989–90	1990–91	1991–92
# Farms resistant	34	72	33	77	27	7	26
# Farms susceptible	187	180	164	399	154	155	188
% Farms resistant	15	29	17	16	15	4[a]	12

[a]an experimental error resulted in test exposures of 24 hours rather than 20 hours.

Table 2. Discriminating dosages used in phosphine resistance testing.

Species	Dose (mg/L)	Exposure period (hours)
Oryzaephilus surinamensis	0.050	20
Rhyzopertha dominica	0.030	20
Sitophilus granarius	0.070	20
Sitophilus oryzae	0.040	20
Tribolium castaneum	0.048	20
Tribolium confusum	0.050	20

Table 3. Grain insect infestation levels on Western Australian farms.

	Heavily infested (%)	Moderately infested (%)	Lightly infested (%)	Insect free (%)	Farms infested (%)	No. of farms inspected
Handling	0.39	0.52	1.47	97.62	3.42	4414
Sealed	0.74	0.70	2.68	95.87	13.75	4435
Unsealed	6.44	7.63	14.91	71.03	38.69	4380
All storages	7	8	17	65	38	4547

Table 4. Summary of phosphine resistance in grain insect species.

	Species						
	Oryzaephilus surinamensis	*Rhyzopertha dominica*	*Sitophilus granarius*	*Sitophilus oryzae*	*Tribolium castaneum*	*Tribolium confusum*	Total
No. of trains resistant	102	53	18	8	188	0	369
No. of strains susceptible	553	296	30	77	910	3	1869
% strains resistant	16	15	38	9	17	0.00	16

Table 5. Percentage of farms with resistant grain insects collected from farm storages.

	Species							
Storage type	*Oryzaephilus surinamensis*	*Rhyzopertha dominica*	*Sitophilus granarius*	*Sitophilus oryzae*	*Tribolium castaneum*	*Tribolium Confusum*	% farms resistant	No. of farms inspected
Handling	0.11	0.05	0.05	0.05	0.20	0.00	0.43	4414
Sealed	0.38	0.11	0.02	0.05	0.32	0.00	0.83	4435
Unsealed	1.83	1.05	0.34	0.09	3.77	0.00	6.51	4380
All storages	2.13	1.14	0.40	0.18	4.05	0.00	7.15	4547

Table 6 Summary of phosphine resistance in grain insects collected from farm storages.

	Storage type			
	Handling	Sealed	Unsealed	Total
No. of strains resistant	20	39	310	369
No. of strains susceptible	111	207	1551	1869
% strains resistant	15	16	17	16

resistance during a 1985–86 survey in 15% of *R. dominica*, 8% of *T. castaneum and* 5% of *T. confusum*, 18% of *S. oryzae*, 14% of *S. granarius* and 2% of *O. surinamensis* samples. Herron (1990) commented that, while phosphine resistance levels had not changed markedly between these two surveys, the increase in the incidence of resistance in *R. dominica* over this period would be of concern in Western Australia.

Data collected during this Western Australian survey are summarised in Table 4. Although frequency of resistance in *R. dominica* is similar to that found by Herron (1990), the higher resistance in *T. castaneum* and *O. surinamensis* is of concern as both these pests are of high pest status in Western Australia. Resistance in *T. confusum* and *S. granarius* is of lesser consequence in Western Australia where these pests are relatively rare in grain stores intended for delivery to the central handling system. The high figure of 37% resistant *S. granarius* was attributed to the discriminating dose being too low for this species. This resulted in tolerant rather than resistant individuals surviving the test. Therefore, the discriminating dose for *S. granarius* needs to be reviewed in future trials.

Resistance on farms

The incidence of resistance presented as a fraction of strains tested may initially appear somewhat daunting (16% of all strains tested). However, Table 5 shows that, on the basis of number of farms inspected, only 7% of properties were infested with resistant insects. Continued vigilance by Western Australian farmers, combined with APB inspections and extension campaigns, are required to maintain infestations of grain insects at the current low level and to continue to minimise the chances of resistant insects contaminating the central handling system.

Effect of storage equipment on resistance

Resistant strains were found in 15% of samples collected from grain handling equipment (Table 6).This figure is similar to sealed and unsealed storages and may warrant further investigation because phosphine is rarely used to disinfest grain-handling equipment. This could indicate that either resistance has developed rapidly following a small number of treatments or that cross-infestation by resistant insects from grain storages is a problem.

Concerns that the continued inefficient use of phosphine in unsealed storages will inevitably increase the frequency of phosphine resistance have lead the Western Australian Grain Protection Committee (GPC) to consider restricting the use of phosphine to sealed storages only in this State. This would leave Western Australian farmers without sealed storages with few alternatives other than purchasing sealed storages or converting old storages because the application of contact insecticides to farm stored grain in Western Australia is highly restricted. However, Table 6 shows that phosphine resistance occurred in 17% of strains collected from unsealed storages compared with 16% from sealed storages. It would seem unlikely that 1% higher incidence of resistance in unsealed storages is sufficient to warrant action by the GPC.

Further work is required to determine if the level of resistance (i.e. resistance factor) rather than frequency of resistance, is higher in strains collected from sealed storages. Strains which have survived the discriminating dose tests during this survey have been kept in culture and a series of graded concentration tests to calculate resistance factors is planned should funds become available in the future.

Summary

The data collected during this survey could be presented in a variety of formats with alternative criteria for resistance. Interested workers are encouraged to contact the author should they require additional information.

While sealed storages had little effect on the frequency of phosphine resistance in grain insects, this type of storage is almost three times as effective in minimising the incidence of infestations.

Resistance to phosphine in grain handling equipment should be investigated further to determine if it is developing more rapidly in this situation than in grain storages.

This survey has provided base-line resistance data. Funds will be sought for another survey in the mid-1990s which will indicate any change in the status of phosphine resistance.

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