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Control of Phoma exigua in Witloof chicory

Efficacy evaluation of post harvest treatment of Tecto SC to control *Phoma exigua* in Witloof chicory taproots

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Praktijkonderzoek Plant & Omgeving (Applied Plant Research), part of Wageningen UR
Business Unit AGV
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This document reports on research on the efficacy of treatment with the fungicide Tecto SC to control *Phoma exigua* attack in Witloof chicory taproots. This research was conducted by Applied Plant Research (PPO-AGV) and was done by order of:

Syngenta Crop Protection AG
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CH-4058 Basel
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Applied Plant Research (Praktijkonderzoek Plant & Omgeving), part of Wageningen UR
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1 Introduction

Phoma exigua can cause severe root rot in taproots of Witloof chicory. *Phoma exigua var. exigua* is a widespread fungus and can cause in potato the so-called 'thumb rot'. *Phoma exigua var. foveata* causes gangrene or dry rot in potato. Both types are closely related and are often studied in relation to potato. These studies formed a source of information for the mode of action of *Phoma* root rot in chicory.
Attack by *Phoma exigua* in chicory is characterized by a black-brown discoloration of the root tissue with a sharp boundary between diseased and healthy tissue. *Phoma* is called a "cold mold" because the fungus develops well in cold and wet conditions. The probability of a *Phoma* attack increases when the soil structure of a plot is bad.
The fungus develops during root cultivation in particular on withered leaves. The fungal spores can infect the roots before or during harvest. The damage begins to injury, usually near the root tip, or by dying leaves on the head causing a disordered chicon formation during forcing. In the storage room *Phoma* grows in the affected taproots slowly. Infection can spread by direct contact with other roots.

1.1 Research aim

Applied Plant Research (AGV Research Unit) will test after root harvesting and during forcing experiments the efficacy of the fungicide Tecto SC (active ingredient: thiabendazole) of Syngenta Crop protection against *Phoma exigua* in witloof chicory (chicon) production. The trial is carried out in three different dosages with one fungicide Tecto SC as a post harvest treatment on taproots. The fungicide Rovral SC (active ingredient: Iprodione) will act as reference. The results will be compared to a non-contaminated and a contaminated reference in one experiment.

2 Materials & methods

2.1 Trial data

Start of forcing	:	March 4, 2013 in Mini-forcing system at Applied Plant Research location Lelystad-NL (Photo 1)
Cultivar	:	Baccara
Root type	:	Taproots, 3.5 until 5.5 cm diameter
# roots/plot	:	Circa 80
Efficacy Guidelines	:	Protocol for Efficacy Evaluation of Fungicides against *Phoma exigua* in taproots of Witloof Chicory (Annex 1)
# objects & replicates	:	6 Objects: A, B, C, D, E & F in 4 replicates: I - IV
Experimental layout	:	Completely randomised design
Temperature water/air	:	17 ^{0}C water & 14 ^{0}C air
Water pH	:	6.5-7.5
Water Electric Conductivity	:	2.0-2.5 mS/cm
Infection *Phoma*	:	Spraying spore suspension on crushed roots with 5 x 10^6 conidia/ml on February 4, 2013
Application Tecto SC	:	Rate 40-60-80 ml/1000 kg roots in 20 litres spraying solution on February 6, 2013
Application Rovral SC	:	Rate 35 ml/1000 kg roots in 20 litres spraying solution on February 6, 2013
Chicon harvest		March 26, 2013
Pesticide testing	:	According GEP, annex 3

Photo 1:.**Chicon production in mini-forcing installation. Each treatment and repetition in separated containers and hydroponic solution. March 2013.**

2.2 Assessments

At start of forcing and directly after forcing the roots were scored on *Phoma* infection, calculated as Phoma-index. Chicon production in Class I, II and III was determined. Relative length of the axe was measured and aberrations of the axe were noticed: brown axe, red discoloration and hollow axe. For further information on evaluated observations see Annex 1.

2.3 Statistical methods

The trial was carried out as a completely randomized design. Analysis of variance was conducted using Genstat 14 (VSN International, 2011). All objects were part of the statistical analysis. The means (medians) and the probability according to the F-test from these analysis are presented. Separation of the means is provided and corresponding least significant differences (LSD) based on the t-distribution, with probability 0.05 are presented. Means without a common letter are significantly different. An extra analysis was performed to detect contrasts between the average of the objects Tecto 40, 60 and 80 and the other objects (see table 2).

3 Results

Application of Tecto SC showed a significant reduction of the *Phoma*-index after forcing, however the differences between the rates of application (40-60-80 ml per 1000 kg roots) are not significant (table 1, photo 2a-b). The effect of Tecto SC on reduction of Phoma attack is stronger than at application of Rovral SC. The natural infection of the used tap roots from biological origin was quite high, artificial infection by a spore suspension increased the Phoma attack after forcing slightly. With regard to the chicon production the effects are limited, although it appears that at the highest dose of Tecto SC (80 ml) the chicon production decreased, is this not different from the control treatments (photo 3a-b). See Annex II for the datasheet with results per experimental unit.

Table 1. **Efficacy of different rates of post harvest Tecto SC treatment on *Phoma* attack of taproots (cv. Baccara) and chicon production in relation to treatment with Rovral SC and non contaminated or contaminated control. Start of forcing 4 March, harvest 26 March 2013.**

Object code	Description	Treatment	Phoma-index Start forcing	Phoma – index After forcing	Chicon production per 100 roots	Class I (%)
A	Control	Not contaminated roots	0	56.8 c	14.7	89.7
B	Control	Contaminated roots	0	71.9 c	16.1	89.1
C	Rovral SC	35 ml/1000 kg roots	0.8	39.2 b	15.9	95.1
D	Tecto SC	40 ml/1000 kg roots	0	21.7 a	16.8	90.3
E	Tecto SC	60 ml/1000 kg roots	0	30.8 ab	16.7	89.4
F	Tecto SC	80 ml/1000 kg roots	0	23.6 ab	15.0	89.5
	lsd (p=0.05)		0.9	16.5	2.5	9.8
	F pr.		n.s.	<0.001	n.s.	n.s.

Statistical analysis as average over the different rates of Tecto SC shows a significant lower *Phoma* attack of the roots after forcing in relation to Rovral SC (table 2).

Table 2. **Efficacy of Tecto SC on *Phoma* attack of taproots (cv. Baccara) and chicon production in relation to treatment with Rovral SC and non contaminated or contaminated control. Start of forcing 4 March, harvest 26 March 2013.**

Object code	Description	Treatment	Phoma-index Start forcing	Phoma – index After forcing	Chicon production per 100 roots	Class I (%)
A	Control	Not contaminated roots	0	56.8 c	14.7	89.7
B	Control	Contaminated roots	0	71.9 c	16.1	89.1
C	Rovral SC	35 ml/1000 kg roots	0.8	39.2 b	15.9	95.1
DEF	Tecto SC	40-60-80 ml/1000 kg roots	0	25.4 a	16.2	89.7
	F pr.		n.s.	<0.001	n.s.	n.s.

Photo 2a-b: **Assessment index of Phoma attack of the taproots after forcing. Left: contaminated control, right: Tecto SC 80 ml/1000 kg roots. Lelystad-NL, March 2013.**

With regard to the relative length of the axe in the chicon, no significant differences between the treatments were noticed (table 3). The aberrations of the axe (brown, hollow, red) were also not influenced by the different treatments.

Table 3. **Influence of Tecto SC on relative length of the axe in the chicons (cv. Baccara) and aberrations of the axe in relation to Rovral SC and not contaminated or contaminated control. Start of forcing 4 March, harvest 26 March 2013.**

Object code	Description	Treatment	Axe (%)	Brown index	Hollow index	Red index
A	Control	Not contaminated roots	39.6	1.50	4.25	0.00
B	Control	Contaminated roots	41.9	5.75	0.75	0.00
C	Rovral SC	35 ml/1000 kg roots	40.3	0.75	7.50	0.00
D	Tecto SC	40 ml/1000 kg roots	38.5	3.25	6.75	0.00
E	Tecto SC	60 ml/1000 kg roots	39.0	6.75	7.50	0.00
F	Tecto SC	80 ml/1000 kg roots	39.4	6.00	7.50	0.75
	lsd (p=0.05)		6.8	5.122	8.629	0.910
	F pr.		n.s.	n.s.	n.s.	n.s.

Photo 3a-b: **Asssesment of chicon production. Left contaminated control. Right Tecto SC, 80 ml per 1000 kg roots. Lelystad-NL, 26 March 2013.**

4 Discussion and conclusions

1. The taproots (cv. Baccara) from biological origin had a natural high infection pressure of *Phoma*. Contamination by a spore suspension increased the *Phoma* attack only slightly.
2. Treatment with different rates (40-60-80 ml/1000 kg of roots) of Tecto SC after several months of root storage was effective in reducing *Phoma* attack during forcing.
3. Remarkeble is the low Phoma-index of the roots at start of forcing. Apparently the latent Phoma infection became active during forcing.
4. As average over the application rates, Tecto SC was more effective is reducing *Phoma* attack than Rovral SC.
5. Spraying fungicides to prevent diseases can reduce production because of a phytotoxic effect, however until a rate of 80 ml per 1000 kg of roots, Tecto SC was not influencing chicon production.
6. Tecto SC and also Rovral SC did not influenced the development of the axe or had impact on the assessed aberrations: brown axe, hollow axe or red discoloration of the axe.
7. The used protocol for efficacy evaluation was updated in cooperation with the Research Institutes *Inagro* in Roeselare (Belgium) and *Station Expérimentale* in Arras (France) and can be used for further experiments.

Appendix 1 Protocol for efficacy evaluation

PROTOCOL FOR EFFICACY EVALUATION OF FUNGICIDES (POST-HARVEST TREATMENTS) AGAINST *PHOMA EXIGUA* IN TAPROOTS OF WITLOOF CHICORY (*CICHORIUM INTYBUS L. VAR. FOLIOSUM HEGI)*

According to GAP-rules (Good Agricultural Practice) and EPPO Guidelines, not meant for residue studies

1. ACQUIRED INFORMATION & MATERIALS AT START OF VALIDITY ASSESSMENT:

1.1. Directions for use of fungicides
1.2. Environmental hazards
1.3. Safety precautions
1.4. Cultures of 7 days old *Phoma exigua* isolates
1.5. Healthy taproots of a sensitive Witloof chicory variety (for instance cv. Focus or cv. Baccara) to be used in this test:
- Number of sorted roots acquired for non contaminated and *Phoma* contaminated control: 800
- Number of sorted roots acquired for testing one chemical in 3 doses: 1200 (3 x 4 repetitions à 100 roots)
- Number of sorted roots acquired for testing X chemicals: 800 + X*1200

2. GROWING *PHOMA EXIGUA* ON PDA-PLATES AND PREPARING PYCNIDIOSPORE SUSPENSION

1. Inoculation of PDA-plates (39.5 g/l water + 100 ppm streptomycine) with pycnidospore suspension or agar plugs (harvesting pycnidiospores is going faster after inoculation with spore suspension)
2. Incubate PDA-plates for at least 7 days at 20 °C in normal daylight
3. After growing mycelium over complete surface PDA-plates, pycnidia are clearly visible
4. Adjust 10 ml physiological water (9 g/l NaCl) per plate and scrape with sterile spate over PDA-plate
5. Suck the spore suspension and repeat when PDA-plate consist a lot of black pycnidia
6. Estimate concentration of spore suspension by using a haemocytometer or Bürker counting chamber
7. Place spore suspension at a temperature of 2 °C (store for maximum 48 hours)
8. Adjust the spore suspension just before root inoculation to a concentration of at least $1x10^6$ conidia per ml.

3. ARTIFICIAL CONTAMINATION OF WITLOOF CHICORY ROOTS, FRESHLY HARVESTED OR FROM STORAGE, WITH *P. EXIGUA*.

3.1 Contamination directly after harvest

In case of starting the validity test with freshly harvested roots, harvest should take place under heavy cleaning intensity of the harvest machine or treating with metal pins to induce extra damage to the roots in order to facilitate artificial contamination with *P. exigua.* Sort out the total acquired number of healthy roots (at least 2000 for testing 1 fungicide) with a diameter of 3 ½ - 5 ½ cm. Contaminate within 24 hours after harvest at least 1600 roots by spraying the spore suspension over de roots, 10 ml suspension per kg roots (10 liter per ton) at a pressure of 1-2 bar. Place 80-100 roots in EPS-boxes at a temperature of 6 °C under perforated plastic film at > 95 % RV for 48 hours. Place the not contaminated roots in EPS-boxes accordingly.

3.2 Contamination during storage

Roots from storage are healed and should therefore be damaged in order to facilitate artificial contamination with *P. exigua.* These roots must have been harvested under normal conditions and cleaning intensity and kept in storage for 6 months at most. Sort out at least 2000 healthy roots with a diameter of 3 ½ - 5 ½ cm. Make fresh wounds by cutting off the root tip to a root length of 15 à 16 cm and/or crush the roots with metal pins. Contaminate at least 1600 roots within 24 hours after cutting or crushing by

spraying the spore suspension over de roots, 10 ml suspension per kg roots (10 litres per ton) at a pressure of 1-2 bar. Place 80-100 roots in EPS-boxes at a temperature of 6 °C under perforated plastic film at > 95 % RV for 48 hours. Place the not contaminated roots in EPS-boxes accordingly.

4. VALIDITY ASSESSMENT OF ONE CHEMICAL AND ONE REFERENCE AGAINST *P. EXIGUA*.

4.1. Fungicide treatment

Treat roots 48 hours after contamination. Spraying of 20 ml solution of fungicide per kg roots (20 litres per ton). Spraying in 3 concentrations at a pressure of 1-2 bar in 4 repetitions à 1 EPS-box filled with 80-100 roots (ca. 20 kg).

4.2. Review of treatments

1. *Control not contaminated*
2. *Control contaminated*
3. *Reference Rovral 35 ml per 1000 kg roots*
4. *Tecto 40 ml/1000 kg roots*
5. *Tecto 60 ml/1000 kg roots*
6. *Tecto 80 ml/1000 kg roots*

4.3. Storage conditions

Place the EPS-boxes after fungicide treatment during 7 days at a temperature of 6 °C under perforated plastic film at > 95 % RV (fogging advisable). Separate vaporous fungicide treated boxes from controls and other treatments to prevent re-disposition through vapour movement.
After 1 week, infestation period is complete and roots can be stored under normal conditions at 3 °C under perforated plastic film for 3 weeks.

4.4. Scoring Phoma infestation

Score *P. exigua* index 4 weeks after fungicide treatment:[100 * (0*clean + 1*light + 2*moderate + 3*heavy) / 3*total] = Pe-index
0 = clean; 1 = light (necroses < 2 mm diameter); 2 = moderate (necroses 2-10 mm diameter); 3 = heavy (necroses > 10 mm diameter)

4.5 Forcing experiment

When necessary for registration purposes: forcing of the roots for witloof chicory production according to normal principles and determination of marketable yield and quality. Forcing of fungicide treated roots must be preferably separated from non treated roots. In preference each experimental unit (1 EPS-box) should be forced on separate reservoirs. After forcing score final *P. exigua* index of 30 roots per experimental unit as mentioned under 4.4.

4.6 Assessment of chicon yield after forcing

Per experimental unit chicons are broken off or cut from the roots, cleaned up and graded in quality Class I, II or III according EC rules. Class I and II are divided in short (9-15 cm) or long (> 15 cm) chicons. The chicons in each quality and grading class are counted and weighted. Total production per 100 roots and percentage production in Class I, II and III is calculated. Number of roots without formation of a chicon is counted with the cause. From Class I the axe and chicon length is measured of 10 chicons after longitudinal cutting. The relative length of the axe as percentage of the chicon length is calculated. Aberrations of the axe: brown axe, red discoloration and hollow axe are noted in a scale of 0 = clean; 1 = light (aberrations < 2 mm diameter); 2 = moderate (aberrations 2-10 mm diameter); 3 = heavy (aberrations > 10 mm diameter). Indexes of the aberrations are calculated.

Appendix 2 Results per experimental unit

Plot code	Object	Phoma-start	Phoma-after	Production	Cl. I (%)	Axe (%)	BI	HI	RI
K1B1	A	0	47	15,5	99	36	0	0	0
K1B8	A	0	46	14,4	89	40	0	0	0
K2B5	A	0	73	11,5	79	41	3	0	0
K3B3	A	0	62	17,6	92	43	3	17	0
K1B6	B	0	64	16,2	94	36	0	0	0
K2B4	B	0	72	15,9	89	45	10	0	0
K2B8	B	0	81	15,7	76	41	13	3	0
K3B1	B	0	70	16,6	97	46	0	0	0
K1B5	C	0	52	17,8	96	37	0	7	0
K1B7	C	3	34	14,4	99	37	0	13	0
K2B6	C	0	38	16,9	87	47	0	0	0
K3B4	C	0	32	14,6	99	41	3	10	0
K1B2	D	0	29	15,9	95	29	7	10	0
K2B1	D	0	16	15,8	86	38	3	10	0
K3B6	D	0	3	17,1	93	43	3	7	0
K3B8	D	0	39	18,2	88	44	0	0	0
K1B4	E	0	16	14,6	93	33	7	7	0
K2B2	E	0	39	17,8	85	40	3	10	0
K2B7	E	0	42	17,8	90	43	10	0	0
K3B5	E	0	27	16,7	90	40	7	13	0
K1B3	F	0	23	13,0	95	34	3	3	3
K2B3	F	0	33	13,8	81	39	7	3	0
K3B2	F	0	22	16,5	88	43	7	7	0
K3B7	F	0	16	16,7	95	41	7	17	0

Objects	Description
A	Control not contaminated
B	Control contaminated
C	Reference Rovral 35 ml per 1000 kg roots
D	Tecto 40 ml/1000 kg roots
E	Tecto 60 ml/1000 kg roots
F	Tecto 80 ml/1000 kg roots

Legends

Phoma-start = Phoma index at start of forcing
Phoma-after = Phoma index at end of forcing
Production = Chicon production (kg/100 roots)
Cl. I = Class I in % of production
Axe (%) = relative axe lenght
BI = brown axe index
HI = hollow axe index
RI = red axe index

Appendix 3 Recognition for efficacy testing

Plantenziektenkundige Dienst
Ministerie van Landbouw, Natuur en Voedselkwaliteit

This is to declare that, in conformity with the request of 7 December, 2009

Praktijkonderzoek Plant en Omgeving, Akkerbouw, Groene ruimte en Vollegrondsgroenten

Residing Edelhertweg 1, Lelystad, the Netherlands

HAS OFFICIALLY BEEN RECOGNISED AS AN ORGANISATION FOR EFFICACY TESTING

as has been laid down in the 'Regeling gewasbeschermingsmiddelen en biociden' (Regulation Crop Protection Products and Biocides) of September 26, 2007 (Staatscourant 2007, 386)

This recognition will commence on February 2, 2010 and expire on February 2, 2016

Wageningen, February 11, 2010

For the Minister of Agriculture,
Nature and Food Quality,

H.A. Harmsma LL. M., Bsc,

Acting Director Plant Protection Service