# Efficacy of entomopathogenic fungi against larvae of the horse chestnut leafminer *Cameraria ohridella DESCHKA & DIMIC, 1986* (Lepidoptera, Gracillariidae)



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#### Introduction

The larvae of the horse chestnut leafminer *C. ohridella* still causes remarkable leaf damages annually. In spite of intensive investigations in Europe, no lasting strategy to control the moth has been found. Trials were conducted to test the biocontrol potential of entomopathogenic fungi *Lecanicillium muscarium* ZARE, GAMS 2000 and *Paecilomyces fumosoroseus* APOPKA against endophytic larvae of the chestnut leafminer *C. ohridella* within mines.



Fig 2 Plastic cages with seedlings of chestnut in climatic chamber

#### **Material and Methods**

Each treatment was composed of 5 to 7 fully grown leaves on each of 3 seedlings. Each seedling, 9 ml suspension (1\*10<sup>7</sup> conidia/ml) of *L. muscarium* strain V24 or *P. fumosoroseus* strain 6, respectively, or 9 ml water in the control were applied, using a manual sprayer. The treatments were kept separately in plastic cages (fig. 2) in a growth chamber for 28 days with the day/night parameters: light regime: L/D 16/8h, temperature 25/21 °C, RH 90%/98%. The dimensions of the mines were determined 3, 7, 10, 14, 17, and 21 days past application (dpa) (n=212 mines). After opening the mines 28 dpa, the stage of development, the mortality and the mouldiness were determined.



Fig. 1 Damage of chestnut leafminer Cameraria ohridella

## Results

# 1. Development of mines

At 3 dpa the development of mines in the fungal treatment seemed to be slower, than in the control. However the difference was not significant. In the following days the development of mines were quicker in the fungal variants than in the control, so that later examinations showed similar development in all variants (fig. 3).

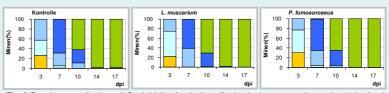


Fig. 3 Development of mines of C. ohridella after leaf applikation in the untreated control and the fungitreatments

## 2. Development of larvae within mines

The natural mortality of the control was significantly lower (12%) than in the fungal treatments. The control-population consist of 91% pupae altogether (of these 9% dead) 5% alive moths and 4% dead larvae (fig. 4). Mortality in *L. muscarium* was 98%, and in *P. fumosoroseus* 96%, respectively (tab. 1). Only 4% of all larvae pupated successfully in both fungal treatments.

All larvae cadavers, even the dead pupae from the treatment with *L. muscarium*, were moulded. In some cases the mycel grew out of the mines (fig. 5). No mycelia growth was observed on the dead larvae from control treatment.

**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 %	а	b	b
mouldy larvae/pupae (number)	0	48	84
moulding rate %	0	98	100
significance %	а	b	b

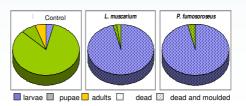






Fig. 5 Mouldy larva and mine with mycelial growth

Fig. 4 Stages of *C. ohridella* 28 d after application of *L. muscarium, P. tumosoroseus* and control, temperature 25/21 °C, RH 90/100%, light 18/6h

## Conclusion

Both fungal strains showed primarily high pathogenicity on larvae of *C. ohridella* in their mines. Mine activity showed that the fungi became effective at the end of larval development, and inhibited the larvae pupation. Fungi grew through the epidermis into the mines and infect the larvae inside. When the larvae were dead, the fungi grew through the epidermis of the mines to the leaf surface again. The present results show the effectivness of entomopathogenic fungi against larvae of endophytic leaf mining moths. The fungi could inhibit complete development of the larvae, thereby reducing generational propagation and overall pathogen load on horse chestnut tree.