Investigations on alternative substances for control of apple scab - results from sanitation trials[1]

Untersuchungen zum Einsatz alternativer Stoffe zur Regulierung des Apfelschorfes Ergebnisse aus den Falllaubversuchen[1]

Pfeiffer B.[2], Alt S.[3], Häfner C.[4], Hein B.[2], Schulz C.[3], Kollar A.[3]

Abstract

The intention of this research project, which was supported within the "Bundesprogramm Ökologischer Landbau", was to look for alternatives in organic fruit growing to control apple scab, *Venturia inaequalis.* One important part of the investigations was the application of different substances like microbiological nutrient media, enzymes usually used for production of fruit juices and organic fertilizers on their effect on the ascospore potential on depots of fallen leaves. Some substances like TRYPTIC SOY BROTH increased the decomposition of the leaves significantly, other like BACTOFIL B and HUMOFIX showed nearly no effect on the decomposition, but reduced the ascospore potential in early spring by 80 % compared to the untreated control.

Keywords: Apple, organic fruit-growing, *Venturia inaequalis*, leaf decomposition, ascospore potential, TRYPTIC SOY BROTH, YEAST EXTRACT, BACTOFIL B, HUMOFIX

Introduction:

Venturia inaequalis is overwintering mainly on infected leaves - except for superficial scab - , which fall down to the ground in late autumn. During the winter pseuothecia are initiated, and asci with ascospores mature during early spring. Possibilities to reduce the ascospore potential are the shredding of leaves in autumn and spring, the removal of leaves by "vacuum cleaners", and the application of substances like Urea, which is not registered for organic fruit-growing, or the application of antagonists of *Venturia inaequalis* (see **literature cited**). The idea of the project was to find alternatives for leaf decomposition and to increase activity of earthworms respectively in order to reduce the ascospore potential.

Material and Methods:

First experiments for screening of suitable nutrient media and enzymes were started at BBA Dossenheim in March 2002. Leaf samples of 5 g were treated two times (at March 28th 2002 and at April 12th 1002). The titer of ascospores was determined according to Kollar (2000) on April 29th . The dried leaves of the samples were shaken with 50 ml aqua dest per g for 60 minutes at 100 revolutions per minute. The ascospores were enumerated in 0,5 ml Kolkwitz-Plankton-cytometer (Hydro-Bios, Germany). The total weight of the leaves was taken at the end exponation in the orchard.

In November 2002 two trials were started, one at BBA Dossenheim, the other at LVWO Weinsberg. In Dossenheim 30 different nutrient media in varying concentrations, partly in combination with pectin destroying enzymes (**table 1**) were tested on scabbed leaves. For each treatment aliquots of 70

[1] supported by the BMVEL within the "Bundesprogramm Ökologischer Landbau"

[2] BBA Dossenheim, Schwabenheimer Str. 101, D-69 221 Dossenheim/LVWO Weinsberg, Traubenplatz 5, D-74189 Weinsberg

[3] BBA Dossenheim, Schwabenheimer Str. 101, D-69 221 Dossenheim

[4] FH Weihenstephan, Institut für Obstbau, Am Staudengarten, D-85 350 Freising-Weihenstephan

g dried leaves were exposed in the orchard in small sowing-boxes without contact to soil. The leaves were treated on November 11th 2002, December 4th 2002, January 17th 2003, March 10th 2003 and April 8th 2003. Samples for determination of ascospore potentials were taken between March 5th 2003 and April 30th 2003 every fortnight. Total weight of all the samples was removed and determined on April 30th.

The experiment in Weinsberg was started in cooperation with the FH Weihenstephan as diploma thesis of Häfner (2003). 9 treatments (see **table 1**) were compared with an untreated control. Each treatment was replicated three times, every leaf depot started with 170 g heavily scabbed dried infected leaves collected from the control of a scab trial in Katzental, the organically grown orchard of LVWO Weinsberg. The depots had contact to the soil and were sprayed on December 12th 2003, February 10th 2003, March 21st 2003, April 18th 2003 and May 11th 2003. The portable burning device TR 111 (produced by REINERT) was only used twice (December 18th 2002 and February 27th 2003). In spring 2003 mixed samples were taken out from every depot at 13 days between March 5th and May 23rd and the ascospores were counted according to the method of Kollar (2000). At the end of the experiment on May 23rd the weight of the remaining leaves was determined. In Weinsberg the weather 2003 was extremely dry, from February to May about 60 mm rain less then the average fell.

Table 1: Enzymes, media and concentrations in the leaf decomposition trials 2002/2003 in Dossenheim and Weinsberg

Dossenheim*	**Medium**	**Weinsberg**
water control		Untreated control
PDB 3x	POTATO DEXTROSE BROTH (Difco)	Nettle dung water 3 % ("Brenn")
ME 3x	Malt extract (Difco)	Vinasse 3 %
TSB 3x	TRYPTIC SOY BROTH (Sigma)	HUMOFIX 0,01 %
YE 3x	Yeast extract (Difco)	Demeter-Malt-extract 3 %
MS 3x + 0,5 % glucose	MURASHIGE AND SKOOG MEDIUM (Sigma)	ROHAMENT PL 1 %
NB 3x	NUTRIENT BROTH	BIOSTIMULATOR 0,2 %
Glucose 6 %		BACTOFIL B 2 %
Saccharose 6 %		DIGESTER (conv.) 0,4 %
MS - ohne Glucose 3x	MURASHIGE AND SKOOG MEDIUM (Sigma)	Burning device ("Abfl.")
ROHAMENT PL 10 %	Pectinase preparationj	
PDB 3x + ROHAMENT PL 1%		
ME 3x + ROHAMENT PL 1%		
TSB 3x + ROHAMENT PL 1%		
YE 3x + ROHAMENT PL 1%		
MS 3x + ROHAMENT PL 1%		
NB 3x + ROHAMENT PL 1%		

* "3x": triple concentration of usual concentrations for nutrient media in microbiology

Nettle dung water and Vinasse normally are used as leaf or soil fertilizers, HUMOFIX supports the process of composting, BIOSTIMULATOR and BACTOFIL B are supposed to activate the soil microorganisms. DIGESTER was chosen as a conventional control. Use of burning device is common in organic vegetable-growing.

Results:

No differences in decomposition of the leaves by the two treatments could be detected in the screening trial at BBA Dossenheim in spring 2002. The evaluation of the ascospore dose on April 29th showed a reduction of the ascospore potential of 30 to 40 % by Rohament PL, YE and TSB 3x, of 44 % by ME 3x and of 54 % by PDB 3x. The combination of NB + 0,5 % pectinase + 0,5 % cellulase achieved the best effect.

Due to the dry weather in spring 2003 the leaf decomposition in the orchards was retarded until the rain-period in the beginning of May. Obvious differences between some treatments and the untreated control could already be detected in Dossenheim at the beginning of March and there were no further changes up to end of the experiment on April 30th. There was no regular positive correlation between the decay and the reduction of ascospore potential. 15 from 16 tested media showed a clear reduction of the ascospore potential (**figure 1**). The greatest effect was seen at the last date. The most effective medium was TRYPTIC SOY BROTH (TSB), which reduced the potential to 7 % of control.

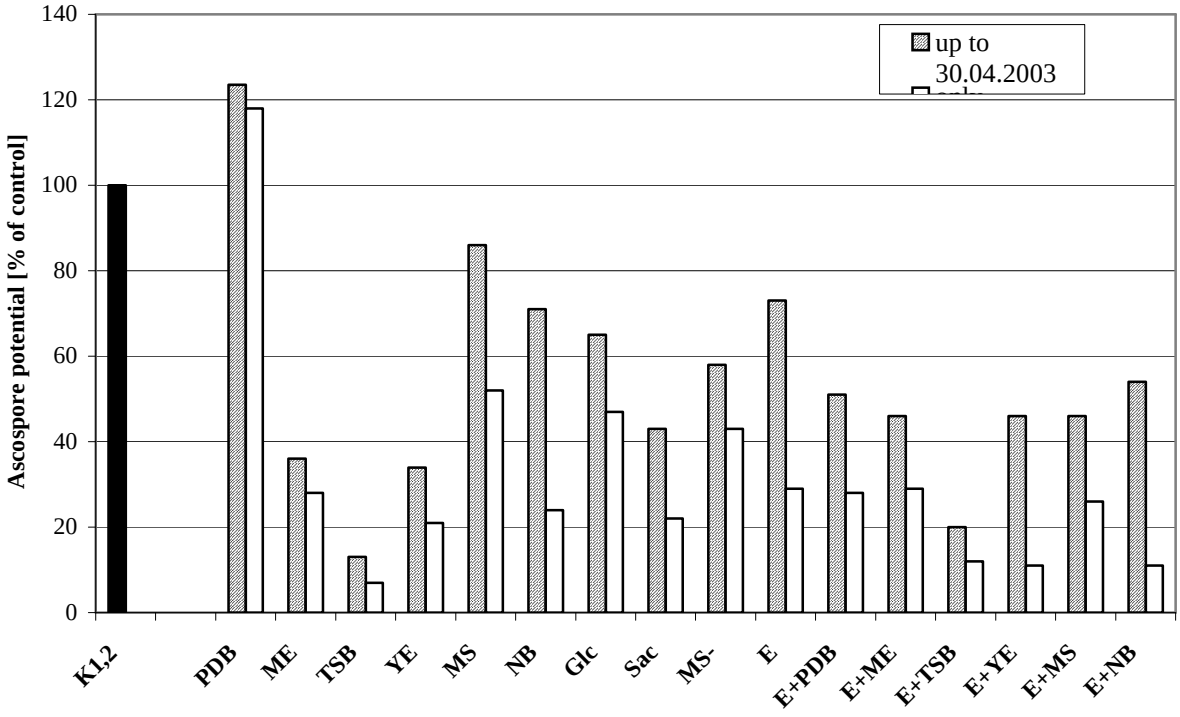

Figure 1: Leaf decomposition trial 2002/2003 Dossenheim. Cumulated ascospore potential (//////) in % of control (black) up to April 30th . White: results of April 30th. "E": addition of enzyme Rohament PL

In contrast to the results from Dossenheim reductions of the ascospore potential could already be seen in Weinsberg at the first evaluation dates. At the best treatments (**figures 2 and 3**) the ascospore potential was low during the whole time of the experiment. 50 % reduction of the cumulated

ascospore potential was caused by the treatments of Demeter malt extract, about 80 % by HUMOFIX and BACTOFIL B (**figure 4**). Only small differences could be found between HUMOFIX, BACTOFIL B and DIGESTER (conv.) or burning device ("Abfl.").

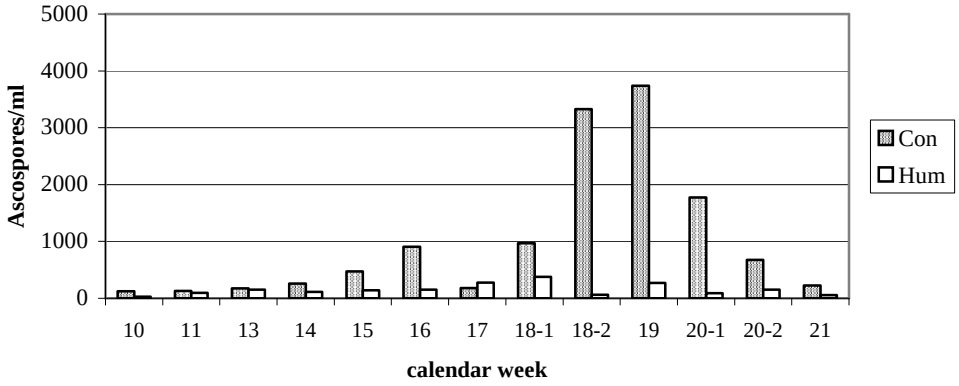

Figure 2: Ascospores/ml of untreated control in comparison with HUMOFIX

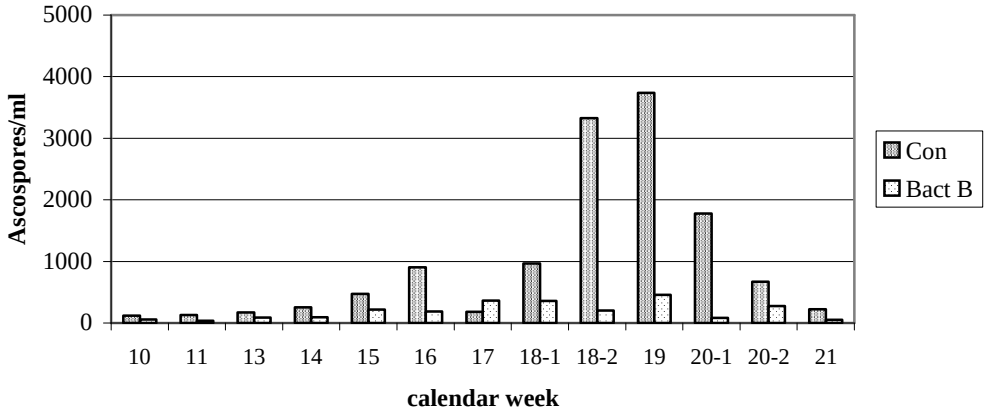

Figure 3: Ascospores/ml of untreated control in comparison with BACTOFIL B

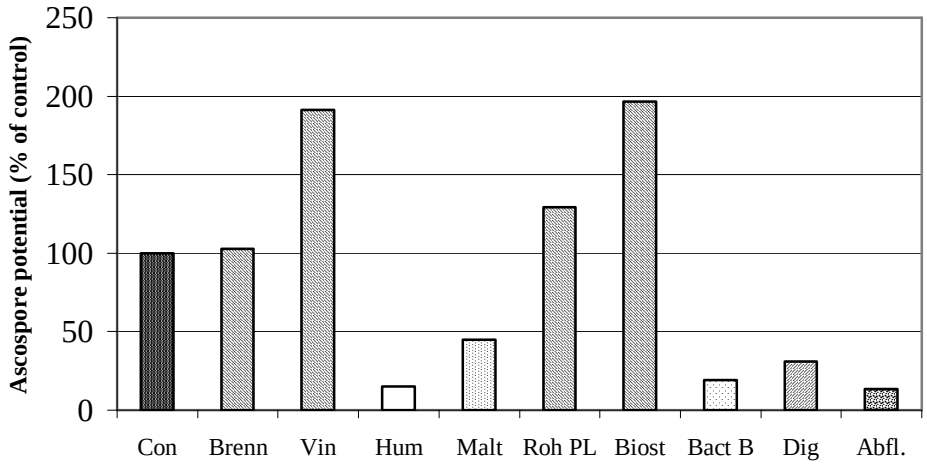

Figure 4: Leaf decomposition trial in Weinsberg 2002/2003. Cumulated ascospore potential in % of the control (black), cumulation of 13 dates up to May 23rd.

On the other side treatments like fermented water extract from *Urtica sp.* ("Brenn" in **Fig. 4**), Vinasse, Rohament PL and Biostimulator seemed to increase the cumulated ascospore potential. There were no effects on the weight of the leaves.

Discussion

Reducing ascospore potential on fallen leaves as primary inoculum for scab infections in spring will be very important in future. This is especially the case under the circumstances of uncertain development of registration of plant protecting substances and their availability for the organic fruit growers in Germany (e. g. sulphur and copper). The presented results based on one ascospore season under very dry weather conditions in March and April 2003. Nevertheless there are promising possibilities, if they can be reproduced in the experiments on frequency and optimal application date started in November 2003 at Dossenheim and Weinsberg. In comparison to methods where the fallen leaves are removed by big "vacuum cleaners" from the orchard, the treatment of leaf litter can be done with simple available techniques. Use of burning device showed a high efficacy, however it needs a lot of energy and damages of the bark of the trees cannot be excluded. Calculations of Häfner (2003) showed, that the advantage to the fruit-grower exceeds the costs, if the marketable yield (apples without scab) is increased to about 5 % by effective reduction of ascospore potential.

Literature Cited (Selection)

Creemers, P. (2002): Sanitation practice to reduce apple scab inoculum in orchards. In: 6th International IOBC/WPRS Workshop of Pome Fruit Diseases.

Golba, B. (2001): Alternativen zum Einsatz von kupferhaltigen Präparaten im Apfelanbau, Ergebnisse einer Literaturrecherche im Auftrag der BLE, TU-München, Fachgebiet Obstbau.

Häfner, Claudia (2003): Untersuchungen zur Wirkung von Falllaubbehandlungen im Ökologischen Apfelanbau auf die Abbaugeschwindigkeit und den Verlauf der Ascosporenreife des Apfelschorfes Venturia inaequalis (Cooke) Winter. Diplomarbeit FH Weihenstephan.

Kollar, A. (2000): A waterbath method for detection of potential ascospore discharge of *Venturia inaequalis.* Bulletin IOBC wprs, Vol.23 (12), 53-60.

Krüger, E., Rasim, S., Rehmet, T., Zwermann, P. (2000): Reduzierung des Ascosporenpotentials von *Venturia inaequalis* durch Förderung der Falllaub-Zersetzung. In: 9. Internationaler Erfahrungsaustausch über Forschungsergebnisse zum Ökologischen Obstbau. Fördergemeinschaft Ökologischer Obstbau e.V., Weinsberg.

MacHardy, W. E.(2002): A scab-risk/ mechanical sanitation action threshold to reduce fungicide input based on 90 % reduction of ascospore dose. In: 6th International IOBC/ WPRS Workshop of Pome Fruit Diseases.