Journal of Stored Products Research 70 (2017) 25–36

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Monitoring resistance to phosphine in the lesser grain borer, *Rhyzopertha dominica*, in Australia: A national analysis of trends, storage types and geography in relation to resistance detections

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A R T I C L E I N F O

Article history:
Received 6 May 2016
Received in revised form
15 September 2016
Accepted 13 October 2016

Keywords:
Phosphine resistance
Insects
Bayesian
Monitoring
Survey

A B S T R A C T

Data on incidence of resistance to phosphine over the last 20 years and factors associated with insect sample collection are stored in the Australian Grain Insect Resistance Database. The database was analysed using descriptive statistics, linear trend analysis and Bayesian hurdle modelling to gain insights into factors contributing to the development of strong resistance in *Rhyzopertha dominica*. Descriptive statistics indicated that strong resistance was significantly more common in central storages, particularly bunker storages, than on farms. Strong resistance in *R. dominica* was also associated with wheat, barley and sorghum but there was no significant link to any grain protectant or storage treatment chemical, other than phosphine. Highest frequency of strong resistance was found in northern New South Wales and no detections were made in Western Australia. In eastern Australia, trend analysis indicated that strong resistance detections increased steadily from the first detection in 1997 to about 8% of samples containing resistant insects in 2014. Weak resistance was detected in about 10% of samples in eastern Australia in the early 1990s but this increased rapidly to 40–50% by 1990, at the same time that industry use of phosphine greatly increased, and then to about 80% in 1995. Strong resistance was first detected in this species when weak resistance was diagnosed in close to 80% of population samples. The Bayesian hurdle model identified bunkers, silos and unsealed storages as being associated with development of strong resistance and sheds with a lower frequency. This model also identified an accelerated increase in resistance frequency of strong resistance from 2011 to present. The information gained from this analysis is being used to inform current and future management of resistance to phosphine.

1. Introduction

The lesser grain borer, *Rhyzopertha dominica*, is a serious, cosmopolitan pest of stored cereal grain and other products (Hagstrum and Subramanyam, 2009). Chemical tactics are generally applied to control this pest; however, it is a highly adaptive species that has developed resistance to a range of contact insecticides (Opit et al., 2012a) and to the fumigant phosphine (Collins, 1998b Resistance to the latter is particularly alarming as storage facilities world-wide rely heavily on this fumigant to disinfest their grain. In Australia, *R. dominica* is a frequent, major pest both in central storages (Rees, 2004) and on farms (Collins, 1998a). This insect was the first pest in which strong resistance to phosphine was recorded in Australia (Collins, 1998b). This detection was made as part of a national resistance monitoring program (Collins et al., 2002; Emery et al., 2011) that has been carried out systematically across Australia since 1996. The monitoring program undertakes random and targeted sampling of farms, grain merchants and central storages throughout the grain growing regions of Australia. Samples of the major pest species are collected or

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http://dx.doi.org/10.1016/j.jspr.2016.10.006
0022-474X/

received and tested with two discriminating dosages of phosphine, a lower dosage in which survivors are diagnosed either resistant or susceptible to phosphine, and a higher dosage used to test for presence of the strong phosphine resistance phenotype (Daglish and Collins, 1999). The results of bioassays are recorded in an internet-based data warehouse called the Australian Grain Insect Resistance Database, AGIRD (Emery and Tassone, 1998; Emery et al., 2011) along with other sample or collection site related information including latitude and longitude, grain type, storage type, application method, previous treatments and other data.

The information contained in AGIRD is used to provide the grain industry with continuous updates on the distribution, frequency and strength of resistance that underpins a national approach to resistance management (Collins, 2009). AGIRD now contains detailed information on the incidence of resistance to phosphine in Australia for the past 20 years from over ten thousand sites. The existence of AGIRD provides a unique opportunity for an analysis of the possible contribution of broader scale biological and environmental factors, management practices and the agricultural context, on the occurrence of resistance that cannot be evaluated in local scale projects aimed at developing management tactics. We have undertaken an analysis of AGIRD to gain insights into the development of phosphine resistance by identifying the risks, if any, associated with broader scale industry factors, such as position in the supply chain, storage type, site type, commodity, and chemical treatment history, as well as spatial-temporal elements. This understanding benefits all parts of the Australian grain supply chain by contributing to the development of more targeted and effective national resistance management strategies.

In this paper, we explore some modelling approaches and use statistical analyses to understand, estimate and predict the trends and significant variables influencing the development of strong resistance to phosphine in *R. dominica*. A similar approach has also been used to analyse resistance to phosphine in *Sitophilus oryzae* (Holloway et al., 2016). We have focussed on analysing the occurrence of strong resistance since this phenotype is the major threat to effective control of this species.

2. Materials and methods

Our approach to investigating strong resistance was to use both standard exploratory statistics and trend analysis, and also to make use of a recent extension of the hurdle modelling approach (Falk et al., 2014). The hurdle model is appropriate as it accommodates for rare events such as detection of the strong resistance phenotype. Standard modelling approaches may miss influential variables and/or give misleading trends, since the large number of not strongly resistant observations may swamp the strongly resistant observations. As such, we supplement our standard statistical analyses with results from the hurdle model.

2.1. Sampling field populations

Insects were collected as adults from farms, grain merchants and other grain accumulators by sieving samples taken from stored grain, seed, and other likely sites of infestation such as machinery, grain spills and residues. Insects from central storages were generally collected by storage staff during routine inspections and from control failures and forwarded to the laboratory. Less often, central storages were sampled by project staff. Sample sizes were as large as 500 but generally ranged 60-100 adult insects.

2.2. Resistance testing

As mentioned, bioassays consisting of two discriminating dosages of phosphine were used to diagnose insect population samples as either susceptible, weak resistant or strong resistant. Assays were undertaken using a modified version of the recommended FAO method (FAO, 1975) as described by White and Lambkin (1990). Unless there was a requirement for a rapid screening, population samples were cultured on whole wheat at 25–30 °C and 55% r.h. in the laboratory, usually for one generation before testing. Progeny were used rather than parents because many insects had already been exposed to insecticide in the field and because field populations are comparatively fragile and were detrimentally affected when sieved from grain several times. Adult progeny were sieved from each sample and up to 350 adult insects removed from the sample without conscious bias. The number of adult progeny varied among samples and where less than 350 progeny were available, all progeny were used. These insects were then grouped into seven batches of up to 50 insects. Three batches were exposed to the low phosphine dosage (0.03 mg/L for 20 h), three more to the high dosage (0.25 mg/L for 48 h) and the remaining batch served as the control. Insect survival of the high dosage indicated presence of strong resistance phenotypes in the sample, survival of the low dosages indicated presence of resistance (undefined) while survival at the low dosage but not the high dosage diagnosed presence of weak resistance but not strong resistance. Replicates were generally conducted and reference strains of known resistance phenotype were included in the assays to ensure accuracy. Replicates were always conducted to confirm strong resistance diagnosis. Only unique strains and a representative diagnosis were used in this analysis.

2.3. Australian Grain Insect Resistance Database

Substantial cleaning of AGRID was undertaken which required removal of duplicate strains, geocoding sites and extraction of records of bioassays related to the correct dose-exposure combination for determining strong resistance. The final dataset contained 4402 unique observations, 141 of which indicated presence of strong resistance.

The variables considered in the analysis were a binary response variable indicating presence or absence of strong resistance, state (that is, federated state of Australia), region within state, site type, storage type, commodity, and 16 binary variables indicating whether or not a particular treatment had been applied to the grain while in storage. Possible treatment variables included aeration cooling, diatomaceous earth, contact insecticides applied to grain (pirimiphos-methyl, chlorpyrifos-methyl, fenitrothion, bioresmethrin, methoprene, dichlorvos), storage fabric treatments (carbaryl, deltamethrin, fenitrothion, pyrethrins), fumigant (phosphine), none, other and unknown. Contact insecticides have not been permitted on stored grain in Western Australia since the early 1980's and therefore no data from that region has been used.

2.4. Statistical analysis and Bayesian hurdle model

Statistical analyses using descriptive statistics, standard parametric tests and linear trend analyses of both strong and weak resistance phenotype detections were performed using the R statistical software (R Core Team, 2015). This was accompanied by a generalized additive model (GAM) (Wood, 2006), which was used to fit a smoothed curve for the trend using the R package MGCV.

We also applied the Bayesian hurdle model (Falk et al., 2014) to search for links between variables and the presence of the strong resistance phenotype. This model is appropriate due to the large number of absences of strong resistance in the dataset relative to the number of presences. In these situations, traditional logistic regression may miss any underlying trends and significant