

Evolution of knock-down resistance to pyrethroids in grain aphids (*Sitobion avenae*) in the UK

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Summary

We report the discovery of the target site mechanism termed knock-down resistance (kdr) to pyrethroids in the grain aphid, *Sitobion avenae*, in the UK. A DNA-based diagnostic designed specifically to identify the kdr mutation was developed and used to screen samples of grain aphids collected from different sites in England. This showed that the frequency of kdr has increased steadily through 2012 and by the autumn had reached greater than 50% of the aphids tested in certain areas.

Key words: Grain aphids, knock-down resistance, pyrethroids

Background

Insecticide resistance is an example of a dynamic evolutionary process in which chance mutations conferring protection against insecticides are selected for in treated populations. Over the past 30 years, rapid advances have been made in the characterisation and understanding of such adaptations. These have provided valuable insights into the origin and nature of selection and micro-evolution in agricultural environments.

In practical terms, the evolution of insecticide resistance has undoubtedly contributed to overall increases in the application of chemicals to crops with significant implications for the environment. Despite this, resistant insects continue to affect our agricultural productivity. As a result, the phenomenon imposes a huge economic burden upon much of the world. Only by monitoring, characterising and predicting the appearance and spread of resistance can we hope to use existing chemical tools in a sustainable manner.

Of the thousands of aphid species that exist globally, only a few have been reported as having developed insecticide resistance (Foster *et al.*, 2007). However, some of these are ranked among the most problematic pests worldwide. Some species are highly polyphagous while others are virtually confined to a single crop type. They damage agricultural and horticultural crops through the transmission of plant viruses, direct feeding and by their impact on the aesthetic value of crops (by depositing honeydew or simply by being present). This has resulted in them being subjected to intense selection by aphicides which has led to the evolution of a variety of resistance mechanisms.

Until recently, the only aphid species known to carry significant pyrethroid resistance in the UK was the peach-potato aphid, *Myzus persicae* (Eleftherianos *et al.*, 2008). However, in response to growing concerns that the grain aphid, *Sitobion avenae*, was becoming difficult to control in this country, we initially tested samples collected in June 2011 from several wheat fields in Cambridgeshire where there had been control problems with the pyrethroid, lambda-cyhalothrin.

This was done by sequencing a small fragment of the nerve membrane sodium channel, which is the target site for pyrethroids, in order to look for mutations (amino acid substitutions) that are known to confer resistance in other insect pests. Analysis of the sequence from the Cambridgeshire samples identified a single mutation involving a change from leucine to phenylalanine at a residue (1014) known to confer moderate levels of pyrethroid resistance in *M. persicae* and other pests (Davies *et al.*, 2007). This is the first time that this mutation, termed *kdr*, had been identified in grain aphids and its discovery in this pest could have serious implications for *Barley yellow dwarf virus* (BYDV) transmission, as the grain aphid is an important vector of this damaging virus. Indeed, in spring 2012 high levels of BYDV were found in cereals across England which may in part have been caused by the presence of the pyrethroid-resistant aphids. Fortunately, much of the remaining season was not conducive to an aphid epidemic, although the very wet summer weather then created a 'green bridge' (grass weeds and volunteers) that can persist in cereal stubbles and act as a source of both aphids and virus in the autumn if not adequately destroyed prior to drilling a succeeding cereal crop. Furthermore, aphids may migrate into newly-emerging autumn cereal crops in larger numbers than usual if the weather is conducive to flight.

Recent bioassays have shown that *S. avenae* with the *kdr* mutation are approximately 30-fold more resistant to deltamethrin than non-*kdr* forms. We have also developed a sensitive DNA-based diagnostic test to quickly and effectively screen large numbers of aphids (alive or dead) to investigate the presence of the *kdr* mutation. This has enabled us to gain a clearer picture of the frequency and spread of *S. avenae* carrying *kdr* during 2012. The results have shown that *kdr* has steadily increased in frequency through the spring and summer, with the frequency of the mutation exceeding 50% in *S. avenae* samples collected from more intensive cereal growing regions. An interesting observation is that all of the *kdr* samples identified so far carry a single copy of the mutated gene (i.e. are heterozygotes). The absence of any homozygote *kdr* aphids is perhaps surprising but may be due to this species primarily reproducing asexually in the UK. Alternatively, this may indicate that homozygote-resistant aphids suffer a fitness cost in a similar fashion to homozygote *kdr* *M. persicae* (Foster *et al.*, 2011).

Our findings are enabling us to give up-to-date information to growers and agronomists for managing grain aphids, including best practice measures to limit the risk of resistance build up and strategies that can be deployed if resistance is suspected during the autumn spraying period.

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