Susceptibility of *Cameraria ohridella* to the entomopathogenic fungus *Lecanicillium muscarium* under outdoor conditions

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Abstract: The adaptation of the most strains of the fungus *Lecanicillium muscarium* ZARE & GAMS to moderate temperatures and humidity higher 80% as well as the sensitiveness of the spores to UV-radiation explains, that the application regime mainly takes place under protected conditions in greenhouses. Laboratory trials with a very effective strain for the use as biological control agent (strain V24, section Phytomedicine) were successfully carried out in the tritrophic system on *Cameraria ohridella* DESCHKA & DIMIC. Following trials showed the susceptibility of the host even under outdoor conditions. The application of the fungus as strain V24 as well as Mycotal® led to infection of the host and moulding of the fungus on the cadavers. Differences between the variants were detectable. The results were discussed.

Introduction:

The use of entomopathogenic fungi within the European Union is inhibited. Reasons of this situation are inter alia the development of biological procedures as well as processing of required documents for admission is more expensive than the expected profit. To race the incentive for commercial production of a very promising strain of the fungus *Lecanicillium muscarium* ZARE & GAMS from our section Phytomedicine (strain V24) we tried to enlarge the area of application. The effectiveness of the strain against important pest insects was already proofed under glasshouse conditions (Wolff 1998; Alavo 2001; Hetsch 2004; Meyer et al. 2005). Now we conducted trials to examine the ability of strain V24 for use under outdoor conditions. The horse chestnut leafminer moth *Cameraria ohridella* DESCHKA & DIMIC was chosen as a model object because in laboratory trials the susceptibility of the host to our strain was shown (Kalmus 2008).

Material and methods:

The trial took place on horse chestnut seedlings 3 years old. The fungus *L. muscarium* was sprayed as spore suspension Mycotal® or as strain V24, respectively. In different variants were tested several spore concentrations of the suspension, different intervals of application and the influence of an oil-containing adjuvant named Addit (Koppert, NL), which is used with Mycotal® in greenhouses to improve the effect of the fungus (tab.1). A water applied variant (control) was carried out to compare the results.

Each variant contained 12 seedlings and was settled with an initial population of *C. ohridella* (April 2008). The first application of the fungus took place on the 7th of May with 500 ml suspension per variant and was repeated at intervals of 7 or 14 days up to the 15th of September.

Table 1. Variants of the trial with application of *L. muscarium*

L. muscarium as:	Addit	Spore concentration per ml		Interval of application		Name of the variant
		$1{,}5x10^7$	$1{,}5x10^8$	7-days	14-days	
Mycotal®		x			x	My 14d
Mycotal®	x	x			x	MyA 14d
Strain V24		x		x		L7 7d
Strain V24	x	x		x		LA7 7d
Strain V24		x			x	L7 14d
Strain V24	x	x			x	LA7 14d
Strain V24			x		x	L8 14d
Strain V24	x		x		x	LA8 14d

On the 28th of June plastic bags with openings covered with fleece were put over leaves on the tree, one leave per bag, 3 leaves per variant. Three weeks later these leaves were picked up. The emerged adults from the bags were disinfected and incubated in wet chambers (20°C, 7 or 14 days). After transferring the leaves in water filled Erlenmeyer flasks they were put in plastic cages with openings covered with fleece. On the 5th of August all new emerged adults were incubated in wet chambers, too. The parameters of counting were: dead moths, dead moths moulding with *L. muscarium*, dead moths moulding with other fungi. When the emerging was finished all closed mines were opened and the viability, mortality and moulding of the juvenile stages of host were determined. Living insects and dead ones without moulding were incubated in wet chambers (20°C, 7 or 14 days).

Results:

The results proofed a direct impact of the fungus *L. muscarium* to the host population; the moulding rate of the emerged adults was different between the variants. Additionally the oil-containing additive showed a positive influence to the development of the fungus, which was plainest at the variants with Mycotal.

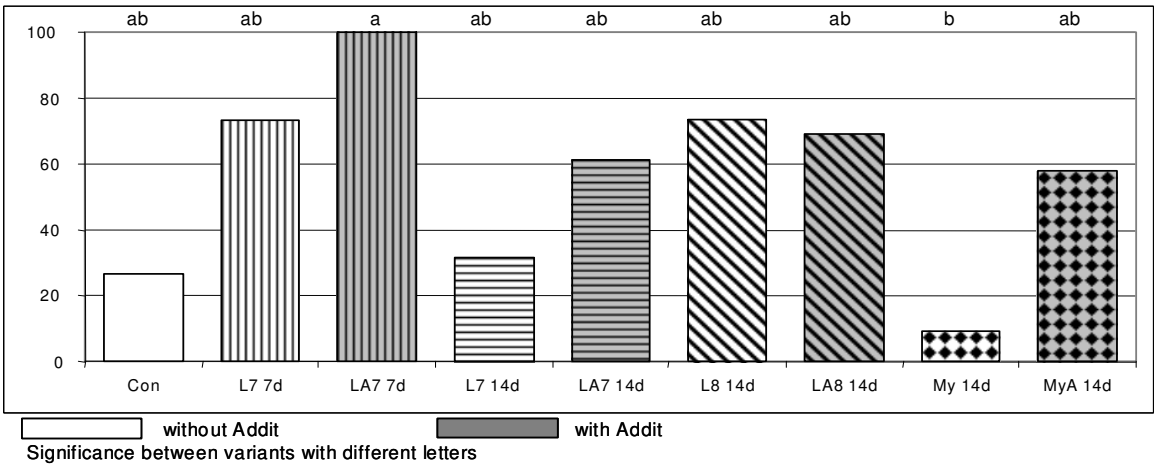

Figure 1. Moulding rate on the emerged adults of *C. ohridella* after application of *L. muscarium* on leaves of *Aesculus hippocastanum* L. and incubation

The effectiveness of the fungus could be detected on the juvenile stages within the mines, too. At the time of opening the mines differences between the variants were detectable (tab. 2). The highest rate of viability of the host could be observed in the control.

Table 2. Viability and mortality of endophytic stages of *C. ohridella* as well as moulding rate of *L. muscarium* on the host in the variants without incubation

Variants	Viability (%)	Mortality (%)	Moulding rate (%)	
			Other fungi	*L. muscarium*
Control	90,7	9,3	0,0	0,0
L7 7	30,0	33,3	0,0	36,7
LA7 7	4,2	33,4	0,0	62,5
L7 14	0,0	22,3	0,0	77,8
LA7 14	45,0	5,7	8,3	40,0
L8 14	19,6	8,5	3,7	68,3
LA8 14	60,4	4,2	0,0	35,4
My 14	75,0	16,6	8,3	0,0
MyA 14	22,2	55,8	0,0	22,2

The ability of the fungus, to infect the host within the mines was detectable in all fungal variants except My 14 (fig. 2).

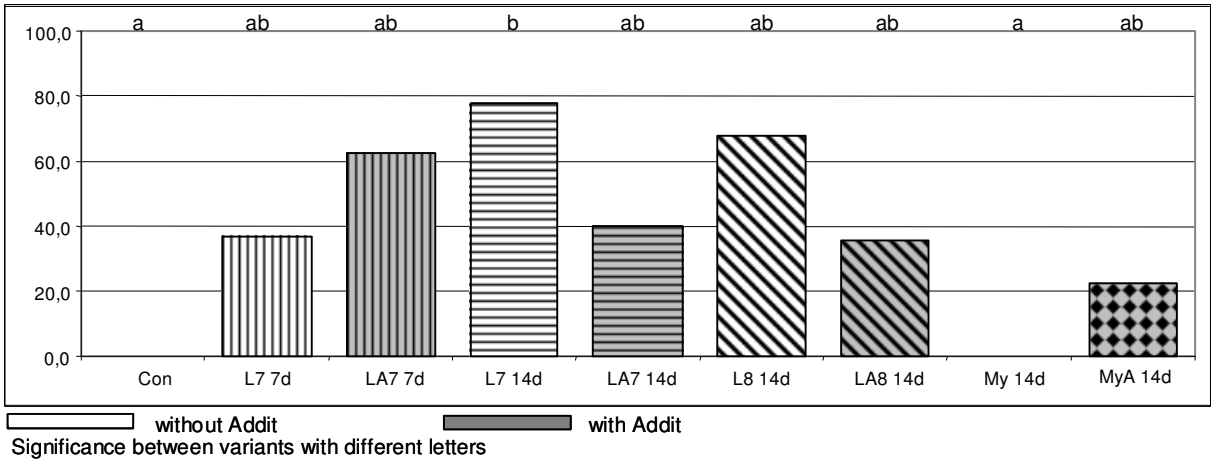

Figure 2. Moulding rate of *L. muscarium* on juvenile stages of *C. ohridella* in the mines on the leaves

After incubation of the pupae, the mortality of the fungus variants was significant higher, except My 14d, compared to the control. While *L. muscarium* was not detected in the control, the moulding rate in the fungus variants was between 40 and 78%. Even after incubation, the juveniles of the variant My 14d didn't showed any mycelia growth.

Discussion:

Usually the fungus *L. muscarium* is used in greenhouses. It is adapted to moderate temperatures and humidity above 80%. The spores are sensitive to UV-radiation. (Braga et al. 2000; Lee et al. 2006). Therefore the results from the outdoor trial are remarkable. Despite unfavourable conditions, the fungus was able to infect the hosts within the mines and led to a visible growth of mycelia and sporulation. The additive has a positive influence on the

effectiveness of the fungus. The reason for this isn't yet settled. The oily substance could be increasing the germination rate as well as the time.

The increasing of the spore concentration to the 10^{th} power didn't lead to a higher mortality. In former laboratory examinations the spores of strain V24 set up clusters in higher concentrated suspensions (Wolff 1998). In the case of Mycotal® such clusters were already found at concentration of $1{,}5x10^7$ sp./ml. Maybe the effective inoculum will be decreased by clusters. That could explain the similarity of the results of L7 14d and L8 14d as well as the low effectiveness of My 14d.

Even under unfavourable conditions the host *C. ohridella* is susceptible to the entomopathogenic fungus *L. muscarium* in the mines under outdoor conditions, too.

Further experiments follow.

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