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ERADICATION OF A STRAIN OF A STRONG PHOSPHINE RESISTANT INSECT POPULATION FROM STEEL FARM SILOS IN WESTERN AUSTRALIA

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ABSTRACT

Phosphine has been readily available to Australian farmers for control of grain insects since the late 1940s. The original product label stated that it could be used in unsealed grain storages by admixture with the grain stream. Repeated use in these unsealed storages created selection pressure on local grain insect populations resulting in escalating phosphine resistance frequency and resistance factors.

Western Australia (WA) exports over 80 percent of the annual grain crop into discerning international markets and, after insecticides were banned in the mid 1980s, the state has relied on phosphine at export to meet the mandated nil-tolerance standards for exported grain. Phosphine fumigation is also the main insect control on farms in WA and to protect the export market it is imperative to limit further development of resistance and to prolong the useful life of this important grain treatment.

A phosphine resistance monitoring, mapping and management program was established in WA during the early 1980s to collect samples of grain insects from farms, merchants and central storages for phosphine resistance testing. This program has studied over 19,000 insect strains using a combination of quick screening assays with follow-up confirmatory international standard FAO tests allowing phosphine resistance to be traced to individual premises. In 1996 WA joined an industry-funded national grain insect resistance monitoring program and now shares data and techniques with laboratories in Queensland and New South Wales.

Weak phosphine resistance frequency continues to increase in WA at the rate of about five percent of strains tested each year. However it was the first detection of strong phosphine resistance on a farm in eastern wheatbelt of WA in June, 2008 that initiated a management program to assess the practicality of eliminating the strain of rust red flour beetle, *Tribolium castaneum* (Herbst).

Surrounding farms were initially surveyed for strong resistance to provide confidence that an eradication program on the property would be worthwhile. No additional strong resistance was found nearby and the eradication project commenced in late 2009 and was considered a success when follow up insect samples taken from the farm through 2011 were tested using the FAO protocol at the Department of Agriculture and Food WA (DAFWA) and determined to be no longer strongly resistant.

Key words: resistant grain insects, grain storage, phosphine, monitoring, eradication, fumigation, sealed silos.

INTRODUCTION

Since aluminium phosphide (AIP) was developed and released for stored grain protection worldwide it has been misused in many storages that are not compatible with good fumigation practice. For example early product labels in Australia indicated that the AIP could be used in unsealed storages; this meant that phosphine was well-received by grain storage managers because there were few, if any, gas tight storages used in Australia at that time and phosphine provided an apparently quick fix for an intractable grain insect problem.

Doubtless there were survivors from each of these poor fumigations and, where the grain needed to be treated multiple times due to reappearance of grain insects, selection of insects with a genetic tolerance to phosphine quickly occurred. Over the last 40 years this continued selection pressure has resulted in elevated incidence of phosphine resistance. Over the last 10 years in association with elevated use of phosphine as exporters moved away from protectants, some phenotypes of grain insects have progressed to very strong resistance. Collins (2006) reported that from 1997 there appeared a quantitative change in resistance levels in four of the five major species and he predicted that strong resistance will become a major problem in Australia unless appropriate management is enacted.

A critical prerequisite to resistance management is anticipation of resistance before control measures actually fail (Brattsten, 1986) and with this in mind the Western Australian Department of Agriculture initiated monitoring of both farms and central storages for phosphine resistance in 1984 (Emery, 1994). Resistance monitoring should serve a purpose whether it is a contribution to a resistance management strategy or to support eradication/containment. Where initial resistance is localised, early warning allows eradication to be implemented before the infested bulk is moved or placed into the market. In addition resistance monitoring programs can provide useful information for a grain storage extension program by highlighting situations and practices that may be selecting for resistance and provide localised advice to remediate the practice.

In Western Australia (WA) the development of phosphine resistance has been slower than the other states. This is likely due to several factors:

- the WA central storage system moved to gas tight storage in the 1980s;
- farm silo manufacturers commenced producing gas-tight silos for growers at the same time as the bulk handlers and the majority of farms now have one or more sealable silos (Newman 1996);
- there is less grain stored on WA farms than in other states;
- a strong extension campaign promoted effective fumigation practice and improved stored grain management by farmers through education;
- high intensity grain insect sampling from farms for resistance testing kept the need for sealed storage at the forefront.

A broad extension campaign has been underway since the early 1980s in WA (Newman 2010) which focused on the need to improve management of grain stored on farms, principally to receive the maximum economic benefit from the treatment, but also to slow the escalation of phosphine resistance.

Phosphine is vital to WA export grain and has been exclusively used by CBH Group (the major bulk grain handling company in WA) since 1990. The difficulty WA faces is that this product is also freely available for use by grain growers who store grain for sale, feed or seed in their farm silos. There is concern across the industry that strong resistance that develops on farms through ongoing inadequate treatments, may inadvertently be transported

to the central handling system, merchants and exporters thereby increasing the cost of controlling the pests.

Phosphine resistance monitoring of farms and central storages in Western Australia since 1985 has shown a steady rise in the frequency of weak resistance status across all species and Fig. 1- highlights the increase in weak resistance in *Tribolium castaneum* from under 20 percent in 1985 to more than 75 percent in 2012. Research and experience in other states has shown that when the frequency of weak phosphine resistance in a grain insect population reaches ~80 percent it can be expected the population will soon become strongly resistant (Collins, 2006b). Genetic analysis revealed that weak resistance is controlled by one major gene and that it was this gene plus the selection of a second resistance gene (that has little effect on its own) that produced the strong resistance phenotype (Schlipalius et al., 2002).

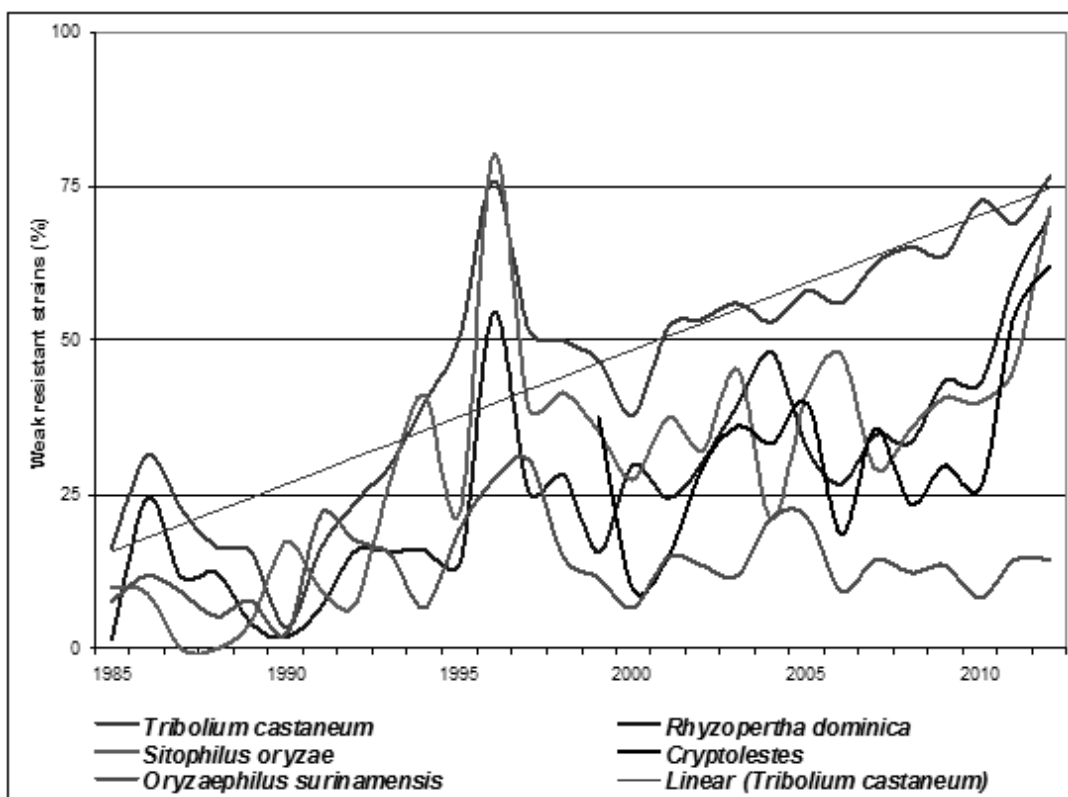


Fig. 1- Frequency of phosphine resistance in major stored grain insect species in Western Australia (Note: 1996 peaks are attributed to defective test gas source).

By 2008 some farms had resident populations of *T. castaneum* that were 100% weak resistant and the overall frequency of weak resistance in WA was approaching 70 percent of strains tested; strong resistance was expected. In June, 2008 there was a detection of strong resistance in *T. castaneum* at Dalwallinu (220km north-east of Perth, Western Australia, 30.2833° S, 116.6667° E).

The strong resistance was initially detected through a random survey that collected seven *T. castaneum* strains all of which tested positive for weak resistance with the rapid screening test (518 individuals, mean weak resistance 73%). There were sufficient numbers of one strain to permit an FAO 20 hour discriminating dose test for weak resistance (80 individuals, 93% weak resistant).

Three strains were immediately tested for strong resistance and all were positive (240 individuals, mean strong resistance 3.3%). These three strains were cultured in the laboratory for confirmatory strong resistance assays but interestingly the resistance was no longer present in any of the cultured strains. The farm was sampled for fresh field insects between July and October 2009 and three additional *T. castaneum* strains were tested with the rapid test and proved to have an average resistance of 57 percent. Two of these strains showed strong resistance at a frequency of one percent.

Laboratory cultured strains were retested for strong resistance in February and August 2010 and again in July 2011 with 33 additional assays. Strong resistance again could not be confirmed despite 3,610 laboratory reared individuals being assayed.

Sub-cultures of the two confirmed strongly resistant strains were sent to DAFWA's sister laboratory at Department of Agriculture, Forestry and Fisheries, Queensland, in north-eastern Australia, however their laboratory testing was also unable to replicate positive tests for strong resistance on these cultured strains.

As the property was relatively isolated and field strains exhibited strong resistance in repeated tests, eradication through correct management principles was deemed to be the most responsible action to protect the Western Australian grain industry.

MATERIALS AND METHODS

In Western Australia a network of about 40 field Biosecurity Officers conduct random and targeted inspections of farms to collect grain insect strains for resistance monitoring. Targeted samples are taken from sites with a history of poor storage practice or resistance while data from random inspections can be used to compare resistance frequencies between storage methods and regions. Collected strains are sent to the DAFWA South Perth Entomology laboratory under bio-secure packaging (Glock and Hall, 2010) for resistance testing.

Follow-up visits to the infested Dalwallinu property and grower interviews determined that AIP in tablet formulation had been applied annually for approximately 11 years using the ineffective and dangerous method of punching holes in the product container and hanging it in the silo headspace (Fig. 2). The gas evolves very slowly from the container due to limited air moisture penetration and is most likely to be lost from the silo quickly through poor seals on the top lids. This practice provided ideal conditions for selection of resistance in the storage.

T. castaneum strains collected in this study underwent a preliminary phosphine resistance screening rapid test following the method described by Reichmuth (1992) which exposes insects to 1mg/L gas for a period of 30 minutes. The test is scored as 'knock-down' for any insect that is incapable of co-ordinated movement. Insects not knocked down in this test were considered to have some level of resistance and, if sufficient insects were present in the field sample, an additional test using the United Nations Food and Agriculture Organisation (FAO) prescribed procedure of 0.05mg/L phosphine for an exposure time of 20 hours followed by 14 days holding time to confirm resistance (Anon, 1975).

Strong resistance was determined using an extension of the FAO method outlined by Dalglish and Collins (1999). For *T. castaneum* this discriminating dose test is 0.25mg/L

phosphine for an exposure time of 20 hours, followed by seven days holding time. Survivors of this test that behave normally are assessed as strong resistant.

Confirmation of the emergence of strong resistance was a major concern for WA, so only FAO strong discriminating dose tests were conducted on subsequent strains in order to maximise numbers of fresh field insects available for testing. Where possible test gas concentrations were verified using a Varian gas chromatograph to measure correct assay dosages.



Fig. 2- Silos fumigated by punching holes in AIP containers and hanging in the headspace.

Properties contiguous with the target property were inspected and insects collected to determine their resistance status, none were found to have strong resistance indicating an eradication program would have some chance of success.

The infested Dalwallinu property had nine 40-70 tonne silos of which eight were found to be sealable, but had various faults due to ongoing lack of maintenance, and one unsealable silo. Remedial work was undertaken on the sealable silos by replacing rubber seals in the inlet and outlet ports and refilling the pressure relief valves with oil. The remaining older silo was not manufactured as a sealed unit and would need special treatment.

A hygiene program was enacted cleaning up spilt grain, disposing of derelict grain and removing dry grass around the silos. (Fig 3) The concrete silo pads were cleaned and silos were sprayed with Deltamethrin. Care was taken not to contaminate any grain because contact insecticides have been banned from farm use on farm-stored grain in Western Australia since 1993 (Dean 1994).

Preparation was made to eradicate by applying correct fumigation practice. Previous studies by Collins et.al. (2000) showed strong resistance in *Rhyzopertha dominica* (Fabricius) could be controlled provided the registered rates of 0.3 mgL^{-1} for 10 d $<25^{\circ}\text{C}$ and 1.0 mg L^{-1} for 7d $>25^{\circ}\text{C}$ were maintained.

Pressure testing of the sealable silos ranged between 5 and 180 seconds. Previous fumigation studies by Newman et al (2004) showed that silos exhibiting a pressure half-life of 180 seconds were needed for an effective fumigation and therefore the lower figure was not acceptable for effective fumigation. However, the eradication fumigations were conducted under these conditions because the cost and time required for full retro sealing of the poor silos was restrictive and it was reasoned that this fumigation soon after harvest would quickly eliminate any adult insects that were present.



Fig. 3- Clean away of spilt grain.

Fumigation of all silos commenced mid December 2009 using tablet formulation AIP at $1.5\text{g}/\text{m}^3$ on warm grain that was over 30°C . The gas concentration readings were taken on day 7 with a Canary Company SiloChek electronic monitor at the base of the silos. Three silos showed zero phosphine and they were recharged with $1.5\text{g}/\text{m}^3$ of AIP. The other 6 silos returned gas concentrations between 7 and 1738 ppm with the higher concentration found in the best sealed silo that achieved a half-life pressure test of 180 seconds.

Commercially available Storgard® pitfall traps and in-house developed pitfall traps made by; drilling 1 mm holes in pvc tubes and open drink cans covered in 1 mm flywire mesh were inserted into the headspace of all silos two months post-fumigation. It was hoped that these traps would verify the effectiveness of the treatment.

Seven of the silos were found to be free of insects however, some *T castaneum* were trapped in the unsealed silo as well as one of the poorer silos. Both silos were re-fumigated and the grain out-loaded for seed. A tub (Fig. 4) containing approximately 25 kg of whole grain was installed under the silos, to attract and trap any insects that may have flown onto the property. Smaller perforated containers holding flour and rolled oats were buried in the whole grain contained in the tub.

There was some uncertainty the strong resistant strain had been eliminated in the first year because of survival in the unsealable silo and the presence of insects in the tub trap under the silos. Accordingly, two months prior to harvest 2010 a follow-up hygiene procedure was conducted on the silo area in October. All empty silos were dusted inside with ~300g of Dryacide® according to the recommended practice, (GRDC 2010). Follow up fumigations were conducted soon after loading in December 2010 using bag chain formulation of AIP at ~1.5g/m³. The unsealable silo was not fumigated with phosphine based on the experience of the first year when insects survived. Monitoring of the fumigations was not conducted but pitfall traps were installed in February 2011. Insects were found in one silo in April it was re-fumigated with a bag chain of AIP at 1.5g/m³.



Fig. 4- External insect trap.

The older unsealable silo contained some insects which indicated that a residual population of potentially strong resistant insects remained on the farm and likely to move into other silos. This is a dilemma when attempting to eliminate a potentially damaging population of resistant insects or quarantine pests. We were aware that Ethyl Formate had been used with some success in other unsealed silo situations (Ren, 2006) to control insect pests so an experimental treatment was set up to use Ethyl Formate in this silo in May 2011 to eradicate the last of the insects. (Newman 2012 – these Proceedings)

Insect samples were removed from the external tub trap under the silos twice and in October 2011 all grain was removed and all insects submitted to the DAFWA grain insect resistance testing laboratory for strong resistance analysis. No strong resistance was detected and the eradication was considered complete and the response team stood down.

CONCLUSION

The property was declared free of strong resistance after 18 months of follow-up inspections, sampling and resistance testing and we concluded that this strain had been created on-farm through poor fumigations in unsealed silos over many years.

The steady development of phosphine continues to be a major threat to the Australian stored grain industry. The national resistance monitoring work currently conducted through the Cooperative Research Centre for National Plant Biosecurity and supported by Australian farmers through the Grains Research and Development Corporation is unique in the world and provides a detailed resistance picture. This information provides the opportunity for strategic planning and tactical response.

The Dalwallinu eradication demonstrated that an effective insect resistance testing program can provide early warning of strong resistance infestations and that application of standard label rate of AIP and recommended grain management principles can eliminate strongly resistant strains of *T. castaneum*. The farm will continue to be visited under the ongoing DAFWA grain insect resistance monitoring program.

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