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# Systemic acropedal influence of endophyte seed treatment on *Acyrthosiphon pisum* and *Aphis fabae* offspring development and reproductive fitness

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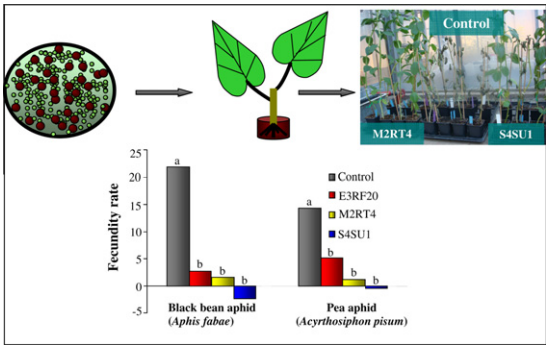
## HIGHLIGHTS

- Fava bean seeds soaked in endophyte spore suspension for 4 h in tween/water solution.
- Seedlings grown in the greenhouse for 21 days and then root ball re-inoculated with the endophyte.
- Aphid development and fecundity was monitored following aphid infestation.
- Endophyte seed treatment slowed offspring development and reduced fecundity rate by a factor of 1.6–14.6.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Most terrestrial plants harbor endophytic fungi, and many of them could directly or indirectly influence insect behavior and community structures by altering plant defense mechanisms. Therefore we evaluated the systemic effects of endophyte seed treatment on aphid population growth rate, offspring performance and fecundity and its effects on *Vicia faba* in response to aphid feeding. Overall, endophyte treated fava beans had a significantly lower number of *Acyrthosiphon pisum* when compared to the untreated controls. The highest reduction effects were observed among plants treated with *Trichoderma asperellum, Gibberella moniliformis* and *Beauveria bassiana* isolates, while all *Metarhizium anisioplaie* and *Hypocrea lixi* isolates had the least effects on *A. pisum* population growth. Similarly, endophyte seed treatment had a detrimental effect on offspring fitness, development and fecundity. Irrespective of aphid species, the birth rate of all offspring arising from females fed on endophyte treated plants for two generations were significantly lower than those arising from females fed on control plants. As a result, all endophyte treated plants had between 1.6–14.6 and 3.7–11.0 times less number of *Aphis fabae* and *A. pisum* nymphs, respectively, compared to untreated controls at the final assessment day. Concurrently, endophyte seed treatment enhanced seedling survivorship with a survival rate of 20–100% compared to none in the control treatment at 20 days post infestation. The present study demonstrates that endophyte seed treatment can offer a protective role by enhancing the competitiveness of *V. faba* towards aphids, which can be manipulated as a tool in IPM systems.



## 1. Introduction

The soil macro- and micro-organisms in terrestrial agro-ecosystems are important regulators of ecological processes and functions. Apart from maintaining soil fertility and recycling nutrients they have positive impact on agricultural productivity

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by regulating pest populations (Lupwayi et al., 2011). In field crops and in greenhouse production, aphids (Homoptera: Aphidae) are considered a major constraint to crop production worldwide. They affect crop productivity by consuming nutrients from the phloem sap of the plant, consequently resulting in stunting and deformation as well as premature leaf death, low pod and seed production and small grain sizes (Pegadaraju et al., 2005). Aphids also cause indirect damage by transmitting plant viruses (Radcliffe and Ragsdale, 2002). Pesticides have been used to combat aphid problem but potential harm to the environment and high costs renders this method unsustainable for most smallholder farmers.

Mutualistic endophytes, usually bacterial or fungal microbes that live inside the vegetative tissues of living plants throughout or at some time of their life cycle without provoking any apparent disease symptoms (Petrini, 1991; Wilson, 1995; Saikkonen et al., 1998), have been shown to be able to reduce insect herbivory in colonized plants. For instance, their presence in many plants were shown to protect host plants from boring, sucking, chewing and leaf mining insects (Latch, 1993; Bing and Lewis, 1991; Cherry et al., 2004; Jallow et al., 2008; Vega, 2008).

Among the sucking insect pests, fungal endophytes have been reported to influence the fitness, development, fecundity and oviposition preference of whiteflies and aphids (Menjivar, 2010). For example, a non-pathogenic *Fusarium oxysporum* (Fo162) isolate inoculated to the rhizosphere of squash or melon and pepper seedlings significantly reduced the reproductive rate of *Aphis gossypii* (Glover) and *Myzus persicae* (Sulz.), respectively (Menjivar, 2010). Conversely, mutualistic endophytes that are limited to the shoot were also shown to have negative effects on sucking insects (Wilson et al., 1991; Gurulingappa et al., 2010). Wilson et al. (1991) in a series of feeding tests showed that the presence of a clavicipitaceous endophyte inside the shoots of perennial ryegrass seedlings negatively affected the Russian wheat aphid (*Rhopalosiphum padi* L.) population increment, consequently leading to endophyte colonized shoots supporting fewer numbers of aphids than endophyte free plants. Additionally, endophyte free plants were noted to support aphid reproduction while nymphs were not found on endophyte infected plants. In another study, aphid reproduction was higher when they were subjected to tall fescue plants treated with ergot alkaloid free endophytic isolates compared to aphids that were fed on plants treated with high ergot alkaloid producing isolates (Bultman et al., 2003, 2004). Feeding of *A. gossypii* on cotton leaves colonized by either *Beauveria bassiana* or *Lecanicillium lecanii* (Zimm.) have also been shown to slow aphid reproduction (Gurulingappa et al., 2010).

The above studies suggest that the use of such beneficial organisms may indirectly alter the nutritional composition of host plants, and this not only help regulate aphid populations but also enhance host plant competitiveness in response to aphid attack. However, different endophyte species contain dissimilar traits including differential alkaloid production and sporulation abilities (Cheplick, 1998; Bultman et al., 2004), which affect their impact on the levels of herbivory and damage caused to plants (Clement et al., 2005; Tintjer and Rudgers, 2006). For example, both the stroma forming and stroma lacking endophytic strains have been shown to increase or lower herbivory rates, respectively (Tintjer and Rudgers, 2006). In other studies, the endophytic effects were shown to depend on the pest species, origin and plant genotype among others (Barbosa et al., 1991; Gehring and Whitham, 2002).

Whereas these findings provide insights into alternative means of combating aphid pest population, the research done by far has focused more on clavicipitaceous endophytes. To date, there is still limited information on the role of non-clavicipitaceous endophytes on the performance of greenhouse aphids like *Acyrthosiphon pisum* (Harris) and *Aphis fabae* (Scopoli). Additionally, it is still unknown whether factors listed above can influence the performance of fungal endophytes that colonize the roots and induce systemic resistance in the shoot to different legume aphids and their host plants. In the present investigation, the influence of endophyte seed treatment on aphid performance was studied to explore four main questions: (i) does fungal endophyte seed treatment affect the performance and population build up of *A. pisum* in the shoot of fava beans, (ii) can endophyte treatment of fava bean seeds affect the competitiveness of bean seedlings in response to *A. pisum* feeding, (iii) what effect does endophyte seed treatment have on the development, fitness and reproductive rate of *A. pisum* and *A. fabae* and (iv) and can the grass fungal endophytes colonize the endosphere of bean seedlings?

## 2. Materials and methods

### 2.1. Experimental design

Three greenhouse experiments were undertaken at the Institute of Crop Science and Resource Conservation (INRES), University of Bonn, Germany to investigate the effects of fungal endophytes isolated from cereals on: (i) *A. pisum* population increment and host plant susceptibility, and (ii) the development and fecundity of *A. pisum* and *A. fabae* offspring. In the first experiment, the treatments included eight fungal isolates and a water control treatment while in the second experiment only three of the best performing isolates were used. The third experiment was established to assess the ability of the best performing isolates to form an endophytic association with fava beans.

### 2.2. Aphid rearing

Colonies of *A. pisum* and *A. fabae* were originally obtained from Bayer CropScience (Bayercode: APHIGO, Bayer CropScience Deutschland GmbH, Langenfeld). The aphids were mass produced on 2 week old Scirocco fava bean seedlings, with colonies of each aphid species maintained separately in different growth chambers at 25 ± 2 °C, 60% relative humidity and a photoperiod of 16:8 h light/dark.

### 2.3. Fungal endophytes

Eight Kenyan fungal isolates belonging to five different genera, *Hypocrea lixii* (1), *Trichoderma asperellum* (1), *Gibberella monliformis* (1), *F. oxysporum* (1), *Metarhizium anisopliae* (2), and *B. bassiana* (2), were used in the study. The fungi were originally isolated from surface sterilized healthy maize and sorghum tissues during the short rainy season of 2009 (Akello, unpublished). The isolates tested included: E3RF20 and M2RT4 (root isolates), F3ST1, S4ST7, S4SU1 and M7SF3 (stem isolates) and G1LU3 and N1LT6 (leaf isolates). With the exception of S4ST7, S4SU1, G1LU3 and N1LT6 that were cultured on malt extract peptone agar (MEA, Merck, Darmstadt, Germany), all isolates were grown on potato dextrose agar (PDA, Merck, Darmstadt, Germany). The cultures were incubated in the dark at 25 ± 1 °C for 10 days. At harvest, conidia of S4ST7, S4SU1, G1LU3 and N1LT6 were scraped off the agar surface and suspended in sterile 0.1% Tween 80. For the remaining isolates (E3RF20, M2RT4, F3ST1 and M7SF3), the spores were harvested by flooding the plates with sterile water and then scrapping gently with a sterile loop. The resulting suspension was filtered with sterile Mira cloth. For each isolate, spore concentration was determined using improved Neubauer hemocytometer and adjusted to $1 \times 10^8$ spores/ml.

### 2.4. Fava bean

Bean seeds were surface sterilized in 70% ethanol for 1 min followed by a 1.5% sodium hypochlorite for 3 min. These were then