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Effects of *Lecanicillium longisporum* infection on the behaviour of the green peach aphid *Myzus persicae*

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Abstract

The effects of the entomopathogenic fungus *Lecanicillium longisporum* (Zimmerman) Zare & Gams on three parameters of behaviour (feeding, reproduction and movement) of the green peach aphid *Myzus persicae* (Homoptera: Aphididae) were investigated in the laboratory. Visual analysis of video tapes established that honeydew excretion events of mycosed aphids gradually declined from 2 d post inoculation and reproduction rate was significantly reduced 2 d prior to death (which occurred on day 6); both parameters were stable in controls over the same period. A detailed comparison was made between mobility of aphids during infection with two isolates of *L. longisporum*, using image analysis of video recordings. Both isolates caused an increase in activity at the beginning of mycosis (during fungal germination and cuticle invasion) though the intensity and the duration of this behaviour varied with the isolate. The possibility that increased movement in early mycosis helps disseminate disease is discussed in the light of the observation that saprophytic surface growth occurs on living *M. persicae* as it does in at least some other *Lecanicillium* spp–insect interactions.

Keywords: *Lecanicillium longisporum*; *Verticillium lecanii*; *Myzus persicae*; Entomopathogen; Aphid; Fungus; Mobility; Behaviour; Migration; Honeydew; Reproduction

1. Introduction

Microbial pathogens of insects have profound effects on the physiology and biochemistry of their hosts (see e.g., reviews by Charnley (2003) and Gillespie et al., 2000). As a consequence they produce consistent, recognisable symptoms that define disease states. Less well characterised, in many cases, are alterations of host behaviour, caused directly or indirectly by the pathogen, that contribute to disease (see Roy et al., 2006).

Among fungal pathogens 'summit disease' syndrome is the most well known and dramatic of pathogen-induced behaviours. Insects expressing symptoms climb to the tops of host plants prior to death. The syndrome has been observed during a variety of mycoses including those caused by *Entomophthora muscae* in flies, *Pandora neoaphidis* in aphids and *Cordyceps* sp. in ants (Krasnoff et al., 1995; Harper, 1958; Evans, 1989). It is presumed to be adaptively favourable to the pathogen as it promotes dissemination of propagules over the widest possible area. However, climbing behaviour is commonly observed in insects infected with parasitoids and viruses also, suggesting a general insect response to parasitism rather than pathogen manipulation of the host (Horton and Moore, 1993).

Reduced feeding and reproduction are common features of insect mycoses (e.g., Blanford and Thomas, 2001; Moore et al., 1992). Not all pathogen-induced "behaviours" are detrimental to the host. In particular, high body temperatures generated by behavioural fever prevent pathogen

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growth in flies (Watson et al., 1993), grasshoppers (Carruthers et al., 1992) and locusts (Blanford et al., 1998) infected with fungi.

Recent studies on aphids have revealed subtle influences of fungi on host behaviour. Jensen et al. (2001) showed altered microhabitat selection by pea aphid *Acyrthosiphum pisum* mycosed with *P. neoaphidis* while Roy et al. (1999) established reduced host sensitivity to alarm pheromone in the same aphid–pathogen interaction. In the present work effects on honeydew secretion, reproductive rate and mobility have been determined in peach potato aphids *Myzus persicae* (Homoptera: Aphididae) infected with two isolates of the generalist pathogen *Lecanicillium longisporum* (Zimmermann). Image analysis of video recordings of individual aphids has enabled for the first time a systematic, detailed description of key elements of aphid behaviour during the development of a fungal disease.

2. Materials and methods

2.1. Aphids

Green peach aphids *M. persicae* (clone 794JZ), provided by Rothamsted Research, UK, were reared on potted green pepper plants *Capsicum annuum*, v. California Wonder in muslin-covered wooden-framed cages (1 m^3) in an air-conditioned room at 21 ± 1 °C and a photoperiod of 16 h light: 8 h dark.

2.2. Fungus

The *L. longisporum* isolates KV42 and KV71 (active constituent of the mycoinsecticide Vertalec®), provided by Koppert B.V. (The Netherlands) were grown routinely on bactopeptone (2% (w/v) Malt extract (Oxoid), 5% (w/v) Bacteria peptone (Difco Laboratories)) in 2%(w/v) Agar (Oxoid) at 24 °C in the dark. Spores were harvested from 8 d cultures in 9 cm Petri dishes with 10 ml of sterile distilled water (dH_2O). Spore suspension was vortexed, filtered through a sterile mesh and resuspended in sterile (dH_2O). Preliminary work established that a surfactant was not necessary during the manipulation of spores in aqueous suspension.

2.3. Infection protocol with fungal pathogen

All experiments were conducted in vented Petri dishes (5 cm diameter, 1 cm depth) with 6 ml of 1% (w/v) water agar and a leaf disk, abaxial surface uppermost, placed on top. This set up served as the experimental arena for the behaviour experiments described in the following sections.

Five adult aphids were placed in the arena. In all cases the arena and the insects were sprayed *in situ* using a Potter Tower (Burkard Ltd., UK) (Potter, 1952). Compressed nitrogen gas (10 lb/in^2) was used as a propellant. Untreated insects (control) were sprayed with 1.5 ml sterile distilled H_2O and treated insects were sprayed with 1.5 ml of 10^6 sp/ml *L. longisporum* spores suspension (LC_{100} dose) (isolates KV71 and KV42). Spore concentration was estimated using a haemocytometer with an Olympus BH2 microscope (400 ×). The arena received 8.7% of the 1.5 ml inoculum, the rest was retained within the tower. Approximately 45 spores landed on the dorsal surface of each aphid and 15 spores on the ventral surface, determined using spores labelled with the fluorescent dye Uvitex (see Section 2.6). The method of application was consistent, e.g., log spores landing on the aphids plotted against log spore concentration ml^{-1} sprayed gave the following regression equations: aphid dorsal surface, $y = 1.28x–6.78$, $r^2 = 0.94$; aphid ventral surface $y = 0.88x–4.81$, $r^2 = 0.77$ ($n = 4$, in each case). Before and after use, the Potter Tower was cleaned with 70% alcohol and then rinsed with sterile distilled H_2O.

2.4. Visual analysis of behaviour

The filming method has been previously described by Roditakis et al. (2000). Briefly, the equipment was placed in an incubator (Gallenkamp, Compenstat, illuminated, cooled incubator) at 24 °C and 16 h L: 8 h D photoperiod (see Fig. 1). Light was provided by six fluorescence tubes (GE, F8W/35) placed two at 10 cm from the arena, two at 25 cm and two at 45 cm, providing luminosity of 1200 Lux. Filming was paused in the scotophase.

Both fungus-treated and untreated (control) Petri dishes were sealed with a strip of parafilm® with six ventilation holes (punctured with sterile pin) to ensure high RH with

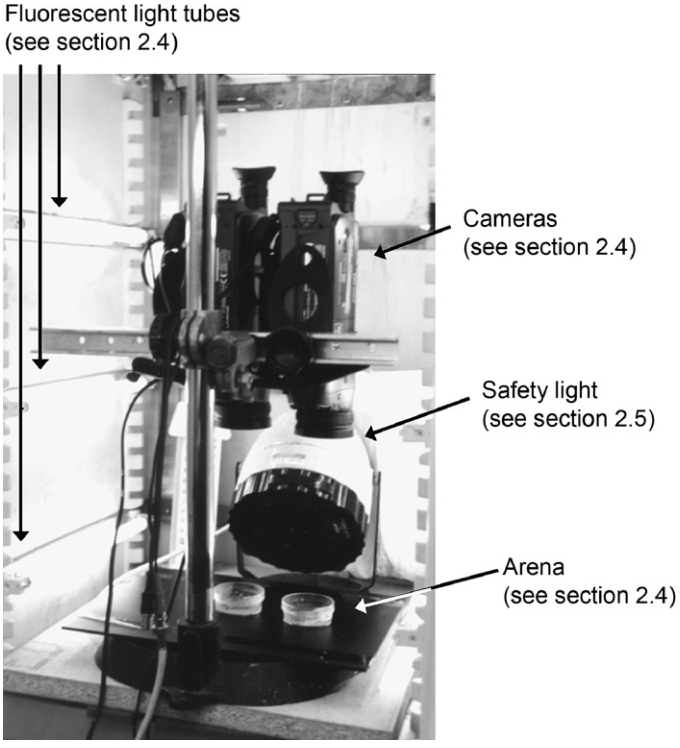

Fig. 1. The filming set up (see Sections 2.4 and 2.5 for full details). The apparatus is inside an incubator. The picture is taken with the door open.