

Control of *Venturia inaequalis* (Apple Black Spot) with biostimulants Digester, Mycorrcin, Foliacin

Karin Watson BioCult Cambridge

March 2000

Key words

Apple Black Spot, Digester, Mycorrcin, Foliacin

Overview

A number of biological fermentation products from Bio-Start Ltd. were tested for potential activity against apple black spot (*Venturia inaequalis*), *in vitro* as well as *in vivo* on detached leaves. Two of the test products were additionally investigated on apple trees in the field. The results show that these fermentation products can provide a highly active biological treatment against apple black spot in the field.

Materials and methods

In-Vitro Tests

Compound Application

Using a sterile cork borer, ca. 0.8 mm wide holes were punched out from the center of fresh potato dextrose agar (PDA) plates. These holes were filled with the test solutions, using ca. 0.3 ml in each hole. Then the test solutions were allowed to diffuse into the agar for ca. 2 hours.

Compound application was conducted either as a protectant treatment (before inoculation), or as a curative treatment (after inoculation).

Two (curative test) to three (protectant test) replicate plates were prepared per treatment.

Fungal Culture

A stock culture of *Venturia inaequalis* (isolated from apple leaves from Gisborne) was grown on potato dextrose agar (PDA) plates in near UV light (16 hours day) at 20 degree Celsius.

Inoculation

Stock plates were flooded with sterile water and the conidiospores were dislodged by gentle scraping and shaking. Using sterile water, the inoculum was then adjusted to a spore density of ca. 250000 spores per ml.

This inoculum was sprayed onto the freshly prepared PDA plates for the tests (with compound filled center holes), applying ca. 0.5 ml per plate evenly across the agar surface.

Test Incubation

After inoculation, the petri dishes were closed and carefully transferred into an incubator with near UV light irradiation (16 hour day) at 20 degree Celsius.

Assessments

Spore germination rates were assessed microscopically at 4 days after inoculation. For assessment, all spores in 3 to 5 microscopical sight fields were counted per plate. The results of these counts were then averaged for each plate, and further on the mean was calculated from all three replicate plates for each treatment.

Subsequently, the plates were returned into the incubator.

After a minimum of 10 days of incubation time, the very slowly growing fungus had developed to a growth density which allowed for a visual assessment of zones of inhibition. The size of the zones of inhibition was measured as radius in mm, starting at the margins of the centre holes.

Detached leaf Study

Plant Material

Detached leaves (c.v. Gala) of a susceptible age were placed into petri dishes filled with water agar, upside facing up.

Compound Application

Test compounds were applied by dipping the detached leaves into the test solutions, ensuring full coverage of the upper leaf surface. Two replicate leaves were used per treatment. Untreated controls were dipped into sterile water. The petri dishes were then left open for 2 hours until the treatment had dried off. Then the petri dishes were closed and placed into an incubator with white light (16 hour day) at ca. 23 degree Celsius in the day and ca. 16 degree Celsius in the night. The leaves were allowed a period of 24 hours to adapt to this environment and regain full turgescence.

Fungal Culture

A stock culture of *Venturia inaequalis* (isolated from apple leaves from Gisborne) was grown on potato dextrose agar (PDA) plates in near UV light (16 hours day) at 20 degree Celsius in the day and ca. 16 degree Celsius in the night.

Inoculation

Stock plates were flooded with sterile water and the conidiospores were dislodged by gentle scraping and shaking. Using sterile water, the inoculum was then adjusted to a spore density of ca. 250000 spores per ml.

This inoculum was sprayed onto the water agar plates with the detached leaves, which had been treated the day before (24 hours protectant treatment), applying ca. 0.5 ml of inoculum per plate. Subsequently, the plates were closed again and re-incubated as before.

Assessment

Spore germination inhibition was assessed microscopically 4 days after inoculation. Due to a large variation in full disease development on detached leaves, an assessment of fully developed disease symptoms could not be performed.

Field Trial

Crop and trial area

Three large apple trees (c.v. Braeburn), located in the Waikato Research Orchard at Rukuhia, were used for a test on single branches.

At the time of test begin, first signs of natural apple black spot disease infection were visible on one side of the orchard, yet virtually no disease (<1%) was visible on the selected 3 apple trees. The single branches selected for the study were all prominent and located at the lower half of each tree.

Trial Setup

A fully randomised test design was employed. 8 to 9 branches were used per tree, allowing for 5 replicates per treatment.

Compound Application

Spray application was performed to run-off, using a narrow cone nozzle applicator driven by a propane gas sprayer at a pressure of 2 bar. At trial begin, a spray volume of 250 ml was used over all 5 replicate branches per treatment, which was increased to 350 ml later in the season, to adjust for the continued growth of the branches.

Throughout the season, 10 applications were performed with spray intervals of generally 8 to 10 days, in two cases longer due to dry weather conditions, unsuitable for disease development. (Application dates were: 09.12.99, 17.12.99, 27.12.99, 07.01.2000, 22.01.2000, 01.02.2000, 09.02.2000, 17.02.2000, 28.02.2000, 17.03.2000).

Inoculation

Natural disease infection appeared to be sparse and uneven. Therefore, a slight artificial inoculation was placed on 08.01.2000, spraying a spore suspension with ca. 17000 spores per ml evenly over all the lower branches of the 3 test trees. The weather conditions on the day of inoculation were very favourable for infection, with warm temperatures (ca. 20 degree Celsius) and slight rain/drizzle throughout the day.

Assessment

Assessments were performed at weekly intervals. For each branch, the number of infected leaves per total leaves was counted, and also the % leaf area covered with disease symptoms was recorded for each infected leaf. Multiplying these data of disease incidence and severity, a single figure for % disease infection was calculated for each branch. Then the mean was taken for each treatment from the 5 replicates.

Assessments on fruit were not possible, due to an apparent lack of susceptibility.

Results

In Vitro Tests

Spore Germination Inhibition

Application 15.10.99

Assessment 19.10.99 = 4 days after inoculation

In vitro – centre hole application: ~0.3ml of test solution filled into centre agar hole per plate, ~2 hours later inoculated with 0.5ml of spore suspension per plate, ~250,000 spores per ml

	% Spore Germination			% Spore Germination Mean	% Germination Inhibition Mean
	Plate 1	Plate 2	Plate 3		
Foliacin	1	8	3	4	95.2
Foliacin 1:10	32	19	33	28	66.4
Mycorrcin	7	2	5	4.7	94.4
Digester	6	5	8	6.3	92.4
Untreated	85	83	82	83	0

Values for each plate were produced by assessing all spores in 3 to 5 microscopical sight fields and taking the mean from these 3 to 5 separate counts.

Zones of Inhibition

Protectant Test

Application: 15.10.99

Assessment: 24.10.99 = 9 days after inoculation

In vitro – centre hole application: ~0.3ml of test solution filled into centre agar hole per plate, ~2 hours later inoculated with 0.5ml of spore suspension per plate, ~250,000 spores per ml

	Zone of Inhibition (cm)			Mean ZOI (cm)
	Plate 1	Plate 2	Plate 3	
Foliacin	3.5	3.5	2	3
Foliacin 1:10	0.3	0	0	0.1
Mycorrcin	3.5	3.5	2.5	3.2
Digester	3.5	3.5	3.5	3.5
Untreated	0	0	0	0

The maximum possible size for the zones of inhibition is 3.5cm. A value of 3.5cm means that the complete plate was free of growth of the test organism.

Curative Test

Applications: 19, 20, 21.11.99

Assessment 27 11.99 = 9 days after inoculation

In vitro – centre hole application: Curative test method.

On day one 6 fresh PDA plates were inoculated with 0.5ml of spore suspension per plate ~250,000 spores per ml. On day two ~0.3ml of test compound was applied into the centre hole of 2 of the 6 plates -> 1 day curative. On day three the test compound was applied to the next 2 plates -> 2 day curative. On day 4 the compound was applied to the third set of 2 plates -> 3 day curative.

Digester	Zone of Inhibition (cm)			Mean ZOI (cm)
	Plate 1	Plate 2	Plate 3	
1 day curative	3	2.5	2.8	2.8
2 day curative	1.8	1.3	1.6	1.6
3 day curative	1.5	1.5	1.5	1.5
Untreated	0	0	0	0

The maximum possible size for the zones of inhibition is 3.5cm. A value of 3.5cm means that the complete plate was free of growth of the test organism.

Detached Leaf Study

In vivo – Detached young apple leaves were dipped into the test solutions, and then immediately placed into water-agar plates. After drying off, the petri dishes were closed and incubated for 24 hours (16 hour day, white light, day temperature ~23 degree C, night temperature 16 degree C). Then they are spray-inoculated with an apple black spot spore solution at ~250,000 spores/ml and are reincubated as before. Two replicate leaves per treatment.

	% Spore Germination	% Spore Germination Inhibition
Foliacin 1:10	7	89.5
Foliacin 1:100	10	85
Untreated	67	0

Values for each plate were produced by assessing all spores in 3 to 5 microscopical sight fields and taking the mean from these 3 to 5 separate counts.

Field Trial on single branches

Applications: 09.12.99, 17.12.99, 27.12.99, 07.01.00, 22.01.00, 01.02.00, 09.02.00, 17.02.00, 28.02.00, 17.03.00.

% Natural disease visible at first application <1%

Artificial inoculation with ~17,000 spores/ml on 08.01.00

Tree variety Braeburn, 3 trees using 8-9 separate branches per tree.

Application rates are only approximate values, derived from volume per tree area estimates.

With single branch treatments exact application was difficult.

For each treatment 250ml of spray solution was applied onto 5 replicate branches

(~50ml/branch), achieving full coverage to runoff (300ml from 5th application on, 350ml from 8th application on).

	% Disease on foliage at assessment date				
	09.02.00	17.02.00	06.03.00	15.03.00	29.03.00
Digester 2lt/ha	1.1	0.8	1.8	0.7	1.1
Digester 4/ha	1.3	0.9	1.3	0.7	1.1
Foliacin 2lt/ha	2.4	2.4	2.6	1.8	1.9
Foliacin 4lt/ha	0.5	0.5	1.0	0.8	0.7
Digester 2lt/ha + Foliacin 2lt/ha	1.5	0.5	1.7	0.7	1.3
Untreated	5.4	3.5	7.7	5.0	6.7

For assessment disease was rated on single leaves for each branch (~25-55 leaves per branch), recording disease incidence on foliage, as well as disease severity on each infected leaf. The given values for % disease are derived combining both, disease incidence and disease severity

DISCUSSION

All tested solutions performed extremely well against *Venturia inaequalis* in all the studies, *in vitro* as well as *in vivo* on detached leaves and in the field. In order to verify the tests, it has to be mentioned that these results are only an extract from a whole range of tests, in which other test solutions demonstrated clearly less potential (results not shown here). A very exciting transfer of the excellent *in vitro* activity to *in vivo* situations has been demonstrated, with the laboratory based detached leaf study and even a full activity transfer into the field was shown with the apple branch trial.

The *in vitro* data demonstrate excellent protectant activity, expressed as an inhibition of spore germination. The zones of inhibition show that the active ingredient is water soluble, diffusing from the centre hole into the agar.

The detached leaf data shows good activity of the diluted Foliacin on the leaf, although the *in vitro* activity of Foliacin at a 1:10 dilution was markedly lower. This effect has also been observed in numerous other *in vitro* studies (results not shown here), and is most probably due to the fact that the active ingredient becomes very diluted when diffusing into the agar.

The field data show some variation throughout the season, which is due to ongoing growth of the branches, accompanied by new infections as well as “loss of infection” due to loss of infected leaves. However, in all assessments the treatments show clearly less disease than the untreated control, a result that gets increasingly prominent at the end of the season.

Conclusion

The tested biostimulant products from Bio-Start Ltd. have proven excellent potential for the biological treatment of apple black spot, one of the most important and damaging fungal apple diseases in the world.