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MICROBIOLOGICAL CONTROL OF *CARPOCORIS FUSCISPINUS* (HEMIPTERA: PENTATOMIDAE), A PEST OF ONION AND LEEK SEED CROPS

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ABSTRACT

Any agricultural production requires the prior production of seeds, carried out by specialized companies, which own in-depth knowledge of seed crops as well as of their pathogens and pests. These pathogens and pests often remain unknown to the agricultural world. They are very little studied and control strategies do not exist. The present study is typical of such a situation: Zollinger Bio, an organic seeds producer, regularly deals with seed crops health problems. Over the last years, the production of seeds of onion (*Allium cepa*) and leek (*Allium ampeloprasum var. porrum*) has been reduced by 50% by a bug, morphologically and genetically identified as *Carpocoris fuscispinus*. Their piercing-sucking mouthparts allow these insects to empty the maturing seeds. *Carpocoris fuscispinus,* a native bug in Europe, has never been considered as a pest, although observed once as a cereal pest in Iran. The pest has already caused damage during flowering making the use of insecticides not possible, because of their harmful effect on pollinating insects. Entomophagous fungi could be an alternative. Two fungal isolates, *Beauveria bassiana* UASWS 1427 and *Paecilomyces fumosoroseus* UASWS 1457, were tested against *C. fuscispinus*. The experimental units consisted of five bugs in a box with water and food (leek flower, spelled crop seeds). The mortality increased quickly between day four and day eight and reached 100% adults for *B. bassiana* and 90% for *P. fumosoroseus* at day eight. Similar results were obtained on larvae. These results are promising of a possible microbiological control against *C. fuscispinus*.

Keywords: *Beauveria bassiana, Carpocoris fuscispinus, entomophagous fungi, organic agriculture, Paecilomyces fumosoroseus.*

INTRODUCTION

The production of any agricultural crop requires farmers to know what their plants need in order to grow. Additionally, they also need to know how to protect their plants from potential pests that could ruin their crops. Pests come in a multitude of shapes and sizes, be it tiny nematodes destroying root systems or bigger mammals grazing on crops. Over the last decades, a new group of pest has emerged and has become a growing problem in agriculture: stink bugs (Hemiptera: Pentatomidae) (Panizzi, 2015). These insects are the cause of many headaches for several reasons. First, most stink bugs are generalists, which means that they have a wide range of host plants that they can feed from (Lee et al., 2013). Species like the Brown marmorated stink bug (*Halymorpha halys*) or the Southern green stink bug (*Nezara viridula*) have been observed on over 100 different plant hosts (Kamminga et al., 2012; Rice et al., 2014). If no suitable host plant is available, stink bugs can adapt on a short time scale and feed on plants that were not part of their diet (Panizzi, 2015). Second, stink bugs seem to be more resilient to pesticides than other insects. Several studies testing the survival rates of different stink bug species after being exposed to synthetic insecticides commonly used in agriculture show that the mortality rates are often not convincing. Stink bugs also seem to be able to rapidly develop resistances against some insecticides (Castellanos et al., 2018; Tugwell et al., 2017). Insecticides that are homologated for organic agriculture (according to Swiss standards) perform fair no better than their synthetic counterparts and don't achieve a convincing mortality rate as well (Joseph, 2018; Kamminga et al., 2009).

A Swiss organic seed production company has also observed the emergence of a new stinkbug pest. Starting in 2017, seed harvest of onion (*Allium cepa*) and leek (*Allium ampeloprasum var. porrum*) has drastically dropped by 50 percent. A closer inspection of the two crops in the following years has shown that a stinkbug, identified later as *Carpocoris fuscispinus*, was feeding on maturing seeds. Using their rostrum, the stinkbugs systematically pierced through the maturing seeds and sucked the contents out, leaving an empty pulp behind. *Carposcoris fuscispinus* is native to Switzerland, with a wide distribution area across Europe and the Middle East (Lupoli et al., 2013). The stinkbug is not considered a pest in Europe, but some reports from Iran mention *C. fuscispinus* as being a minor pest of cereals (Hassazadeh, et al. 2009). No records mention any feeding on onions or leeks. Since the feeding damage occurs during or shortly after the crops bloom, many pollinators are present on the fields. Due to this, commonly used insecticides homologated for organic agriculture (neem, pyrethrin, spinosad) could not be used to control *C. fuscispinus*, as they would have had harmful non-target effects on these pollinators (Lopes et al., 2015; Miles, et al., 2012; Soni, 2014).

Bearing in mind the poor efficacity of insecticides against stinkbugs and the effects they would have on non-target pollinators, a new solution had to be sought in order to control the pest and reduce harvest loss. Microbiological control methods, such as the use of entomophagous fungi, seemed to be an interesting alternative that could solve the issues encountered with insecticides (Gouli et al., 2012; Ihara et al., 2001; Todorova et al., 2002)

The goal of the present study was to test the mortality rate of nymphs and adults of *C. fuscispinus* after being exposed *in vitro* to two entomophagous fungi strains, *Beauveria bassiana* (UASWS[1] 1427) and *Isaria fumosorosea* (UASWS 1457), applied at two different concentrations, 10^7 and 10^8 conidia per millilitre.

MATERIAL AND METHODS

The two fungal strains, *B. bassiana* and *I. fumosorosea*, were selected because their virulence was successfully tested in a previous research (Eckert, 2017). Fungal strains were cultivated in 96 mm Petri dishes on diluted Sabouraud medium (2% agar instead of 4%) with 0.3% chitin. Petri dishes were kept in a dark incubator at 24°C and fungi were transplanted once a month into fresh medium. After sporulation, conidia were extracted from the Petri dishes using a solution of distilled water and Tween®20 (0.4%) and were stored in a sterile flask. Conidia concentration was then calculated for each strain using a Thoma counting cell. Based on these concentrations, the initial solutions were diluted by factors six and seven in order to perform a viability test. For each strain and solution, three 96 mm Petri dishes of diluted Sabouraud medium were inoculated and were kept in a dark incubator at 24°C. Sporulation was analysed after three days.

In order to test the mortality rate of the stinkbugs, two solutions containing 10^7 and 10^8 conidia per ml were created for each fungi strain. A control solution consisting of distilled water mixed with Tween®20 at 0.4% was also created. These treatments were named $Bb10^7$, $Bb10^8$, $If10^7$, $If10^8$ and C. The solutions were tested on nymphs of the 2nd and 3rd instar as well as on adults.

For the nymphs, each experimental unit consisted of five nymphs, reared in the laboratory, placed in a 30 mm Petri dish. A 30 mm filter paper soaked with 100 µl of sterile water was placed at the bottom of each Petri dish in order to provide moister. Five leek flowers were placed in the Petri dish as well. Three experimental units were assigned for each of the five treatments (2 strains*2 concentrations+control). Nymphs were inoculated by applying 2 µl of the treatments onto their abdomen. Experimental units were kept in climatic chamber. Mortality of the nymphs was noted on D+1, D+4 and D+7. Sporulation was noted on dead nymphs seven days after death. Kruskal-Wallis test was applied in order to compare the mortality rate of the nymphs and the sporulation rate to the control at a significance level of 10%.

For the adults, each experimental unit consisted of five adults placed in a plastic box (10*8*6 cm) with a perforated cover. Adults were captured 10 days prior to the experiment and kept in a rearing cage. Each experimental unit was provided with a watering station, a leek flower head and a maturing spelt ear. Food and water were replaced every three days. Adults were inoculated by submerging five insects at a time in 20 ml of each treatment suspension for 10 seconds. Three experimental units were assigned for each of the five treatments (2 strains*2 concentrations+control). Experimental units were kept in a climatic chamber.

[1] UASWS: University of Applied Sciences Western Switzerland

Mortality of the adults was noted on D+1, D+2, D+3, D+4, D+5, D+8, D+9, D+12 and D+15. Sporulation was noted on dead adults 14 days after death. Kruskal-Wallis test was applied in order to compare the mortality rate of the adults and the sporulation rate to the control at a significance level of 10%.

RESULTS AND DISCUSSION

On D+7, mean mortality rate for the nymphs were 100% for $Bb10^7$, 93% for $Bb10^8$, 86% for $If10^7$ and $If10^8$, and 53% for the control (Fig. 1). Mean mortality rose faster for $Bb10^8$ during the first four days of experiment than for the other treatments. P-Value on D+7 was 0.045 and median mortality rate was significatively different from the control for $Bb10^7$.

Mean sporulation rate of dead nymphs after 10 days was 80% for $Bb10^7$, 83% $Bb10^8$, 0% for $If10^7$, 16% for $If10^8$ and 0% for C. Sporulation of *B. bassiana* were noticed three days after nymphs died.

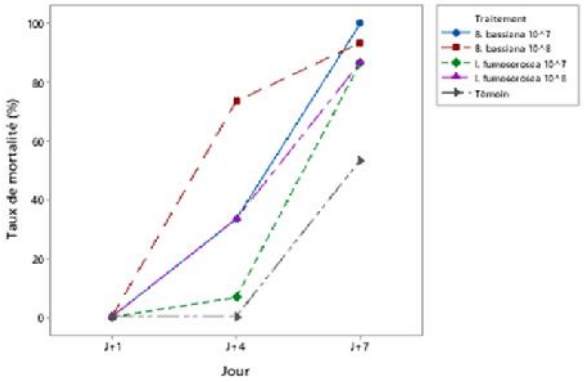

Figure 1. Mean mortality rate of nymphs over one week

For the adults, mean mortality rate on D+15 was 100 % for $Bb10^7$, $Bb10^8$ and $If10^8$. For $If10^7$ it was 93% and 46% for C (Fig. 2). Mean mortality rate increased mostly between D+4 and D+8 for the fungi treatments. Mean mortality rate for the control was high since the first day of experiment and evolved slowly over the next two weeks. On D+15, P-Value was 0.022. Median mortality rate was significantly higher, with a 95% certainty, for $Bb10^7$, $Bb10^8$ and $If10^8$ compared to C. Mean sporulation of dead adults after 14 days was 100% for $Bb10^8$, 93% for $Bb10^7$ and $If10^7$, 86% for $If10^8$ and 8% for C (Fig. 3). One adult from the control treatment sporulated.

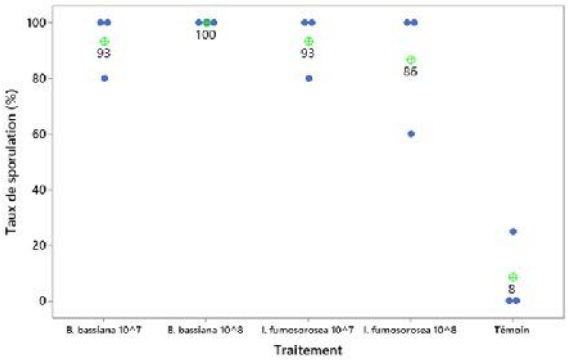

Figure 2. Mean mortality rate of adults over two weeks

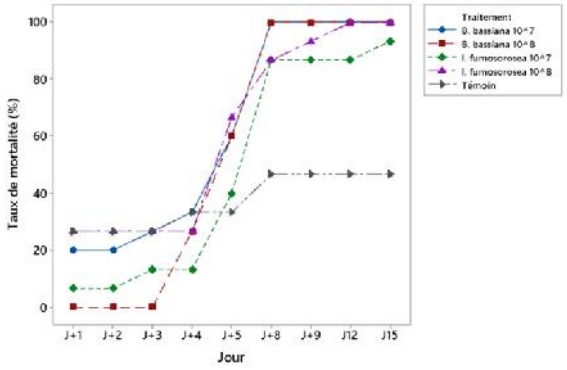

Figure 3. Sporulation rate of each experimental unit (blue) and mean sporulation rate (green)

These results show, that the fungal strains *B. bassiana* (UASWS 1427) and *I. fumosorosea* (UASWS 1457) can infect the stink bug *C. fuscispinus* in laboratory conditions. Sporulation on insects showed that both strains can successfully reproduce themselves in this host and create new conidia. The high sporulation rate observed on adults could indicate that mortality is mainly caused by the entomophagous fungi used in this experiment. This could also be the case for the nymphs treated with *B. bassiana* at both concentrations, were mean mortality and mean sporulation are high. On the other hand, mean mortality of nymphs was high for both *I. fumosorosea* treatments, but sporulation was low or completely absent. After day four of the experiment, a gradual drying out of the filter paper that was at the bottom of the 30 mm Petri dishes was noted. Since nymphs inoculated with *I. fumosorosea* seemed to die after day four, sporulation would have occurred in drier conditions than it occurred for *B. bassiana* which sporulated during the first four days of experiment. Several sources claim that sporulation of entomophagous fungi depends strongly on water availability (Arthurs & Thomas, 2001; Borisade, 2018).

This could explain why mortality rate of both *I. fumosorosea* treatments was higher compared to the control and why sporulation was low.

Mortality rate of the control was an important issue in this experiment. The nymphs were reared in laboratory conditions and were thus formed a homogenous population. Mortality appeared after day four, when the filter paper started to dry out. The mortality could result from this lack of moisture. In further experiments, this issue should be addressed in order to get better results. For the adults, laboratory rearing proved to be difficult and wild insects had to be collected in order to conduct the experiment. The population used for the test was thus not homogenous, which could explain the high mortality rate.

CONCLUSIONS

The emergence of this pest shows that new control methods have to be studied in order to protect crops. This experiment showed that the two tested entomophagous fungi strains, *B. bassiana* (UASWS 1427) and *I. fumosorosea* (UASWS 1457), could potentially be interesting solutions to control the stink bug *C. fuscispinus*. Mortality rates were high for adults and nymphs for both fungi at concentrations of 10^7 and 10^8 conidia per ml. Since both fungi could reproduce and create new conidia, these entomophagous fungi could potentially infect new individuals and thus protect crops over longer periods.

Even though these first results look promising, further experiments have to be conducted. The effectivity of both strains has to be tested in an *in situ* experiment, for evaluating their potential in a less controlled environment.

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