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# Effect of three isolates of *Pandora neoaphidis* from a single population of *Sitobion avenae* on mortality, speed of kill and fecundity of *S. avenae* and *Rhopalosiphum padi* at different temperatures

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ABSTRACT

We studied the effect of three *Pandora neoaphidis* isolates from one *Sitobion avenae* population, three temperatures, and two aphid species namely *S. avenae and Rhopalosiphum padi* on (i) aphid mortality, (ii) time needed to kill aphids, and (iii) aphid average daily and lifetime fecundity. A total of 38% of *S. avenae* and 7% of *R. padi* died and supported fungus sporulation. *S. avenae* was killed 30% faster than *R. padi*. Average daily fecundity was negatively affected only in *S. avenae* inoculated with, but not killed by, *P. neoaphidis*. Nevertheless, lifetime fecundity of both aphid species inoculated and sporulating with *P. neoaphidis* was halved compared to lifetime fecundity of surviving aphids in the control. Increased temperature resulted in higher mortality rates but did not consistently affect lethal time or fecundity. Results suggest that (i) temperature effects on virulence differ between isolates, even when obtained within the same host population, and (ii) even though an isolate does not kill a host it may reduce its fecundity. Our findings are important for the understanding of *P. neoaphidis* epizootiology and for use in pest-natural enemy modelling.



## 1. Introduction

Plant pests (weeds, pathogens, arthropods) and their natural enemies (microorganisms, predators and parasitoids) interact with each other directly and indirectly through the plant. These interactions are affected by abiotic factors such as temperature, pesticides, relative humidity, water, and light (Klingen and Westrum, 2007; Asalf et al., 2012; Caballero-López et al., 2012; Holland et al., 2012; De Castro et al., 2013). An example of non-target effects of plant protection measures are insecticides killing predators and parasitoids, leading to a resurgence of secondary pests (Fernandes et al., 2010). This can also work across pest categories, as observed when fungicides used against plant pathogens also kill beneficial fungi (Klingen and Westrum, 2007), leading to higher levels of pest arthropods and subsequent pesticide use. The effects of natural enemies are sometimes included in decision support systems. In USA, farmers withhold insecticide application for the cotton aphid during epizootics of the aphid-killing fungus *Neozygites fresenii* to enhance control of the aphid population by this beneficial fungus (Hollingsworth et al., 1995). More strategies such as this are needed. However, to build pest-natural enemy models to serve as a basis for such strategies, detailed studies on biotic and abiotic factors affecting these interactions are needed.

Entomopathogenic fungi in the phylum Entomophthoromycota, such as *N. fresenii,* are important natural enemies of foliar pest insects and may cause natural epizootics that can contribute to the control of these pests (Hollingsworth et al., 1995; Pell et al., 2001; Barta and Cagáň, 2006). The major drawback of Entomophthoromycota as biocontrol agents is their primarily biotrophic lifestyle and close association with their insect- or mite-host, which prevents mass-production on artificial media for most species (Jaronski and Jackson, 2012). There are few successful cases of their use in inundation- or inoculation-biological control (Lacey et al., 2001; Shah and Pell, 2003). The use of Entomophthoromycota in

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conservation biological control, as described above for the cotton aphid-killing fungus *N. fresenii,* represents a promising strategy that we would like to develop for *Pandora neoaphidis* (Syn. *Erynia neoaphidis*) (Entomophthoromycota: Entomophthorales) as well.

*Pandora neoaphidis* is an important fungal pathogen on aphids in temperate agroecosystems (Keller, 1991; Ekesi et al., 2005; Steinkraus, 2006). It has the ability to infect several species of aphid pests on different host plants (Pell et al., 2001; Shah et al., 2004; Barta and Cagáň, 2006; Scorsetti et al., 2007) including the English grain aphid *Sitobion avenae* (Pell et al., 2001; Shah et al., 2004; Chen et al., 2008) and the bird cherry-oat aphid *Rhopalosiphum padi* (Nielsen and Steenberg, 2004; Shah et al., 2004; Chen et al., 2008; Manfrino et al., 2014). In Europe, both *S. avenae* and *R. padi* are important pests in cereals and can co-occur in the same field (Blackman and Eastop, 2007). *P. neoaphidis* penetrates the aphid cuticle, develops inside its host as hyphal bodies, kills the host, breaks through the cuticle, and then produces primary conidia on conidiophores. Primary conidia are then actively projected if conditions (humidity, temperature, light, *etc.*) are favourable. They can then start another infection cycle if they land on the integument of a suitable host. However, if the primary conidia land on unsuitable surfaces (e.g., leaf or soil), secondary conidia may be projected. These are also infective and may infect new aphids or form new infective tertiary or quaternary infective conidia (Shah et al., 1998).

In insect pathology, virulence is defined as "the disease producing power of an organism, *i.e.,* the degree of pathogenicity within a group or species" (Shapiro-Ilan et al., 2005). The virulence of *P. neoaphidis* varies with aphid host species (Shah et al., 2004), the aphid host genotype (Milner, 1982; Stacey et al., 2003; Parker et al., 2017), the geographic origin of the isolate (Shah et al., 2004; Barta and Cagáň, 2009) and even between isolates co-occurring in one aphid metapopulation (Rohel et al., 1997; Sierotzki et al., 2000; Barta and Cagáň, 2009). Because *P. neoaphidis* is a biotrophic fungus, it kills its hosts at the end of the infection process, prior to sporulation. This time between initial host infection and death, *i.e.* lethal time (LT) can dramatically influence the epizootiology of the disease in a host population (Bonsall, 2004). Further, the time between when the infected host dies and the onset of sporulation (becoming infectious) is probably also an important factor in the epidemic development. In Entomophthoromycota sporulation can start at host death, but it can also be delayed if conditions are not suitable. In that case, cadavers may dry and not start sporulating again until a few hours at high humidity triggers the sporulation (Sawyer et al., 1997). Before the infected aphid dies, it may be able to reproduce and contribute to population increase (Schmitz et al., 1993; Baverstock et al., 2006; Chen and Feng, 2006). Consequently, studies of the effect of fungal isolates should also include effect on host fecundity. Lambrechts et al. (2006) highlight the role of both host and pathogen in the expression of various epizootiological traits, including virulence. They emphasise that most epizootiological traits of host-parasite relationships are not host- or parasite-specific but rather the result of complex interactions between the two organisms and, therefore, studying such interactions is encouraged.

Temperature can have complex non-linear effects on host-pathogen interactions such as virulence (e.g. Thomas and Blanford, 2003). Temperature can influence (i) the host mortality caused by a pathogen (Milner and Bourne, 1983; Blanford et al., 2003; Stacey et al., 2003; Eliasova et al., 2004), (ii) the LT (Schmitz et al., 1993; Nielsen et al., 2001; Shah et al., 2002) and (iii) the host's susceptibility (Stacey et al., 2003; Linder et al., 2008; Wojda, 2017; Doremus et al., 2018). Interactive effects between temperature and fungal isolates have also been shown on virulence of Entomophthoromycota; more specifically on the lethal concentration of *Zoophthora radicans* infecting the diamondback moth *Plutella xylostella* (Morales-Vidal et al., 2013) and on the prevalence of *Furia gastropachae* infecting the forest tent caterpillar *Malacosoma disstria* (Filotas et al., 2006). Temperature effects on virulence and sub-lethal effects on the host fecundity may vary between isolates. Exploring the effect of a realistic range of temperatures on several naturally co-occurring isolates would help to reveal the importance of these processes in the disease epizootiology.

In continental Europe (Slovakia) *P. neoaphidis* infects aphids from April to the first frost in mid-November (Barta and Cagáň, 2006). Further, Nielsen et al. (2001) report that a Danish isolate can infect and kill *S. avenae* from 2 to 25 °C. *Pandora neoaphidis* may, therefore, be active from early spring to late summer even at climatic locations similar to Northern Europe (e.g. Agrometeorology Ås, Norway, 2000–2016). Studies on European isolates (from Denmark, Slovakia, UK, and France) suggest that *P. neoaphidis* optimal temperature for vegetative growth, LT and host mortality ranges between 15 and 25 °C (Schmitz et al., 1993; Morgan et al., 1995; Nielsen et al., 2001; Stacey et al., 2003; Barta and Cagáň, 2006). As the virulence of *P. neoaphidis* increases, its LT decreases until the temperature approaches the fungus optimal temperature range (Milner and Bourne, 1983; Schmitz et al., 1993). Although, *P. neoaphidis* infection has been reported to decrease the fecundity of infected aphids compared to uninfected ones for the pea aphid *Acyrthosiphon pisum* (Baverstock et al., 2006; Parker et al., 2017) and the peach-potato aphid *Myzus persicae* (Chen and Feng, 2005, 2006), to our knowledge, no studies have been conducted on the effect of the interaction between temperature and *P. neoaphidis* isolate on aphid fecundity.

The objective of our study was, therefore, to reveal the influence of interactions between three *P. neoaphidis* isolates, two host aphid species (*S. avenae* and *R. padi*), and three temperatures relevant for northern Europe (7.5, 14.0, 18.0 °C) on three fungal virulence traits: (i) aphid mortality *i.e.* the success of the infection, (ii) lethal time (LT), and (iii) decrease of the host fecundity.

## 2. Materials and methods

### 2.1. Aphid cultures

*Sitobion avenae* and *R. padi* cultures were established from a single individual collected on winter wheat (*Triticum aestivum*) in May 2015 in Ås, Norway (59.6607 N, 10.7506 E), and on bird cherry (*Prunus padus*) in 2012 in Toten, Norway (60.5536 N, 10.9309 E), respectively. They were maintained on winter wheat (*T. aestivum* var. Ellvis) at 18 °C, 70% relative humidity and 16:8 h light:darkness. Only 1-3-d-old adult apterae were used in the experiment. The age of the aphids was ensured by controlling nymph production by transferring four apterous adults into a 50 mL plastic vial containing 7.5 mL 1.5% water agar and 6 pieces of winter wheat leaves stuck into the agar. A total of 40 vials were prepared for each aphid species. Adults were left in the vial for 3 d to produce nymphs. In order to avoid the formation of winged individuals among nymphs, each vial was only allowed to contain eight *R. padi* nymphs or five *S. avenae* nymphs. They were maintained until adulthood (10 d for *S. avenae* and 8 d from *R. padi*) at 18 °C, 70% relative humidity and 16:8 h light:darkness. We started adult production of *R. padi* 2 d after *S. avenae* since the two species have different developmental times and we wanted simultaneous adult emergence of both species at the start of the experiment.

### 2.2. Pandora neoaphidis isolates and production of fungal cadavers

The three *P. neoaphidis* isolates (NCRI 459/15, NCRI 460/15 and NCRI 461/15) used in our experiment were collected from three

*S. avenae* individuals from a spring wheat (*T. aestivum*) field in Ås, Norway (59.6607 N, 10.7506 E) in August 2015. Isolate NCRI 459/15 and NCRI 460/15 were collected 3 m apart from each other and NCRI 461/15 was collected 30 m apart from the two other sites. The three isolates were identified morphologically to *Pandora* spp. according to Keller (1991) and Humber (2012), and to species level as *P. neoaphidis* by the use of molecular methods as described by Thomsen and Jensen (2016). Cadavers of each isolate obtained from the field were individually incubated on a glass slide at 18 °C and high relative humidity (>95%) to trigger sporulation. These spores were used to inoculate new *S. avenae* so that we could establish an *in vivo* culture for each of the three isolates on their original host. This was done by placing 20 apterous *S. avenae* adults from our laboratory culture directly in contact with the spores on the glass slide with a paint brush. The inoculated *S. avenae* were then transferred to a Petri dish (8.6 cm diameter) with wet filter paper and 15–20 wheat leaf pieces. The Petri dish was then covered by a lid with 50–70 holes (3 mm diameter) covered with insect net. Aphids were then allowed to reproduce, and the production of winged individuals was not controlled. Petri dishes were kept at 18 °C, 70% relative humidity and 16:8 h light:darkness. They were monitored twice a week in order to clean the cultures and collect suitable cadavers for this experiment. Only non-sporulating cadavers (generally situated on the underside of the lid close to the holes) were collected by the use of a paint brush from the *in vivo* culture. Collected cadavers where then placed on top of dry filter paper in a Petri dish to dry and be stored in the refrigerator at 7 °C for up to 4 months before use in the experiment. Only cadavers of apterous big nymphs and adults were used in the experiment.

### 2.3. Fungal inoculation and experimental set up

For each isolate, seven dry non-sporulating cadavers were rehydrated for 24 h in a Petri dish (8.6 cm diameter) with 1.5% water agar at room temperature (23–25 °C) under constant light to trigger sporulation. All rehydrated cadavers sporulated well and spores were present on the bottom and in the lid of the Petri dishes. One inoculation replicate consisted of transferring 40-50 *S. avenae* and 40-60 *R. padi* individuals into a Petri dish with sporulating cadavers and they were kept there for 3 h. Aphids were walking throughout most of the exposure time. Consequently, both aphid species and all individuals of one inoculation replicate were assumed to be exposed to the same amount of fungal inoculum. Control aphids were treated similarly except that no sporulating cadavers were present in the Petri dishes they were transferred to. After inoculation, aphids were individually transferred using 30 mL plastic vials containing 5 mL 1.5% water agar and a piece of winter wheat leaf stuck into the water agar. To ensure high humidity in the vials and good conditions for infection during the first 24 h of incubation, only four holes (1 mm diameter) were made with a pin in the lid. After 24 h, another four holes were made to reduce humidity and the risk of growth of saprophytic fungi. The experimental units were then placed at 70% relative humidity and 16:8 h light:darkness at three different temperatures: $7.5 \pm 1$, $14.0 \pm 1$ or $18.0 \pm 1$ °C. The temperatures were selected based on average spring and autumn temperatures (6 °C) and the range in average summer temperatures (14–18 °C) in Ås, Norway between 2000 and 2016 (Agrometeorology Norway, 2000–2016). Aphids were monitored daily for fecundity, mortality and fungal sporulation from cadavers. Every day, the newly produced nymphs were removed from the vials. Aphids were categorized as follows: (i) Inoculated dead sporulating aphids, that were inoculated with, died from and sporulated with *P. neoaphidis*; (ii) Inoculated surviving aphids, that were inoculated with *P. neoaphidis* but survived and were still alive at the end of the experiment; and (iii) Inoculated dead non-sporulating aphids, that were inoculated with *P. neoaphidis* and died but without any signs of fungal growth. Inoculated dead non-sporulating aphids from the two first replicates were dissected to look for fungal growth (hyphal bodies, conidia or other fungal structures) under the microscope. No sign of fungal infection was found. Therefore, inoculated dead non-sporulating aphids from replicates 3 to 6 were only observed under binocular microscope without dissection. Again, no sign of fungal infection was found. In the control, aphids were scored as: (i) control surviving aphids, that were alive at the end of the experiment; and (ii) control dead aphids, that died for unknown reasons before the end of the experiment. Aphids that died 1 d and 2 d after inoculation were considered killed during transfer and removed from the dataset. No sporulating cadavers were found in the control. Based on pilot studies, the treated aphids were monitored for at least 180 degree-days (DD), which corresponds to 10 d at 18 °C, 13 d at 14 °C and 24 d at 7.5 °C. If a sporulating cadaver was observed at the end of the initial 180 DD monitoring period, the treatment was observed for three more days to ensure that all potentially inoculated dead sporulating aphids had died and sporulated. We aimed for a total of 70 individuals per treatment (temperature, isolate and aphid species). Therefore, we conducted six replicates of the protocol described above. To optimize the production of 1-3-d-old aphids for the experiment, we conducted the replicates two by two, with one day difference between the paired replicates. Replicates 3 and 4 started 3 weeks after Replicates 1 and 2. Finally, Replicates 5 and 6 started seven weeks after Replicates 3 and 4. Fecundity was recorded only in the first four replicates (corresponding to 572 *S. avenae* and 597 *R. padi*) due to the extensive work load.

### 2.4. Statistical analysis

R program version 3.4.2 (R Core Team, 2017) and R studio (R Studio Team, 2016) were used for statistical analysis. Because the host species effect was clearly the dominant trend in the data (data not shown), we present the results separately for each aphid species to allow visualization of the interactive effects of temperature and isolate.

#### 2.4.1. Pandora neoaphidis virulence

Generalised Linear Mixed Models (GLMM, random effect: replicate, family: binomial) were used to investigate the effect of fungal isolate, temperature and their first order interaction on: (i) the number of sporulating cadavers produced through the mortality of inoculated dead sporulating aphids, and (ii) the mortality of inoculated dead non-sporulating aphids compared to the mortality of dead aphids in the control for each aphid species. We used the R. package *lme4* (Bates et al., 2015) for this and we compared the different isolates and temperatures with estimated marginal means (*post hoc* analysis, R package *emmeans*, Lenth (2017)).

#### 2.4.2. Lethal time (LT) of Pandora neoaphidis

We modelled the cumulative percentage of sporulating aphid cadavers per DD as a sigmoid Gompertz equation (Batschelet, 1976) (Equation (1)), referred to as the LT distribution in the following.

$$Y_i = \alpha \left( e^{-\beta e^{-k\ DD_i}} \right) \quad (1)$$

Where $Y_i$ is the cumulative percentage of sporulating cadavers at day $i$; $DD_i$ is the degree-day accumulation at day $i$; $\alpha$ is the asymptote *i.e.* the maximal number of sporulating aphid cadavers (here fixed at 100%); $\beta$ is the curve displacement: the higher, the more DD are needed for the first sporulating cadavers to occur. Finally, $k$ is the curve slope or growth rate: the higher the growth rate, the faster the fungus kills all the infected hosts. For a more

flexible fit (Equation (1)), the parameters (displacement: $\beta$ and growth rate: $k$) were subdivided according to the different values of the studied factor (two species, three temperatures or three isolates) (Equation (2)).

$$\begin{cases} \beta = \beta 0 + \beta 1 * X1 + \beta 2 * X2 \\ k = k0 + k1 * X1 + k2 * X2 \end{cases} \quad (2)$$

*X1* and *X2* are binary variables (*X1* equals 1 for the second value of the tested factor and *X2* equals 1 for the third value). The LT models (Equations (1) and (2)) were fitted to test (i) species effect on LT by pooling all temperatures and isolates together (one parameter per species in Equation 2), (ii) temperature effect on LT by pooling all the isolates together (one parameter per temperature in Equation (2)) (Because there were only four *R. padi* sporulating cadavers at 7.5 °C, we compared only 14.0 and 18.0 °C for this host species.), (iii) isolate effect on LT by pooling all the temperatures together (one parameter per isolate in Equation (2)) and finally, (iv) for *S. avenae*, we tested the temperature effect on the LT distribution of each isolate by fitting one model per isolate with one parameter per temperature in Equation (2). This model was impossible to fit for *R. padi* due to low numbers of sporulating cadavers per isolate and temperature (insufficient replication). The standard LT50 (defined as time needed to reach 50% sporulating cadavers) can be derived from Equation (1). The LT distribution models (Equation (1)) were fitted with nonlinear least-square estimators (R package *minpack.lm*, Elzhov et al. (2016)). The difference between the parameter $\beta 1$ and $\beta 2$ and between $k1$ and $k2$ were tested with the Delta method (*post hoc* analysis, R package *car*, Fox and Weisberg (2011)).

#### 2.4.3. Aphid fecundity

We ran GLMMs (random effect: replicate, family: Poisson) to investigate the explanatory power of *P. neoaphidis* inoculation (by comparing all inoculated aphids to the aphids in the control), temperature and their first order interaction on aphid daily fecundity and lifetime fecundity of (i) inoculated dead sporulating aphids compared to surviving aphids in the control, and (ii) inoculated surviving aphids compared to surviving aphids in the control. Ongoing infection processes could have been hidden by the fact that mortality of inoculated dead non-sporulating *R. padi* occurred quickly and before mortality of inoculated dead sporulating *R. padi*. To investigate this possibility, we also studied the effect of the factors listed above on the fecundity of inoculated dead non-sporulating *R. padi* compared to dead *R. padi* in the control. If there was a significant effect of *P. neoaphidis* inoculation (all isolates pooled together versus the control), we further studied the effect of each of the three fungal isolates compared to the control. Results are shown for the pooled data in the case of no effect, and for individual isolates where an inoculation effect was detected. Because fecundity depends on the longevity of the aphids, log-transformed longevity was included as a co-variable in all GLMM in order to study the average daily fecundity. For both average daily fecundity and lifetime fecundity, we compared the different isolates and temperatures to each other with estimated marginal means (*post hoc* analysis, R package *emmeans*, Lenth (2017)).

## 3. Results

### 3.1. Effect of Pandora neoaphidis isolate and temperature on aphid mortality and fungal sporulation

For all three *P. neoaphidis* isolates tested, significantly more *S. avenae* (38%) than *R. padi* (7%) died and developed into sporulating cadavers ($Chi^2 = 123.140$, $df = 1$, $p < 0.001$).

#### 3.1.1. Sitobion avenae

Isolate significantly influenced the number of *S. avenae* sporulating cadavers ($Chi^2 = 6.779$, $df = 2$, $p = 0.034$) (Fig. 1A). Isolate NCRI 461/15 caused significantly more sporulating cadavers than NCRI 459/15 ($p = 0.030$, *post hoc* comparison), while no significant difference was found between the other isolates ($p > 0.05$). Further, temperature also significantly influenced the number of sporulating cadavers ($Chi^2 = 17.895$, $df = 2$, $p < 0.001$) (Fig. 1B). An increase in temperature from 7.5 to 18.0 °C resulted in a significant increase in sporulating *S. avenae* cadavers ($p < 0.001$, *post hoc* comparison). No interaction between temperature and isolate was found ($Chi^2 = 3.879$, $df = 4$, $p = 0.423$). Only 6% of the inoculated *S. avenae* (all isolates together) were dead non-sporulating aphids

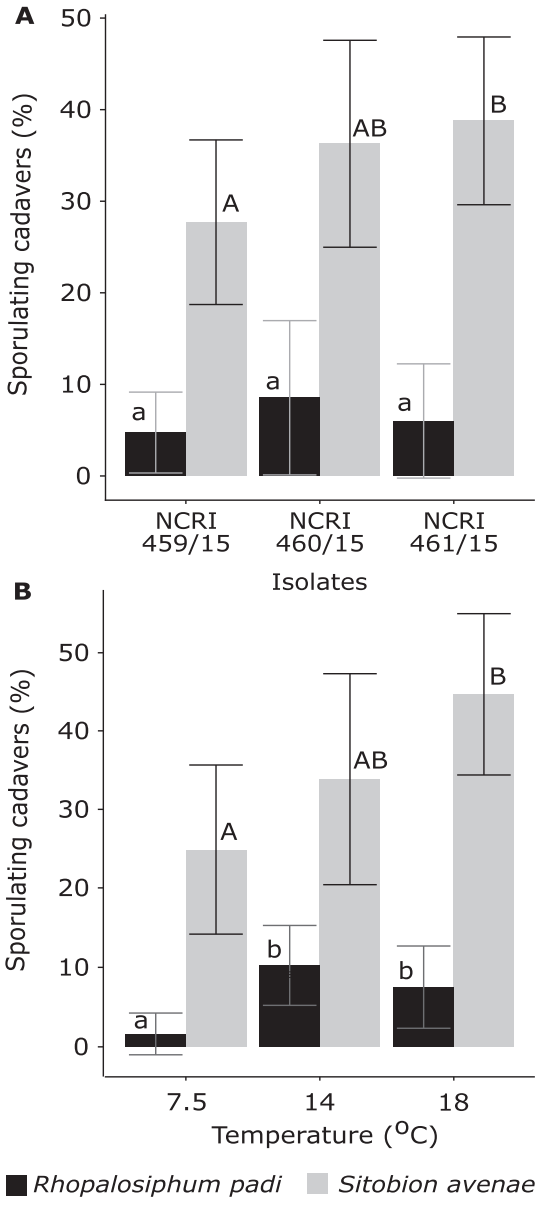


**Fig. 1.** *Effect of Pandora neoaphidis* isolates (A) and temperature (B) on mean percentage sporulation (±SD) of two inoculated aphid species, *Sitobion avenae* and *Rhopalosiphum padi*. Means with different letters are significantly different based on GLMM and *post hoc* estimated marginal means analysis ($p < 0.05$). Uppercase letters indicate comparisons among *S. avenae* and lowercase letters among *R. padi*. The experiment was repeated six times and a total of 68–75 individuals for each treatment were tested.

(died without fungal growth). In the control, the mortality was 4% and was not significantly different to the mortality of the inoculated dead non-sporulating aphids ($Chi^2 = 0.462$, $df = 1$, $p = 0.497$). Neither the temperature ($Chi^2 = 1.720$, $df = 2$, $p = 0.423$), nor the interaction between the inoculation and the temperature ($Chi^2 = 3.728$, $df = 2$, $p = 0.155$) significantly influenced the mortality of inoculated dead non-sporulating *S. avenae*.

#### 3.1.2. Rhopalosiphum padi

There was no significant difference between *P. neoaphidis* isolates in sporulation of *R. padi* cadavers ($Chi^2 = 1.459$, $df = 2$, $p = 0.482$) (Fig. 1A). However, the temperature significantly influenced it ($Chi^2 = 10.992$, $df = 2$, $p = 0.004$) with significantly higher numbers of sporulating cadavers occurring at 14.0 and 18.0 °C than at 7.5 °C ($p = 0.003$ and $p = 0.025$ respectively) (Fig. 1B). There was no significant interaction between temperature and isolate ($Chi^2 = 7.463$, $df = 4$, $p = 0.113$). Only 19% of the inoculated *R. padi* (all isolates together) were dead non-sporulating aphids (died without any fungal growth). In the control, the mortality was 26% and not significantly different to the mortality of inoculated dead non-sporulating aphids (all isolates together) ($Chi^2 = 3.752$, $df = 1$, $p = 0.053$). However, there was a temperature effect on the inoculated dead non-sporulating *R. padi* mortality ($Chi^2 = 21.471$, $df = 2$, $p < 0.001$) and it was significantly higher at 7.5 °C compared to 14.0 and 18.0 °C ($p < 0.001$, $p < 0.001$, respectively). The temperature effect on the mortality of the inoculated dead non-sporulating *R. padi* was not significantly dependent on the isolate ($Chi^2 = 3.086$, $df = 2$, $p = 0.214$).

### 3.2. Effect of Pandora neoaphidis isolates and temperature on lethal time

Each model describing the LT distribution fitted the data well with an $R^2$ value exceeding 0.96.

*Pandora neoaphidis* killed all inoculated dead sporulating *S. avenae* significantly faster ($T = 5.419$, $p < 0.001$) than all inoculated dead sporulating *R. padi,* with an estimated growth rate that was 30% higher for *S. avenae* (LT curve slope *k,* Equation (1)). However, the time needed for the first sporulating cadavers to occur (curve displacement $\beta$, Equation (1)) was not significantly different between the two host species ($T = 0.785$, $p = 0.434$). The estimated LT50 was 116.2 DD for *S. avenae* and 147.7 DD for *R. padi*.

A significant effect of *P. neoaphidis* isolate on LT was detected for both *S. avenae* and *R. padi* (Fig. 2A). Isolate NCRI 460/15 killed both aphid species slower than isolate NCRI 459/15 (parameter *k*, $T = -3.004$, $p = 0.003$ for *S. avenae* and $T = -5.047$, $p < 0.001$ for *R. padi*, Fig. 2B). However, NCRI 460/15 resulted in a significantly shorter time—to-first-sporulating-cadavers compared to the isolate NCRI 459/15 (parameter $\beta$, $T = -2.173$, $p = 0.031$ for *S. avenae* and $T = -2.610$, $p = 0.010$ for *R. padi*, Fig. 2C). The difference in LT between the two isolates was more than twice as big for *R. padi* compared to *S. avenae* (Fig. 2B and C). The estimated decrease in growth rate was 21% for *S. avenae* and 43% for *R. padi*. For *R. padi*, isolate NCRI 460/15 resulted in a significantly shorter time (about 10 times) for the first sporulating cadaver to be observed than for NCRI 461/15 (parameter $\beta$, $p = 0.009$, *post hoc* comparison, Fig. 2C). However, isolate NCRI 460/15 killed significantly slower (30%) than NCRI 461/15 (parameter *k*, Equation (1), $p < 0.001$, *post hoc* comparison, Fig. 2B).

The temperature did not influence LT distribution for any of the aphid species when all the isolates were pooled. Neither how fast the inoculated aphids were killed (the growth rate of the LT distribution: curve slope k), nor the time needed for the first sporulating cadavers to appear (curve displacement $\beta$) were significantly influenced ($p > 0.05$) by temperature. When the isolates were studied separately, the time needed for the first sporulating *S. avenae* cadaver to occur (parameter $\beta$) did not depend on temperature for any of the isolates ($p < 0.05$). However, temperature significantly influenced how fast *S. avenae* were killed (LT growth rate, parameter *k*) by each isolate. Isolate NCRI 459/15 (Fig. 3A) killed *S. avenae* significantly faster at 14.0 and 18.0 °C than at 7.5 °C ($T = 3.886$, $p < 0.001$ and $T = 3.138$, $p = 0.003$, respectively) with an estimated increase in *k* of 60 and 47% respectively. Isolate NCRI 460/15 (Fig. 3B) killed *S. avenae* faster at 7.5 °C ($T = -2.366$, $p = 0.023$) than at 14.0 °C with an estimated increase in the growth rate of 34%. Finally, the isolate NCRI 461/15 (Fig. 3C) killed *S. avenae* faster at 18.0 °C than at 14.0 °C ($p = 0.020$, *post hoc* comparison) with an estimated increase in the growth rate of 30%.

### 3.3. Effect of Pandora neoaphidis isolates and temperature on aphid fecundity

#### 3.3.1. Sitobion avenae

The fecundity of 124 inoculated dead sporulating *S. avenae* and 139 surviving *S. avenae* in the control were monitored. Average daily fecundity of inoculated dead sporulating *S. avenae* was not significantly different from average daily fecundity of surviving *S. avenae* in the control ($Chi^2 = 0.051$, $df = 1$, $p = 0.821$ all isolates combined). However, it was influenced by temperature ($Chi^2 = 139.073$, $df = 2$, $p < 0.001$). Fecundity increased with temperature ($p < 0.001$ for all pairwise comparisons). The temperature effect on *S. avenae* fecundity was not influenced by isolate ($Chi^2 = 3.012$, $df = 2$, $p = 0.222$).

Lifetime fecundity of inoculated dead sporulating *S. avenae* was significantly influenced by isolate ($Chi^2 = 37.221$, $df = 3$, $p < 0.001$), and its interaction with temperature ($Chi^2 = 54.759$, $df = 6$, $p < 0.001$). Lifetime fecundity of inoculated dead sporulating *S. avenae* was lower than for surviving aphids in the control ($p < 0.001$ for all pairwise comparisons) with a mean decrease of 51%. At 7.5 °C, lifetime fecundity of sporulating *S. avenae* inoculated with NCRI 460/15 and NCRI 461/15 was lower than lifetime fecundity of surviving *S. avenae* in the control ($p < 0.001$ and $p = 0.002$ respectively), with a mean decrease of 44 and 29% for NCRI 460/15 and NCRI 461/15 respectively (Fig. 4A). At 14 °C, the life time fecundity of sporulating *S. avenae* inoculated with the three isolates was lower than the lifetime fecundity of surviving aphids in the control ($p < 0.001$ for all pairwise comparisons), with a mean decrease of 65, 54 and 51% for NCRI 459/15, NCRI 460/15 and NCRI 461/15 respectively. Furthermore, the lifetime fecundity of dead sporulating *S. avenae* inoculated with NCRI 459/15 was lower than for dead sporulating *S. avenae* inoculated with NCRI 461/15 ($p = 0.047$) with a mean decrease of 29%. We found the same pattern for 18 °C as for 14 °C, and the lifetime fecundity of sporulating *S. avenae* inoculated with the three isolates was lower than the lifetime fecundity of the surviving aphids in the control ($p < 0.001$ for all pairwise comparisons). At 18 °C, the mean decrease in lifetime fecundity was 55, 56 and 64% for NCRI 459/15, NCRI 460/15 and NCRI 461/15 respectively. However, at 18 °C the lifetime fecundity of sporulating aphids inoculated with NCRI 461/15 was lower than for NCRI 461/15 ($p = 0.021$), with a mean decrease of 24%. Finally, the aphid lifetime fecundity was not influenced by the temperature ($Chi^2 = 4.982$, $df = 2$, $p = 0.083$).

Fecundity of 270 inoculated surviving *S. avenae* was recorded. Their average daily fecundity was significantly lower compared to surviving aphids in the control ($Chi^2 = 4.334$, $df = 1$, $p = 0.037$, all isolates combined). This decrease in fecundity was different depending on the isolate ($Chi^2 = 18.672$, $df = 3$, $p < 0.001$). *Post hoc* comparisons showed that average daily fecundity of inoculated surviving *S. avenae* decreased when inoculated with isolate NCRI 460/15 compared to surviving aphids in the control and the

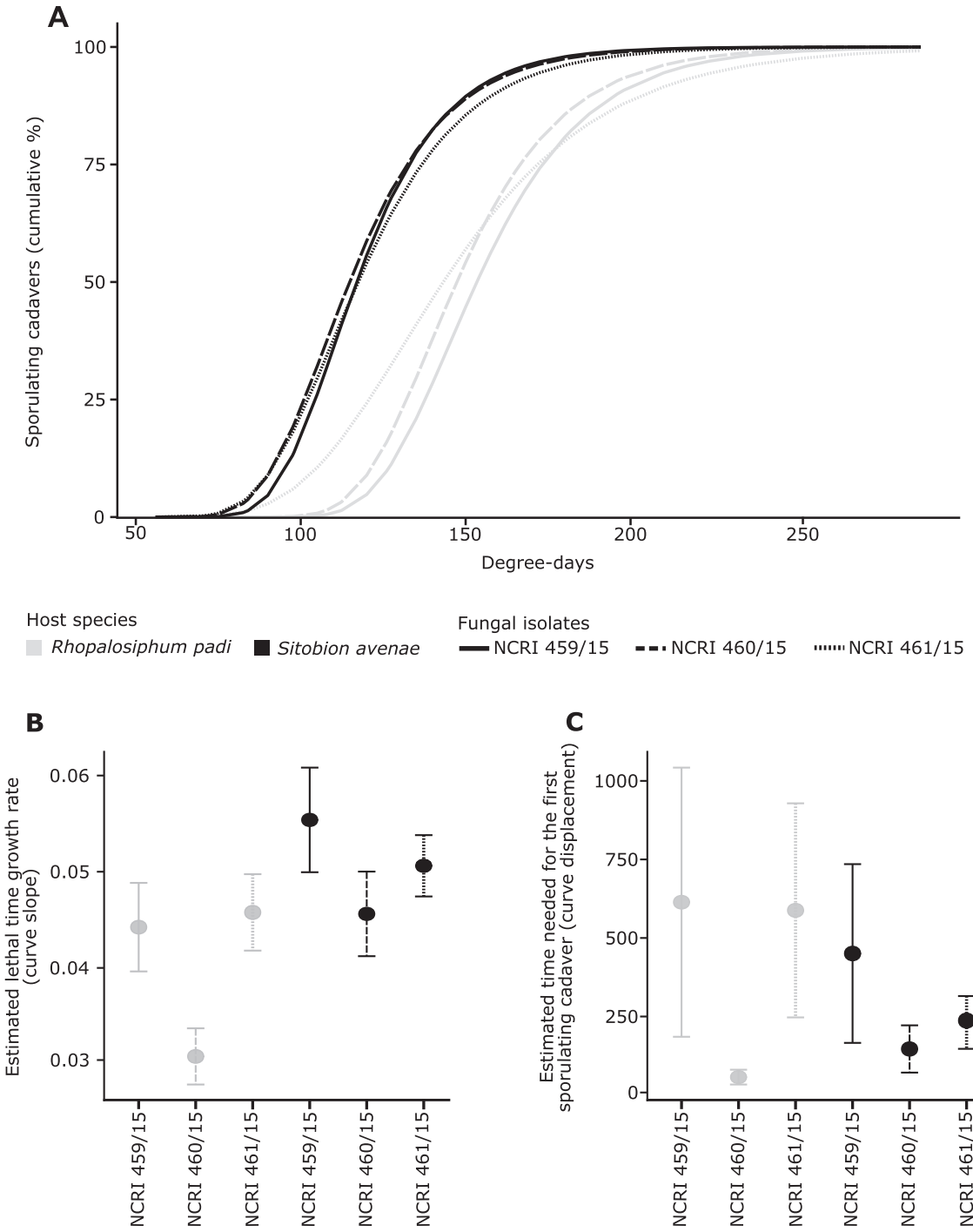


**Fig. 2.** (A) Fitted lethal time distribution of *Pandora neoaphidis* isolates to *Sitobion avenae* (black lines) and *Rhopalosiphum padi* (grey lines) expressed in cumulative percentage of sporulating cadavers. For each host species, the three *P. neoaphidis* isolates NCRI 459/15, NCRI 460/15 and NCRI 461/15 are represented. Corresponding mean estimates and their 95% confidence interval of (B) the curve slope (growth rate k, Gompertz equation) and (C) the curve displacement (parameter β, Gompertz equation) for each fitted lethal time distribution.

surviving aphids inoculated with NCRI 459/15 ($p = 0.022$ and $p = 0.047$, respectively). Further, temperature influenced average daily fecundity of inoculated surviving *S. avenae* ($Chi^2 = 520.590$, $df = 2$, $p < 0.001$). The higher the temperature the higher the fecundity was observed ($p < 0.001$ for all of the pairwise comparisons). However, the effect of temperature depended on the *P. neoaphidis* isolate aphids were inoculated with ($Chi^2 = 31.042$, $df = 6$, $p < 0.001$) (Fig. 5A). At 7.5 °C, the fecundity of the surviving *S. avenae* inoculated with isolate NCRI 460/15 was lower than the fecundity of surviving *S. avenae* in the control and the surviving aphids inoculated with NCRI 459/15 and 461/15 ($p < 0.001$, $p < 0.001$ and $p = 0.044$ respectively). At 14.0 °C, no significant difference was observed in the average daily fecundity between surviving *S. avenae* in all combinations, inoculated or not. At 18.0 °C, the fecundity of surviving *S. avenae* inoculated with NCRI 461/15 was lower than the fecundity of surviving aphids in the control and surviving aphids inoculated with NCRI 459/15 ($p = 0.004$ and $p = 0.010$ respectively).

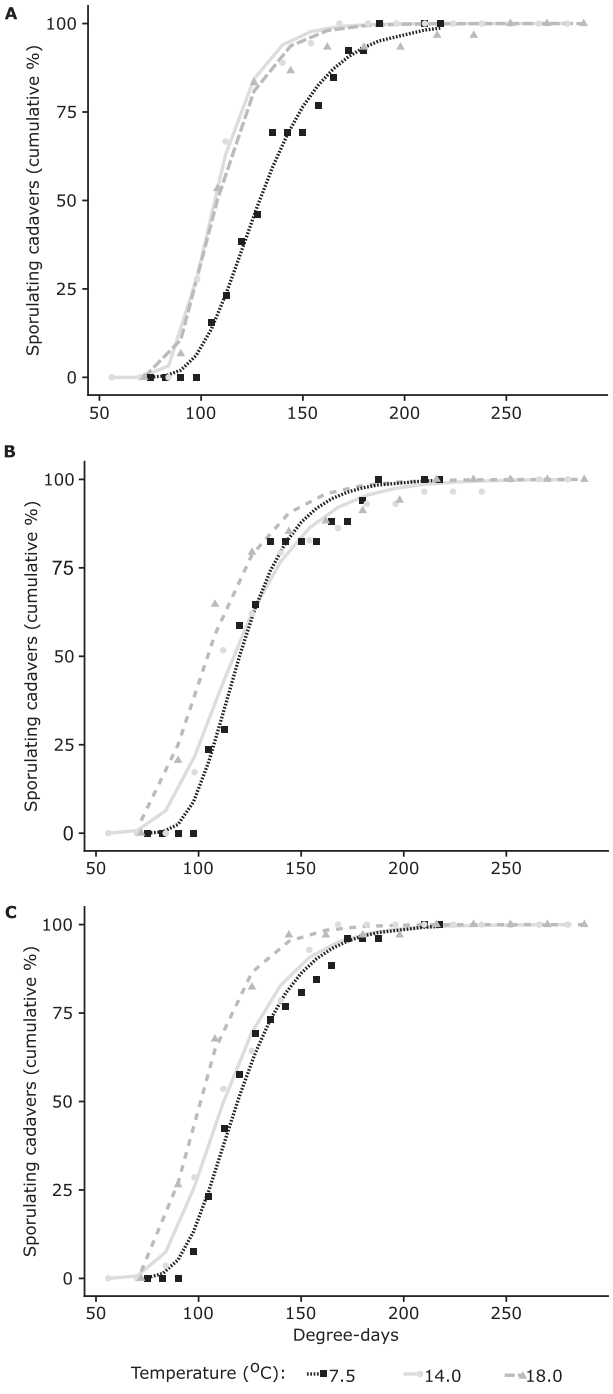


**Fig. 3.** Effect of temperature per degree-day on cumulative sporulation percentage of three *Pandora neoaphidis* isolates (A) NCRI 459/15, (B) NCRI 460/15, and (C) NCRI 461/15 from fungus killed *Sitobion avenae*. The lines represent the fitted Gompertz equations and the points represent the observed values.

Lifetime fecundity of inoculated surviving *S. avenae* was significantly influenced by isolate ($Chi^2 = 19.005$, df = 3, $p < 0.001$), temperature ($Chi^2 = 186.572$, df = 2, $p < 0.001$) and their interaction ($Chi^2 = 25.461$, df = 6, $p < 0.001$). Lifetime fecundity of surviving *S. avenae* inoculated with NCRI 460/15 was lower than for surviving aphids in the control and for NCRI 459/15 and NCRI 461/15 ($p < 0.001$, $p < 0.001$ and $p = 0.035$ respectively) with a mean decrease in lifetime fecundity of 16, 13 and 8% respectively. At 7.5 °C, the lifetime fecundity of surviving *S. avenae* inoculated with NCRI 460/15 and NCRI 461/15 was lower than the lifetime fecundity of surviving *S. avenae* in the control ($p < 0.001$ and $p = 0.002$ respectively), with a mean decrease of 20 and 5% for NCRI 460/15 and NCRI 461/15 respectively (Fig. 4B). At 14 °C, no significant differences were found between the different treatments ($p > 0.05$). At 18 °C, the lifetime fecundity of surviving *S. avenae* inoculated with NCRI 461/15 was lower than for inoculated *S. avenae* with NCRI 459/15 and for surviving *S. avenae* in the control ($p = 0.020$ and $p = 0.041$, respectively), with a mean decrease in lifetime fecundity of 19 and 12% for respectively.

### 3.3.2. *Rhopalosiphum padi*

Fecundity of 17 inoculated dead sporulating *R. padi* and 98 surviving *R. padi* in the control was monitored. Average daily fecundity of all the inoculated dead sporulating *R. padi* (all isolates together) was not significantly different from fecundity of the surviving *R. padi* in the control ($Chi^2 = 1.282$, df = 1, $p = 0.258$). However, average daily fecundity was influenced by the temperature ($Chi^2 = 210.539$, df = 2, $p < 0.001$). The higher the temperature, the higher the fecundity ($p < 0.001$ for all pairwise comparisons).

Lifetime fecundity of inoculated dead sporulating *R. padi* was significantly lower than for surviving aphids in the control ($Chi^2 = 101.540$, df = 1, $p < 0.001$), with a mean decrease of 51%. Furthermore, *R. padi* lifetime fecundity was significantly influenced by the temperature ($Chi^2 = 130.050$, df = 2, $p < 0.001$) and was lower at 7.5 °C than at 14 °C and 18 °C ($p < 0.001$ for both comparisons). For both analyses, interaction between temperature and the inoculation of *P. neoaphidis* (all isolates together) and the effect of each isolate was not investigated due to low *R. padi* cadaver numbers.

The fecundity of 256 inoculated surviving *R. padi* was monitored. Temperature significantly influenced the fecundity ($Chi^2 = 228.082$, df = 2, $p < 0.001$). The higher the temperature the higher the fecundity ($p < 0.001$ for all pairwise comparison). Neither *P. neoaphidis* inoculation ($Chi^2 = 3.403$, df = 1, $p = 0.065$), nor interaction between temperature and inoculation ($Chi^2 = 4.477$, df = 2, $p = 0.106$) significantly affected average daily fecundity of inoculated surviving *R. padi* compared to surviving *R. padi* in the control (Fig. 5B).

Lifetime fecundity of surviving *R. padi* was also not significantly influenced by either the fungal inoculation ($Chi^2 = 0.092$, df = 1, $p = 0.762$) or by the interaction between inoculation and temperature ($Chi^2 = 5.869$, df = 2, $p = 0.053$; Fig. 4C). However, it was significantly influenced by temperature ($Chi^2 = 409.352$, df = 2, $p < 0.001$). Aphid lifetime fecundity was lower at 7.5 °C than at 14 °C and 18 °C ($p < 0.001$ for both comparisons).

When the first sporulating *R. padi* cadavers occurred, 70% of inoculated dead non-sporulating *R. padi* had died (Fig. 6A). Therefore, the fecundity of 173 inoculated dead non-sporulating *R. padi* was compared to the fecundity of 53 *R. padi* that died in the control in order to reveal a possible ongoing infection processes hidden by the early death of the inoculated dead non-sporulating *R. padi*. Isolate effect on the average daily fecundity of inoculated dead non-sporulating *R. padi* was not significant ($Chi^2 = 0.370$, df = 3, $p = 0.946$) when compared to average daily fecundity of dead *R. padi* in the control. However, average daily fecundity of inoculated dead non-sporulating *R. padi* was influenced by temperature ($Chi^2 = 132.343$, df = 2, $p < 0.001$) and the interaction between isolates and temperature ($Chi^2 = 41.763$, df = 6, $p < 0.001$). There was a similar fecundity at 7.5 °C between inoculated dead non-sporulating and dead *R. padi* in the control ($p > 0.05$) (Fig. 6B). At 14.0 °C, the fecundity of dead non-sporulating *R. padi* inoculated with NCRI 459/15 and NCRI 460/15 was significantly lower than for the control ($p = 0.048$, $p = 0.001$, respectively) and at 18.0 °C, the fecundity of dead non-sporulating *R. padi* inoculated with NCRI

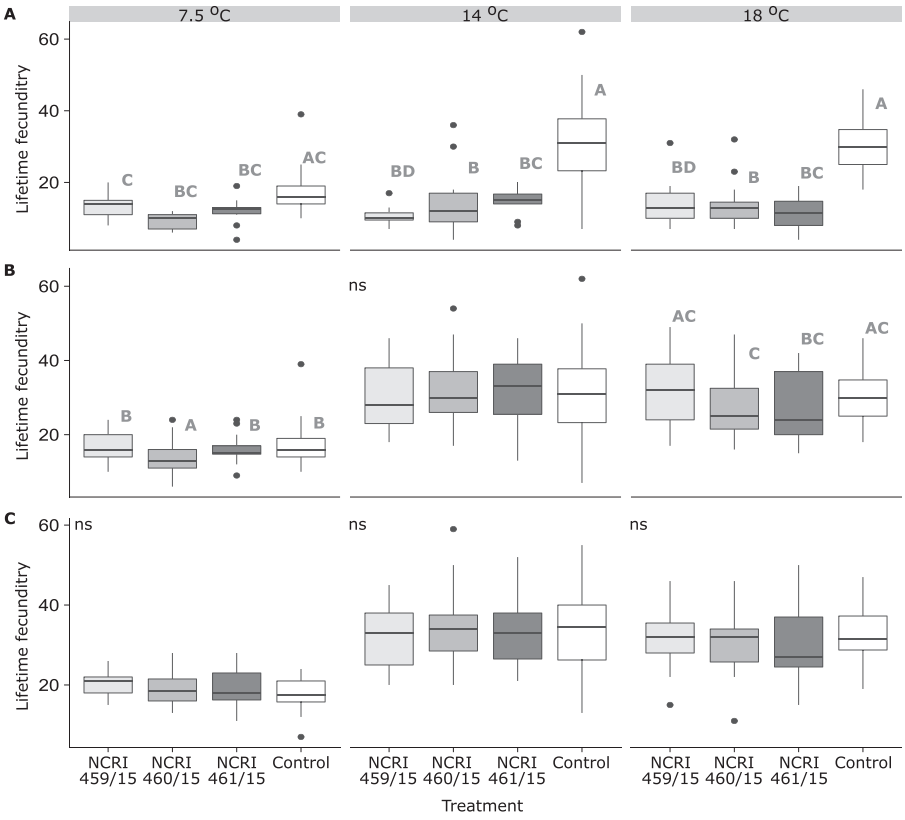


**Fig. 4.** Interaction effect of temperature and *Pandora neoaphidis* isolates on lifetime fecundity of (A) *Sitobion avenae* that became sporulating cadavers (inoculated dead sporulating) compared to aphids still alive at the end of the experiment in the control (control surviving), (Similar analysis of this interaction could not be investigated for *Rhopalosiphum padi* due to low sporulating cadaver numbers.), (B) *S. avenae* that survived the inoculation (inoculated surviving aphids) compared to aphids still alive at the end of the experiment in the control (control surviving), and (C) *Rhopalosiphum padi* that survived the inoculation (inoculated surviving aphids) compared to aphids still alive at the end of the experiment in the control (control surviving). The boxplots represent the interquartile range (distance between 25 and 75% quantiles), the black line the median and the vertical lines span the largest and smallest value no further than 1.5 x interquartile range. Dots indicate outliers. Results are based on GLMM and uppercase letters indicate comparisons obtained by *post hoc* estimated marginal means analysis ($p < 0.05$). "ns" indicates non-significant difference among all the groups tested.

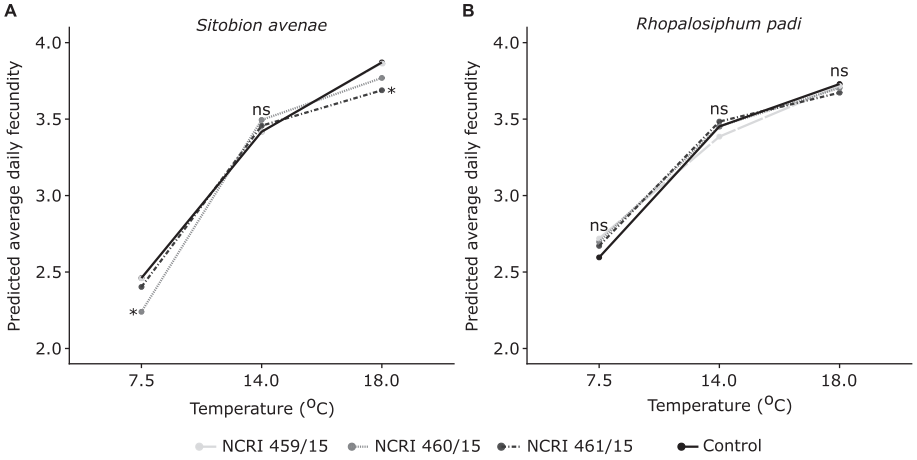


**Fig. 5.** Interaction effect of temperature and *Pandora neoaphidis* isolates on estimated average daily fecundity of aphids that survived the inoculation and are still alive at the end of the experiment (inoculated surviving aphids). (A) *Sitobion avenae* and (B) *Rhopalosiphum padi*. Estimations are based on GLMM and comparison between estimates based on a *post hoc* estimated marginal means analysis. * indicates a significant difference between the isolates and the control ($p < 0.05$). "ns" indicates a non-significant difference.

460/15 and NCRI 461/15 was lower than for NCRI 459/15 and for the control ($p < 0.001$ for both comparisons). Temperature influenced both the fecundity of inoculated dead non-sporulating *R. padi* and dead *R. padi* in the control ($p < 0.001$). The higher the temperature, the higher the fecundity ($p < 0.001$ for all pairwise comparisons).

## 4. Discussion

We showed that *P. neoaphidis*, collected from *S. avenae*, can infect and kill both *S. avenae* and *R. padi*. However, *P. neoaphidis* was much less virulent to *R. padi*. Firstly, more *S. avenae* sporulating

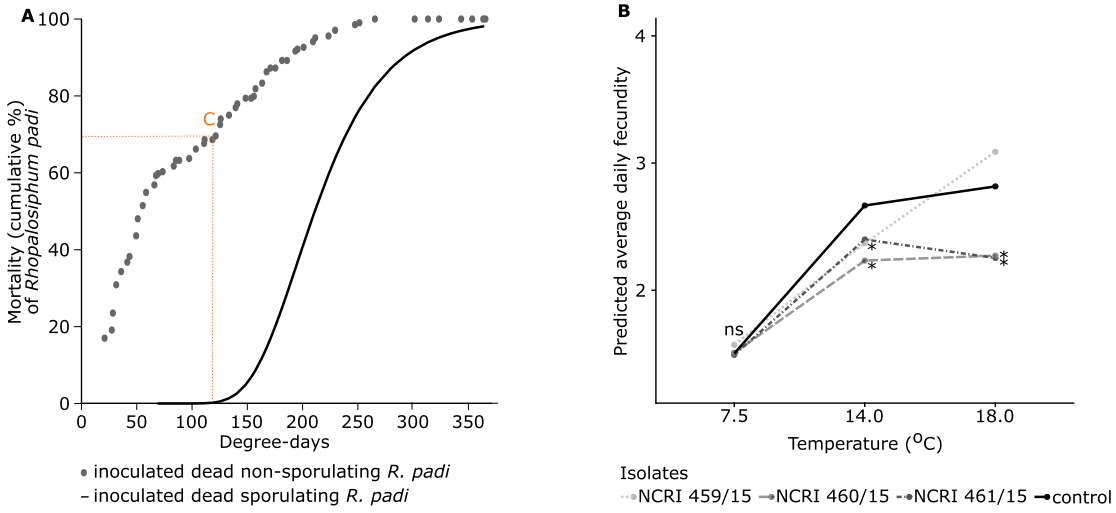


**Fig. 6.** (A) Cumulative percentage of inoculated *Rhopalosiphum padi* that died without fungal growth (inoculated dead non-sporulating aphids) and estimated cumulative percentage of inoculated dead sporulating *R. padi* (all isolates together) per degree-days. Point C indicates that 70% of the final mortality was achieved in all inoculated *R. padi* when the first sporulating cadavers occurred. (B) Interaction effect of temperature and *Pandora neoaphidis* isolate on estimated average daily fecundity of inoculated dead non-sporulating *R. padi*. Estimations based on GLMM and comparison between estimates based on a *post hoc* estimated marginal means analysis. * indicates a significant difference between the isolates and the control ($p < 0.05$). "ns" indicates a non-significant difference.

cadavers were produced. This is consistent with Shah et al. (2004), who studied aphid susceptibility and median lethal concentration of 20 mostly European *P. neoaphidis* isolates collected from diverse pest and non-pest aphids. They reported *R. padi* to be among the least susceptible aphid species compared to *S. avenae* and five other species (*A. pisum*, the black bean aphid *Aphis fabae,* the rose-grain aphid *Metopolophium dirhodum* and *M. persicae*). Secondly, we showed that *P. neoaphidis* killed *S. avenae* 30% faster (LT growth rate) than *R. padi* and resulted in a lower LT50 for *S. avenae* (116.2 DD) than for *R. padi* (147.7 DD). To our knowledge, no comparison of LT modelled with Gompertz equations has been done among the species we studied. Nielsen et al. (2001) infected *R. padi* and *S. avenae* at 18 °C with a Danish *P. neoaphidis* isolate collected from *R. padi*. This isolate resulted in a lower LT50 for *R. padi* (81 DD (our calculation)) than for *S. avenae* (93.6 DD (our calculation)). This may indicate that *P. neoaphidis* isolates from *R. padi* are more virulent to *R. padi,* but the authors did not conduct any statistical comparisons to confirm this. Thirdly, it is only when comparing inoculated surviving *S. avenae* (inoculated with, but not killed by *P. neoaphidis*) to surviving aphids in the control that we found a small decrease in average daily fecundity. The small decrease in average daily fecundity resulted in a small decrease of the lifetime fecundity of inoculated dead sporulating *S. avenae*, which produced on average 7% less offspring than surviving aphids in the control. A decrease in host average daily fecundity can be interpreted as an effect of the energy the host loses due to the infection, called the immune response cost (*e.g.* Parker et al., 2017). This may indicate that inoculated aphids that did not apparently die from the fungus may have allocated energy to defence responses to the disease rather than using energy to produce progeny. However, since we cannot distinguish aphids surviving the infection from those who escaped it entirely, the immune response cost could be higher than our study indicates. Grell et al. (2011) and Gerardo et al. (2010) suggest that aphids have a reduced immune repertoire and respond only weakly to *P. neoaphidis.* Parker et al. (2017) stabbed *A. pisum* with a needle coated with heat-killed fungal spores and mycelia (non-infectious) of one *P. neoaphidis* isolate collected from *A. pisum* before measuring aphid fecundity. They showed a decreased fecundity in inoculated alate *A. pisum* compared to surviving aphids in the control. As the fungus was non-infectious (heat-killed), the inoculated *A. pisum* in their study could maybe be compared to inoculated surviving *S. avenae* in our study. On the other hand, the complex hierarchy of defence mechanisms to a live and a dead pathogen is probably quite different.

Despite the differences mentioned above, the effect of *P. neoaphidis* was similar for both host species when it came to: (i) the fecundity of inoculated dead sporulating aphids and (ii) the mortality of inoculated dead non-sporulating aphids. Indeed, no effect on the inoculated sporulating aphid average daily fecundity was found for *S. avenae* or *R. padi.* Our results contrast with Baverstock et al. (2006), who calculated the lifetime fecundity over the infection period of inoculated dead sporulating *A. pisum* apterous adults killed by a *P. neoaphidis* isolate, collected from *A. pisum*, at 18 °C. Inoculated dead sporulating *A. pisum* produced 35% less offspring than surviving aphids in the control over the same period of time. However, we showed that for both species there was a significant decrease in lifetime fecundity between inoculated dead sporulating aphids and surviving aphids in the control. Offspring production by inoculated dead sporulating *R. padi* and *S. avenae* was halved compared to surviving aphids in the control (all isolates together). These differences are probably caused by differences in longevity between fungus-killed aphids and surviving aphids in the control since no difference between their average daily fecundity was observed. A similar decrease in lifetime fecundity was shown by Chen and Feng (2006) for inoculated dead sporulating *M. persicae* alates infected with an isolate from unknown origin. The fecundity of inoculated dead sporulating *M. persicae* was reduced by 59% (our calculation) compared to surviving *M. persicae* in the control after 7 d. Also the study by Chen and Feng (2006) suggests that the difference in longevity between inoculated dead sporulating aphids and surviving aphids in the control was the main reason for reduced lifetime fecundity. This decrease in lifetime fecundity could have dramatic importance on the infected aphid capacity to build up colonies and disseminate the disease (e.g. Chen and Feng, 2006). Finally, in our experiment the mortality of inoculated dead non-sporulating aphids was similar to the mortality in the control for both *S. avenae* and *R. padi*. Fungi in the phylum Entomophthoromycota have no or minimal

saprophytic growth since they are considered to be obligate or semi obligate-pathogens. Therefore, our study is consistent with the hypothesis that deadly toxins are probably not produced by *P. neoaphidis* prior to fungal growth in host (*e.g.* Pell et al., 2001).

In our work, *R. padi* exhibits substantial lower susceptibility to *P. neoaphidis* isolates collected from *S. avenae*. However, *R. padi* has been recorded to be infected with *P. neoaphidis* both in cereals (Hatting et al., 2000; Barta and Cagáň, 2006; Chen et al., 2008; Manfrino et al., 2014) and on bird cherry (Barta and Cagáň, 2004; Nielsen and Steenberg, 2004). This suggests a significant variability in *R. padi* susceptibility to *P. neoaphidis*. This may be supported by Parker et al. (2014) who showed that different clones of *A. pisum* vary in susceptibility when infected with a single *P. neoaphidis* isolate. Even though *P. neoaphidis* isolates do not cluster molecularly according to their original host species (Rohel et al., 1997; Tymon et al., 2004; Tymon and Pell, 2005), genetic intra-specific variation among isolates collected from different host species has been identified (Sierotzki et al., 2000; Tymon et al., 2004; Tymon and Pell, 2005; Fournier et al., 2010). This may suggest a variation in virulence between isolates (Tymon and Pell, 2005). We and Shah et al. (2004) did not study isolates originating from *R. padi*. Reyes-Rosas et al. (2012) showed variability in the virulence of isolates collected from the corn aphid *Rhopalosiphum maidis* to the cabbage aphid *Brevicoryne brassicae*. Further, other studies also show both high (e.g. Milner, 1982; Shah et al., 2004) as well as low (e.g. Milner, 1982; Morales-Vidal et al., 2013) virulence of entomopathogenic fungi if tested on other species than the original host. Therefore, *P. neoaphidis* cross-infection between *S. avenae* and *R. padi* could be asymmetrical and needs further investigation to determine if *R. padi* is generally more resistant to *P. neoaphidis* or if it depends on the fungal isolate origin. Information on this would allow estimation of the importance of *R. padi* in the epizootic of the pathogen *P. neoaphidis* in crops with mixed aphid species populations such as cereals.

Regarding the variability of our *P. neoaphidis* isolates, we did not find any difference in numbers of sporulating cadavers between the three *P. neoaphidis* isolates tested for *R. padi*. However, isolate NCRI 461/15 was more virulent to *S. avenae* than isolate NCRI 459/15. Differences in virulence between *P. neoaphidis* isolates have been shown for *A. pisum* by Barta and Cagáň (2009). They reported different median lethal concentration among *P. neoaphidis* isolates collected (i) at the same time in one *S. avenae* population and (ii) at two different dates in one common nettle aphid *Microlophium carnosum* population. Furthermore, our isolates from one fungal population expressed different speeds for killing their aphid hosts, as demonstrated by the LT growth rate. Interestingly, in our study the pattern of LT differences between isolates was consistent between host species, although the magnitude of the difference in *R. padi* was twice that of *S. avenae*. As shown by Bonsall (2004), such differences in LT could have dramatic consequences for the epizootic development of a pathogen in a host population. To our knowledge, no similar study on the effect of temperature to the LT distribution variability among isolates has been conducted on the species studied here. Finally, the suggested immune response cost of inoculated surviving *S. avenae*, shown through a decrease in average daily fecundity and consequently the decrease in lifetime fecundity of inoculated dead sporulating *S. avenae* and *R. padi*, depended on the isolates tested. To our knowledge, no studies have been conducted on the variability of host fecundity among isolates of the same entomophthoromycotan pathogen. However, *P. neoaphidis* isolates collected in one aphid metapopulation have been shown to express different (i) conidia size and fungal biomass production in liquid media ($g.l^{-1}$) (Sierotzki et al., 2000; Barta and Cagáň, 2009), and (ii) germination rate and sporulation capacity in *in vitro* culture (Sierotzki et al., 2000). These variations could be linked to the variability in virulence that we show in our study. Studying the natural variability of *P. neoaphidis* population in the field in more detail could potentially contribute to estimating its importance in the epizootiology of this fungal species.

Regarding temperature effect on virulence of different *P. neoaphidis* isolates, firstly, we found that *P. neoaphidis* produced more *S. avenae* and *R. padi* sporulating cadavers at 18 and 14 °C, respectively. Temperatures between 18 and 14 °C represent average Norwegian summer temperatures. These results are consistent with other studies that suggest that *P. neoaphidis* is a mesophilic fungus with an optimal temperature around 15–25 °C (Schmitz et al., 1993; Morgan et al., 1995; Stacey et al., 2003; Barta and Cagáň, 2006). Further, our isolates reacted similarly to different temperatures when studying sporulating cadaver production. To our knowledge the effect of the interaction between isolate and temperature on mortality of aphids inoculated with *P. neoaphidis* has not been studied previously. Nevertheless, temperature has been reported to influence differently isolates in the Entomophthoromycota from different geographical origins for *Z. radicans* infecting the diamondback moth *P. xylostella* (Morales-Vidal et al., 2013) and for *Furia gastropachae* infecting the forest tent caterpillar moth *M. disstria* (Filotas et al., 2006). The lack of interaction between temperature and isolate in our study, could be due to the fact that our isolates are from the same geographical origin (same population in one field).

Secondly, when pooling all isolates, no temperature effect on LT was found for any of the aphid species, neither on the LT growth rate (parameter $k$), nor on time needed for the first sporulating cadavers to occur (parameter $\beta$). This is in conflict with other studies that show that the LT50 of *P. neoaphidis* infecting *S. avenae* and *Acyrthosiphon kondoi* decreases when the temperature increases from 2 to 20–25 °C (Milner and Bourne, 1983; Schmitz et al., 1993; Nielsen et al., 2001). However, these authors expressed LT in days and not in DD, and did not use Gompertz equations to model LT distribution. Using DD allows us to focus on the infection process without considering the direct influence that the temperature has on ectotherm species. For instance, if one infection process needs 100 DD to be completed, it should take 10 d at 10 °C or 5 d at 20 °C. If the time in DD changes with temperature then we reveal a temperature effect on the process itself. When our isolates are studied separately, we report a temperature effect on the growth rate of LT with a minimum variation of 30% depending on the temperature. Our *P. neoaphidis* isolates killed *S. avenae* faster under different temperatures. These results suggest that our isolates react differently to temperature.

Finally, we showed that the decrease in aphid average daily fecundity and in their lifetime fecundity depended on the interaction between temperature and isolate, the host species and its health status. The average daily fecundity and lifetime fecundity of inoculated surviving *S. avenae* were slightly reduced at 18 °C when inoculated with NCRI 461/15. At 7.5 °C the average daily fecundity and the lifetime fecundity was reduced only when inoculated with isolate NCRI 460/15. In both cases, the decrease in fecundity occurred at the same temperature as the highest LT growth rate. We, therefore, hypothesize that the immune response cost is higher under more optimal conditions for the fungus to kill its host. Interestingly, when studying *P. neoaphidis* influence on the average daily fecundity of inoculated dead non-sporulating *R. padi*, we also found a significant non-linear temperature effect depending on the isolate. Baverstock et al. (2006), Blanford et al. (2003) and Stacey et al. (2003) found a significant interaction between *A. pisum* clones and temperature on inoculated dead sporulating apterous adult fecundity. Together, these results suggest that *P. neoaphidis* virulence and the host recovery depends on (i) the host and the fungal genotypes as suggested in Lambrechts et al. (2006) and (ii)

their interaction with the temperature as explained in Thomas and Blanford (2003). Consequently, the non-linear effect of temperature on the variability in virulence between isolates could potentially trigger seasonal shifts in the fungal population. It would, therefore, be interesting to study the effect of temperature on *P. neoaphidis* isolates collected from one aphid population but at a different time in the season in order to understand the development and progression of an epizootic.

## 5. Conclusion

Our study demonstrates three main findings: (i) *P. neoaphidis* collected from one *S. avenae* population infected and killed both *S. avenae* and *R. padi* but it was much less virulent to *R. padi*. Indeed, it produced fewer *R. padi* sporulating cadavers, killed it slower and did not decrease average daily fecundity for either inoculated dead sporulating or inoculated surviving aphids. (ii) *P. neoaphidis* infection caused a decrease in the average daily fecundity of those *S. avenae* that survived the inoculation. This may suggest that *S. avenae* is using energy to combat the infection rather than producing progeny. However, lifetime fecundity of inoculated dead sporulating and inoculated surviving aphids was halved for both host species. (iii) The variability in production of sporulating cadavers between isolates did not depend on temperature but depended on host species. The lowest LT growth rate and decrease in host fecundity occurred at different temperatures according to the isolate and the host species studied. These differences suggest different spread dynamics of the isolates into the two host populations, which can have dramatic consequences for the epidemic development of the pathogen. The non-linear temperature effect on the isolate virulence and sub-lethal effect on the host fecundity emphasises the importance of studying (i) the influence of a realistic range of temperatures on the infection process and (ii) the variability of the isolates present in one fungal population. This information could be useful to understand and model the population dynamics of *P. neoaphidis* and its hosts through the season in order to increase our understanding of its epizootics and its potential use in biological control.

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