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DNA Testing for Phosphine Resistance ——The Future of Resistance Monitoring and Management

David I. Schlipalius[1,2], Rajeswaran Jagadeesan[3], Yosep Mau[3], Patrick J. Collins[1,2] and Paul R. Ebert[3*]

Abstract: In recent years, monitoring programs have detected increasing levels of resistance to phosphine among pest insect species. Effective management of resistance requires knowledge of the frequency and distribution of resistance genes within populations. Such knowledge-based management strategies will require rapid, high-throughput resistance assays. Our research is focussed on genomic approaches to identifying phosphine resistance genes in two of the major worldwide pests of stored products, *Tribolium castaneum* and *Rhyzopertha dominica*. These genes will then be used in rapid diagnostics to provide data on resistance gene distribution.

Our results to date indicate that in both *T. castaneum* and *R. dominica*, high-level resistance is mediated by two major genes, each of which confers weak resistance, butwhich interact synergistically to provide high-level resistance. For *R. dominica*, the genes appear to be the same in all outbreaks yet studied, which suggests they are conserved in most (if not all) cases of high level resistance. This observation enhances the validity of a DNA based test for phosphine resistance, at least in a great majority of cases.

Data produced by DNA testing can be used to model the spread of resistance, to indicate pest management strategies that may be deemed at "high-risk" of promoting resistance as well as to assess the probability of success or failure of future resistance management strategies. As DNA testing and the more traditional resistance bioassays have complementary advantages and limitations, it will be most effective to apply both techniques in a program for resistance monitoring

Key words: phosphine resistance, monitoring, genomics

Introduction

In 2002, worldwide annual post-harvest crop losses directly due to insects and mites in food storage were conservatively estimated at approximately 5% translating to approximately 120 – 160 million tons of cereal product each year. This is despite the widespread use of chemical treatments to control these pests[1]. In 2008 dollars and using the current wheat price of approximately US $320/ton, this translates to a global loss of more than US $38 billion per year due to insects in cereals alone. Given the recent rate of increase in the cost of food, especially grain crops, this estimated annual cost is set to increase.

One major chemical treatment, phosphine fumigant, has been in use for more than 50 years. This is remarkable longevity for an insecticidal compound. However, the increase in the use of phosphine in recent decades is associated with a parallel increase in both the frequency of occurrence and the absolute level of resistance. Even so, when used appropriately, phosphine is the most economically and environmentally sound routine commercial treatment for stored grain. Since no alternative fumigants match the value of phosphine, the arguments for maintaining the effectiveness of this important chemical is clear.

Since the 1980's, Australia has had the unique distinction of maintaining a long-term program for monitoring resistance to phosphine and grain protectants. The result of this long-term program has enabled the detection, culturing and characterisation of a number of resistances in various pest species from across Australia[11-14].

Currently, the technique most often used in resistance monitoring involves a bioassay of resistance in which insects are exposed to an insecticide for a set period of time at a dose that discriminates between resistant and sensitive strains of insect. Usually this discriminating

1. *Queensland Department of Primary Industries and Fisheries*, 80 *Meiers Rd. Indooroopilly*, *Queensland*, *Australia*, 4068;

2. *Cooperative Research Centre for National Plant Biosecurity*, *Canberra*, *Australian Capital Territory*, *Australia*;

3. *School of Integrative Biology*, *University of Queensland*, *St Lucia*, *Queensland*, *Australia*, 4072

* presenting author: p. ebert@ uq. edu. au, FAX + 617 33651655

dose is marginally higher than the $LD_{99.9}$ of a susceptible strain of pest insect. Sometimes, such as with phosphine in the Australian resistance monitoring program, a second discriminating dose may be used to distinguish between-weak' andstrong' resistances. In this way the occurrence of resistance can be detected in the field and the number of strains exhibiting a given level of resistance can be documented.

In Australia, resistance 20 – 25 times higher than the basal level of tolerance of fully susceptible beetles, was first reported in the lesser grain in the mid – 1970s[2] and the frequency of weak resistance has gradually increased from that time[7], whereas strong resistance was not detected until 1997[8]. Resistance has also been reported to be increasing in many countries around the world[9,10] but especially in Asia and the Indian subcontinent[11-14].

One limitation of the bioassay is that it is a slow, labour-intensive process that is unable to detect heterozygous carriers of resistance genes. However, it is currently the only method that is able to detect unknown resistances in field – collected populations. In contrast, DNA testing of resistance genes is rapid, high-throughput, and is able to detect resistance genes in heterozygous carriers who do not express the resistance phenotype. The DNA assay can even be used to detect the presence of the resistance gene in dead insects. The two techniques are complementary, however, as the DNA test cannot detect novel resistance mutations, whereas the slower bioassay can be used to assess the resistance levels of any insects.

Recent Work

The majority of our recent work has focussed on the genetic basis of phosphine resistance. We have shown that in the Australian strains of *R. dominica* the majority of the strong resistance trait was controlled by two independently assorting genes, named *rph*1 and *rph*2, that individually conferred a weak resistance(– 25X and – 12. 5X respectively), but together act synergistically to confer a strong resistance(> 250X)[15,16]. This unique situation in which no individual gene can confer high-level resistance provides an opportunity for effective management of resistance in the field. One potential barrier to resistance management is that laboratory experiments reveal no detectable fitness costs associated with the resistance genes[15], which has consequences for modelling of resistance outbreaks.

We have also found through complementation analysis with several independent phosphine resistance outbreaks across Australia, the genes were the same in each case[17]. In fact, the phenomenon of limited numbers of resistance genes appears to be the case for insecticide resistances generally[18]. This indicates that the number of phosphine resistance genes is likely to be limited and that genetic tests should be robust.

We have also extended our search for resistance genes to *Tribolium castaneum*, a pest insect for which the genome sequence has just recently been published[19], an advance that will greatly facilitate isolation of resistance genes. Our recent results have shown that, as with *R. dominica*, two genes act synergistically to confer high-level resistance(R. Jagadeesan, *unpublished*), although we have yet to determine whether the same genes confer resistance in each of the two species. Identification and comparison of the resistance genes in the two species will allow us to make more broadly relevant predictions about phosphine resistance.

Implications

Onstad[20] highlights three issues that are critical for the evaluation of all resistance monitoring plans: goals, precision and cost. We believe that application of DNA testing to monitoring will greatly increase the scope of the first and make significant improvements to the logistics of the latter two of these critical issues. To highlight these improvements, we provide a brief overview of current and proposed methods and discuss their relative merits.

Monitoring

Current bioassay techniques for monitoring resistance have a major strength in that they can detect resistance genes in field – collected populations of insects regardless of whether those particular resistances have been documented or characterised previously. It also requires a minimal amount of specialist training to set up and uses basic, non-specialist laboratory equipment. However, bioassays are a slow, labour intensive process and require a minimum number of insects collected from the field, which are usually bred through to F_1 or F_2 generations to generate enough material for robust confirmation of resistance. This process takes months to do. Also, since bioassays rely on a 'phenotype'(i. e. the actual expression of resistance in the insect) and insecticide resistances are generally reces-

sive, this type of assay does not detect insects that are heterozygotes for resistance gene alleles (i. e. 'carriers' of the trait). Heterozygotes only have one copy of the resistance gene/allele, whereas expression of resistance requires two copies, one from the female parent and one from the male parent. This means that if resistance is rare or uncommon in a population, then the bioassay technique will likely be unable to detect it.

Looking ahead, diagnostic DNA tests will able to distinguish phosphine resistance genotypes from field-collected insects without a requirement of breeding or exposure to the chemical. In fact, the insects to be assayed need not be of any particular age and dead insects can be assayed just as readily as living insects. DNA tests are also rapid (requiring only hours or days) and many samples can be processed in parallel. This can save months of work and provide high-quality, uniquely informative data at the same time.

The data from DNA tests are unambiguous, which facilitates information sharing between laboratories as the assays are not influenced by the age of the insects or the culturing conditions. Also, DNA itself can be transferred over large distances, eliminating the need to transport living cultures, which avoids quarantine restrictions between countries. DNA tests are also scalable, in that they can be performed in small labs, or in large centralised labs depending on the research model adopted. In fact, they provide much more flexibility for small labs since no culturing of insects is needed, thus reducing labour costs and space requirements (eg. constant temperature cabinets).

While DNA tests are very good at rapidly and effectively identifying insects that carry known resistance genes, the tests cannot readily detect novel resistances. Bioassays, however, are good for detecting novel resistances, despite being slow and expensive for routine resistance monitoring. Therefore, we envisage a synthesis of the two methods, whereby the molecular tests are augmented with periodic bioassays to ensure that new resistance genes do not escape detection.

Future Directions

The utility of our DNA tests will extend beyond the very practical identification of resistant insects in commercial settings. We will also use the tests to gather data on the distribution and frequency of individual resistance genes in the field. This information will be integrated into resistance monitoring programs and pest ecology projects that seek to identify the primary factors that select for resistance as a means of assessing the effectiveness of various resistance management strategies. Our goals in using DNA tests can also be stated as a series of questions that they will be used to answer, namely: Where, when and how many resistance genes or resistant individuals exist in a particular setting This information allows one to answer the question: Are resistance management strategies working Ultimately, we wish to answer: What are the factors that pose the greatest risk of generating or increasing phosphine resistance in insect populations on farms and in bulk storages Identifying the genes directly responsible for resistance is the first step in developing the tools to answer these questions.

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