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Insecticide resistance and genetic composition of *Myzus persicae* (Hemiptera: Aphididae) on field potatoes in New Zealand

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Abstract

The prevalence of insecticide resistance mechanisms in populations of *Myzus persicae* on potatoes in New Zealand was determined in five parthenogenetic aphid lineages raised from individuals collected from Pukekohe in the North Island, and 66 from Canterbury and one from the West Coast in the South Island during mid-summer (January) to early autumn (March) 2005. For each lineage, the genotypes, determined using four microsatellite loci, were compared with the presence or absence of resistance mechanisms assessed using a combination of biochemical tests, polymerase chain reaction-based allelic discrimination and diagnostic dose bioassays. The 72 lineages comprised 23 genotypes (defined using microsatellite markers), with 60% containing one or more mechanisms that confer resistance to between one and three of any of four insecticide classes. In all, 38% contained high or extreme elevated carboxylesterases (E-Carb-R_2 and -R_3) that confer resistance primarily to organophosphates, and some resistance to carbamates and pyrethroids; 19% contained modified acetylcholinesterase (MACE) that gives strong resistance to the di-methyl carbamates, pirimicarb and triazamate; 54% contained a mutation in a voltage-gated sodium channel gene, called knockdown resistance (kdr), that confers resistance to pyrethroids; and 36% contained a second mutation of the gene (super-kdr) that gives enhanced resistance to pyrethroids. In all, 10% of the lineages also showed low-level resistance to neonicotinoids, as shown in imidacloprid diagnostic dose bioassays. Most of the microsatellite genotypes appeared to disclose cycles of sexual reproduction, the clones of which were susceptible to all classes of insecticide chemistry, except two that had acquired MACE possibly by breeding with an invasive MACE genotype or an individual carrying a locally derived MACE mutation. However, lineages carrying multiple resistance mechanisms belonged to two resistant genotypes that formed widely distributed clones. Behavioural bioassays on selected lineages from New Zealand and Europe showed that *M. persicae* clones from both regions carried E-Carb R_3, or kdr/s-kdr, and showed consistently low responses to the aphid alarm pheromone (*E*)-*β*-farnesene. Methods are discussed for exploiting such putative fitness costs of resistance traits in *M. persicae* aphids to minimise the development of resistance and restore efficacy to insecticide programmes.

Keywords: Potato; Aphids; *Myzus persicae*; Alarm response; Insecticide resistance; Carboxylesterase; Modified acetylcholinesterase; kdr; Neonicotinoid resistance; Microsatellite; Genotypes

1. Introduction

In New Zealand, insecticides are applied to potatoes to control potato tuber moth (*Phthorimaea operculella*) (Foot, 1979) and several species of virus-transmitting aphids, especially the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Stufkens and Teulon, 2001; van Toor and Teulon, 2006). The requirement to keep potatoes, especially seed potatoes, virus-free necessitates a thorough and exacting aphid control programme often resulting in multiple insecticide applications. Potato seed crops are often treated with a neonicotinoid (imidacloprid) seed treatment, followed by up to 11 applications of insecticides from organophosphate, carbamate, pyrethroid and other chemical groups to control aphids and thereby limit

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infection of potatoes by viruses (van Toor and Teulon, 2006). Many crops receive three or more foliar applications of an organophosphate (methamidophos) and a pyrethroid (λ-cyhalothrin). Insect control programmes that rely on multiple uses of the same or related insecticides can lead to insecticide resistance in the absence of insecticide resistance management strategies (Martin et al., 2005).

Insecticide resistance to aphids on potatoes has already been documented in New Zealand. Fellowes and Fergusson (1994) considered that insecticide resistance was responsible for failure to control *M. persicae* in field trials in potatoes. Insecticide resistance to several organophosphates, carbamates and pyrethroids has been confirmed in *M. persicae* through laboratory studies (Cameron and Walker, 1988).

Elsewhere in the world, insecticide resistance in the most viruliferous aphid, *M. persicae* (Kennedy et al., 1962), is conferred by three genetically distinct mechanisms (Devonshire et al., 1998). A detoxification mechanism (E-Carb) confers resistance to most ester-containing insecticides, including organophosphates and to a lesser extent carbamates and pyrethroids, by increased production of closely related E4 and FE4 carboxylesterases in cells (Devonshire and Moores, 1982). The increase in carboxylesterase synthesis and production is caused by amplification of genes encoding these enzymes (Field et al., 1988). Based on the amount of carboxylesterase produced, individual aphids can be classified as susceptible (S), moderately resistant (R_1), highly resistant (R_2) or extremely resistant (R_3) (Devonshire et al., 1986).

The other two resistance mechanisms are based on modifications to insecticide target sites. One involves changes in acetylcholinesterase, called modified acetylcholinesterase (MACE), which specifically confers resistance to di-methyl carbamates, pirimicarb and triazamate (Foster et al., 2000; Moores et al., 1994). The MACE phenotype is associated with a single amino acid substitution (serine to phenylalanine, S431F) within the active site of the enzyme (Andrews et al., 2002; Nabeshima et al., 2003). The second target site-based mechanism, called knockdown resistance (kdr), involves mutations in a voltage-gated sodium channel gene from leucine to phenylalanine L1014F, and confers resistance to pyrethroids. Aphids with this mutation can possess a second mutation from methionine to threonine M918T, called super-kdr (s-kdr), which enhances the resistance phenotype (Eleftherianos et al., 2002; Martinez-Torres et al., 1999). A proportion of *M. persicae* also show low-level resistance ($<$20-fold increase in the dose for LD50) to the commonly used neonicotinoid insecticide, imidacloprid, although the mechanism in aphids remains unknown (Foster et al., 2003a).

A combination of PCR assays to diagnose kdr mutations (Anstead et al., 2004), biochemical assays for detecting elevated carboxylesterases (E-Carb) levels and MACE (Field and Foster, 2002), and dose–response assays for detecting imidacloprid resistance (imid-R) was used to test aphid lineages raised from individual aphids collected from potatoes in New Zealand. This information was used for the first time to determine whether resistance mechanisms to several insecticide classes occur in *M. persicae* in this country. The resistance profile for each clone was related to its genotype at polymorphic microsatellite loci used previously to investigate the clonal composition of insecticide-resistant *M. persicae* in the UK (Fenton et al., 2005).

The effect of two insecticide resistance mechanisms on the ability of aphids to respond to alarm pheromone was also assessed. The aphid alarm pheromone (*E*)-β-farnesene is released from cornicle secretions exuded by aphids when they are physically disturbed (Edwards et al., 1973). Previous studies on *M. persicae* lineages from individuals collected from the UK and mainland Europe suggested that kdr and extreme E-Carb (R_3) resistance have negative pleiotropic effects on various aspects of aphid behaviour, including response to aphid alarm pheromone (Foster et al., 1999). Foster et al. (2003b) hypothesised that the suppressed alarm response was caused by a deleterious impact of these resistance mechanisms on nerve and/or biological function. An alternative explanation is that the resistance genes are closely associated with other genes affecting behaviour, i.e. resistance is found in a ‘low alarm response’ genetic background. The alarm response of *M. persicae* lineages containing no resistance mechanisms or varying levels of E-Carb and/or kdr, raised from individuals collected from New Zealand, was compared with the lineages of individuals collected from the UK and Europe. Since these two groups of aphids were genetically isolated, a similar set of responses to the alarm pheromone by resistant aphids collected from two geographically isolated regions would support the hypothesis that pleiotropic effects were attributable to genes conferring resistance.

By convention, in this paper, an individual refers to one aphid that was collected in the field. The parthenogenetic offspring of an individual are clones that form a parthenogenetic lineage. Genotype refers to alleles at all loci borne by clones, described as the genotype of that clone or lineage.

2. Methods

2.1. Aphids

Individual aphids of *M. persicae* were collected from potato (*Solanum tuberosum*) from 31 sites in New Zealand between mid-January and March 2005 (Table 1). One aphid was collected from each plant ($>$4 m apart) within a potato crop to reduce the chance of sampling the same clone. Five aphids were collected from Pukekohe in the North Island and 66 aphids were collected from locations within Canterbury in the South Island. Within Canterbury, 19 aphids were collected from Christchurch, either from the northern part, in which market gardens predominate and therefore the aphids may have come in contact with pesticides, or from the southern part, dominated by home