The use of molecular markers to detect predation by beetles on viruliferous aphids in cereals.

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Introduction

Yield losses in winter barley due to Barley Yellow dwarf virus (BYDV) can be substantial (Kennedy and Connery, 2001). The disease can only be spread by the feeding activity of aphids. In Ireland, the most important vector of the disease is the grain aphid (*Sitobion avenae*) and the most important strain of virus is MAV. The fauna of cereal fields include many pest predators among them the Carabidae – ground beetles. Evidence of aphid predation by ground beetles is well known in cereal fields (Vickerman & Sunderland, 1975; Kennedy *et al.,* 1986) but is difficult to quantify. The objective of this study was to determine if predation on aphids by beetles reduced the incidence of virus.

Materials and methods

MAV primer design

MAV primers were selected by aligning published sequences of the coat protein genes of the three virus strains MAV, PAV and RPV using the sequence alignment tool clustalW. Two primer pairs were picked from the MAV coat protein sequence (Genbank accession No. X17260). The primers were subjected to a gradient to determine the optimum annealing temperature.

Testing of beetles for virus

Thirty ground beetles *Nebria brevicollis* (common in cereal fields in Ireland) were collected from the field. These were kept individually in petri-dishes and starved for 7 days. Six beetles were frozen as negative controls. The remaining 24 beetles were each fed one single virus infected aphid and were frozen in liquid nitrogen immediately following aphid consumption.

Total RNA was extracted from whole beetles using Trizol. Primers were designed targeting the aphids actin RNA to confirm the RNA extraction process was successful. The total RNA extracted from the beetle was then tested for viral RNA.

Aphid primer design.

Primers were designed targeting the aphids mitochondrial gene cytochrome oxidase with the aim of detecting the aphid material in the gut of the predatory beetle. A primer amplifying a product size of 103 bp was successfully designed. This primer was tested for cross reactivity with other cereal aphids (*Rhopalosiphum padi* and *Metopolophium dirhodum*) found in cereal fields.

Detection of predation

Thirty *Nebria brevicollis* were sampled from the field and fed aphids as already described. Six beetles were frozen as negative controls. The remaining 24 beetles were subdivided into 6 treatments of 4 replicates. Beetles were killed by freezing following various time intervals post aphid consumption. These intervals were 0h, 1h, 3h, 5h, 24h and 48h. Six hundred and fifty seven carabid beetles were collected from plots of winter barley. Beetles were captured daily using pitfall traps. Following collection beetles were identified and frozen.

Results and Discussion

Two MAV specific primers were successfully designed. These were tested on MAV infected plants and plant tissue free of the virus. Primers were also tested on viruliferous and virus free aphids. The primers successfully detected virus.

Aphid actin RNA was successfully amplified from beetles. However, viral RNA was not detected in beetles. It was concluded that viral RNA was degraded by the digestion process and was therefore not detectable.

Primers targeting *Sitobion avenae* mitochondrial gene cytochrome oxidase were used to detect aphid DNA. The specificity of these primers for *S. avenae* only was established by screening against other cereal aphid species.

Detection of predation

Aphid DNA was detected in beetles in the laboratory up to 48 hours following consumption of aphids. No aphid DNA was detected in starved beetles (negative controls). The investigation of field captured beetles, using the aphid primers, is given in Table 1. This table gives results for seven of the 17 of the most common species encountered. Investigations on beetles and beetle larvae is continuing.

Table 1. The number of field captured beetles examined and the percent testing positive for *Sitobion avenae* using 103 bp product size

Ground beetle species	No. beetles examined	% positive for 103bp product
Nebria brevicollis	181	5.52
Bembidion tetracolum	62	12.9
Bembidion lampros	28	14.28
Pteros. melanarius	17	11.76
Amara spp.	83	15.60
Notiophillus biguttatus	179	13.4
Trechus quadristiatus	50	16.0

Conclusion

Aphid DNA is detectable in predators but not viral RNA.

References

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