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Targeted Dispersal of the Aphid Pathogenic Fungus *Erynia neoaphidis* by the Aphid Predator *Coccinella septempunctata*

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The potential of adult and larval C. septempunctata *to vector the aphid-specific entomopathogenic fungus* E. neoaphidis *was assessed through a series of laboratory and field experiments. The ability of coccinellids to vector conidia from a colony of* E. neoaphidis*-infected pea aphids,* Acyrthosiphon pisum*, to a colony of uninfected* A. pisum *was demonstrated in a laboratory study. Adult coccinellids which had previously foraged on plants infested with different densities of sporulating cadavers (1, 5, 15, 30 cadavers per plant) initiated infection in a proportion of uninfected pea aphids (4, 0, 2 and 8%, respectively) when subsequently allowed to forage on* A. pisum *infested bean plants. Further laboratory studies demonstrated that fourth instar larvae and adult coccinellids artificially inoculated with conidia initiated infection in 11 and 13% of an* A. pisum *population in which they foraged, respectively. Furthermore, a proportion of* A. pisum *placed on bean plants which had previously been foraged on by inoculated larval and adult coccinellids also died from infection (3 and 10% of* A. pisum*, respectively). However, although coccinellid adults inoculated with conidia initiated infection in 19% of* A. pisum*, cereal aphids,* S. avenae*, exposed to the inoculated coccinellids did not become infected. A further laboratory study demonstrated that infection of* A. pisum *only occurred if inoculated coccinellids were transferred to* A. pisum *populations immediately post inoculation. However, a proportion of* A. pisum *placed on bean plants which had been foraged on by inoculated coccinellids transferred 0, 4 and 24 h post inoculation died from infection (9, 3 and 7%, respectively). A field study further demonstrated the potential of coccinellids to vector* E. neoaphidis. *Single spring sown field bean plants (Long Hoos Experimental Plots, IACR-Rothamsted Farm) were enclosed within nylon mesh bags and 25 adult* A. pisum *were added to each bag with one of the following treatments: no further addition (control), coccinellid adult (control), inoculated coccinellid adult, inoculated* A. pisum *or sporulating* A. pisum *cadavers. No aphids died of* E. neoaphidis *in the control treatments; 5, 16 and 33% of aphids were infected with* E. neoaphidis *on the other treatments, respectively.*

Keywords: Erynia neoaphidis, Coccinella septempunctata, Acyrthosiphon pisum, Sitobion avenae, *dispersal, vector*

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INTRODUCTION

Many studies have considered the potential of the aphid-specific entomopathogenic fungus *Erynia neoaphidis* Remaudiére and Hennebert (Zygomycetes: Entomophthorales) to control aphid populations (Perrin, 1975; Wilding, 1981; Wilding *et al*., 1986). The development of an epizootic is fundamental to the use of *E. neoaphidis* as a biological control agent and this is dependent on properties of both the host and the pathogen. Epizootics are usually host-density dependent, developing as the host population increases, however, an epizootic can develop at low host densities if the pathogen is widely distributed within the host habitat (Fuxa & Tanada, 1987). Generally, pathogen populations are distributed at low densities or discontinuously in the host habitat and, therefore, pathogen dispersal is essential (Fuxa & Tanada, 1987; Richards *et al*., 1994).

Entomophthoralean fungi (except *Massospora*), including *E. neoaphidis*, produce conidiophores that forcibly discharge primary conidia. The forcible discharge of conidia by fungal pathogens enables the conidia to traverse the boundary layer (Ingold, 1971; Steinkraus *et al*., 1996). However, dispersal of conidia over a greater distance often requires physical factors such as rain (Wilding, 1975; Fitt *et al*., 1989; McCartney, 1990, 1994; Pedersen *et al*., 1994; Steinkraus *et al*., 1996) or non-physical factors such as the movement of entomopathogens by host and non-host vectors such as invertebrate natural enemies.

Dispersal by forcible discharge or by weather factors are considered as random dispersal. In contrast, the movement of entomopathogens by host and non-host invertebrate natural enemies is a targeted mode of dispersal. These modes of transmission are more likely to target the host species than dispersal by physical factors, although wind may also be important in the dispersal of infected hosts and contaminated non-hosts (Mann *et al*., 1995). The movement of infected hosts is considered to be one of the most important ways in which a pathogen is transmitted and dispersed to new habitats (Fuxa & Tanada, 1987). It is also a way in which we can encourage the development of epizootics for pest management (Pell *et al*., 1993, Furlong *et al*., 1995). Pathogens are also dispersed by non-hosts, for example invertebrate natural enemies of the host that forage in the environment of infected host populations where they become contaminated with the pathogen. Insect vectors are important dispersal agents of many plant pathogens and this too has been exploited in biological control of plant pathogens (Nemeye *et al*., 1990; Peng *et al*., 1992; Gillespie & Menzies, 1993). The transmission of entomopathogens by non-host vectors has also been demonstrated (Schabel, 1982; Poprawski *et al*., 1992; Pell *et al*., 1997). The potential of adult seven-spot ladybirds, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) to vector *E. neoaphidis* has been demonstrated (Pell *et al*., 1997) in a laboratory study, in which it was shown that *C. septempunctata* adults contaminated artificially with *E. neoaphidis* initiated infection in 10% of the target population. In this paper, these studies are expanded to incorporate the assessment of both larval and adult *C. septempunctata* as potential vectors of *E. neoaphidis*. In addition to artificially-contaminating coccinellids with *E. neoaphidis* a more natural approach was employed, whereby *C. septempunctata* adults foraged on plants infested with *E. neoaphidis* infected aphids before foraging within colonies of uninfected aphids.

The use of non-host invertebrate natural enemies (vectors) to encourage the transmission of pathogens within an agroecosystem could be developed within a manipulative (Pell *et al*., 1993; Furlong *et al*., 1995) or conservation-based strategy. The results from the studies outlined in this paper will begin to address these possibilities.

MATERIALS AND METHODS

Insect, Fungal and Plant Cultures

Two aphid species were used in these studies: *Acyrthosiphon pisum* Harris (Homoptera: Aphididae) and *Sitobion avenae* F. (Homoptera: Aphididae). Both were maintained under

controlled conditions of 18ºC and a 16-h photoperiod. *A. pisum* were cultured on 3–4-week old dwarf broad bean plants (*Vicia faba* L. cultivar The Sutton) and *S. avenae* were cultured on one to 2-week old wheat plants (*Triticum aestivum* L. cultivar Beaufort). The aphids were transferred to new plants every one to two weeks. Aphids were regularly harvested to feed the *C. septempunctata* colony and for maintenance of *E. neoaphidis*. *C. septempunctata* larvae were derived from eggs laid by adults collected in the field.

Two *E. neoaphidis* isolates were used in these studies: isolate X4 (from the IACR-Rothamsted collection, original host = *A. pisum*) and isolate NW305 (from the Royal Veterinary and Agricultural University collection, Copenhagen, Denmark, original host = *S. avenae*).

An *in vivo* culture of isolate X4 was maintained by regular passage through adult apterous *A. pisum* as described by Wilding (1973). Recently-killed, infected aphids were dried and the resulting cadavers stored at 20% relative humidity (RH) and 4ºC. For further aphid inoculation, cadavers were rehydrated at 10ºC and 100% RH. After 15 h the emerging fungus was sporulating profusely.

An *in vitro* culture of isolate NW 305 was maintained on a sterile medium of Sabouraud dextrose agar supplemented with milk and egg yolk (SEMA) as described by Wilding and Brobyn (1980). To encourage sporulation, a 2 cm^2 section of fungus was removed from the SEMA and placed on a moistened filter paper disc in a 5 cm Petri dish for 16 h. The fungus was never subcultured more than three times after isolation from the aphid host to maintain pathogenicity.

Plants used in experiments with *A. pisum* were three to four-week old dwarf broad bean plants (*V. faba* cultivar The Sutton) and, in experiments with *S. avenae*, one to two-week old wheat plants (*T. aestivum* cultivar Beaufort) were used.

Assessment of Aphid Mortality

After experimentation, aphids used in experiments were placed in controlled conditions (18ºC and 16-h photoperiod) and assessed for *E. neoaphidis* infection daily for up to nine days. Mortality due to *E. neoaphidis* was confirmed by collecting and identifying conidia. *E. neoaphidis* conidia are a characteristic lemon-shape (Wilding & Brady, 1984). The proportion of aphids infected and the standard errors of the mean values based on the binomial distribution were calculated. Unless otherwise stated, the data were not analysed further because of the large number of zero values.

Adult *C. septempunctata* as Vectors of *E. neoaphidis* to Adult *A. pisum*

Sporulating *E. neoaphidis* (isolate X4)-infected cadavers were evenly distributed on bean plants at five different densities (each replicated four times): no cadavers (control); one cadaver; five cadavers; 15 cadavers and 30 cadavers.

One coccinellid was added to each plant. After 4 h, each coccinellid was transferred to a bean plant infested with 25 aphids. Four bean plants infested with 25 aphids to which no coccinellid was added were used as controls. After 22 h at high humidity the coccinellids were removed and humidity reduced to ambient.

Adult and Fourth Instar Larval *C. septempunctata* as Vectors of *E. neoaphidis* to Adult *A. pisum*

Thirty-six sporulating *E. neoaphidis* (isolate X4)-infected cadavers were evenly distributed over the surface of two moistened 70 mm filter paper discs in the lid of a 90 mm Petri dish. Five fourth instar coccinellid larvae were placed in one Petri dish and five 3 week old coccinellid adults in the other Petri dish. The coccinellid adults and larvae were inoculated with the *E. neoaphidis* conidia discharged from the cadavers for 2.5 h, at room temperature. Five coccinellid larvae and five adults held under similar conditions, but with no cadavers, were used as controls.

After the inoculation period, each coccinellid adult or larva was placed individually onto

a bean plant infested with 25 *A. pisum*. An additional control consisted of plants (five replicates) infested with aphids but with no coccinellids. Plants were maintained in controlled conditions (10% RH, 18°C and 16-h photoperiod) and coccinellids were removed after 22 h. Aphids remaining on these plants were transferred to clean bean plants enclosed with lamp glasses and nylon mesh.

Three of the original plants in each treatment were each infested with a further 20 aphids and maintained at high humidity (approximately 100% RH) for 24 h.

The proportion of aphids infected were analysed using one-way analysis of variance (ANOVA) after a log transformation.

Adult *C. septempunctata* as Vectors of *E. neoaphidis* to Fourth Instar *A. pisum* and *S. avenae*

Ten adult coccinellids were placed in a 50 cm Petri dish and were exposed to conidia discharged from *in vitro* cultured *E. neoaphidis* (isolate NW305) for 4 h, at room temperature. A further 10 adult coccinellids were held under similar conditions but with no fungus as controls. After the inoculation period, each individual coccinellid was either placed on a bean plant with 15 fourth instar *A. pisum* or a wheat plant with 15 fourth instar *S. avenae*. After 22 h, the coccinellids were removed and the polythene covers replaced with nylon mesh. The aphids were not transferred to clean plants.

Delaying the Introduction of Adult *C. septempunctata*; Effect on Their Potential to Vector *E. neoaphidis* to Adult *A. pisum*

Adult coccinellids were inoculated with *E. neoaphidis* (isolate X4). Thirty-six sporulating *E. neoaphidis* (isolate X4)-infected cadavers were evenly distributed over the surface of two moistened 70 mm filter paper discs in the lid of a 90 mm Petri dish. Eight 3-week old coccinellid adults were placed in each Petri dish. The coccinellid adults were inoculated with *E. neoaphidis* conidia discharged from the cadavers for 4 h, at room temperature. Sixteen coccinellid adults held under similar conditions, but with no cadavers, were used as controls.

Immediately after the inoculation period, four inoculated coccinellid adults were placed individually onto separate bean plants infested with 20 *A. pisum* adults. Similarly, four control (uninoculated) coccinellid adults were placed on bean plants infested with 20 *A. pisum*. The remaining coccinellids were placed on bean plants (six coccinellids per plant) with excess *A. pisum* and kept under controlled conditions (18°C and a 16-h photoperiod). Four hours post-inoculation, a further four of the inoculated coccinellid adults were placed individually onto separate bean plants infested with 20 *A. pisum*. Again four control (uninoculated) coccinellid adults were placed on bean plants infested with 20 *A. pisum*. This procedure was repeated 24 and 72 h post-inoculation.

Plants were maintained in controlled conditions (100% RH, 18°C and 16-h photoperiod) and coccinellids were removed after 22 h. Aphids remaining on these plants were transferred to clean bean plants enclosed with lamp glasses and nylon mesh.

The original plants on which coccinellids had foraged in each treatment were each infested with a further 10 aphids and maintained at high humidity (approximately 100% RH) for 24 h.

Potential of Adult *C. septempunctata* to Vector *E. neoaphidis* to Adult *A. pisum* in the Field

Field site. The experiment was conducted on the Long Hoos experimental plots, IACR-Rothamsted Farm. The experimental plot (76 m by 22 m) of field beans (*Vicia faba* cultivar Alfred) was sown in March 1996. Standard fertiliser and herbicide applications were applied to the plot but no insecticides or fungicides were applied. The plot was separated from an established hedgerow by a 5 m grass headland and from the surrounding crops (spring wheat and lupins) by a 1 m wide path.

Inoculation of C. septempunctata *adults and* A. pisum *with* E. neoaphidis *conidia.* Twenty coccinellids were inoculated with *E. neoaphidis* (isolate X4) conidia as described previously

(with the 20 coccinellids evenly distributed between four Petri dishes each with 36 sporulating cadavers). On this occasion, the coccinellids were exposed to the fungus for 6 h. A further 20 *C. septempunctata* adults were held under similar conditions without sporulating cadavers for use as controls. Five hundred adult *A. pisum* were also inoculated with *E. neoaphidis*.

Experimental design. The upper 300 mm of individual bean plants (102 plants) was enclosed within a 1-m circumference nylon mesh bag. Each bean plant used in the experiment was separated by at least 1 m from adjacent experimental bean plants. The following treatments were added to the bagged plants (20 replicates of each treatment) on 1 July 1996 in a completely random design: 25 aphids; 25 aphids and one coccinellid; 25 aphids and one inoculated coccinellid; 25 aphids and an additional 25 inoculated aphids; 25 aphids and eight sporulating cadavers.

The following day (2 July 1996), all coccinellids were removed from the bags. Four replicates of each treatment were destructively sampled and aphid mortality was assessed. All surviving adult aphids were placed on 3-week old bean plants, enclosed with lamp glasses at high humidity under controlled laboratory conditions (18ºC and 16-h photoperiod). Humidity was reduced to ambient after 24 h. Mortality of these remaining aphids was monitored daily for a further 4 days.

Further destructive samples (four replicates per treatment) were made after 24, 48, 72 and 144 h (3, 4, 5, 8 July 1996) and treated as described above.

The mortalities (proportion of infected aphids) were analysed using logistic regression via the generalized linear model facilities of Genstat 5 (Genstat 5 Committee, 1995).

Site temperature and humidity. Tiny Talk data loggers (Orion Software, RS components, UK) were placed in two additional treatment bags. A temperature data logger was added to one bag and a humidity data logger to the other. A further temperature data logger was used to monitor the temperature outside the bagged plants. These data loggers monitored conditions for the duration of the experiment at 15-min intervals.

RESULTS

Adult *C. septempunctata* as Vectors of *E. neoaphidis* to Adult *A. pisum*

The proportion of aphids which became infected with *E. neoaphidis* after exposure to adult coccinellids which had foraged on bean plants infested with sporulating cadavers was greatest if coccinellids had previously foraged on plants with 30 cadavers (Table 1). The proportion of aphids infected was very small in all of the other treatments. The majority of aphids which succumbed to *E. neoaphidis* infection died four days after exposure to the contaminated coccinellids.

Aphids in the presence of uninoculated coccinellids or in the absence of coccinellids did not become infected.

TABLE 1. Percentage of adult *A. pisum* infected with *E. neoaphidis* after direct exposure to coccinellid adults which had previously foraged on plants with different numbers of sporulating *E. neoaphidis* cadavers. Standard errors are based on the binomial distribution

Number of cadavers on plant	Percentage of infected *A. pisum*
1	4 ± 3
5	0 ± 0
15	2 ± 2
30	8 ± 3

TABLE 2. Percentage of adult *A. pisum* infected with *E. neoaphidis* after either direct exposure to a coccinellid (adult or larva) inoculated with *E. neoaphidis* conidia (X4) or to plants which had been foraged on by a coccinellid (adult or larva) inoculated with *E. neoaphidis* conidia (X4). Standard errors are based on the binomial distribution

	Percentage of infected *A. pisum*	
	Direct exposure	Exposure to plants
Coccinellid adult	13 ± 4	10 ± 4
Coccinellid larva	11 ± 4	3 ± 2

Adult and Fourth Instar Larval *C. septempunctata* as Vectors of *E. neoaphidis* to Adult *A. pisum*

Direct exposure of aphids to either adult or larval coccinellids inoculated with fungal conidia resulted in *E. neoaphidis* infection in 13 and 11% of the aphids, respectively (Table 2). Infection occurred on days four and five after exposure to contaminated coccinellids. Aphids in the presence of an uninoculated coccinellid or in the absence of a coccinellid did not become infected.

There was no difference in the proportion of aphids which became infected after exposure to either adult or larval coccinellids inoculated with *E. neoaphidis* ($F_{1,8} = 1.6$; $P > 0.05$).

Aphids placed on bean plants, on which an adult or larval coccinellid inoculated with fungus had foraged, also died from *E. neoaphidis* infection (Table 2). On plants previously foraged on by adults and larvae, 10 and 3% of aphids became infected, respectively. Infection occurred on days four and five after exposure to contaminated leaves. There was no mortality of aphids placed on plants exposed to uninoculated coccinellids.

There was no significant difference in the proportion of aphids which became infected with *E. neoaphidis* after exposure to plants foraged upon by either adult or larval coccinellids inoculated with fungus ($F_{1,4} = 2.4$; $P > 0.05$).

Adult *C. septempunctata* as Vectors of *E. neoaphidis* to Fourth Instar *A. pisum* and *S. avenae*

Acyrthosiphon pisum exposed to coccinellid adults inoculated with *E. neoaphidis* conidia (isolate NW305) became infected with the pathogen (19 ± 5%). Infection of *A. pisum* occurred between six and nine days after exposure to the inoculated coccinellids, whereas *S. avenae* exposed to inoculated coccinellid adults did not become infected. Aphids in the presence of uninoculated coccinellids or in the absence of coccinellids did not become infected.

Delaying the Introduction of Adult *C. septempunctata*; Effect on Their Potential to Vector *E. neoaphidis* to Adult *A. pisum*

Adult coccinellids inoculated with fungal conidia and transferred directly to a population of aphids resulted in *E. neoaphidis* infection in 8% of the aphids (Table 3). No aphids were infected when exposed to coccinellids inoculated 4, 24 or 72 h previously. Aphids in the presence of an uninoculated coccinellid or in the absence of a coccinellid did not become infected. Infection occurred on days four and five after exposure to contaminated coccinellids.

Aphids placed on bean plants, on which an adult coccinellid inoculated with fungus had foraged, also died from *E. neoaphidis* infection (Table 3). Nine, 3 and 7% of aphids became infected on plants on which adult coccinellids had foraged 0, 4, or 24 h post-inoculation, respectively. No infection occurred when aphids were placed on bean plants on which a coccinellid had foraged 72 h post-inoculation. Infection occurred on days four and five after exposure to contaminated leaves. There was no mortality of aphids placed on plants exposed to uninoculated coccinellids.

TABLE 3. Effect of delaying the introduction of coccinellids inoculated with *E. neoaphidis* (isolate X4) by 0, 4, 24 and 72 h on the percentage of adult *A. pisum* infected with *E. neoaphidis* after either direct exposure to inoculated coccinellid adults or exposure to plants which had been foraged on by inoculated coccinellid adults. Standard errors based on the binomial distribution are given

Time delay after inoculation	Percentage of infected *A. pisum*	
	Direct exposure	Exposure to plants
0	8 ± 8	8 ± 6
4	0 ± 0	3 ± 3
24	0 ± 0	6 ± 7
72	0 ± 0	0 ± 0

Potential of Adult *C. septempunctata* to Vector *E. neoaphidis* to Adult *A. pisum* in the Field

The addition of either inoculated aphids, sporulating cadavers or inoculated coccinellids resulted in *E. neoaphidis* infection in a proportion of aphids (Table 4). There was no significant difference in the proportion of aphids infected in samples collected from the field at different times post-inoculation. The proportion of infected aphids was significantly different between treatments ($F_{2,45} = 29.78$; $P < 0.001$). The addition of aphids inoculated with *E. neoaphidis* to bagged plants resulted in the greatest overall proportion of E. *neoaphidis* infection (33% infection). This was significantly greater than the overall proportion of *E. neoaphidis* infection on plants with the addition of sporulating cadavers (16% infection). However, both these treatments resulted in a significantly greater proportion of *E. neoaphidis* infection than the addition of coccinellids inoculated with *E. neoaphidis* (5% infection). There was no significant interaction between the time of sampling from the field and the treatment type (inoculated aphids, sporulating cadavers or inoculated coccinellids).

No aphids became infected with *E. neoaphidis* on the plants where either only aphids or an uninoculated coccinellid was added.

The time taken for aphids to die from *E. neoaphidis* infection was variable between treatments. The first infection was observed in aphids sampled from plants that had inoculated aphids added. In general, most aphids in this treatment died of *E. neoaphidis* on day three post-inoculation. The addition of either sporulating aphids or a coccinellid inoculated with *E. neoaphidis* resulted in mortality of aphids due to *E. neoaphidis* after day five post-inoculation.

TABLE 4. Percentage of adult *A. pisum* infected with *E. neoaphidis* on field beans destructively sampled at different times post-inoculation after the addition of different treatments (inoculated aphids (day 0 post-inoculation), sporulating cadavers or inoculated coccinellid adults). Standard errors based on the binomial distribution are given

Sampling day post-inoculation	Treatments		
	Inoculated aphids	Sporulating aphids	Inoculated coccinellid
Day 1	38 ± 5	10 ± 4	12 ± 4
Day 2	37 ± 4	27 ± 6	3 ± 2
Day 3	39 ± 4	17 ± 5	0 ± 0
Day 4	18 ± 4	15 ± 5	6 ± 3
Day 7	34 ± 6	8 ± 8	5 ± 4
Overall average	33 ± 2	16 ± 2	5 ± 1

Site temperature and humidity. Temperatures inside and outside the bag ranged from 7 to 30ºC (the average temperature over the study period was 14ºC). The RH in the bag ranged from 40 to 100% (the average humidity over the study period was 82%).

DISCUSSION

Adult coccinellids which had foraged on plants with different densities of sporulating cadavers became naturally contaminated with *E. neoaphidis* conidia which were vectored to aphids on whole plants, initiating infection in a proportion of those aphids. Although transmission was greatest when coccinellid adults had foraged on plants with 30 aphid cadavers, even coccinellids which had foraged on plants with only one cadaver became contaminated with sufficient fungal conidia to vector those conidia to a proportion of the uninfected aphids and initiate infection. Therefore, coccinellids may contribute to pathogen dispersal from aphid populations even when the pathogen density is low. In contrast, the number of conidia produced by an individual sporulating cadaver may not be sufficient for aerial dispersal because aerial dispersal is random and non-targeted. The ability of coccinellids to aggregate in areas with large aphid densities (Kareiva & Odell, 1987) would contribute to their role in pathogen dispersal by targeting the pathogen to aphid populations.

The increase in transmission observed when coccinellid adults had foraged on plants with 30 cadavers, compared to one, five or 15 cadavers, could be related both to the increase in inoculum density and the increase in spatial distribution of the cadavers over the plant. Each cadaver can produce thousands of mucous-coated conidia which could potentially contaminate foraging *C. septempunctata*. A foraging *C. septempunctata* has a greater probability of contacting a cadaver and becoming contaminated with conidia if there are many cadavers distributed over the plant.

Adult and larval coccinellids artificially contaminated with *E. neoaphidis* (X4) conidia also passively vectored the conidia to *A. pisum*. However, adult coccinellids inoculated with an isolate of *E. neoaphidis* (NW305) originating from *S. avenae* did not vector the pathogen to *S. avenae*, despite 19% of *A. pisum* becoming infected in the same conditions. Aphid species and even clones of the same species are differentially susceptible to different isolates of *E. neoaphidis* (Milner, 1982, 1985; Pickering & Gutierrez, 1991). It is possible that *A. pisum* is more susceptible to isolate NW305 than *S. avenae*, even though previous experiments demonstrated that this *S. avenae* clone was highly susceptible to this isolate (Dromph *et al*., 1996).

The increase in proportion of *A. pisum* infected after exposure to coccinellids contaminated with isolate NW305, compared to those contaminated with X4 could be a consequence of increased susceptibility of *A. pisum* to isolate NW305 compared to X4. However the studies are not directly comparable, because the *C. septempunctata* were inoculated with an *in vitro* culture of the isolate NW305 and an *in vivo* culture of X4, and this could have influenced the results.

In addition to aphid susceptibility, differences in the architecture and surface characteristics of wheat and bean plants may affect the ability of coccinellids to vector *E. neoaphidis* to *S. avenae* on wheat plants compared to *A. pisum* on bean plants. Positive phototaxes and negative geotaxes result in aphidophagous coccinellids searching the terminal parts of plants where aphids are most likely to be found (Dixon, 1959). Differences in the surfaces and architecture of different plant species can modify these basic taxes and thus the searching efficiency of coccinellids (Carter *et al*., 1984; Ferran & Deconchat, 1992; Frazer & McGregor, 1994). If the inoculated coccinellids foraged for less time on wheat plants than bean plants then their probability of contacting an aphid would be reduced on the wheat.

The different behavioural escape responses of *A. pisum* and *S. avenae* to foraging coccinellids may also account for the difference in transmission of the pathogen from inoculated coccinellids; in general, *A. pisum* exhibits a more dramatic escape response to predators than *S. avenae*. The increased movement of *A. pisum* in the presence of a foraging

inoculated coccinellid would increase the transmission of the pathogen by increasing the probability of the aphid coming into contact with conidia vectored by the coccinellid (Roy *et al*., 1998). In a comparable study, Furlong and Pell (1996) demonstrated that the presence of a foraging parasitoid increased the movement of *P. xylostella* larvae and consequently the transmission of the entomopathogen *Z. radicans*, although there was no evidence that the parasitoid vectored the pathogen to new *P. xylostella* populations. The interaction between plant structure and aphid behaviour is likely to contribute to the differential capacity of *C. septempunctata* to vector to *A. pisum* and *S. avenae*.

The potential of coccinellid adults to vector *E. neoaphidis* in the field was demonstrated. Inoculated coccinellids resulted in approximately 5% of aphid mortality due to *E. neoaphidis*. In controlled laboratory studies, inoculated coccinellids resulted in greater proportions of target aphids dying of *E. neoaphidis* infection. Most Entomophthoralean fungi generally have temperature optima around 20ºC (Morgan *et al*., 1995). During the course of the field experiment the temperature ranged from 7 to 30ºC (the average was 14ºC), therefore, the temperature was low in terms of optimal development of the pathogen. Suboptimal temperatures may also reduce the infectivity of conidia (Morgan *et al*., 1995). However, 5% infection may still be sufficient to initiate an epizootic. Roy *et al*. (1998) have demonstrated that even one cadaver on an aphid infested plant can result in a high proportion of aphids becoming infected.

The transmission of *E. neoaphidis* from inoculated coccinellids appeared to occur over the same time period as from sporulating cadavers. In contrast, aphids which had been inoculated directly with *E. neoaphidis* conidia, prior to introduction in the field, died from *E. neoaphidis* earlier. Morgan *et al*. (1995) demonstrated that the highest rates of germination of *E. neoaphidis* conidia were obtained at 18 and 21ºC for primary conidia and at 20ºC for secondary conidia. Therefore, the aphids inoculated with *E. neoaphidis* in the laboratory were at the optimal temperature for germination of primary and secondary conidia. In contrast, aphids that received inoculum in the field (either from the sporulating cadavers or an inoculated coccinellid) were at suboptimal temperatures for conidia germination. The reduction in germination rate of the conidia may explain the delay in mortality due to *E. neoaphidis* in these treatments.

The complex interaction of biological and environmental factors in the development of fungal epizootics is widely recognized (Wilding & Perry, 1980; Steinkraus *et al*., 1996). It is often speculated that environmental conditions are rarely optimal for epizootic development; however, a recent field study has shown that night environmental conditions are unlikely to limit conidia production (Hemmati, 1998). Biological factors such as limited conidia dispersal and low host density are more likely to impede the development of an epizootic than environmental conditions (Wilding & Perry, 1980; Hemmati, 1998). Wilding and Perry (1980) examined the prevalence of entomophthoralean fungi in populations of aphids on field beans and demonstrated that even a small reservoir of fungal inoculum can produce a rapid epizootic if sufficient infected alates are present. Migrating infected alates rapidly dispersed the fungus. Therefore, the movement of coccinellids contaminated with fungal conidia could also contribute to the dispersal of *E. neoaphidis*.

The ability of *E. neoaphidis* conidia to persist and remain viable on an artificially or naturally-contaminated coccinellid will undoubtedly affect the potential of coccinellids as vectors. No aphids became infected when exposed directly to adult coccinellids inoculated with *E. neoaphidis* conidia 4, 24 or 72 h previously. However, aphids placed on bean plants, on which an adult coccinellid inoculated with fungus 0, 4 or 24 h previously had foraged, did die from *E. neoaphidis* infection. This could be explained partly by the length of time the aphids were exposed to a source of conidia. Those aphids in direct contact with the inoculated coccinellid were only exposed to conidia for 22 h. In contrast, aphids placed on plants previously foraged on by an inoculated coccinellid would be exposed to conidia for longer (3 to 4 days). Furthermore, at 18ºC all primary conidia would have given rise to secondary conidia after 20 h (Morgan *et al*., 1995). Therefore, aphids placed on plants

previously foraged on by an inoculated coccinellid would be exposed to secondary (or tertiary) conidia whereas aphids in direct contact with an inoculated coccinellid would encounter both primary and secondary conidia. The secondary conidia of *E. neoaphidis* are more important in host infection than the primary conidia (Morgan *et al*., 1995) and this may partly explain the differences in infection rates. In addition it has been shown that stressed aphids (removed from their host plant for prolonged periods) are less susceptible to infection by *E. neoaphidis* than unstressed aphids (Pell, unpublished observation). Therefore, aphids exposed to a foraging coccinellid, and therefore stressed, may be less vulnerable to *E. neoaphidis* infection than those aphids on plants in the absence of a coccinellid. The ability of coccinellids to successfully transmit *E. neoaphidis* 24 h after exposure to the pathogen is an important finding. Storing coccinellids at cooler temperatures after inoculation would decrease the rate of secondary conidia production and, therefore, potentially increase the time that artificially- or naturally-inoculated coccinellids remain viable as vectors.

Erynia neoaphidis does not produce resting spores; however, reservoirs of pathogen could be established in the form of conidia or cadavers. Nettle aphids (*Microlophium carnosum*) on nettles (*Uritica dioica*) in field margins and hedgerows are often an early source of *E. neoaphidis* inoculum and may act as a pathogen reservoir. The dispersal patterns of coccinellids throughout a season will inevitably dictate their potential to vector *E. neoaphidis* from such reservoirs. The main overwintering habitats of *C. septempunctata* are woodland (Zhou & Carter, 1992), and adults disperse from these woodland habitats to non-crop habitats particularly nettles early in the spring. By late spring, *C. septempunctata* individuals generally move onto crops to breed (Hodek, 1973). Coccinellids foraging in nettle patches may become contaminated with *E. neoaphidis* conidia which they may subsequently vector to crop aphids. Therefore, it is possible that retaining nettle patches within an agroecosystem may conserve *E. neoaphidis* and enhance transmission to crop species.

The development of more manipulative strategies is also important if the potential of *C. septempunctata* as a vector of *E. neoaphidis* is to be realized. A pheromone trap has been developed for the autodissemination of the fungal pathogen *Z. radicans* by *P. xylostella* (Pell *et al*., 1993; Furlong *et al*., 1995). It is possible that a similar approach could be developed for the transmission of *E. neoaphidis* by *C. septempunctata*.

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