

TRADE SECRET

Kobe organic and Kobe non-organic: 10% RHEUM OFFICINALE BAILL PLANT EXTRACT: Efficacy on the tomato powdery mildew *Oidium lycopersici* - Semi-field Test in 2011

Test Guideline(s)

Based on EPPO guideline 57 (2) for *powdery mildew on cucurbits and other vegetables*, semi-field trials

Author(s)

Guido MKM Sterk, Master in Zoology

Study Completion Date: 01/03/2012

Test Facility

Test Facility Name: **IPM Impact**

Test Facility Address : **Gierkensstraat 21 3511 Hasselt Belgium**

Sponsor

Onze Livre BV BV

Wim Duisenbergplantsoen 29 4/F,
Office 04, 6221 SE Maastricht The
Netherlands .

Study Number: OIDILY12-01A



IPM Impact

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IPM Impact

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

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Wim Duisenbergplantsoen 29 4/F,
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Study Director:

Study Director's Name, degree Lic. Guido Sterk
Test Facility Name: IPM Impact

Date 01/03/2012

Test Facility Management:

Manager's Name, degree Lic. Guido Sterk
Manager's Title
Test Facility Name : IPM Impact

Date 01/03/2012

Applicant/Sponsor

Onze Livre BV :
Wim Duisenbergplantsoen 29 4/F,
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Netherlands

Onze Livre BV Representative

Date



IPM Impact

QUALITY ASSURANCE STATEMENT

Study Number

OIDILY12-01A

Study Title

Kobe Organic and Kobe Non-Organic: Efficacy on the tomato powdery mildew *Oidium lycopersici* in a Semi-field Test in 2012

First, Middle Initial, Last Name
Quality Assurance Unit Auditor's Title
QAU Auditor's Company Affiliation

Date

CERTIFICATION OF AUTHENTICITY

Indicate Full Study Title Here

**Kobe Organic and Kobe Non-Organic : Effects on the tomato powdery mildew
Oidium lycopersici in a Semi-field Test in 2012**

We, the undersigned, declare that the work described in this report was performed under our supervision, and that this report provides an accurate record of the procedures and results.

Report by: Lic. Guido Sterk

Study Director's Name Guido Sterk
Study Director's Title Lic.

Date 01/03/2012

Approved by: Lic. Guido Sterk

Manager's Name Guido Sterk
Manager's Title Lic.

Date 01/03/2012

Study Monitor's Name

Date

Study Initiation Date:

01 02 2012

Date Study Completed:

09 03 2012

Sponsor:

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**Kobe Organic and Kobe Non-Organic : Efficacy on the tomato powdery mildew
Oidium lycopersici in a Semi-field Test in 2012**

Guido MKM Sterk

1.0 SUMMARY

Materials and Methods:

Test Item, Control and Reference Item

Test Item

The test item and the information concerning the test item were provided by the sponsor:

Name:	Kobe Organic Kobe Non-organic
Active Ingredient Content	10% RHEUM OFFICINALE BAILL PLANT EXTRACT
Type of Formulation:	SL
Water Amount in this Study:	25 mL/plant (Spraying just before beginning of run-off)
Target Amount in this Study:	Three dose levels for each formulation of Kobe. The dose rates are documented in the raw data and reported in the final report
Storage:	Stored at low humidity, out of direct sunlight at a temperature less than 40 °C, in special agro-cabinets, Asecos, developed for the storage of plant protection compounds
Safety Precautions:	Routine safety and hygienic procedures were applied

Control

Control: Tap water
Water Amount in this Study: 25 mL/replicate (plant) = 200 mL/object (Spraying just before beginning of run-off)

Reference Item

The information on the reference item according to the test item container label and data sheet:

Name: Fungaflor 100 EC
Manufacturer: Certis Europe
Active Ingredient Content: Imazalil
Type of Formulation: 100 EC
Type: Fungicide
Water Amount in this Study: 25 mL/plant (Spraying just before beginning of run-off)
Target Amount in this Study: One dose level
Storage: Store at low humidity, out of direct sunlight at a temperature less than 40 °C
Safety Precautions: Routine safety and hygienic procedures

Test System

Taxonomic Group: Tomato powdery mildew (Deuteromycetes)
Species: *Oidium (neo)lycopersici* (= *Oidium lycopersicum*)
Origin: IPM Impact
Stage at Delivery: Infested plants
Stage at Test Start: Infested plants

Test Units

Type and Size: Test units:
The trial were made on tomato (*Solanum lycopersicum*, var. Moneymaker).

- 1 plant/replicate
- 8 replicates

Identification: Each test unit was uniquely identified with study number, treatment and replicate number.

Test Conditions

Test Environment:	Greenhouse compartment
Temperature:	25 °C ± 5 °C
Relative Humidity:	Ca. 70 ± 10 %
Cropping considerations	<ol style="list-style-type: none">1. Tomato plants are potted into separate pots (pot surface size = 81 cm², 1 plant per pot. They were infested with infested tomato leaves.2. BBCH Stage: 55 (first signs of individual flowers)3. Each replicate was placed on separate tables. Treatments were separated by plastic holding large foam shields to avoid spray drift or are kept in a separate places.4. Trial treatments were prepared according to protocol.

Food

Food: Not relevant. Fungus disease.

Application of the Test Item, the Control and the Reference Item

Application: Single application with a “Birchmeier Super Star 1.25 L” hand-held sprayer

Reason for this Route of

Administration: Worst case scenario

Application rate of the Test Item: Kobe Organic was be sprayed with a defined concentration of 0.1, 0.2 and 0.3 % formulated compound.
 Kobe Non-Organic was be sprayed with a defined concentration of 0.1, 0.2 and 0.3 % formulated compound.
 The spray volume was 25 mL per test plant achieving spray coverage just before beginning of run-off.

Concentration of the Test Item

Spraying Dilution: 0.1, 0.2 and 0.3 % formulated compound

Concentration of the Reference Item

Spraying Dilution: 0.2 % formulated compound Fungaflor 100 EC

Spraying Scheme: 1. control, 2. Three dose rates of each test item, 3. Standard

Application Rate: 8x25 ml spraying solution was applied on 8x1 plant.

Documentation: A technical report with all the details of the trial in an Excel file is stored at IPM Impact.

Course of the Test

Individuals: High infestation

Introduction Procedure: Infested leaves

Exposure Time: 12 days

Test Parameters

Population density: Degree of infestation according to official EPPO guideline

Phytotoxicity: Any observations on phytotoxicity would be recorded, but no phytotoxicity was observed.

DATA TO COLLECT:

Assess plant growth in treated and untreated plants by direct measurement of height or foliage density and by digital photographs.

Record phytotoxicity as % of total leaf area affected by chloroosis and necros

Record any other symptom or plot differences observed using a scale appropr the symptom.

Record changes in vegetative behavior when present.

Result Evaluation: degree of infestation

$$\text{Corrected \%} = \left(1 - \frac{n \text{ in T after treatment}}{n \text{ in Co after treatment}} \right) * 100$$

n=degree of infestation T = test compound Co = control



Degree of infestation was determined 15 days after exposure to the test item and the reference item, respectively. It was corrected according to the corresponding results of the control group by the following formula (Abbott, 1925)

Statistical Analysis: Not performed.

Validity Criteria of the Study

Control Mortality: High infestation of powdery mildew (>4 on the EPPO scale)
Standard: >90 % control in the standard, compared to the control



Conclusion:

- **Kobe non-Organic and Kobe Organic at all dose rates had a high efficacy against powdery mildew.**
- **Fungaflor 100 EC had a high efficacy, erasing the powdery mildew from the leaves. The trial is valid.**
- **The mean degree of infestation of powdery mildew in the control was more than 4 on the old leaves. The trial is valid.**

2.0 GENERAL STUDY INFORMATION

2.1 *Study Objectives*

The purpose of this study was to measure the effects of a spraying treatment of Kobe Organic and Kobe Non-Organic on powdery mildew on tomatoes.

2.2 *Test System Justification*

Based on EPPO guideline PP1/57(2) Powdery mildew on cucurbits and other vegetables

2.3 *Study Personnel*

Test Facility Name Study Personnel

Management: Guido Sterk
Study Director: Guido Sterk
Gierkensstraat 21
3511 Hasselt
Belgium

Principal Investigator: idem

Technical Personnel: idem

2.4 *Proposed Study Execution Dates*

Experimental Start Date: 02 2012

Experimental Completion Date: 03 2012

3.0 MATERIALS AND METHODS

Summary

The trial was carried out at IPM IMPACT trial site in Linter Belgium . Tomatoes of the variety Moneymaker were placed on tables in the greenhouse at a spacing of 100 cm between the crop and 50 cm between the rows. Plots existing of 1 plant and measuring 1 m by 1 m replicated 8 times were used in a randomized complete block design. Irrigation on the table was given during the first 8 weeks after planting thereafter drip irrigation continued throughout the growing season.

Assessment of the disease levels were done on a weekly basis a day before the treatments application starting from November 2012. All leaves were checked once after treatment. The treatments were applied using handheld sprayer to deliver an equivalent spray volume of 1000 litres per hectare. There was only one treatment.

Phytotoxicity was checked for 7 days from each treatment after the application according to the official EPPO guideline. The rest of the cultural practices were done as usual. Data was analyzed using the Abbott formula and according to the official EPPO guideline. The trial was completed on 9th of March 2012

3.1 *Test Guidelines*

This study was designed to comply with the following methods: EPPO guideline : PP1/57(2)
Powdery mildew of cucurbits and other vegetables

3.2 *Test System*

3.2.1. Chemical System

3.2.1.1 *Test Item*

Name:	Kobe Organic and Kobe Non-Organic
Active substance(s)	RHEUM OFFICINALE BAILL PLANT
Product Use:	EXTRACT Fungicide

3.2.1.2 *Reference Items*

Control

Name:	water
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Toxic Standard

Name:	Fungaflor 100 EC
Formulation:	100 EC
Product Use:	Fungicide

3.2.1.3 *Test Vehicle*

Tap water

3.2.1.4 *Application Information*

Test item application rates were based on the results of range finding study(ies) and consultation with the Sponsor's Study Monitor.

Spray Solution Table

Nominal Rate (g prod/ha)	Product Concentration (mg/mL)	Total Spray Solution Made (mL)
Kobe Organic	1,5, 2 and 3 mL/L	8x25 ml = 200 ml
Kobe Non-Organic	1,5, 2 and 3 mL/L	8x25 ml = 200 ml
Fungaflor 100 EC	2 mL/L	8x25 ml = 200 ml
Water		8x25 ml = 200 ml

Application Details

Application Order:	<ol style="list-style-type: none"> 1. Control 2. Kobe Organic (tests item): 3 dose rates 3. Kobe Non-organic (test item) : 3 dose rates 4. Fungaflor 100 EC (standard)
Method of Application:	Spraying.
Application Equipment:	Birchmeier Super Star
Number of Nozzles:	1
Spray Nozzle Type:	Swivel
Spray Pressure:	2 bar
Application Speed:	+/- 50 ml/min.
Height above Target:	10 cm
Calibration Procedure:	
Spray Equipment Clean-up:	Rinsing with tap water – every active ingredient, water included, has its own Birchmeier Super Star sprayer
Documentation:	All application data are documented in the study records.

3.2.2 Biological System

3.2.2.1 Test Organism

Taxonomic Group:	Deutomycetes
Species:	<i>Oidium lycopersici</i>
Sex:	Not applicable
Source:	<i>IPM Impact</i>
Stage at Delivery:	Heavy infestation
Life Stage at Treatment:	Heavy infestation
Age at Treatment:	Not applicable

3.2.3 *Physical System*

3.2.3.1 *Acclimatisation (Pre-Test)*

Pre-Test Location: Not applicable

Temperature:

Relative Humidity:

Light Intensity:

Light Regime:

3.2.3.2 *Test Units*

Exposure Units: 1 tomato /replicate was placed in a plastic recipient (30x20x12)

Identification of Test Units: Each test unit was uniquely identified with study number, treatment and replicate number

3.2.3.3 *Test Conditions*

Test Location: Controlled-environment room

Exposure period: Temperature: $25 \pm 5^{\circ}\text{C}$
Relative Humidity: 70 – 10% Light
Intensity: 5000 – 10000 lux

Post-exposure period: Temperature: $25 \pm 5^{\circ}\text{C}$
Relative Humidity: 70 – 10% Light
Intensity: 5000 – 10000 lux

Light Regime: 16 light, 8 hr dark (see climatic conditions during the trial in appendix)

Instrumentation: Hobo U12 data logger Temperatur/Relative humidity/Light

Documentation: Test conditions were recorded with suitable instruments and documented in the raw data.
The source, preparation date, and storage conditions of the food are documented in the raw data.

3.3 *Test Design*

Treatment Groups: Water Control, test items, standard

Exposure Period

Length of Exposure Period: 12 days

Sample Size: All leaves

3.4 *Test Conduct*

3.4.1 Exposure Period

12 days

3.5 *Parameters Observed*

Degree of infestation	The number and degree of infested leaves were counted directly on the leaves.
Fytotoxicity measurement	Assess plant growth in treated and untreated plants by direct of plant height or foliage density. Record phytotoxicity as % of total leaf area affected by chlorosis and necrosis. Record any other symptom or plot differences observed using a scale appropriate to the symptom. Record changes in vegetative behavior when present.

3.6 *Result Analysis*

3.6.1 Parameter 1

Degree of infestation formula	Evaluation in the population density by using the Abbott Corrected mortality = $(1 - (N \text{ in } T \text{ after treatment} / N \text{ in } Co \text{ after treatment})) * 100$ N=Degree of infestation T = test compound Co =
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control

IPM Impact

3.6.2 Parameter 2

Phytotoxicity No Phytotoxicity was observed in this trial

3.6.3 Documentation

Statistical procedures and computer program used to analyze the study data are referenced in the study records.

3.7 ***Validity Criteria of the Study***

Control Mortality: Mean value >4 in the control
The test is valid.

Standard >90 % control in the standard, compared to the control.
The trial is valid.

3.8 ***Study Plan Deviations***

Deviation 1 Study Plan:
Deviation: None
Reason: /
Impact on Study: /

4.0 RESULTS AND DISCUSSION

4.1 Parameter 1: Degree of infestation

Nb.	Product	Application n	Dose rate %	Total degrees of damage	Abbott (%)
1	Kobe Non-organic	Spraying	0,1	7	82
2	Kobe Non-organic	Spraying	0,2	7	82
3	Kobe Non-organic	Spraying	0,3	4	89
4	Kobe Organic	Spraying	0,1	11	71
5	Kobe Organic	Spraying	0,2	3	92
6	Kobe Organic	Spraying	0,3	2	95
7	Fungaflor 100 EC	Spraying	0,2	0	100
8	Control	Spraying		38	

Nb.	Product	Application n	Dose rate %	Total degrees of damage	Abbott (%)
1	Kobe Non-organic	Spraying	0,1	3	92
2	Kobe Non-organic	Spraying	0,2	0	100
3	Kobe Non-organic	Spraying	0,3	0	100
4	Kobe Organic	Spraying	0,1	7	82
5	Kobe Organic	Spraying	0,2	0	100
6	Kobe Organic	Spraying	0,3	0	100
7	Fungaflor 100 EC	Spraying	0,2	0	100
8	Control	Spraying		15	

4.2 *Parameter 2: Phytotoxicity*

	Plant height	% leaf area chlorosis	% leaf area necrosis	Other
Control	Normal	None	None	Negative
Kobe Non-Organic all dose rates	Normal	None	None	Negative
Kobe Organic all dose rates	Normal	None	None	Negative
Fungaflor 100EC	Normal	None	None	Negative

5.0 CONCLUSIONS

- Kobe Non-Organic and Kobe Organic at the tested dose rates are very effective against this disease. Also the new leaves were protected.
- No signs of *Phytotoxicity* on tomato, var. Moneymaker were observed.
- Fungaflor 100 EC was highly effective against powdery mildew in this trial.

6.0 RETENTION OF RECORDS

For the periods demanded by guidelines and specific country requirements, study documents and materials will be stored in the archives of IPM Impact (Gierkensstraat 21 3511 Hasselt Belgium) , including but not limited to:

- study plan;
- any study plan and/or report amendments or addenda or SOP deviations;
- all raw data;
- comments of the sponsor on the draft report;
- one original signed copy of the final report;

Documents and materials are archived according to the principles of Good Laboratory Practice in the organization of the testing facility. If Test Facility name wishes to purge their files of the study records, they will contact the sponsor. Test Facility name must receive written permission from Onze Livre BV to either send these study records to Onze Livre BV for archival or to discard study records.

Copies of the signed original report, study plan, and any study plan amendments were sent to Onze Livre BV upon finalization of the study. These documents are retained in the archives at: Wim Duisenbergplantsoen 29

4/F, Office 04, 6221 SE Maastricht
The Netherlands

7.0 DISPOSAL OF TEST ITEM

After issuance of the final report, the remaining test item will be stored at Test Facility name until its expiration date and then destroyed in accordance with local regulations, unless other arrangements are made between the sponsor and the Test Facility name .

8.0 REFERENCES

1. Chemikaliengesetz der Bundesrepublik Deutschland (ChemG), Anhang 1, in der Fassung der Bekanntmachung vom 25. Juli 1994 (BGBl. I S. 1703) mit Änderungen vom 27. September 1994 (BGBl. I S. 2705) und 14. Mai 1997 (BGBl. I S. 1060).
2. EC Agrochemical Registration Directive (DS 65) (Directive 91/414/EEC).
3. OECD Principles of Good Laboratory Practice, adopted by Council on 26th November 1997 [C(97)186/Final], Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998.
4. Abbott W.S. 1925: A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265 – 267



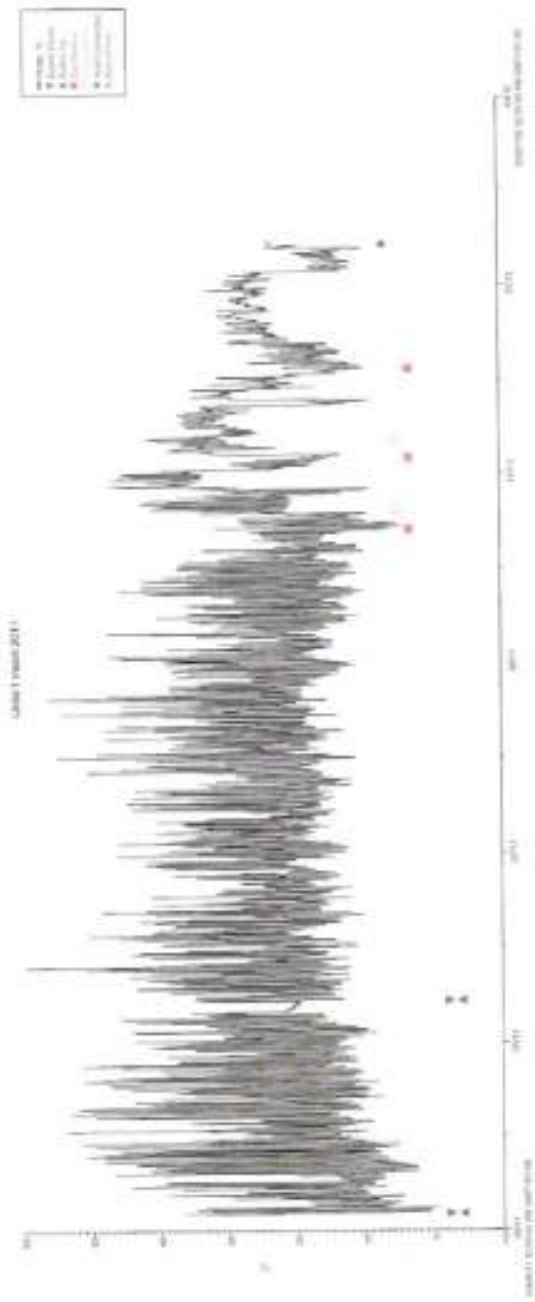
5. EPPO (2006) Standard 1/135 (3): Guideline on phytotoxicity assessment.
6. EPPO (2006) Standard 1/57(2): Guideline on powdery mildew on cucurbits and other vegetables.

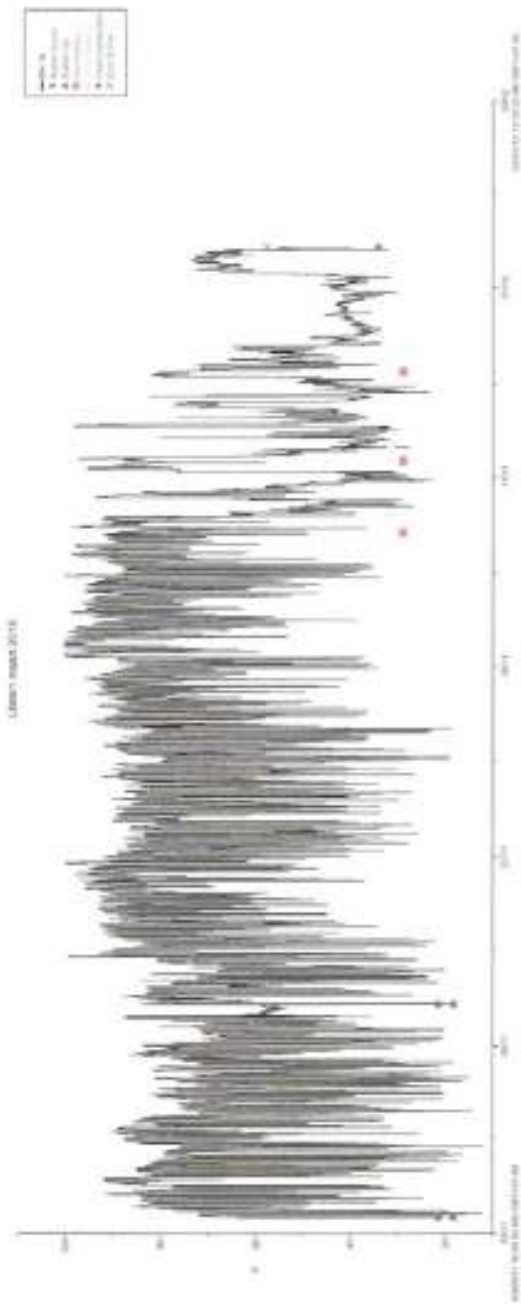
APENDIX 1 RAW DATA ASSESSMENT

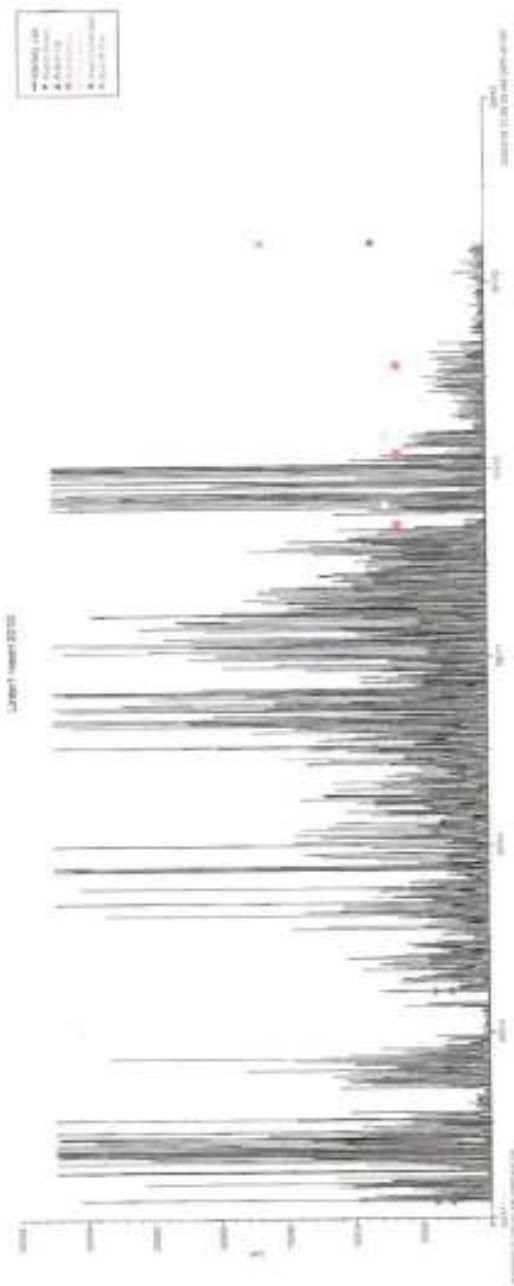
Old leaves				Replication Nb.								Total degrees of damage
Nb.	Product	Application	Dose rate %	1	2	3	4	5	6	7	8	
1	Kobe Non-organic	Spraying	0,1	2	0	0	2	0	0	2	1	7
2	Kobe Non-organic	Spraying	0,2	2	2	0	1	0	0	2	0	7
3	Kobe Non-organic	Spraying	0,3	2	0	2	0	0	0	0	0	4
4	Kobe Organic	Spraying	0,1	2	2	1	1	1	2	1	1	11
5	Kobe Organic	Spraying	0,2	1	0	0	1	0	0	0	1	3
6	Kobe Organic	Spraying	0,3	1	1	0	0	0	0	0	0	2
7	Fungaflor 100 EC	Spraying	0,2	0	0	0	0	0	0	0	0	0
8	Control	Spraying		5	5	5	5	4	4	5	5	38

New leaves				Replication Nb.								Total degrees of damage
Nb.	Product	Application	Dose rate %	1	2	3	4	5	6	7	8	
1	Kobe Non-organic	Spraying	0,1	0	1	1	0	1	0	0	0	3
2	Kobe Non-organic	Spraying	0,2	0	0	0	0	0	0	0	0	0
3	Kobe Non-organic	Spraying	0,3	0	0	0	0	0	0	0	0	0
4	Kobe Organic	Spraying	0,1	1	0	0	1	1	2	1	1	7
5	Kobe Organic	Spraying	0,2	0	0	0	0	0	0	0	0	0
6	Kobe Organic	Spraying	0,3	0	0	0	0	0	0	0	0	0
7	Fungaflor 100 EC	Spraying	0,2	0	0	0	0	0	0	0	0	0
8	Control	Spraying		0	3	2	3	2	1	2	2	15

Appendix 2 Climatic conditions Linter







APPENDIX CERTIFICATE OF TESTING FACILITY

To whom it concerns,

IPM Impact is a company specialized in efficacy trials with biological compounds on pests and side-effect trials on beneficial organisms. Although it also carries out tests for registration under GEP and GLP, in cooperation with its partners, IPM Impact is mainly specialized in trials for Integrated Pest Management under practical conditions.

The tests are done under the same stringent conditions that are required for GEP trials.

The results from this research are published on the website that offers the most comprehensive database in the world on effects of pesticides on pollinators (bumblebees), predators and parasitoids.

The company does also a lot of work on the effects of plant protection compounds, mainly fungicides, on entomopathogenic fungi and zoophagous nematodes.

The study director Guido Sterk was responsible for doing the efficacy trials for registration of insecticides and acaricides in orchards and soft fruit for the Ministry of Agriculture in Belgium for more than 10 years.

He was also founding member of the ecotox committee for registration in Belgium.

Guido Sterk was for many years co-convenor of the IOBC working group 'Effects of Pesticides on Beneficial Organisms' where he was responsible for the joint testing programs.

He's an active member of the International Commission for Plant-Bee Relationships, bee protection group.