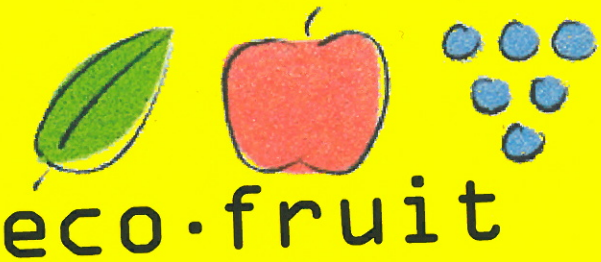
Proceedings of the 17th International Conference on Organic Fruit-Growing

from February 15th to February 17th, 2016
University of Hohenheim, Germany



Edited by
Foerdergemeinschaft Oekologischer Obstbau e.V.

Patronage: IFOAM EU Group

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However, the study was conducted on the cultivar Cripps Pink, and it is well-known that in organic farming, on this cultivar, apple scab control is extremely challenging.

It must be pointed out that only a limited number of products that have been developed within the research project, has also been tested in the open field. The likelihood exists that at least some of the tested new products did not provide adequate disease control in the open field due to the fact that formulations were not advanced enough by the time of testing. For other products, companies were not able to up-scale production in time, and thus to produce the amounts required for practically relevant field trials(i.e. applications with spray equipment, very similar to that commonly used by growers). In some trials, CO-FREE agents showed promising and comparable results to copper (CoFree A2, CoFree 24-2 and CoFree19/19), but further development appears necessary for final use in practice. Finally, The sector Organic Farming of the Research Centre Laimburg and ITAB/GRAB are still willing and interested in evaluating new plant protection products for the control of fungal diseases, and in developing new methods and tools (including cropping systems and cultivar susceptibilities), which allow for a reduction in the use of copper compounds.

Acknowledgements

This project has received funding from the European Union´s Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 289497.

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Effect of Strategic Irrigation on infection of Apple scab (*Venturia inaequalis*)

M. Korsgaard[1]

Abstract

*Strategic irrigation is a method to prevent Apple scab (*Venturia inaequalis*). It is performed by irrigating the orchard floor in dry periods during spring, 24 hours before rain forecast. Irrigating the old leaves on the orchard floor will elicit the release of ascospores, but due to the dry trees and the dry weather, the ascospores dry out without causing infections.*

The method relies on the occurrence of dry periods during the period of primary infection from April to mid-June.

Experiments were carried out at the University of Copenhagen and in an unsprayed Danish organic orchard in 2014 and 2015. Apple scab on fruit and leaves was reduced by strategic irrigation in 'Elshof' at the University field in both years. In the organic orchard the strategic irrigation reduced the infection of leaves by apple scab and Elsinoe leaf and fruit spot in some cultivars and some years.

Studies of the ejection of ascospores took place in 2015 using a glass slide trap. The reaction of ascospores to rain and to the amount of irrigated water was studied.

Keywords: *Venturia inaequalis*, ascospore release, strategic irrigation, prevention, organic apple production.

Introduction

The acreage of organic apples in Denmark 2014 was 327 ha, which is 21.6 % of the total apple area in Denmark (Ministeriet, 2015). App. 57 % of the Danish organic apple area is cultivated without fungicides (Pedersen, 2013). Apple scab (*Venturia inaequalis* (Cke.) Wint.) is a major problem in organic apple production and methods to prevent apple scab is a big desire among all apple growers but especially the growers who have chosen not to spray with fungicides.

Strategic Irrigation is a new method to prevent apple scab. It was inspired by the dry springs in 2008 and 2009, where apple scab became a minor problem in Denmark due to the dry spring and short showers of rain depleting the stock of ascospores.

Strategic irrigation is carried out by imitating rain in dry periods during spring, when many ascospores are ripe, and the weather forecast promises at least 24 hours with air humidity below 85 % RH. Ejection of the ripe ascospores is provoked by irrigating the old leaves at the orchard floor. Due to the dry tree, the ascospores cannot infect the leaves, but will dry out without infecting. The irrigations are repeated during spring before rain forecast, thus depleting the stock of ripe ascospores that eventually would be ejected during rain.

From 2011 to 2015 the Strategic Irrigation method has been tested in Denmark. The results from 2011-2013 indicated, that it is possible to reduce the infection of scab by strategic irrigation. In the best case, five irrigations during spring reduced the percentage of apple scab on fruits of 'Rubens' from 47 % to 12 %. In other cases though, there were no significant effect of the irrigation, thus the method has potentials, but needs improvement (Korsgaard, 2014).

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Material and Methods

The strategic irrigation was carried out in 2014 and 2015 at two locations and in 5 apple cultivars.

One location was at the University of Copenhagen, the "Pometum" in Taastrup in East-Sealand in the apple cultivar 'Elshof'. The second location was at an organic apple orchard "Lærkehøj" in Regstrup in West-Sealand in the cultivars 'Collina', 'Red Aroma', 'Alkmene' and 'Holsteiner Cox'.

The orchard floor at both locations was irrigated by sprinklers, mounted app. 0.6 m above the ground in the row. The sprinkler type was "Spin-Net UD 160/90 l/h", which spread the water horizontally, only irrigating the orchard floor. The irrigated plots were 35 meter of row in 6 rows at the location Lærkehøj. The irrigated plot at the Pometum was 21 m row. In both locations the irrigated and untreated plots were divided by 7 m of irrigated row.

Climate-data was sampled at both locations with a Davis Weather station. The time of irrigation was decided by using the scab warning model Rimpro at www.fruitweb.info and the weather forecast, primarily the www.yr.no.

Table 1: Irrigation time, duration and amount of water at two trial locations in 2014 and 2015.

Location	Date of irrigation	Time of irrigation	Minutes of irrigation	Mm water at each irrigation
POMETUM	2014.04.02	13:00	7	0.8
		14:00	7	0.8
	2014.04.25	08:05	5	0.6
		09:05	5	0.6
		09:25	10 + rain	1.2 + 0.2 mm rain
	2014.05.02	08:35	10	1.2
		09:35	10	1.2
		14:00	10	1.2
	2015.04.23	10:20	10	1.4
		11:20	10	1.4
	2015.05.15	09:40	10	1.4
		10:40	10	1.4
		11:13	10	1.4
LÆRKEHØJ	2014.04.02	15:00	10	0.8
		16:00	10 + rain	0.8 + 0.4 mm rain
	2014.04.20	06:45	10	0.8
		07:30	10	0.8
	2014.05.03	14:30	10	0.8
		15:45	10	0.8
	2015.04.23	12:40	10	0.8
		13.45	10	0.8
	2015.05.15	07:45	10	0.8
		08:50	10	0.8

It was decided to irrigate, when the following three conditions were fulfilled:

- Rimpro shows a minimum of app. 5 % ripe ascospores
- Dry weather with RH< 85 % is forecasted for the following 24 hours
- Rain is forecasted afterwards

The irrigations took mainly place in the morning, taking advantage of the morning dew. It was irrigated twice with an interval of one hour. The amount of water and the duration of the irrigations differed between years and locations (table 1). In 2014 the weather permitted 3 possibilities for strategic irrigation, while in 2015 it was possible only twice.

Sampling of data

In June, right after the end of the primary infection period of apple scab, the leaves of the apple trees got evaluated. The percentage of infected leaves was registered at respectively the lower and upper part of the tree, divided at app. 1.50 m height above ground. This division was made to detect a suspected difference in the infection pattern in the tree.

At the location Lærkehøj, the orchard suffers from attack of Elsinoe leaf and fruit spot (formerly "Topaz spot"), therefore the attack of this disease was registered as well as apple scab.

The fruits of 'Elshof' were evaluated in December in 2014 and 2015. They were sorted in two classes: saleable and non-saleable (</> 1cm² of scab spots pr. fruit).

Ascospore counts

Studies of the release of ascospores took place in 2015 using 32 glass slide traps. The traps were placed on the ground at 1m´s distance from the row with sprinklers. The traps consist of a layer of scab infected apple leaves, held firmly by a mesh. Glass slides are put on top of this mesh at fixed positions. After rain or irrigation the slides were changed after a minimum of 6 hours after wetting. The slides were examined in a microscope and the number of ascospores was counted. The same section of the slides was examined after each rain-event, picturing the ejection of ascospores from the pseudothecias placed under this slide. At the end of the season, the total number of ejected ascospores from each slide was calculated, and every ejection event was recalculated as a relative ejection: The proportion of the total ejection at this position during the whole season.

Statistical methods

The effect of strategic irrigation on leaves was modelled using a linear mixed model with block as random effect. Outcome was square root transformed before analysis. Estimates and confidence intervals were back-transformed suing the method reported in Laursen *et al.* (2014). The effect of strategic irrigation on fruit were analysed using a logistic regression. All of the analysis was made using open source statistical software R version 3.2.2.

While the data was not normally distributed, the data is presented as medians with inter quartile ranges (IQR).

Results

At the location "Pometum", at the University field in Taastrup, the strategic irrigation had a significant reducing effect on apple scab on both leaves and fruit in 'Elshof' in 2014 and 2015.

The infection level in 2014 was between 50-80 % infected leaves. In 2015 it was much less, between 1-15 % infected leaves (table 2).

The reduction of the infection of leaves was 11.1 percentage points in the upper part of the trees and 16.9 percentage points at the lower part of the trees. On fruits there was a significant but small reduction of discharged apples due to apple scab of 0.7 percentage points (table 3).

Table 2: Data on the effect of strategic irrigation on leaves and fruits of 'Elshof' at the location Pometum in 2014 and 2015. The infection on leaves was evaluated in June on fruits in Dec.

			% leaves with apple scab in lower part of tree in June		% leaves with apple scab in upper part of tree in June		% discharged fruits in December	
Year	Variety	Treat-ment	Median	IQR1-3	Median	IQR 1-3	Median	IQR 1-3
2014	Elshof	Control	80	70-80	50	40-55	36.8	34.3-42
2014	Elshof	Irrigated	60	50-70	30	20-40	26.1	19.4-31
2015	Elshof	Control	10	4.5-15	2	0-2,25	19.7	10.6-31.4
2015	Elshof	Irrigated	2	1-4	0	0-0	18.5	7.7-33.9

Table 3: Statistic estimate of the effect of strategic irrigation on infection of apple scab on leaves and fruits of 'Elshof' in 2014 and 2015. All estimates are significant at 95 % level.

Effects of strategic irrigation	Estimate	Lower	Upper
Percentage points of scab in lower part of the tree	-16.9 %	-27 %	-6.8 %
Percentage points of scab in upper part of the tree	-11.1 %	-21.4 %	-0.9 %
Percentage points discharged apples	-0.7 %	-0.9 %	-0.5 %

At the unsprayed organic fruit orchard "Lærkehøj" in Regstrup, the level of infected leaves by apple scab was very low in both years, only the cultivar 'Collina' , which is not scab resistant in Denmark, showed a substantial level of scab of 25 % in 2015 (table 4).

The strategic irrigation reduced significantly the infection of apple scab on the lower leaves of 'Alkmene' and 'Red Aroma' in year 2015. The reduction was 4.4 percentage points in 'Alkmene' and 1.4 percentage points in 'Red Aroma'. In both years and in all tested cultivars there was a small but significant reduction of scab in the upper part of the tree of 0.44 percent points. Surprisingly the strategic irrigation also had a significant reducing effect on infections of Elsinoe leaf and fruit spot ("Topaz spot") in 2015. This effect was found in all cultivars tested: 'Collina', 'Red Aroma','Holsteiner Cox' and 'Alkmene' (table 5).

Table 4: Data on the effect of Strategic Irrigation on % leaves with apple scab and Elsinoe leaf and fruit spot in the cultivars 'Collina', 'Red Aroma', 'Holsteiner Cox' and 'Alkmene'. At the unsprayed organic apple orchard Lærkehøj 2014 and 2015.

			% leaves with apple scab in lower part of tree in June		% leaves with apple scab in upper part of tree in June		% leaves with Elsinoe leaf and fruit spot in June	
Year	Variety	Treat-ment	Median	IQR 1-3	Median	IQR 1-3	Median	IQR 1-3
2014	Collina	Control	3.5	0-15	0	0-5	30	18.8-52.5
2014	Collina	Irrigated	2	0-5	0	0-0	17.5	5-31.3
2015	Collina	Control	25	22.5-36.3	12.5	10-25	40	15-70
2015	Collina	Irrigated	25	15-36.3	10	5-30	10	5-22.5
2014	Red Aroma	Control	0	0-2	0	0-0	5	5-10
2014	Red Aroma	Irrigated	0	0-0.3	0	0-0	5	2-6.3
2015	Red Aroma	Control	1	1-2	1	1-1.5	2	2-4
2015	Red Aroma	Irrigated	1	0-1	0	0-1	2	1-3
2014	Holst. Cox	Control	0.5	0-2	0	0-0	2	1-2
2014	Holst. Cox	Irrigated	0.5	0-1	0	0-0	1	1-1
2015	Holst. Cox	Control	2	1-4	0	0-2	2	1-3
2015	Holst. Cox	Irrigated	2	1-2	0	0-1	1	1-2
2014	Alkmene	Control	0	0-1	0	0-0	5	2-20
2014	Alkmene	Irrigated	0	0-1.3	0	0-0	2	1.8-10
2015	Alkmene	Control	4	2-10	2	0-3	1	1-4
2015	Alkmene	Irrigated	1	0-1	0	0-1	1	1-2

Table 5: Statistic estimate of the effect of strategic irrigation on infection of apple scab on leaves of 'Collina', 'Red Aroma', 'Holst. Cox' and 'Alkmene' in 2014 and 2015 at the location Lærkehøj. Estimates marked with * are significant at 95 % level or more.

Effect on:	Cultivar	Year	Estimate	Lower	Upper
Percentage points of leaves with scab in low part of tree	Collina	2014	-1.12	-3.78	0.99
Percentage points of leaves with scab in low part of tree	Collina	2015	-0.48	-3.10	2.15
Percentage points of leaves with scab in low part of tree	Red Aroma	2014	-0.68	-2.2	0.86
Percentage points of leaves with scab in low part of tree	Red Aroma	2015	-1.46*	-2.84	-0.08
Percentage points of leaves with scab in low part of tree	Holst. Cox	2014	-0.13	-2.75	2.50
Percentage points of leaves with scab in low part of tree	Holst.Cox	2015	-0.93	-3.28	1.43
Percentage points of leaves with scab in low part of tree	Alkmene	2014	0.23	-2.40	2.86
Percentage points of leaves with scab in low part of tree	Alkmene	2015	-4.40*	-6.76	-2.06
Percentage points of leaves with scab in upper part of the tree	Collina, Red Aroma, H. Cox and Alkmene	2014 and 2015	-0.44*	-0.64	-0.24
Percentage points of leaves with Elsinoe leaf and fruit spots	Collina, Red Aroma, H. Cox, Alkmene	2014	-0.31	-0.77	0.16
Percentage points of leaves with Elsinoe leaf and fruit spots	Collina, Red Aroma, H. Cox and Alkmene	2015	-0.66*	-1.09	-0.24

Ascospore counts

The pattern of ejection of ascospores was followed at the Pometum location in 2015 by counting ascospores in a glass slide trap. It showed that after one session of irrigation with 2 times 1.4 mm within 2 hours, the stock of ascospores was depleted. (Table 6)

When no irrigation took place, a number of ascospores corresponding to 12.4 % of the total stock of the season was ejected after the following 12 mm of rain.

After one strategic irrigation of 1.4 mm the stock of ascospores got depleted, and only 4.0 % of the total stock of ascospores was ejected after the following 12 mm of rain.

After two strategic irrigations with a total of 2.8 mm, the stock of ascospores got even more depleted and only 0.6 % of the total stock of ascospores was ejected after the following 12 mm of rain.

After three strategic irrigations with a total of 4.2 mm, the stock of ascospores got depleted at almost the same level as after irrigation with 2.8 mm, to 0.7 % of the total stock of ascospores. (Table 6) These observations suggest, that an optimal effect of Strategic irrigation is obtained with an amount of irrigation water between 1.4 and 2.8 mm.

Table 6: Percentage of ejected ascospores of apple scab succeeding strategic irrigation the 15th of May. Average of 4 slides counted the 22th of May 2015 after 12 mm of rain.

Ejected ascospores (percentages of total season) after 7 days of rain (12 mm) Control, not irrigated.	12,4 %
Ejected ascospores (percentages of total season) after 7 days of rain (12 mm), but with one strategic irrigation of 1.4 mm the 15th of May.	4,0 %
Ejected ascospores (percentages of total season) after 7 days of rain (12 mm), but with two strategic irrigations, 2 x 1.4 mm the 15th of May.	0,6 %
Ejected ascospores (percentages of total season) after 7 days of rain (12 mm), but with three strategic irrigations, 3 x 1.4 mm the 15th of May.	0,7 %

Discussion

The Strategic Irrigation method relies on the climate during spring. It is necessary to have longer periods of dry weather to find optimal moments to irrigate. In 2014 this was easy, while the spring in Denmark was very dry, and gave three very good opportunities to irrigate. In 2015 the wet spring only gave two opportunities to irrigate. But even in 2015, the two irrigations proved to have a significant reducing effect on apple scab in the cultivars 'Elshof', 'Alkmene' and 'Red Aroma'. This suggests that strategic irrigation is a useful tool even in more wet seasons. But Strategic irrigation is also a tool, which cannot stand alone, while the effect in some cases is rather small.

The amount of water used in the trials differed between locations. The rather low amount of water (2 x 0.8 mm) used for irrigation in the organic orchard was decided on what was possible to bring out with the local water pressure within 2 hours. This was a compromise, while "windows" with dry weather with 24 hours of RH < 85 % are rather few, and therefore the period of the treatment should not be very long. According to the counts of ascospores, the amount of water should be increased, to increase the effect.

At the Pometum location, the orchard floor was irrigated with larger amounts of water from 2 times 0.8 mm to 3 times 1.4 mm, which resulted in larger reducing effects on infection of apple scab on leaves.

The infection level in the upper part of the tree was in all cultivars and both years significantly reduced by strategic irrigation. This could indicate that the irrigation causes a delay in the secondary conidia infection cycle.

Suggestions to improve the method:

The period for using strategic irrigation could be extended by adding irrigations, also in late winter. In 2014 some of the ascospores were released before bud break. This is a favourable situation, while these spores miss their chance to infect. Results from a study in southern France indicate, that rain events above 2.5 mm of rain in late winter accelerate the maturation of ascospores, thus the first release of ascospores occurs earlier after more rain events during winter (Roubal & Nicot, 2015). This suggests that irrigating the orchard floor in late winter could accelerate the season of ascospores, resulting in more wasted ascospores before bud break.

In future trials, the amount of irrigated water should be raised.

Acknowledgements

This work is part of the GUDP-project ProtecFruit, performed in 2014-2017 thanks to the grants from the Ministry of Environment and Food. This work was carried out with the help of the employees at the Pometum, the statistic expert Signe Marie Jensen, the fruit grower Mikael M. Andersen and my colleagues at the section of Crop Science in the department of Plant and Environmental Sciences at the University of Copenhagen.

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Yeast extract applications to reduce the primary ascospore inoculum of *Venturia inaequalis*

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Abstract

Ascospores of Venturia inaequalis, *released from pseudothecia on overwintered infected apple leaves, are the primary inoculum of the pathogen. In this study, a new sanitation strategy to reduce the ascospore inoculum was tested under orchard conditions during four overwintering periods (2011-2015). Apple scab infected leaves were collected at the beginning of leaf fall. Leaves were exposed in plastic trays (JKI Dossenheim) or on the soil (LVWO Weinsberg) and protected by a wire mesh. The leaf litter depots were treated up to four times in late autumn and winter with yeast extract preparations. In all four years the potential ascospore inoculum was reduced. Fourfold 30 % or 60 % yeast extract treatments were the most effective and reduced the ascospore discharge to 99-100 %. Twofold application of a 30 % yeast extract revealed a similar efficacy. Analyses of biological oxygen demand in the leaf litter depots indicated an increased microbiological activity. Additionally, the treatments enhanced the attraction of leaf litter for earthworms, which led to an accelerated ingestion rate. Leaves treated three times with a 6 % yeast extract or two times with a 10 % or 20 % yeast extract were removed completely until the beginning of ascospore maturity in 2013-2015. By comparison, up to 28 % of the untreated leaves were not ingested. The results demonstrated that the performed leaf litter treatments with yeast extracts reduced the primary ascosporic inoculum almost completely, and thus might contribute to a better management of the disease. Obviously, apple scab control was achieved by enhanced microbiological and earthworm activity.*

Keywords: apple scab, sanitation, biological control, sustainable fruit production, copper replacement

Introduction

Apple scab, caused by the fungal pathogen *Venturia inaequalis*, is the economically most important apple disease worldwide (MacHardy, 1996). Scab management in organic fruit growing may require 30 treatments per season depending on weather conditions and disease pressure. In spring, infection is initiated mainly by ascospores released from pseudothecia on overwintered infected apple leaves (Hirst & Stedman, 1962a, b). In integrated fruit production the application of urea 5 % at the beginning of leaf fall is the standard method to reduce the overwintering inoculum of *V. inaequalis*. The application of urea accelerated leaf decomposition significantly (Jones & Aldwinckle, 1990), apparently due to quantitative shifts in microbial populations (Crosse *et al.*, 1968; Burchill *et al.*, 1965). Furthermore, the softening and degradation of leaf litter resulted in increased earthworm activity (Helling & Larink, 1998; Wright, 1972). However, in European fruit growing areas, urea is not registered for organic production. The aim of this study was to develop an alternative sanitation treatment with yeast extract preparations to suppress pseudothecial development and/or limit the discharge of acsospores of *V. inaequalis* by promoting leaf inhabiting microorganisms. The treatments should also stimulate earthworm activity to accelerate the leaf decay.

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ISBN 978-3-9804883-7-2