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A COMPARATIVE STUDY OF PHOSPHINE RESISTANCE LEVELS IN STORED-GRAIN BEETLES COLLECTED FROM SEALED AND UNSEALED FARM STORAGES IN WESTERN AUSTRALIA

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ABSTRACT

Western Australia has established a reputation as an exporter of residue-free grain despite having conditions that are more suitable to the development of grain-insect pests than many other countries. This has been brought about by the extensive use of sealed storage and phosphine (PH_3) fumigation both in the central handling system and on-farm.

The Western Australian grain industry has been concerned for some time that PH_3 resistance could result in control failures due to the inefficient use of PH_3 in unsealed and poorly maintained sealed storage units. To monitor this threat a survey was conducted in 1991 to study the frequency of PH_3 resistance in the rust-red flour beetle, *Tribolium castaneum*; the rice weevil, *Sitophilus oryzae*; the granary weevil, *S. granarius*; the lesser grain borer, *Rhyzopertha dominica*; and the sawtoothed grain beetle, *Oryzaephilus surinamensis*. This study, which used a discriminating dose test, concluded that there was no significant difference between sealed and unsealed storage units with respect to the *frequency* of resistant populations.

There is, however, a need to determine whether sealed or unsealed storage units give rise to either significantly different *levels* of PH_3 resistance or to a different frequency of resistant individuals within a population. This paper reports on a frequency distribution analysis of data collated from an earlier PH_3 resistance survey and on progress toward the establishment of resistance levels of grain-insect strains collected during the same survey.

INTRODUCTION

Since 1990 all Western Australian grain exports have been effected without the use of contact insecticides. This represents 27 million t of residue-free grain. Strategic planning, resistance management and close cooperation between industry and government over the last 20 years has enabled the use of insecticides to be phased out both on-farm and in the central handling system (Dean, 1994).

Cooperative Bulk Handling (WA) has sealed over 65% (7 Mt) of its permanent storage

capacity and is currently using phosphine (PH_3) for grain-insect control, although other controlled atmosphere alternatives could be employed if required.

Sealed storage with PH_3 fumigation is also widely used on Western Australian farms. A recent survey (Newman, 1994) has shown that over 60% of farms have at least one sealed silo on the property.

There is a danger that the indiscriminate use of contact insecticides on-farm and the subsequent delivery of treated grain to the central handling system could jeopardise the residue-free status of Western Australian grain. To protect its marketing advantage, the use of contact insecticides on farms in Western Australia is highly regulated. There are no insecticides registered for application to farm-stored grain, and seed treatments which contain insecticides must be applied in conjunction with a dye to ensure that late deliveries of treated grain will be detected and rejected before the grain bulk is contaminated.

Clearly there is heavy reliance on PH_3 fumigation and sealed storage in the Western Australian grain industry for which protection is needed from the development of resistant grain-insect pests. A major concern is that the type of storage might be influencing resistance; for example, using PH_3 in unsealed storage units could be selecting for high frequencies of low level resistance, whereas sealed storage units could be selecting for low frequency, but high level, resistance.

Monitoring for resistant grain-insects has underpinned the state's PH_3 resistance management strategy and will continue to do so. Emery (1994) reports the results of a PH_3 resistance survey of 4,547 farms in 1991. This survey also looked at the resistance frequency in grain-insect samples collected from sealed and unsealed farm storage units, but it did not consider either the resistance level of strains or the frequency of resistance within a strain.

This paper compares the frequency of resistant individuals in strains of *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), *S. granarius* (L.), *Rhyzopertha dominica* (F.) and *Oryzaephilus surinamensis* (L.) collected from both sealed and unsealed storage units and reports on progress towards establishing resistance levels for these strains.

MATERIALS AND METHODS

Over 4,500 farms were inspected for grain-insect infestation during the 1991/92 financial year. Samples of grain insects were collected, where possible, from infested sealed and unsealed storage units on each property, resulting in over 2,000 discriminating dose-resistance tests. Test methods followed the procedures described by the FAO (Anon., 1975) and are detailed in Emery (1994) along with sampling methods. Discriminating dosages, taken from the FAO method, but with the *T. castaneum* dosage increased by 0.008 mg/L, are shown in Table 1. Susceptible control insects were included in every test to ensure that there were no protocol failures, such as blocked syringes or broken seals, and tests were repeated if any control insects survived. Mortality was assessed 14 d after treatment, and insects were classified as dead if incapable of coordinated movement.

TABLE 1
Discriminating dosages used in phosphine resistance testing

Species	Dose (mg/L)	Exposure period (h)
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

Strains were classified as resistant in the 1991 survey if two or more insects of at least 50 test individuals survived the discriminating dose. This survey tested 2,238 samples and identified 349 resistant strains, 39 from sealed storage and 310 from unsealed storage. A random subset of 126 farms (63 using sealed and 63 unsealed storage) has been chosen for a follow-up survey to establish the resistance level of these strains. A subset was necessary due to the more extensive resistance testing required in order to determine resistance levels. Sealed and unsealed storage on these farms will be inspected for grain-insect infestations and the specimens collected will be retested using the discriminating dose procedure described above. Storages were classified as sealed in this study if they were originally manufactured as sealed storages. If the strain has retained its PH_3 resistance, the survivors of the test will be cultured until sufficient insects are available to test with five graded concentrations and a control. Three groups of at least 50 insects will be exposed at each concentration and probit regressions fitted using the method of Finney (1971).

RESULTS AND DISCUSSION

Emery (1994) showed that there was no significant difference in the frequency of resistant populations collected from sealed and unsealed storages — 16% and 17%, respectively. The frequency of resistance in individuals within a population was not considered in this paper. To determine the frequency of PH_3-resistant individuals in a population, the 1991 data were re-analysed to show the frequency distribution of resistance scores (expressed as the percentage of insects surviving the discriminating dose) for sealed and unsealed storage.

Table 2 shows 21 class limits for resistance frequency, ranging from 0 to 95.1–100% surviving the discriminating dose, the number of scores which occurred in that class limit, and the scores expressed as a percentage of the total scores for that storage type. The latter is required to compensate for the large difference in the number of strains collected from sealed and unsealed storage facilities (246 and 1,861, respectively).

The data from Table 2 are presented as a cumulative frequency polygon (ogive) in Fig. 1 and indicate that there is no significant difference ($p > 0.05$) in the frequency of resistance within grain-insect populations collected from sealed and unsealed farm storage in Western Australia. The error bars have been calculated as standard error for proportions (Zar, 1984).

TABLE 2
Frequency of resistance in grain insects collected from sealed and unsealed storage facilities

Upper limit of class (% insects surviving discriminating dose)	Sealed storage		Unsealed storage	
	Frequency	% of total	Frequency	% of total
0	186	75.61	1392	74.80
5	43	17.48	295	15.85
10	6	2.44	72	3.87
15	3	1.22	33	1.77
20	4	1.63	22	1.18
25	1	0.41	15	0.81
30	0	0.00	12	0.64
35	1	0.41	10	0.54
40	1	0.41	5	0.27
45	1	0.41	2	0.11
50	0	0.00	3	0.16
55	0	0.00	0	0.00
60	0	0.00	0	0.00
65	0	0.00	0	0.00
70	0	0.00	0	0.00
75	0	0.00	0	0.00
80	0	0.00	0	0.00
85	0	0.00	0	0.00
90	0	0.00	0	0.00
95	0	0.00	0	0.00
100	0	0.00	0	0.00
Total	246	100.00	1861	100.00

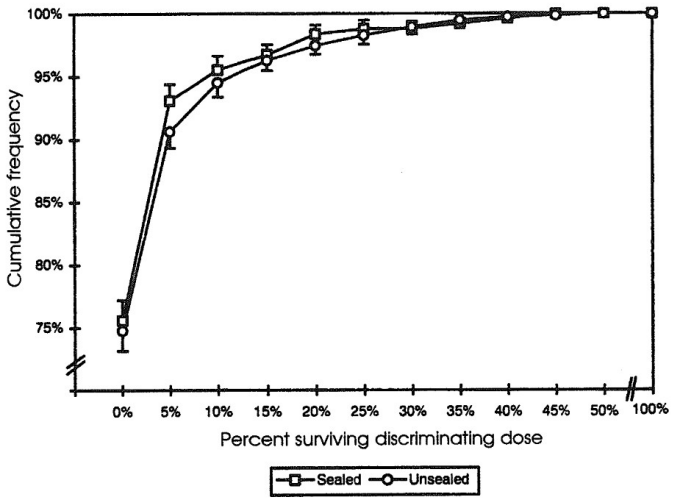

Fig. 1. Cumulative frequency polygon of resistance in grain insects collected from sealed and unsealed storage. Error bars are standard errors for proportions.

Newman (1994) found that 73% of sealed farm storage units were not well maintained and failed the standard pressure decay test for gastightness (a half-life of 5 min from a 25-mm water column). Most of the failures were due to leaking seals. This may have caused some sealed storage units in this survey to behave like unsealed ones in gas holding ability. However, it is unlikely that this could account for the very close correlation between storage types.

Having established that neither sealed nor unsealed storage selects for higher frequency of either resistant populations or individuals, there is a need to study the level of PH_3 resistance, i.e. comparing the resistance factors of strains collected from sealed and unsealed storage. This graded concentration work is in progress. As well as establishing resistance factors, it will measure the gastightness of sealed silos and compare the resulting data with resistance levels. Results of this work are incomplete at the time of writing and will be reported elsewhere.

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