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
PAGE RESERVED

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Date 01/03/2012

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Manager’s Title
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Onze Livre BV Representative
Date
QUALITY ASSURANCE STATEMENT

Study Number
OIDILY12-01A

Study Title
Kobe Organic and Kobe Non-Organic 1.2 SL: 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 1.2 SL: 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  Date 01/03/2012
Study Director’s Title Lic.

Approved by: Lic. Guido Sterk

Manager’s Name Guido Sterk  Date 01/03/2012
Manager’s Title Lic.

Study Initiation Date:
01 02 2012

Date Study Completed:
09 03 2012

Sponsor:
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Wim Duisenbergplantsoen 29 4/F,
Office 04, 6221 SE Maastricht The Netherlands
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Kobe Organic and Kobe Non-Organic 1.2 SL: 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
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**

**Phytotoxicity** Degree of infestation according to official EPPO guideline

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 chlorosis and necrosis
- 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**

\[
\text{Corrected \%} = \left( 1 - \frac{n_{\text{in T after treatment}}}{n_{\text{in Co after treatment}}} \right) \times 100
\]

\(n=\text{degree of infestation T = test compound Co = control}\)
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 1.2 SL non-Organic and Kobe 1.2 SL 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**

<table>
<thead>
<tr>
<th>Test Facility Name</th>
<th>Study Personnel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Management:</td>
<td>Guido Sterk</td>
</tr>
<tr>
<td>Study Director:</td>
<td>Guido Sterk</td>
</tr>
<tr>
<td></td>
<td>Gierkensstraat 21</td>
</tr>
<tr>
<td></td>
<td>3511 Hasselt</td>
</tr>
<tr>
<td></td>
<td>Belgium</td>
</tr>
<tr>
<td>Principal Investigator:</td>
<td>idem</td>
</tr>
<tr>
<td>Technical Personnel:</td>
<td>idem</td>
</tr>
</tbody>
</table>

2.4 **Proposed Study Execution Dates**

<p>| | |</p>
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental Start Date:</td>
<td>02 2012</td>
</tr>
<tr>
<td>Experimental Completion Date:</td>
<td>03 2012</td>
</tr>
</tbody>
</table>

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
- **Formulation:** 1.2 SL
- **Active substance(s):** RHEUM OFFICINALE BAILL PLANT EXTRACT
- **Product Use:** Fungicide

3.2.1.2 **Reference Items**
- **Control**
  - **Name:** water

  **Toxic Standard**
  - **Name:** Fungafior 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

<table>
<thead>
<tr>
<th>Nominal Product</th>
<th>Product Concentration (mg/mL)</th>
<th>Total Spray Solution Made (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kobe Organic</td>
<td>1.5, 2 and 3 mL/L</td>
<td>8x25 ml = 200 ml</td>
</tr>
<tr>
<td>Kobe Non-Organic</td>
<td>1.5, 2 and 3 mL/L</td>
<td>8x25 ml = 200 ml</td>
</tr>
<tr>
<td>Fungaflor 100 EC</td>
<td>2 mL/L</td>
<td>8x25 ml = 200 ml</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>8x25 ml = 200 ml</td>
</tr>
</tbody>
</table>

### Application Details

**Application Order:**
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:** *Oidium lycopersici*
- **Sex:** Not applicable
- **Source:** IPM Impact
- **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 ± 5°C
  - **Relative Humidity:** 70 – 10% Light
  - **Intensity:** 5000 – 10000 lux
- **Post-exposure period:**
  - **Temperature:** 25 ± 5°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 measurement 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

Corrected mortality = (1 – (N in T after treatment/N in Co after treatment))*100

N = Degree of infestation T = test compound Co =
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: None
Deviation: None
Reason: /
Impact on Study: /
### RESULTS AND DISCUSSION

#### 4.1 Parameter 1: Degree of infestation

<table>
<thead>
<tr>
<th>Nb.</th>
<th>Product</th>
<th>Application</th>
<th>Dose rate %</th>
<th>Total degrees of damage</th>
<th>Abbott (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kobe Non-organic</td>
<td>Spraying</td>
<td>0,1</td>
<td>7</td>
<td>82</td>
</tr>
<tr>
<td>2</td>
<td>Kobe Non-organic</td>
<td>Spraying</td>
<td>0,2</td>
<td>7</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td>Kobe Non-organic</td>
<td>Spraying</td>
<td>0,3</td>
<td>4</td>
<td>89</td>
</tr>
<tr>
<td>4</td>
<td>Kobe Organic</td>
<td>Spraying</td>
<td>0,1</td>
<td>11</td>
<td>71</td>
</tr>
<tr>
<td>5</td>
<td>Kobe Organic</td>
<td>Spraying</td>
<td>0,2</td>
<td>3</td>
<td>92</td>
</tr>
<tr>
<td>6</td>
<td>Kobe Organic</td>
<td>Spraying</td>
<td>0,3</td>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td>7</td>
<td>Fungaflo 100 EC</td>
<td>Spraying</td>
<td>0,2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>Spraying</td>
<td></td>
<td>38</td>
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</table>

<table>
<thead>
<tr>
<th>Nb.</th>
<th>Product</th>
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<th>Dose rate %</th>
<th>Total degrees of damage</th>
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<tr>
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<tr>
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<td>Spraying</td>
<td>0,2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>Spraying</td>
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<td>15</td>
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</table>
4.2 **Parameter 2: Phytotoxicity**

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<tr>
<th></th>
<th>Plant height</th>
<th>% leaf area chlorosis</th>
<th>% leaf area necrosis</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal</td>
<td>None</td>
<td>None</td>
<td>Negative</td>
</tr>
<tr>
<td>Kobe Non-Organic all dose rates</td>
<td>Normal</td>
<td>None</td>
<td>None</td>
<td>Negative</td>
</tr>
<tr>
<td>Kobe Organic all dose rates</td>
<td>Normal</td>
<td>None</td>
<td>None</td>
<td>Negative</td>
</tr>
<tr>
<td>Fungaflor 100EC</td>
<td>Normal</td>
<td>None</td>
<td>None</td>
<td>Negative</td>
</tr>
</tbody>
</table>

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**


### Appendix 1 Raw Data Assessment

#### Old leaves

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

#### New leaves

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