Pathogenicity of entomopathogenic fungi to endophytic leaf miner moth

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Introduction

Entomopathogenic fungi are microbial antagonists of different insects. The infection process takes place prevailing over the spores on the integument of hosts. Endophytic species are prevented from spore contact in their habitat through the plant tissue. Still infected individuals are found regularly in the mines. In biotests should be investigated, if a leaf application of entomopathogenic fungi can increase the mortality of endophytic stages of leaf miner moths.

Material and method

Investigations took place with two entomopathogenic fungi L*ecanicillium muscarium* (V 24) and *Paecilomyces fumosoroseus* (P 6) on larvae/pupae of chestnut leaf miner moth *Cameraria ohridella* or eggs, larvae and pupae of black locust leaf miner moth *Phyllonorycter robiniella* respectively.
[1] Biotests were carried out for larvae on chestnut seedling in growth chamber. Nine ml suspension ($1x10^7$ sp./ml) per seedling were sprayed with a hand sprayer (4 replicates). Dead and moulded individuals were counted 21 days past application (dpa).
[2] In case of *P. robiniella* application (10 ml $1x10^9$ sp./ml/twig) took place outdoors on trees (4 replicates). Seven days past application 25 leaf lets were separated in wet chambers (25°C). Six days later the number of moulded individuals were counted.
[3] One kg of leaf litter of chestnut were sprayed (100 ml, $2{,}2x10^8$ sp./ml) in the autumn (4 replicates). After 5 months the number of living and dead pupae of 50 leaflets were counted. All found pupae were incubated in a wet chamber at 12°C. After 14 days the number of mouldy pupae were determined.

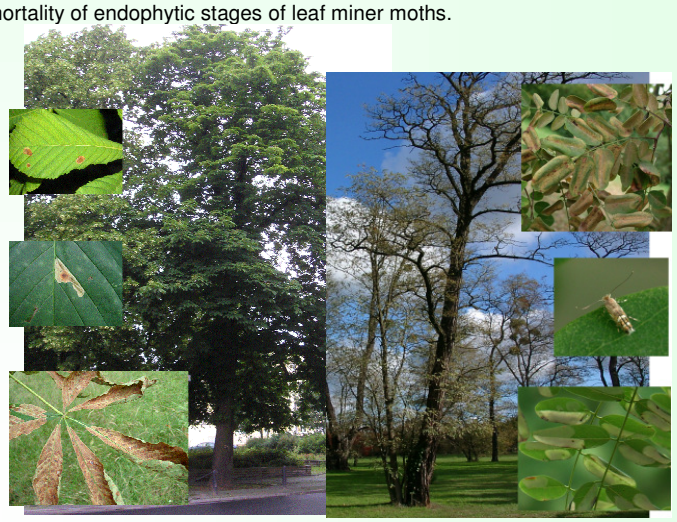

Fig. 1 Damage of *C. ohridella* on chestnut (left) and of *P. robiniella* on robinia (right)

Results

1

Fig. 2 Moulded larva of *C. ohridella* in the mine

The mortality of larvae of *C. ohridella* in mines was nearby 100% in every treatment and appeared on the end of larval development. Only in few cases pupation took place. Each larva or pupa respectively was moulded and mycelium of applied fungi grew out of mines (Tab.1).

Tab. 1: Mortality and moulding of larvae/pupae of *C. ohridella* after leaf application of *L. muscarium* and *P. fumosoroseus*

	control	L. muscarium	P. fumosoroseus
larvae/pupae 21 dpa	57	50	84
alive (number)	51	1	0
dead (number)	6	49	84
mortality %	11	98	100
degree of efficiency %	-	98	100
significance %	a	b	b
mouldy larvae/pupae (number)	0	48	84
moulding rate %	0	98	100
significance %	a	b	b

2

Fig. 3 Moulded pupa of *P. robiniella* in the pupal cell

L. muscarium is able to infect the pupae of *C. ohridella* in their pupal cells at low temperature. In semi field trials this fungus reached 40% mouldy pupae after hibernating and incubation in wet chambers as well. In the further development it was to be seen, that the fungus has an effect on moths, too. The moth died earlier than in control and mycelium appeared quickly. The difference to control was significant. Together pupae and moth reached 60 - 70% mortality in the hibernating population.

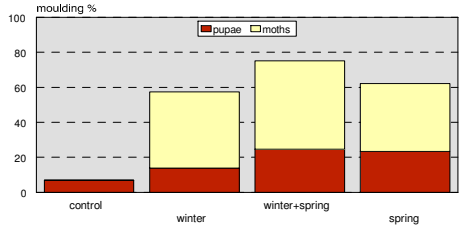

Fig. 4 Percentage of mouldy pupae and moths of *C. ohridella* after application of chestnut leaves with *L. muscarium* in the winter and/or spring and after incubation of pupae at 12°C and 95% humidity (15.5. 06)

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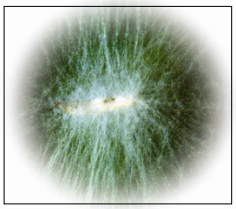

Fig. 5: *L. muscarium* on a larval cadaver of *Ph. robiniella* L3 (25°C, 98% RH).

Larvae and pupae of *Ph. robiniella* were infected from entomopathogenic fungi in their mines, too. In all development stages we found mouldy individuals. The smallest larval stages L1 - L3 were the most susceptible. At all *L. muscarium* reached in this trial nearby 80% mortality.

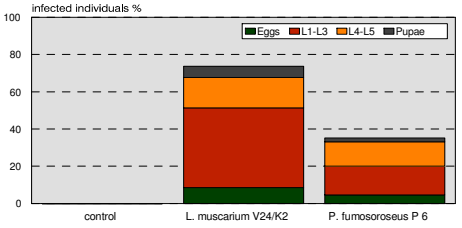

Fig. 6 Efficacy of *L. muscarium* and *P. fumosoroseus* against different stages of *Ph. robiniella* after field application of spore suspension ($1x10^9$ sp./ml) and incubation at 25°C, 98% RH, n=891

Conclusion

In different trials it was proved that entomopathogenic fungi, especially *L. muscarium*, are able to infect larval stages and pupae of leaf miner moths. Infection doesn't come from spores. It is probably that the fungi overcome the epidermis on damaged cells and grow inside of mines. This infection process depends on wetness of leaf. So, it is to explain that on green leafs in early summer more infected individuals were found than in hibernating on high damaged and dry leaves. The results encourage to carry out further experiments in the field.