Persistence of the entomopathogenic fungus *Lecanicillium muscarium* ZARE & GAMS under outdoor conditions

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Abstract: Positive results of laboratory trials, which proofed the effectiveness of the entomopathogenic fungus *L. muscarium* against endophytic larvae of the horse chestnut leafminer moth *Cameraria ohridella* DESCHKA & DIMIC, led to outdoor trials. One aspect of these trials was to determine the persistence of the fungus, which was used as commercial product Mycotal® (Koppert, NL) and as strain V24 from the section Phytomedicine. In different variants several spore concentrations and the influence of an oil-containing adjuvant (Koppert, NL) were tested. The persistence of the fungus was determined through the number of colony forming units (cfu) after impressing the leaves on agar plates. The fungus could be detected until 14 days past application (dpa), with differences between the variants, despite most unfavourable weather conditions, like above-average of temperature and hours with sunshine as well as low humidity and heavy rainfall. In all variants were found dead and moulding larvae within the mines.

Key words: *Lecanicillium muscarium*, entomopathogenic fungus, *Cameraria ohridella*, persistence

Introduction:

In former conducted laboratory trials, the effectiveness of the entomopathogenic fungus *L. muscarium* strain V24 (Section Phytomedicine) against endophytic larvae of horse chestnut leafminer moth *Cameraria ohridella* DESCHKA & DIMIC was proofed successfully. Larvae of the test population were infected and mouldy in there mines. The mortality was 100% (Kalmus 2008). To study the capacities of *L. muscarium* and the strain V24 in the field, a trial followed within the tritrophic system under outdoor conditions. The used strain V24, distinguishes itself by positive characteristics like effectiveness in a wide range of temperature as well as tolerance to low humidity conditions (Hetsch et al. 2005; Meyer et al. 2005; Lerche et al. 2008). The commercial product Mycotal® (Koppert, NL) was chosen to assess the results.

Among other aims of the trial, in this paper the persistence of the fungus on the plant surface will be presented.

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 (tab. 1) were tested several spore concentrations of the suspension 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.

Table 1. Variants of the trial

L. muscarium as:	Spore concentration per ml		Addit	Name of the variant
	$1,5x10^7$	$1,5x10^8$		
Mycotal®	x			My
Mycotal®	x		x	MyA
V24	x			L7
V24	x		x	LA7
V24		x		L8
V24		x	x	LA8

Each variant contained 12 seedlings and was settled with an initial population of *C. ohridella* in April 2008. The first application took place on the 7th of May with 500 ml suspension per variant and was repeated at intervals of 14 days up to the 15th of September. The determination of the persistence of *L. muscarium* took place between the 21st of June and the 5th of July 2008. One, seven and 14 days past application (dpa) the number of colony forming units (cfu) was counted after impressing leaves (12 leaves per variant and time) on plates with selective-agar for entomopathogenic fungi. To notice the influence of direct and indirect sunlight, upper and lower surfaces of leaves were separately examined. After incubation (5d, 20°C) the number of colony forming units (cfu) was counted and the average per cm^2 was determined. The average surface of the leaves conducted 30 cm^2, so the presented results were projected to this size.

The temperature and humidity were measured directly within the trial, the results from the duration of sunlight and precipitation are from a weather station in Berlin-Dahlem.

Results:

The temperature and duration of sunlight were above-averaged ([1], [2]). Therefore the humidity of air was amounted below 80% the most time. This was less than the required minimum for successful germination and development of entomopathogenic fungi. The fungus was not only exposed by the temperature, sunray and humidity but also by precipitation on 6 days.

The fungus could be detected on the plant surface until 14 dpa, with differences between the variants. Already one day past application My and MyA had fewer cfu as the variants of strain V24. In all variants the cfu decreased rapidly till 7 dpa. Remarkable is the following increase of the inoculum in the variants with V24 without the Addit.
An influence of direct or indirect sunlight was visible on the persistence of *L. muscarium*. One day past application the number of cfu from upper and lower surfaces were still similar. Seven days past application cfu were found more frequent on the lower surfaces. Only in LA8 was detected a delay of the decrease of inoculum.

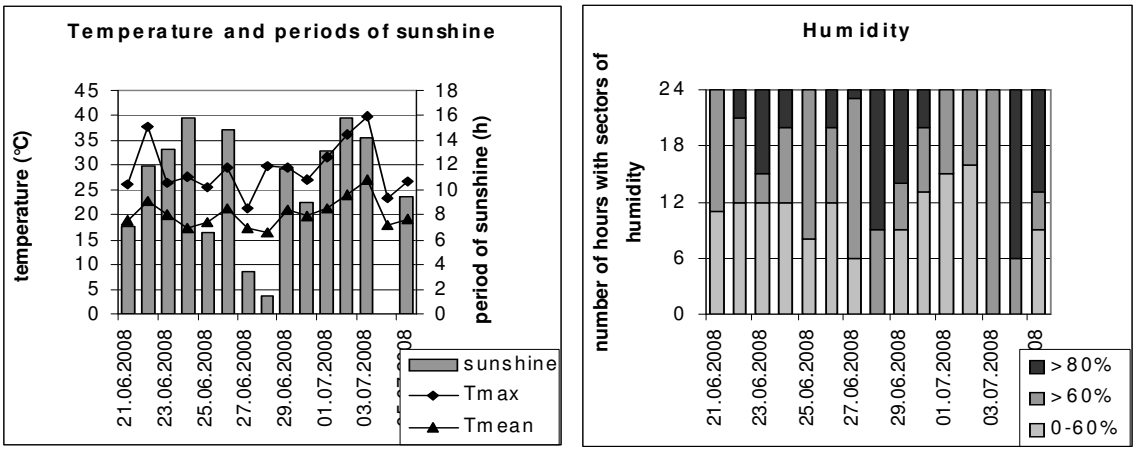

Figure 1. Temperature, period of sunshine and humidity during the carrying out of the test

Despite 14-day direct sunshine cfu were sporadically detectable in the variants L7, My and MyA but L8 and LA8 had a greater amount. At last, it was proofed through cadavers of the host moulding spontaneously with *L. muscarium* in all variants, that the development of the fungus under outdoor conditions was successful.

Conclusions:

The ability of the fungus *L. muscarium* to persist unfavourable weather conditions over a period of 14 days is very remarkable because radiation of UV-light leads to limited viability and low humidity delays the development of the fungus (Braga et al. 2002; Hetsch 2005; Lee et al. 2006) and through rain the fungus will be washed from the leaf (Lerche 2008). So the decrease of the inoculum over 14 days is explainable. Shadow seems to offer some kind of protection on the lower surfaces of the leaves.

The reason for the increase of cfu 14 dpa isn't yet clarified. It could results from a too small random sample as well as growing of the fungus on the cadaver of the host or saprophytic development of the fungus on the surface of the leaf.

The use of the Addit hasn't any positive impact to the persistence of *L. muscarium*. It contains oil and perhaps the negative influence of UV radiation could be amplified.

The spore concentration to the 10^{th} power didn't lead to more cfu during the test. Laboratory examinations show, that the spores of strain V24 set up clusters in higher concentrated suspensions (Wolff 1998). In the case of Mycotal® clusters were already found at concentrations at $1{,}5 \times 10^{7}$. Maybe the amount of inoculum will be decreased by clusters. That could explain the similarity of the variants of V24 as well as the small amount of cfu in the variants of Mycotal® from one day past application.

Even under outdoor and also in case of climatic unfavourable conditions, *L. muscarium* persists on the leaf and is able to germinate, infect and kill larvae of *C. ohridella* within their mines.

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