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

Emmanouil Roditakis[a,b,*], Iain D. Couzin[c,d], Nigel R. Franks[e], Anthony K. Charnley[a]

[a]*Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK*
[b]*Plant Protection Institute of Heraklion, National Agricultural Research Foundation, 71003 Heraklion, Greece*
[c]*Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544, USA*
[d]*Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK*
[e]*Centre for Behavioural Biology, School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK*

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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

*Corresponding author. Plant Protection Institute of Heraklion, National Agricultural Research Foundation, 71 003 Heraklion, Greece. Tel.: +30 281 030 2309; fax: +30 281 024 5858.
E-mail address: e-roditakis@her.forthnet.gr (E. Roditakis).

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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

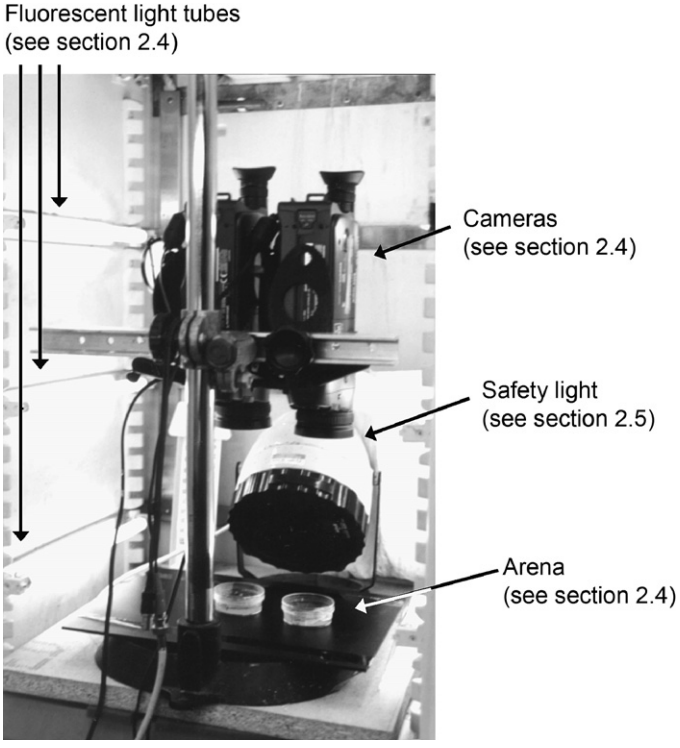

Fig. 1. The filming set up (see Sections 2.4 and 2.5 for full details). The apparatus is inside an incubator. The picture is taken with the door open.

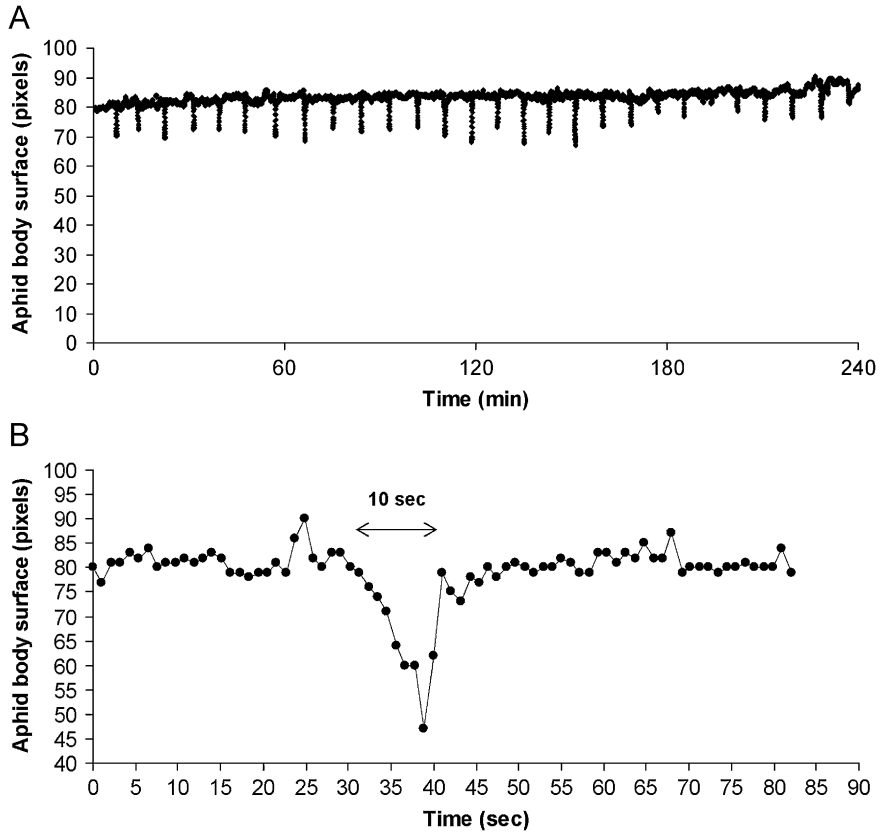

Fig. 2. The body surface of an untreated aphid (in pixels) as recorded by the image analysis programme. (A) Observations over a 4 h period. Brief, apparent reductions in area caused by the lifting of the aphid abdomen recorded as (vertical bars) correspond to honeydew excretion events. (B) A typical excretion event (duration of 10 s); corresponds to the second vertical bar shown in (A).

moderate ventilation. Each dish was then placed under the camera (Panasonic NV-SX30BF). Filming started once the image was centred and focused. The time-lapse method used compressed the recording of 6 d of filming onto a 3-h tape. No condensation was observed on the Petri-dish lid, thus the aphid behaviour was never obscured.

Once recording was complete the tape was played back and aphid behaviour was visually analysed using the computer program Observer^{TM} (Noldus Software, The Netherlands) to record the time and duration of the events. Mobility was determined as the 'number of movement events per day'. A 'movement event' was defined as directional movement (i.e. walking) by the aphid, resulting in the modification of the insect position on the filming arena.

The video recording set up had high resolution. The arena comprised a 768×576 pixel image, which filled ca. the whole of the computer screen. Since each aphid was 24×14 pixels, even movements of individual legs could be seen clearly. Preliminary experiments established that adult aphids lifted the rear part of the abdomen during honeydew excretion. The excreted honeydew drop was immediately observed on the Petri dish lid, usually a few mm away of the aphid body. The alteration of the body angle in relation to the leaf surface during the excretion event resulted in the reduction of the visible surface of the aphid body. This was recorded during computerised analysis of aphid behaviour (see next section for details). In Fig. 2-A, the observed reductions of body surface of an aphid corresponding to the honeydew excretion events are shown over a 4-h period. Excretion events are repeated in fixed intervals (in this case: approximately every 8 min, seven events per hour). The duration of an excretion event was approximately 10 s (Fig. 2-B). Thus this brief, characteristic change in body posture, which was easily detected visually, was recorded as a "honeydew excretion event".

Insects killed by fungal infection were covered by white sporulating mycelium. This symptom was never observed on control insects.

To determine the rate of reproduction, individual adult aphids that had just moulted were placed in a standard arena (prepared as above, but with a single insect per dish). The day that the first offspring was born was considered as day 0. The offspring were removed and the aphids were sprayed *in situ* with water or fungal suspension (see above). The number of offspring produced per day was recorded and defined as the "reproductive rate". Offspring positioned relatively distant from the adult were carefully removed with a fine paint brush to avoid overcrowding. Seven aphids in total were tested for each treatment.

2.5. Set up for computerised analysis of movement

Treated and untreated (control) Petri dishes were filmed at the same time using duplicate equipment. A dark room safe light (Kodak No 1) with a 40 W (Philips) bulb, 10 cm from the arena, was used to allow filming during the dark phase (see Fig. 1) (Nauen, 1995). This allowed only 10% transmission $\geqslant$610 nm. The experimental arena described in the previous section was used. Preliminary experiments established that differences in behaviour between aphids infected with the two isolates occurred during the first 3 d after inoculation and this time frame was focused on. The time-lapse video recorder compressed 23 h of real time into 3 h of videotape. The computer was capable of identifying each aphid individually using background subtraction. The position of each aphid was continuously recorded with the time in data arrays (x, y coordinates) which were saved in text format. The computerised analysis of video tapes and data extraction has been described previously in detail by Roditakis et al. (2000). For behaviour analysis, an aphid was considered mobile when two rules were satisfied: (a) when the path followed had a clear direction and (b) the position of the aphid was modified at least 3 mm from the previous position. This analysis filter excluded artefacts including videotape noise (see Fig. 3). Every 0.9 of a second the elapsed time and the position (x, y co-ordinates) of the aphids were recorded. For a typical 6 h videotape analysis the aphid position in the arena was recorded ca.19,850 times. The beginning and the end of each movement event was identified within the 19,850 data array by using simple mathematical formulae (analysis example illustrated in Fig. 4 and Table 1). Movement events that were interrupted by intermissions (immobile periods) shorter than 1 min were considered as part of the same event. This study focused on the distance covered by the aphids, the duration of movement events and the number of distinct movement events (Table 1). Five aphids were observed per treatment in each experiment. The experiments with isolate KV71 were done three times and with isolate KV42 twice.

2.6. Preparation and observation of Uvitex BHT stained spores

The method employed was essentially that described by Drummond and Heale (1985). Spores were harvested from a 9-d-old colony of *L. longisporum* KV71, as described in Section 2.2. One ml of a 10^9 spores/ml suspension and 1 ml of 1% (w/v) Uvitex BHT (fluorescent dye from Ciba-Geigy, Ltd) in sterile dH_2O were added to 8 ml of sterile dH_2O, to give 10^8 spores/ml in 0.1% Uvitex BHT. The suspension was incubated for 1–2 h. The spores were then washed by centrifugation at 5000 rpm for 10 min, removing the supernatant, and re-suspending them in water. The procedure was repeated. Spores were sprayed onto aphids (see Section 2.3) and located on hosts using an Olympus BH2 microscope equipped with an episcopic fluorescence

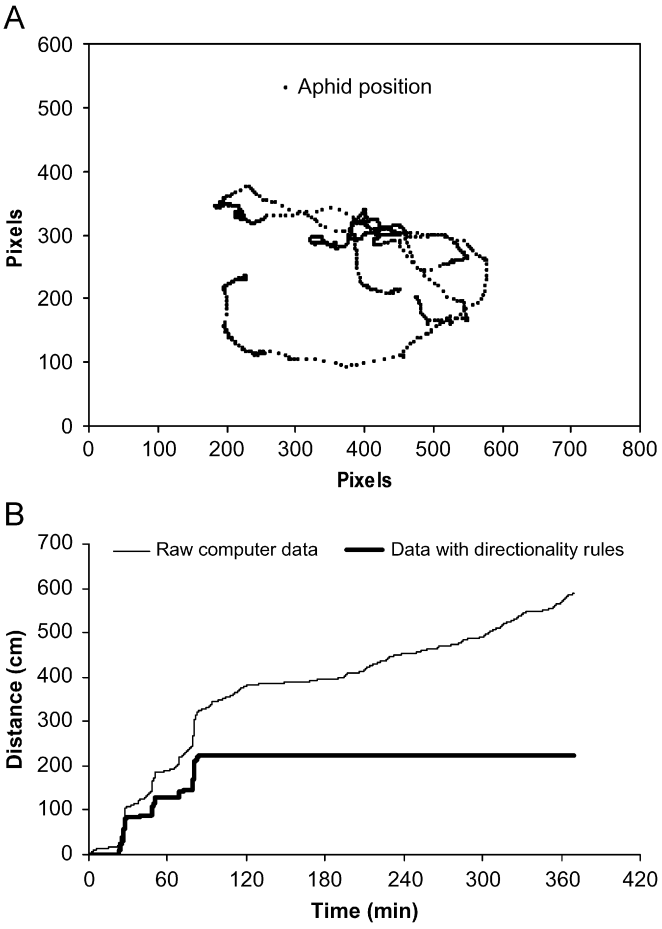

Fig. 3. Graphical illustration of the behavior of one aphid individual as recorded by the tracking program in a 6 h period. Each graph comprises 19,850 points. (A) The actual path followed by the insect in the filming arena. (B) The cumulative distance covered by the insect without (fine line) and with (thick line) the implementation of directionality rules, which exclude videotape noise.

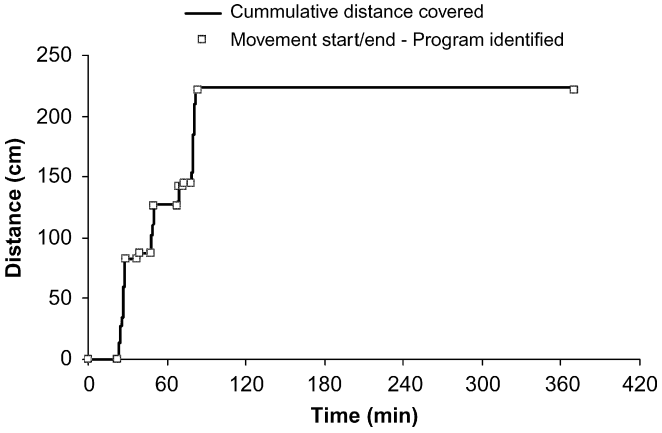

Fig. 4. The data analysis program could identify the initiation and the end of a movement event and describe accurately the activity of the insect. These time points (12 points for six movement events, corresponding to the data displayed on Table 1), are illustrated on this graph as white squares on the cumulative distance (fine line, 19,850 points) covered by an individual aphid in a 6 h period.

Table 1
Movement events by a single adult aphid in a 6-h period, after the analysis of the tracking program data

Movement event	Start time (h:m:s)	Event duration (h:m:s)	Distance (mm)
1	0:22:05	0:06:10	82.5
2	0:37:27	0:02:11	4.1
3	0:47:35	0:02:44	39.3
4	1:08:13	0:01:01	16.3
5	1:12:28	0:00:48	2.1
6	1:18:07	0:05:16	77.1
Total		0:18:09	221.5

For each movement event the initiation time the duration and the distance covered were also recorded. Movement events that were interrupted by intermissions (immobile periods) shorter than 1 min were considerate as part of the same event. These points correspond to the white square marks illustrated in Fig. 3.

illuminator, supplying UV light a from 100 W mercury lamp.

2.7. Scanning electron microscopy

Aphids 4 d after inoculation with *L. longisporum* KV71 (see Section 2.3) were processed for electron microscopy using the method described by Dean et al. (2004).

2.8. Statistical analysis

The data from visual analysis of the aphid behaviour and the reproduction rate experiment were analysed using repeat measure GLM and one way ANOVA in the programme SPSS. Data from the computerised behaviour analysis were analysed by a two factor ANOVA design using Genstat (Payne et al., 1993).

3. Results

3.1. Analysis of three aspects of behaviour in aphid infected with isolate KV71

The movement of five adult aphids of each treatment was recorded over 6 d. Due to high reproductive rate overcrowding caused increased mobility of controls on day 3. Once the offspring were removed, normal mobility was resumed. However, there were no significant differences in mobility between treatments (data not shown). Two out of the five fungus-treated aphids exhibited a distinctive behaviour viz. slow, non-directional movement with shaking, on 4 and 5 d, not observed in control aphids. Their behaviour appears to be a pre-death syndrome because these two aphids did not move again and stopped producing honeydew (criterion of death). All five aphids were dead by day 6.

Honeydew excretion events were monitored through the experiment (Fig. 5). There was no significant change in number of excretion events with time for control aphids (least squares regression, $Y = 1.6837X + 28.619$; $R = 0.0523$). Day 3 data were excluded from the analysis because of the effects of overcrowding. The number of excretion events of infected aphids significant declined with time (least squares regression $Y = -6.083X + 37.987$; $R = 0.789$). The treatments were significantly different (repeat measures GLM, df = 1, $F = 12.398$, $P = 0.013$). In particular, infected aphids had fewer excretion events than controls on days 2, 5 and 6 (ANOVA, df = 9, $F = 5.370$, $P = 0.048$; df = 7, $F = 16.256$, $P = 0.0.007$; df = 9, $F = 391.304$, $P < 0.001$; respectively).

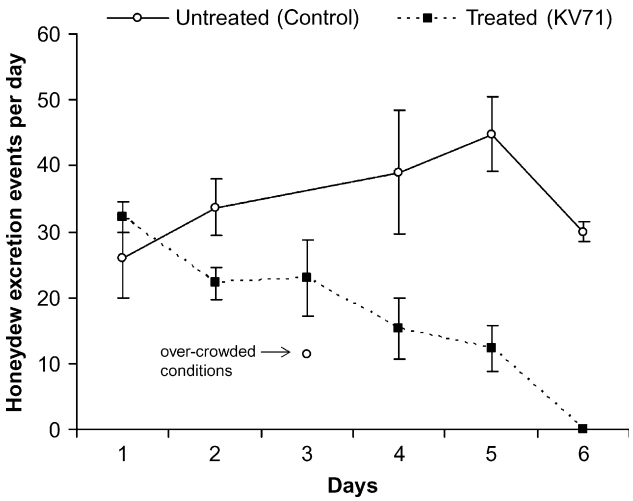

Fig. 5. Average number of honeydew excretion events per day recorded by untreated adults (control) and adults treated *with L. longisporum* isolate KV71 ($n = 5$ aphids per treatment), during a 7-d incubation period. Bars represent standard errors of the mean. On day 3 the adults for control treatment were disturbed by crowded conditions, offspring were removed from the filming arena between day 3 and 4. On day 6, all treated aphids were dead from fungal infection (cadavers covered by white sporulating mycelium on day 7). Treatments were significantly different (repeat measures GLM, df = 1, $F = 12.398$, $P = 0.013$). Infected aphids had fewer excretion events than controls on days 2, 5 and 6 (ANOVA, df = 9, $F = 5.370$, $P = 0.048$; df = 7, $F = 16.256$, $P = 0.0.007$; df = 9, $F = 391.304$, $P < 0.001$; respectively).

Control aphids produced 5.0–6.0 offspring/d throughout the experiment (Fig. 6). For the treated aphids the reproductive rate was stable only in the first 4 d (4.0–5.7 offspring/d). Reproductive rate was significantly different between treatments over the time course of the experiment (repeat measures GLM df = 1, $F = 13.409$, $P = 0.011$) and in particular infected aphids produced fewer offspring on days 5 and 6 (ANOVA; df = 13, $F = 6.811$, $P = 0.023$; df = 7, $F = 13.762$, $P = 0.010$; respectively). Aphid death occurred between day 6 and 7.

3.2. Analysis of mobility in infected aphids: studies with two isolates of L. longisporum

Quantifying movement by visual inspection of video-tapes failed to show differences between control aphids and those mycosed with KV71 (Section 3.1). Therefore the

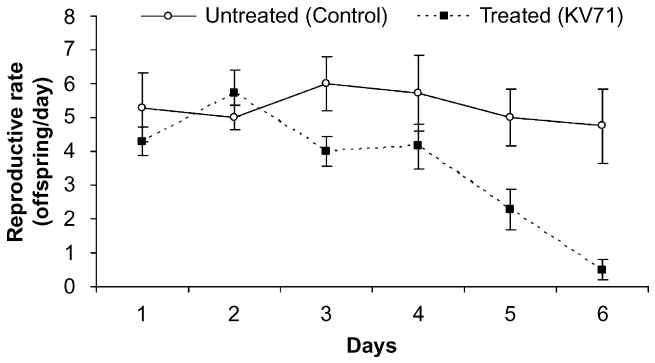

Fig. 6. The reproductive rate of fungus treated (*L. longisporum,* isolate KV71) and control aphids for a 6-d incubation period ($n = 7$ aphids per treatment). Bars represent standard errors of the mean. Treatments were significantly different over the time course of the experiment (repeat measures GLM df = 1, $F = 13.409$, $P = 0.011$). Infected aphids produced fewer offspring on days 5 and 6 (ANOVA; df = 13, $F = 6.811$, $P = 0.023$; df = 7, $F = 13.762$, $P = 0.010$; respectively).

experiment was repeated using computer-aided image analysis that provided more detailed information. The opportunity was also taken to compare the effects of two isolates of *L. longisporum*. Data for three aspects of mobility are presented in Tables 2 and 3. Two factor ANOVA was preformed on $\ln(x+1)$ transformed data. For isolate KV71, significant differences between treated and untreated aphids were observed on days 1 and 2 (Table 2). Infected aphids had more movement events ($P_{\text{Day-1}} = 0.032$ and $P_{\text{Day-2}} = 0.012$), spent significantly more time walking ($P_{\text{Day-1}} = 0.042$) and covered longer distances ($P_{\text{Day-1}} = 0.030$) when compared to control aphids. No differences were observed on day 3. The variance between the experiments was not significant, suggesting homogenous behavioural responses of the insects in the three replicate experiments ($N = 5$ aphids in each case).

For the isolate KV42 significant differences were observed between treated and untreated aphids only on day 1 (Table 3). A highly significant effect of infection on the number of movement events ($P = 0.005$) and the movement duration ($P = 0.021$) was observed. There was no significant difference between treatments in the distance covered ($P = 0.084$). No differences were observed on days 2 and 3. The variance between the experiments on day 1 was significant; however, the interaction between the experiment and treatment was not, suggesting that both experiments indicated the same trend ($N = 5$ aphids in each case) (Table 3). This demonstrated the importance of simultaneous filming when investigating behavioural effects.

Table 2
The computerised behavioural analysis of data from experiments using *L. longisporum* KV71 (five aphids per treatment in each of three experiments) analysed by two factor ANOVA design (df = 29) on $\ln(x+1)$ transformed data

	Day 1		Day 2		Day 3	
	Control	KV 71	Control	KV 71	Control	KV 71
Number of movement events						
Experiment 1	14.8	17.0	2.6	15.6	2.2	0.4
Experiment 2	6.4	50.2	0.4	9.8	5.2	0.0
Experiment 3	4.4	7.6	5.2	14.0	4.6	6.2
SED	(0.751)		(0.772)		(0.602)	
P_{values}						
Treatment	0.032		0.012		0.244	
Experiment	0.128		0.252		0.179	
Interaction	0.210		0.738		0.074	
Cumulative duration of movement events (min)						
Experiment 1	83.2	120.1	6.8	33.8	3.9	4.8
Experiment 2	26.8	371.1	1.3	36.4	15.9	0.0
Experiment 3	40.6	27.5	23.9	40.3	15.1	17.7
SED	(1.122)		(1.088)		(0.913)	
P_{values}						
Treatment	0.042		0.056		0.438	
Experiment	0.167		0.254		0.184	
Interaction	0.095		0.609		0.125	
Distance covered (cm)						
Experiment 1	1432.0	3057.1	104.6	627.2	24.9	41.3
Experiment 2	294.1	10880.6	15.2	848.4	171.5	0.0
Experiment 3	337.2	542.5	427.5	697.1	240.8	156.1
SED	(1.691)		(1.857)		(1.561)	
P_{values}						
Treatment	0.030		0.071		0.384	
Experiment	0.197		0.273		0.180	
Interaction	0.130		0.600		0.154	

Mean values for each experiment are presented in the table.

4. Discussion

Mycosis with *L. longisporum* KV71 had a significant impact on all three aspects of aphid behaviour studied. First, honeydew excretion events of *M. persicae* declined from the 2nd day post inoculation suggesting reduced honeydew production by infected aphids. Fungal infections in insects are commonly associated with a decline in food consumption and excretion. However, such illness-induced anorexia is not confined to mycoses. It has been observed during a variety of microbial infections in insects and other animals (Adamo, 2006). Entomophthoralean fungi often become opaque to the host immune system by removing antigenic cell-wall components (see review by Gillespie et al., 2000). Anorexia is expressed late in these mycoses perhaps at a point when the insect finally detects the fungus and belatedly mounts an ineffectual response. This would be consistent with evidence that anorexia increases immune defence and thus is adaptively favourable to the host (Adamo, 2006). In some hypocrealean infections, such as those caused by *L. longisporum*, reduction of feeding is dose-dependent (see e.g. Moore et al., 1992). This might be expected if the phenomenon is linked in some way to host defence.

Reduced food intake, indicated by declining honeydew excretion, in aphids mycosed with *L. longisporum* KV71, is probably a contributory factor to the drop in reproductive rate. Competition with the fungus for resources and a detrimental impact of the fungus on host physiology, e.g.,

Table 3
The computerised behavioural analysis of data from experiments using *L. longisporum* KV42 (five aphids per treatment in each of two experiments) analysed by two factor ANOVA design (df = 19) on ln(*x* + 1) transformed data

Experiment	Day 1		Day 2		Day 3	
	Control	KV 42	Control	KV 42	Control	KV 42
Number of movement events						
Experiment 1	1.0	5.6	4.8	2.6	4.4	0.0
Experiment 2	7.2	29.8	2.4	5.4	2.6	1.2
SED	(0.531)		(0.689)		(0.628)	
P_{values}						
Treatment	0.005		0.929		0.257	
Experiment	0.000		0.465		0.532	
Interaction	0.480		0.537		0.815	
Cumulative duration of movement events (min)						
Experiment 1	5.1	24.5	9.4	6.1	19.6	0.0
Experiment 2	26.9	117.8	4.8	14.6	10.6	1.8
SED	(0.801)		(0.940)		(0.923)	
P_{values}						
Treatment	0.021		0.866		0.195	
Experiment	0.000		0.520		0.528	
Interaction	0.976		0.602		0.957	
Distance covered (cm)						
Experiment 1	48.1	216.2	84.3	45.0	230.3	0.0
Experiment 2	474.2	1873.4	106.7	180.7	194.9	7.3
SED	(1.275)		(1.678)		(1.543)	
P_{values}						
Treatment	0.084		0.976		0.172	
Experiment	0.000		0.502		0.434	
Interaction	0.822		0.748		0.892	

Mean values for each experiment are presented in the table.

through tissue invasion or secretion of toxic metabolites, may also be important. Reduced fecundity of pea aphids, *A. pisum*, infected with *Beauveria bassiana* and *P. neoaphidis*, occurred within 24 h of infection (Baverstock et al., 2006). However, in the present study reproduction was not affected until 5 d after inoculation; that is 3 d after honeydew production dropped significantly and 1 d before death. This confirms previous observations on the *L. longisporum–M. persicae* interaction (Yokomi and Gottwald, 1988; Hall, 1979).

The key novel finding of this study is that *L. longisporum* infection caused increased activity of *M. persicae* during the initial stages of infection, when the fungus is penetrating host cuticle and just starting to colonise the haemolymph (Graystone and Charnley, unpubl.). Jensen et al. (2001) showed that *P. neoaphidis*-infected pea aphids were more likely to migrate off the plant than healthy aphids and suggested this could be due to increased mobility. Relocation of mycosed aphids on host plants has been shown in a number of studies (e.g. Roy et al., 2002). The present work is the first to quantify such mobility and link it to a particular stage in mycosis. The presence of the fungus *per se* did not cause host behaviour alterations viz. maintaining inoculated aphids at low RH, thus not allowing disease development, did not affect behaviour while aphids did not avoid fungus-contaminated areas of leaf (Roditakis, unpubl.).

Increased movement in mycosed aphids could be fungus-induced to optimise spore dispersal/transmission, or an attempt by the insect to prevent disease spread among close relatives (kin selection) (Roy et al., 2006). It should be distinguished from "summit disease", where infected aphids move to elevated positions during the later stages of infection (see Section 1) (Harper, 1958; Jensen et al., 2001; Rockwood, 1950; Samson et al., 1988, McCoy et al., 1988). Indeed elevation-seeking behaviour has not been reported for the *M. persicae–L. longisporum* interaction in nature. A pre-death behaviour was observed in *M. persicae* infected with *L. longisporum* KV71 viz slow, non-directional movement with shaking. Similar symptoms have been described previously in terminal stages of some mycoses in aphids (Jensen et al., 2001; McCoy et al., 1988). It was observed infrequently in the present work and its relationship to the reproducible, increased movement early in mycosis is not clear.

It is interesting that the increased mobility in the present work occurs so early in the disease process, just 1 or 2 d after inoculation. There have been reports that *Lecanicillium* spp. grow saprophytically on the surface of insect hosts while invasion is underway (e.g. Schreiter et al., 1994; Askary et al., 1999). In this regard it is very different from other hypocrealean infections, where invasion is prompt and surface growth is *post mortem* (see Clarkson and Charnley, 1996). Askary and Yarmand (2007) also observed limited sporulation by *L. muscarium* on the potato aphid, *Macrosiphum euphorbiae*, after as little as 2 d. If saprophytic sporulation occurs in early mycosis on *M. persicae* infected with *L. longisporum*, then increased host movement could reduce vertical transmission, as the mother moves away from recently born offspring n.b. juvenile aphids are not infected during emergence (Roditakis, unpubl). However, at the same time, disease spread among the wider colony might be enhanced if spores were dislodged while the aphid is in transit. We observed saprophytic growth by *L. longisporum* during early mycosis on *M. persicae* (see Fig. 7A) and limited sporulation was seen also on live aphids (Fig. 7B and C). However, spore formation seemed to occur only in the later stages of mycosis mostly on elements of pathogenic fungus re-emerging from the aphid body through intersegmental membranes and segmental leg cuticle (see Fig. 7B and C). This is too late to be influenced by the early increase in movement of mycosed aphids. Note, the pre-death sporulation was sparse in comparison with that on the cadaver.

The early stage increase in mobility among mycosed aphids expressed differently in disease caused by two equally virulent isolates of *L. longisporum*. KV71-infected aphids showed increased activity during days 1 and 2 after inoculation. This presented as more movement events (days 1 and 2), a greater amount of time spent moving (day 1)

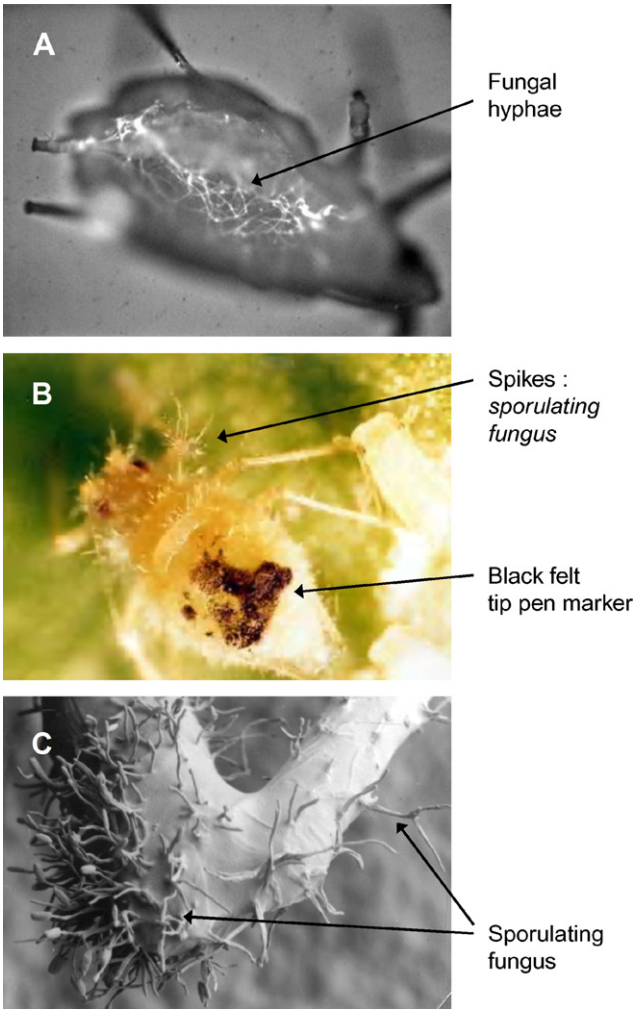

Fig. 7. (A) Saprophytic fungal growth (KV 71) on aphid cuticle 2 d after inoculation. Fungal hyphae, stained with Uvitex (following treatment of the spores, see Section 2.6), appear as a bright web on the aphid surface under UV light. (B) Fungus (KV71) sporulating on an adult aphid prior to death (4 d post inoculation). In this experiment, inoculated aphids were marked for identification with black water-soluble ink on the back of the abdomen. Note offspring close by. Black felt tip market to identify inoculated aphid, used on this experiment only. (C) Scanning electron micrograph showing the hind leg of a live aphid (4 d post inoculation with KV71). Fungus has emerged from the insect and is forming spores.

and a greater distance covered than controls (day 1). By comparison, KV42-infected aphids only expressed significantly enhanced activity over controls during day 1. Furthermore, only the number of movement events and amount of time spent moving were significantly different from control. The fact that the distance covered only increased in mycoses caused by KV71 suggests that only with this isolate might infected aphids change their position on a host plant. However, this would need testing on whole plants rather than the experimental arenas used here. It would be interesting to know whether the saprophytic, surface fungal growth was more intensive in mycoses from KV71 than KV42. If it is, then the extended period of increased mobility of KV71-mycosed aphids may be linked to greater early-stage infectivity. The *caveat* is that we have not yet observed sporulation on aphids during early mycosis with either isolate.

In general, causes of behavioural alterations in microbe–insect interactions are little understood (Horton and Moore, 1993). They could be the result of a coevolutionary arms race between host and pathogen (Roy et al., 2006). However, aphids tend to respond to disturbance with increased activity (i.e. overcrowding, presence of toxic compounds, production of alarm pheromone) (Griffiths and Pickett, 1980; Nauen, 1995; Roditakis et al., 2000). Furthermore, in generalist hypocrealean fungi like *Lecanicillium* spp. the selection pressure may be too weak to favour the evolution of behavioural modifications, though interestingly Sitch and Jackson (1997) established the specificity of KV71 for aphids and *M. persicae* in particular. In conclusion, *M. persicae* responded to infection from *L. longisporum* with increased mobility, though the intensity and the duration of effects varied between isolates, suggesting a quantitative or qualitative difference in signal or its perception. Whether this is truly adaptive remains to be established.

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