

To what extent does dispersal by insect flight influence the spatio-temporal dynamics of phosphine resistance?

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Abstract

Globally, phosphine is relied on as the major tool for managing insect infestations in stored food grain. Phosphine is unique; it is cheap, easy to apply, applicable to multiple commodities, and is accepted by virtually all markets as a residue-free treatment. Alarming, high-level resistance to PH₃ in target pests has developed as an acute and growing problem in both India and Australia and this seriously threatens the long term security of stored food grains globally. Actively dispersing insects may carry resistant genes from storage to storage, and perhaps into populations not exposed to phosphine. Our research aims to investigate aspects of the extent of this movement and to define how this movement impacts upon the development and persistence of phosphine resistance within stored product pest populations. Ecological observations of *Tribolium castaneum* and *Rhyzopertha dominica* have been carried out to establish the pattern of emigration from storage structures under field conditions, the mating status of dispersing beetles and the diversity of genetic material that females may be carrying within their spermathecae. The effects of migration and periodic selection events are also investigated in a laboratory simulation study. Active dispersal of insect pests, and their reproductive and physiological state when dispersing, must be incorporated into resistance management models and strategies. The results of our studies will help provide a mechanistic interpretation of the movement of resistance alleles in populations of these pests and thus contribute to the improvement of resistance management strategies.

Keywords: dispersal, migration, mating status, genetic potential, resistance development, flight pattern

1. Introduction

We are investigating ecological variables associated with the spatio-temporal dynamics of phosphine resistance in *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.). These dynamics are assumed to have species specific features. That means the ecology of each relevant beetle species needs to be investigated in its own right; that is, the investigation of the spatio-temporal dynamics of a species is essentially an autecological study (Walter and Hengeveld 2014). Ultimately, we wish to use this knowledge to help explain the geographical dynamics of phosphine resistance and its local frequency over time.

The dispersal of stored product pests occurs through the anthropogenic movement of grain and by flight. The former has been assumed to be the primary means by which these insects get distributed through the grain storage and distribution network, especially with respect to *T. castaneum*, a species widely considered not to fly to any significant extent (Good, 1933; Campbell and Arbogast 2004; Drury et al., 2009; Campbell et al., 2010). Sampling with pheromone traps at bulk storage sites and in agricultural sites at least one kilometre from such sites, coupled with population genetics analyses on microsatellite markers, has demonstrated

that these beetles fly sufficiently to render beetles genetically homogeneous over an area of at least 40,000km² (Ridley et al., 2011). *Rhyzopertha dominica*, by contrast, is well known for its flight propensity and abilities in the field (Winterbottom 1922, Hagstrum 2001, Edde et al. 2006, Toews et al., 2006, Mahroof et al., 2010). The following research is based on an appreciation of these abilities in the two species. The specific issues we address in this paper are: 1) the pattern of beetle emigration from a resource, typically a grain storage, 2) the mating status and potential fecundity of migrating female beetles, and 3) the impact of migration and selection (phosphine fumigation) on resistance frequencies locally.

2. Materials and Methods

2.1. Flight patterns of beetles leaving storage under field conditions

Specifically, we investigated the peak time of flight activity, how high the beetles fly above the ground, and whether the beetles simply fly in the direction of the prevailing wind. A mixed age culture of beetles reared from field collected adults was placed in a storage bin in which they had access to excess grain, and from which they could leave freely. The storage bin was placed centrally within a circular arrangement of 16 tall (6 m), narrow sticky traps to monitor beetles trapped within 5 m of the initiation of beetle flight.

2.2. Dispersing females – mating status and fecundity

We investigated whether dispersing females are mated or virgin, and if mated, the number of males with which they have mated. Finally, we quantified the potential fecundity of migrating beetles and compared them to females collected from storage. Beetles were intercepted in pheromone traps about 1 km from bulk storage depots that were monitored persistently so arriving beetles could immediately be kept separately from one another (to prevent mating in captivity). A sample of beetles was also collected from the nearby storage for comparison with the beetles trapped in flight. Each female was held for 12 weeks in standard culture medium, to estimate their potential fecundity and to collect offspring for genotyping. Parental female tissue was genotyped with eight polymorphic microsatellite markers, and so were 16 of her offspring. A Bayesian parentage reconstruction estimated the number of males that had contributed to the genotype of the offspring of each trapped female.

2.3. Simulations and laboratory tests of migration and selection

We simulated (with SimuPOP, an individual-based population genetics simulator) the effects of low level migration of mated resistant *T. castaneum* females on resistance frequencies in susceptible populations, and experimentally tested the simulation results. We did the same for the combined effects of low-level migration and selection pressure on susceptible beetles from phosphine fumigation. We also simulated and tested two migration rates, 0.01 and 0.1 per generation for seven generations, and screened beetles for the rph2 resistance gene at each generation. The rph2 gene is one of two major genes responsible for conferring strong resistance (Jagadeesan et al., 2011). Phosphine treatment was applied, at generations three and six, to half of the experimental populations at a dose high enough (and for long enough) to kill all homozygous phosphine susceptible individuals.

3. Results and Discussion

3.1. Flight patterns of beetles leaving storage under field conditions

The peak time of flight for *T. castaneum* was dusk (1600 to 2000h) and the average height of capture was 1.1m (with the release height being 1.2m), and beetles flying predominantly with the wind. Observations were made on 20 days and in half of these virtually no *T. castaneum* beetles (no more than two, but usually none, were trapped). These were all days on which the

wind speed exceeded about 3.5m/sec, so windspeed does influence the emigration rate of these insects. The flight of *R. dominica* was slightly different, with these beetles being trapped at about 1.7m above the ground and flying across the wind to some extent. They did, however, fly predominantly at dusk.

3.2. Dispersing females – mating status and fecundity

Ninety-seven percent of dispersing *T. castaneum* females were mated at the time of being trapped in flight with 87% of the females genotyped to date having mated more than once, with a maximum of 4 matings estimated. The *T. castaneum* females taken from storage differed in this regard, with 77% having been mated, and only 58% of females genotyped so far having mated more than once. The potential fecundity of flying beetles was 296.5 ± 40.4 , which is much the same as that of the beetles from storage, which was 272.3 ± 38.9 ($F = 0.19$, $d.f = 1$, $P = 0.67$).

Rhyzopertha dominica was somewhat different from *T. castaneum*. Again, about 97% of beetles trapped in flight had mated, with 77% of the females genotyped so far having mated twice (with 4 or 5 matings estimated to be the maximum). Although 97% of the beetles from storage had been mated, far fewer had been mated multiple times (54%) than in *T. castaneum*. The other difference across the species is that the mean potential fecundity of flying beetles was 333.5 ± 28.0 , but in storage was 179.7 ± 20.6 ($F = 19.25$, $d.f = 1$, $P < 0.001$).

3.3. Simulations and laboratory tests of migration and selection

Simulations showed that frequency of *rph2* in populations subject to the lower level migration rate grew very slowly and had not reached 0.1 at generation seven. At the higher rate, by contrast, the rate had climbed to just over 0.5 in the same time. The results of screening of the resistance gene in the experimental populations followed the patterns predicted by the simulations.

The fumigation of half of the experimental populations at generation three resulted in the high levels of mortality expected, but the results from generation six were dramatically different. In both the low and high migration treatments, low (virtually zero) levels of mortality were expected because of the elimination of susceptibles in generation three. However, mortality was high in both treatments (~98% in the low migration treatment and ~66% in the high one). Presumably, eggs were not killed in the fumigation and thus could contribute to the following generations, but this is under investigation.

4. Conclusions

In conclusion, we have demonstrated that *T. castaneum* and *R. dominica* beetles readily fly from storages in which abundant resources remain. This movement accounts for the genetical homogeneity of populations (of *T. castaneum* at least) recorded in the field (Ridley et al., 2011). In the initial phase, migration of beetles is directional relative to wind direction, occurs at dusk on days that are relatively windless (wind speeds of < 3.5 m/s), and takes place relatively close to the ground (as opposed to vertical flight to enter wind currents at higher elevation).

Female beetles (of both species) are in a state, at the point when they start their flight, to disseminate genes for phosphine resistance effectively. Specifically, they have a high potential fecundity and may carry the genes of several males. Multiple mating must enhance the chances of any female carrying resistance genes (even if she, herself, is not resistant).

Low levels of immigration of resistant individuals into populations can establish resistant populations even without selection pressure, but selection pressures from fumigation are likely to increase the rate of this process. Fumigations that are not effective may well select

for resistance (Dyte and Halliday, 1985, Ridley et al., 2012) but our results suggest that they also maintain susceptibles in populations, and this aspect warrants further research.

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