MANAGEMENT OF RESISTANCE TO INSECTICIDES IN STORED GRAIN: RESISTANCE RISK AND IMPACT ASSESSMENT

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Abstract - Classical resistance management relies on manipulation and exploitation of biotic and abiotic components of agricultural systems to prevent, delay and combat resistance development. In a grain storage system dependent on insecticides to maintain high standards of control, however, storage management practices are aimed at reducing insect numbers in order to comply with a very low or even nil tolerance for live insects rather than with the manipulation of these components to delay the development of resistance. Many of these practices not only conflict with theoretical strategies for avoiding or delaying resistance, but may be accelerating its development. Thus resistance seems inevitable.

What should be the approach to resistance management in this type of system? In the short term, two areas must be addressed: firstly, if resistance occurs to a chemical in current use we must be able to judge the impact of this resistance on the grain industry; and secondly, we must be able to determine the risk of resistance to a new as yet unused chemical. In the long term, research should be directed towards modifying operational factors relating to pesticide use with the aim of *preventing or delaying the development of resistance. In response to these challenges a research* program that is both reactive and proactive has been developed. Four broad areas of interest are identified: 1. Early detection of resistance, 2. Determination of effective alternative chemical controls, 3. Predication of future development of particular resistances, and 4. Development of recommendations and general theories for delaying the onset on resistance. Details of each of these research areas are outlined and discussed with examples.

INTRODUCTION

Export grain is a major income earner for Australia. To maximise our chances of retaining this income in what is a very competitive market the Australian grain industry maintains very high standards of grain quality. One of the major components of quality is a "*nil tolerance*" for *live* insects. To maintain this "nil tolerance" most cereal grains and a significant proportion of other grains are treated in storage either with a grain protectant (residual insecticide) or with a fumigant or often both. For example, usually more than half of the wheat harvest and about 90% of the barley crop are treated with grain protectants and some of this tonnage is fumigated with phosphine if infestations are detected.

The present system of insect pest control using chemicals relies almost completely on one fumigant - hydrogen phosphide, and on a limited number of residual grain protectants. Compounds available for application to grain are severely limited by strict health standards and by costs. Clearly, the development of resistance to any of these compounds could have disastrous consequences for the Australian grain industries. How then, have they responded to challenge of resistance?

The Australian grain industry's approach to the problem of resistance in insect pests has been simply to develop alternatives - both alternative chemicals and alternative physical control methods. The industry, however, has failed to implement the alternatives to chemical control. And, furthermore, despite this dependence on chemicals, conventional resistance management strategies have not been adopted. The necessity to reduce insect numbers to comply with the "nil tolerance" for live insects at the lowest cost has been achieved at the expense of the operational flexibility needed to implement resistance management.

Conventional resistance management is based almost entirely on the manipulation of biological and operational aspects of the agricultural system in question. Options include, among others, variation in insecticide dose rates and frequency of application, limited area application, inclusion of IPM economic thresholds, use of less persistent pesticides, use of mixtures and rotations, use of synergists, use of pesticides against particularly vulnerable life cycle stages of the pest, use of natural enemies and reintroduction of susceptible pest insects (Leeper *et al.* 1986). Most of these tactics are essentially various ways of minimising the relative fitness of resistant genotypes, either by preserving susceptible homozygotes or by destroying resistant genotypes (Roush, 1989). Most of these tactics also depend on the assumption that the presence of some pest insects can be tolerated at some time in the life of the crop. So how do the grain industry's current insect control practices effect the development of resistance? Lets examine these practices in the light of what is known about resistance development.

GRAIN STORAGE MANAGEMENT PRACTICES IN AUSTRALIA

When grain is brought into storage the total crop is either sprayed with insecticide or fumigated. If infestations occur during the storage period, these are usually controlled by a fumigant (usually phosphine), although sometimes more chemical is added. Storages are cleaned between harvests to remove insect refuges and permanent storages are usually sprayed down with an insecticide when emptied. Grain is accepted only if its moisture content is at or below a certain limit. Some storages are also fitted with aeration ducting so that the grain can be cooled using cool, dry air. Lower temperatures will inhibit insect population growth. The grain in storage is quarantined in the sense that all loads coming in are inspected for insects and rejected if insects are found. During the storage period the grain bulks are inspected at irregular intervals for insect infestations. If infestations are detected samples are sometimes collected for resistance testing.

These management practices have proved to be very successful at maintaining instances of infestations at acceptable levels. However, when these practices are analysed in the context of the evolution of resistance we find that most either have no effect or that they may be accelerating the development of resistance.

Complete coverage of the commodity provides the strongest selection pressure possible. If a resistant individual is present this type of treatment will select for it. In the early stages of resistance development resistant individuals are normally heterozygous at the resistance locus. However, even if the target dosage is high enough to overcome these insects, application of insecticide is uneven so that pockets of lower dosed grain may allow their survival. This will facilitate the selection of individuals homozygous for the resistant gene.

In this type of system there is no escape for susceptible insects. Strict hygiene compounds this effect by removing refugia for susceptibles.

Drying of grain and cooling will lower population growth but will have only a minor effect on resistance development as virtually the whole gene pool is still selected by the insecticide when grain is brought into storage.

Inspection at intake reduces the number of insects entering the storage but research (White, 1981) has shown that an average of 5-10 insects/tonne still enter storages in Queensland, Australia.

Use of fumigants to control infestations in protectant treated grain is a positive step to limit selection for resistance. This is because different mechanisms seem to be involved in resistance to fumigants and protectants.

Thus despite its heavy dependence on chemicals and its high level of organisation the grain industry has failed to integrate any more than fortuitous resistance management into its operations.

What then is the role of an entomologist studying resistance in a system where operational inflexibility seems to inhibit the implementation of resistance management tactics? In the short to medium term there are two issues that must be addressed:

Firstly, if resistance occurs to a chemical in current use we must be able to judge the impact of this resistance on the grain industry.

Secondly, we must be able to predict the risk of resistance to new, as yet unused,chemicals.

These questions apply equally to residual insecticides and fumigants.

In the long term, research should be directed towards modifying pesticide use with the aim of preventing or delaying the development of resistance.

In response to these challenges a research program that is both reactive and proactive has been developed. The research program is divided into four areas. These are:

1. Early Detection of Resistance
2. Determination of Effective Alternative Chemical Control Measures
3. Prediction of Future Development of Particular Resistances
4. Development of General Theories and Recommendations for Delaying the Onset of Resistance.

Details of each of these research areas are listed in Table I.

EXAMPLE - RESISTANCE TO SYNTHETIC PYRETHROIDS IN *TRIBOLIUM CASTANEUM*

As an example of this research program I will describe the studies being undertaken on resistance to synthetic pyrethroids in *Tribolium castaneum.* The story of this resistance is unique in that it was detected and studied before these insecticides were used in the field to control this insect.

1. Early Detection of Resistance

Resistance to synthetic pyrethroids was first detected while monitoring a field trial of the *alpha*-cyano pyrethroid cyfluthrin. Field trials are monitored carefully because they are as close as practicable to full scale resistance selection experiments in the field. Suspect resistant insects were brought into the laboratory and tested with a discriminating dose of cyfluthrin using the impregnated-paper assay (Anon. 1974). Obviously, reliable methods to detect and measure resistance are essential. This means that there must be access to reference susceptible strains and that baseline responses to insecticides should be established before resistance occurs. The likely importance of any suspect resistance can be assessed using treated grain assays. These assays simulate application of grain in the field as closely as is feasible in the laboratory.

2. Determine effective alternative chemical controls

The next stage is to establish the cross-resistance characteristics of the resistance. This involves selecting the resistant population in the laboratory with the failing chemical until all (or most) individuals in the population are homozygous for the resistance mechanism. Selection is necessary for three reasons: firstly, new resistances can be associated with a loss of fitness so that resistance levels may be lost during culturing; secondly, selection of the resistant population allows the full potential of the resistance to be measured and thirdly, it provides the material needed for more detailed genetic studies. The response of the resistant strain to a wide range of chemicals can then be tested - preferably with treated-grain assays. These not only tell us which insecticides are jeopardised by the resistance and which are not, they also provide a reasonable indication of the field doses necessary both to kill adults and to prevent the development of progeny. Thus I found that the pyrethroid resistance mechanism was particularly strong against *alpha*-cyano pyrethroids and carbaryl (Collins 1990) but had little impact on the efficacy of organophosphorus materials.

3. Predict future development of particular resistance

The third stage of the research program is to characterise the resistance in terms of its biochemistry and genetics and to use this information to try and predict the future impact of this resistance on grain storage industries. Almost complete suppression of the pyrethroid resistance in *T. castaneum* with the synergist piperonyl butoxide strongly suggests that the resistance is mediated by cytochrome P-450 dependant monooxygenases. This conclusion was also supported by cross-resistance data that showed that resistance was correlated with structural modifications of the pyrethroid molecule and by biochemical studies (Rose and Visetson, 1990, personal communication).

Testing of piperonyl butoxide using grain assays revealed that only very high doses of this synergist would suppress the resistance in the field. Thus it would be impractical to use addition of this synergist as a tactic to overcome the resistance.

Table I: Research Program - Insecticide Resistance Risk and Impact Assessment

1. EARLY DETECTION OF RESISTANCE
    - 1.1 Maintain susceptible reference strains
    - 1.2 Establish bioassay methods
        - (a) for rapid diagnosis of resistance
        - (b) to predict effects under field conditions
    - 1.3 Surveys to detect new resistances
        - (a) routine monitoring - generally inefficient but important when new chemicals introduced
        - (b) investigate control failures
            - i) current treatments
            - ii) field trials

2. DETERMINE EFFECTIVE ALTERNATIVE CHEMICAL CONTROL MEASURES
    - 2.1 Establish lab R strains from field samples
        - (a) allows full potential of resistance to be studied
        - (b) provides material for genetics
    - 2.2 Determine potential resistance levels to
        - (a) current protectants
        - (b) alternatives
    - 2.3 Recommend alternatives and dosage rates to control resistant populations

3. PREDICT FUTURE DEVELOPMENT OF PARTICULAR RESISTANCE
    - 3.1 Identify and characterise resistance mechanisms
        - (a) synergists
        - (b) biochemistry
        - (c) cross-resistance spectrum
    - 3.2 Elucidate formal genetics
        - (a) number of factors
        - (b) dominance, linkage, etc.
        - (c) functional dominance (relative to field dosages)

    - 3.3 Investigate population genetics
        - (a) gene frequencies
        - (b) relative fitness of genotypes

    - 3.4 Computer simulation modelling

4. DEVELOP GENERAL THEORIES AND RECOMMENDATIONS FOR DELAYING ONSET OF RESISTANCE
    - 4.1 Develop data base from particular cases of resistance
        - (a) resistance mechanisms
        - (b) population genetics
        - (c) formal genetics
    - 4.2 Conceptual models
    - 4.3 Computer simulation models

Studies of the inheritance of this resistance revealed that it is controlled by a single major gene which is almost completely dominant in expression and seems to occur on linkage group VI. Because the insecticides have not yet been used in the field it has been possible to estimate the initial gene frequency before selection. We have also found that there is a significant relative fitness disadvantage associated the resistance gene in the absence of insecticides. Further, using the grain assay technique it has been possible to estimate the relative fitness (and effective dominance) of the three genotypes RR, RS and SS in the presence of various concentrations of insecticide. The characteristics of the heterozygote are particularly important at the beginning of a resistance episode. All of this information is very powerful. It enables us to model the dynamics of resistance development and perhaps to predict the future evolution of a resistance episode. Computer simulations also allow us to "try out" various resistance management options before they are applied in the field.

4. Development of recommendations and general theories for delaying the onset of resistance

One of the most important functions of an entomologist studying resistance is to elaborate tactics and strategies aimed at managing or delaying the development of resistance. So, what should grain industries that rely for the most part on chemical controls, be doing to delay the onset of resistance to grain protectants and fumigants? In the short term, the most viable option available is to alternate non-chemical control techniques with the use of grain protectants and fumigants. This means expanding the use of controlled atmosphere technology, cooling and heating, inert dusts and other non-chemical techniques. In other words, creating a heterogeneous environment that is much more of a challenge to the adaptability of the insects than a simple chemical-soaked one. I advocate the alternation of chemical and non-chemical techniques not the mixing of these techniques for slowing the development of resistance. Mixing chemical and non-chemical methods (such as insecticides and aeration) will not reduce selection for resistance. If the insecticide is killing insects or even causing differential reproduction between genotypes, then an environment suitable for the selection of resistant individuals is provided.

In the longer term, grain industries need to re-evaluate their requirements for a chemical solution to insect problems and the necessity of their rigid management practices such as the requirement for perfect hygiene and the "nil tolerance" for live insects.

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GESTION DE LA RESISTANCE AUX INSECTICIDES DANS LES STOCKS DE GRAINS
LES RISQUES DE RESISTANCE ET LA MESURE DE SON IMPACT

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RESUME

La gestion classique de la résistance repose sur la manipulation et l'exploitation des composants abiotiques et biotiques des systèmes agricoles afin de prévenir, retarder et combattre le développement de cette résistance. Dans les stocks de grains dépendant des insecticides pour maintenir des normes de qualité élevées, les pratiques de stockage visent à réduire le nombre d'insectes afin de répondre à une tolérance très basse, voire nulle, d'insectes vivants plutôt que de manipuler ces composantes pour retarder le développement de la résistance. Plusieurs de ces pratiques sont non seulement en contradiction avec les stratégies théoriques cherchant à éviter ou à retarder la résistance mais peuvent également accélérer son développement. Ainsi, elle devient inévitable.

Quelle serait la bonne approche de cette gestion de la résistance dans un tel système ? A court terme deux possibilités sont offertes : premièrement, si une résistance apparaît envers un certain produit chimique, nous devons être capables d'en juger l'impact sur l'industrie céréalière ; deuxièmement, nous devons être capables de calculer les risques de résistance future à un nouveau produit qui n'a pas encore été utilisé. A long terme, la recherche devrait se tourner vers la modification des facteurs opérationnels en rapport avec l'utilisation des pesticides dans le but de prévenir ou de retarder le développement de la résistance. En réponse à ces défis, on a développé un programme de recherche à la fois réactif et actif et on a cerné quatre vastes zones d'intérêt : 1) La détection précoce de la résistance, 2) La détermination de moyens d'élimination alternatifs efficaces, 3) La prédiction des apparitions futures de certaines résistances, 4) La mise sur pied de recommandations et de théories générales visant à retarder l'apparition de ces résistances.

Les détails de chacune de ces voies de recherche sont définis et discutés en donnant des exemples.