Donahaye, E.J., Navarro, S. and Leesch J.G. [Eds.] (2001) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, Fresno, CA. 29 Oct. - 3 Nov. 2000, Executive Printing Services, Clovis, CA, U.S.A. pp. 679-684

MONITORING FUMIGATION EFFECTIVENESS USING BIOASSAYS OF MIXED AGED CULTURES OF TARGET PESTS

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ABSTRACT

Mixed age cultures of several target insect species of typical resistance levels, were accommodated in specially constructed containers (vials) and placed in the commodity at the top of the storage immediately prior to fumigation. The storage was then fumigated as per normal procedures. Insect vials were removed from the fumigation area periodically during the fumigation process. The cultures were then assessed for survivors. The results were collated with concentrations and time at that concentration and compared against laboratory data. Minimum Ct products were calculated for complete control of adults and immature stages for each time period under fumigation. Results indicated that some of the label rates specified, more precisely those pertaining to low flow fumigation, were inadequate. These were subsequently raised from 35 ppm for 14 days to 100 ppm for 14 days. Bioassays were also used to compare the effectiveness of various control methods. The success of these trials has highlighted the value of mixed age cultures to assessment of pest control strategies against target pests.

INTRODUCTION

Grainco Australia (GA) operates a series of country grain accumulation sites on the eastern seaboard of Australia, as well as several strategically located port facilities. The climate in this area is tropical to subtropical and in this environment insect population growth potential is at its maximum.

GA manages all grain pests, including the full range of grain storage insects, with an Integrated Pest Management Plan (IPM) (Bridgeman, 1999). This plan is a five-year strategy, which is updated annually. The GA IPM has been in place since 1991 (Collins and Bridgeman, 1991). The success of the plan has been previously documented (Bridgeman and Collins 1994).

Resistance Management to all chemical controls is an integral component of the GA IPM (Collins and Bridgeman 1991). The operational environment is conducive to rapid population development and evolution of resistant strains.

The priority placed on thorough population monitoring can not be underestimated.

Shortly after implementing Siroflo into GA storages, it became evident that the label rate was ineffective against the low level of resistance in *Rhyzopertha dominica* found commonly in Queensland (Bridgeman *et al,* 2001). With considerable trial and error in the field it was established that this low resistance required approximately double the recommended rate to achieve control. This strain had been known to exist for some considerable time and it was assumed that the relevant data would have been incorporated in the label directions.

The Farming Systems Institute (FSI) of Queensland verified the field data and a recommendation was made to the Working Party on Grain Protection to change the label. Subsequent to the increase in rates a single occurrence of a higher level of resistance in *R. dominica* was discovered through routine population monitoring (Bridgeman *et al.,* 2001). This "Millmerran" strain required 120 ppm for 14 d to achieve population extinction.

This series of incidents highlighted a deficiency in the GA IPM plan, specifically in the population monitoring program. Although documenting prevalence of insect populations is a critical component in the management of pests, it is also essential to understand fully, the dynamic nature of these pest populations, including their resistance characteristics and what that means in the practicality of controlling resistant strains.

In a laboratory situation, Ct products for fumigants can be made relevant to the practical fumigator by using a variety of mixed-age cultures in a flow-through apparatus (Winks and Hyne, 1997). In this series of trials, the Wink's system has been modified and adapted to field conditions.

MATERIALS AND METHODS

Population monitoring

Population monitoring is undertaken throughout the storage period (pre-intake, during the storage period, on out-turn and in the emptied storages). Insects are collected using a variety of trapping and inspection techniques (probe and sieve, pit-fall traps and during out-turn inspections).

Live specimens of all insect populations detected in GA storages are collected and submitted for analysis. Target pests detected in previously phosphine (PH_3) fumigated products are tested as a priority. All samples submitted are forwarded to the FSI in Brisbane. The FSI also collects and analyses insects detected in other non-GA sites. Samples are developed into significant populations and then tested for PH_3 resistance (Collins *et al.,* 2001).

Matrix construction

For each strain, $LD_{99.9}$ levels are calculated using mixed age cultures exposed to different fumigant concentrations in the "Winks" flow-through system (Winks and Hyne 1997, Collins *et al.,* 2001).

The 99.9% efficacy point from each of these data sets are then set into a data matrix which enables the required fumigation duration to be calculated for each Ct product (Fig. 1). The benefit of this matrix is that it can include all target species, environmental conditions, and all fumigation conditions.

Figure 1 shows the time required for a particular Ct product (ppm/h) to reach $LD_{99.9}$ for the Millmerran Strain (QRD569) of *R. dominica* as set by the Lab data.

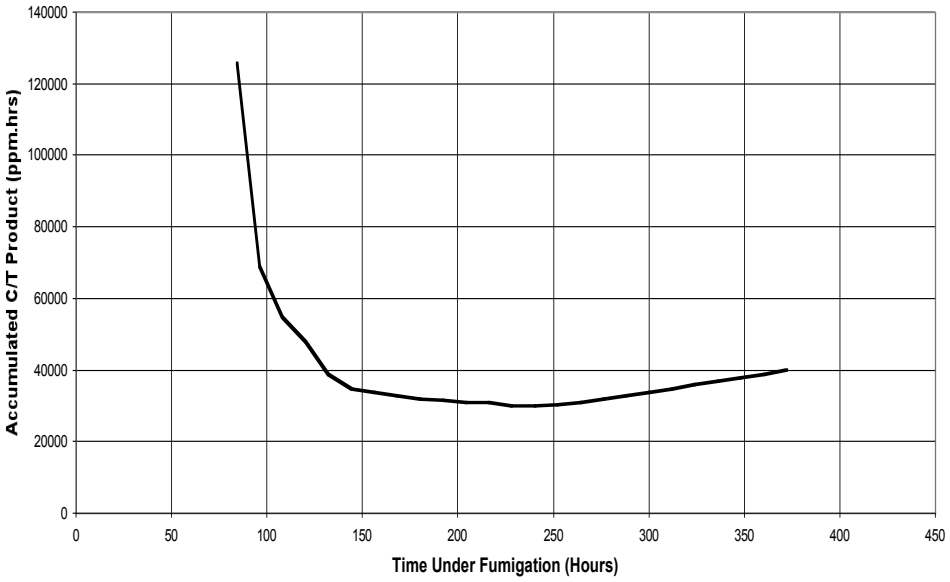

Fig. 1. Data matrix of LD_{99} for Millmeran strain of *Rhyzopertha dominica*.

Field trials

In order to validate the data on the Matrix, a suitable field test had to be designed. The test protocol for the field trials is based on the Winks flow-through test used in the laboratory. The matrix formulated from the lab data indicates the time under fumigation required for every Ct target.

The protocol is as follows: mixed age cultures of target species are placed in specially constructed vials to be placed into the fumigated silo. Four sets of cultures, in duplicate are prepared for each trial fumigation. The fumigations are replicated. The fumigation (target) time is estimated and sets of cultures are marked to be retrieved 3, 2, 1 and 0 days prior to that time being reached. A set of cultures to match each of the retrieved sets is located onsite under conditions as close as possible to those in storage, to provide control batches. Each of the test vials is pushed into the grain at various points within the enclosure. It is

important to sample areas where gas concentrations are suspected of being less than sufficient.

Fumigation "Dosimeters" (United Phosphorus Limited) are placed close to the data sets (some data sets had the "Dosimeters" inside the vials) to measure PH_3 in accumulated concentration. The temperature of the grain in the vicinity of the vials is measured. This temperature is recorded in the fumigation records under Standard Operating Procedure (SOP).

Fumigation

When the samples are in place, the fumigation is carried out as per SOP. The day when the fumigation is due to be complete is calculated and the days for sample extraction are noted. Fumigation monitoring occurs periodically during the process and the readings are recorded as per SOP.

Recovery of sample sets

Three days prior to the end of fumigation the first set of cultures are retrieved and together with the control batch for that set they are forwarded to the FSI lab for determination (Collins *et al*., 2001). The second set is retrieved on the following day and so on until the fumigation is concluded. A sample for residue analysis is taken at the end of the fumigation.

Data analysis

Following full assessment of the mixed age culture sample sets the data is compared with the results from the lab (Collins *et al*., 2001). From this data we can draw conclusions as to the efficacy of dose rates and fumigation methods.

RESULTS

Data collected from the mixed age culture sets in the field confirmed the lab data results. The accumulated Ct products for several of the fumigation were inserted into the Data Matrix to indicate the efficacy predicted in Fig. 2.

The data in Fig. 2 indicate significant overkill in some of the conventional fumigations (recirculation in sealed enclosure at 1 mg/L with liquid and solid formulations of PH_3) and a failure for Siroflo if conducted at 70 ppm for 14 d. Indeed the Matrix indicates that at 50 ppm this fumigation procedure will never control the Millmerran Strain (QRD569) *of R. dominica*.

The Data indicate that there is little advantage in fumigating at high dosages (above 2 mg/L), while fumigation at below 50 ppm will not be effective in controlling low level resistance no matter how long the fumigation lasts.

CONCLUSION

These trials provide conclusive evidence that low concentration fumigations with phosphine are not effective in controlling target pests. Field trials indicated that a 14 day Siroflo fumigation would require a concentration in excess of 100 ppm to be effective against the Millmerran Strain of *R. dominica* and 75 ppm to control the low level resistance of *R. dominica* now commonly found worldwide. It is evident that fumigation at concentrations lower than this will control susceptible insects only, and repeated fumigation at low concentrations will select

for resistance. The results from these trials have resulted in a change in the recommended Siroflo dose rates.

The results of the trials when compiled into the matrix, formed an excellent tool for fumigation practitioners to measure the effectiveness of their fumigation techniques and the dose rates to be used.

Dose rates and exposure times for each Ct product used by Grainco Australia have now been field tested, and procedures have been modified to ensure continued effectiveness. Data is continually being developed and significant changes are added to the matrix. Changes to the Matrix are reconfirmed with field trials.

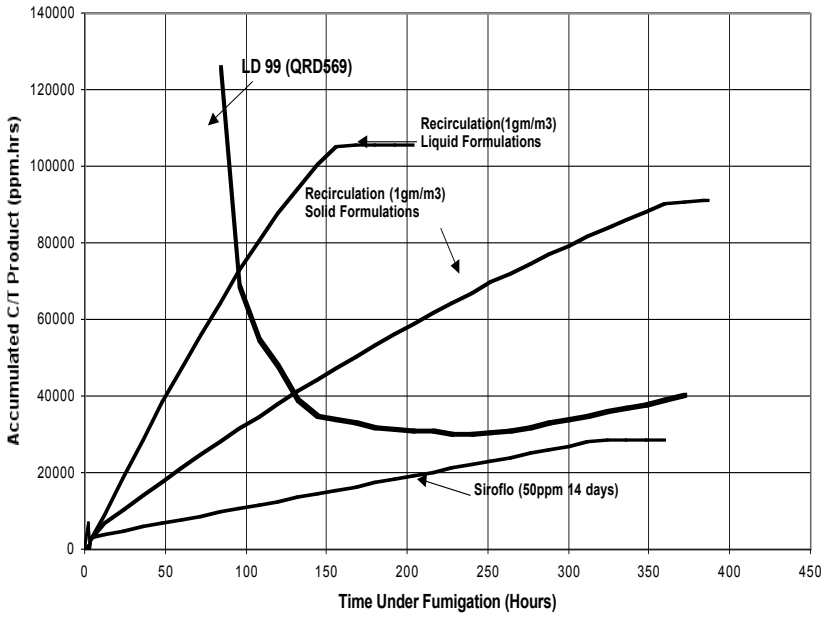

Fig. 2. Comparison of efficacy of phosphine fumigation methods.

GENERAL DISCUSSION

Changes in customer preferences, concerns for OHS and the environment as well as development of resistance to available protectants in target pests forced GA to review protocols, which culminated in the GA IPM plan (Collins and Bridgeman 1991). The IPM plan changed GA's reliance on residual pesticides to a policy based on fumigation. Within 2 seasons (1993) 90-94% of grain being stored in GA stores became “residue free”. This change required the use of novel fumigation techniques such as Siroflo.

Even in the initial year of use, failures to control insects were observed. These were generally blamed on teething problems with the system, power supply

interruption or operator error. It was noted that almost all of these failures involved *R. dominica*.

After repeated failure, Grainco Australia began to suspect under-dosing was the cause of survival. The dose rate was increased above the label rate, which specified a minimum of 35 ppm for a 15-d exposure to a 50 ppm concentration for a 15-d target and this proved much more successful. In 1997/8 failures were experienced using this higher rate. Phosphine resistance was then suspected and investigated.

The flow-through system using mixed age cultures (Winks and Hyne, 1997) was set up at the FSI to facilitate full scale testing. Laboratory results were collated and confirmed that the label concentration did not control the low level of resistance in *R. dominica*, which was exhibited in 95-99% of the tested populations in Queensland. Once this fact was established, efforts were directed to establishing the concentration time products required to maintain control of this species in Siroflo systems and other fumigation methods used in Queensland. The data obtained was used to develop a matrix, which indicated the required Ct product, for each fumigation duration. This data was then used as a guide for new fumigation rates, which were effectively tested in the field to prove efficacy.

ACKNOWLEDGEMENTS

The entire project outlined in this paper has been the work of a focused team. Without their combined efforts the considerable work required to do this study could never have been accomplished. We recognise the considerable efforts of the staff of the Farming Services Institute, QDPI, Indooroopilly; the staff of Grainco Operations, Malu, Brookstead, Dalby West; the Grain Quality staff of Globex Gladstone Port and the Grainco Australia Fumigation staff. We thank you all.

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