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Efficacy evaluation of fungicides against downy mildew *Bremia lactuca* in lettuce.

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Confidential Report (LTO)
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Business Unit Glastuinbouw
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Introduction

In lettuce *Bremia lactuca* is an important disease, especially in autumn and winter when the day-lengths and light intensity decrease and the humidity of the air increases. Frequently new lettuce varieties which a resistance against the latest fysio's of *B. Lactuca* are put on the market. However, this resistance lasts until the fungus makes a new fysio. As far as the new resistant lettuce variety is available growers need fungicides to control this disease.

At the moment two fungicides propamocarb (Previcur) and fosethyl-aluminium (Aliette) are available for the growers. Problem is that in practice both fungicides are not effective enough and resistance of the fungus against those fungicides is quite possible.

The growers organisation LTO want to broaden the number fungicides for lettuce growers by a so called 'derden toelating' in the Netherlands. Therefor an efficacy research is needed.

1 Objective

The aim of this trial was to test the efficacy of three fungicides against *Bremia lactuca* in lettuce *(Lactuca sativa).*

2 Materials & Methods

2.1 Planting and propagation

In this experiment a lettuce variety susceptible to the used *B. lactuca* -fysio was planted in week 51 (25 cm * 25 cm). The *B. lactuca* fysio was obtained from a breeding company.
At 02-01-2003 all plants were sprayed with iprodione (40 g/100 m^2) and thiram (100 g/ 100 m^2) to protect the crop against *Botrytis, Rhizoctonia, Sclerotinia* and *Pythium.*
Weekly the out-of-trial plants were sprayed with propamocarb/ hydrochloride (Previcur) to decrease the infection pressure of downy mildew. Cultural conditions were uniform for all plots of the trial and were standard to local horticultural practise.
When it turned out the fungus had some difficulties to develop the climate settings were changed (22-01-2003). Windows stayed longer closed to achieve higher air humidity. In the last weeks before harvest also water was sprayed to increase the air humidity.

2.2 Climate data

Glasshouse climate and weather conditions may affect the efficacy of crop protection compounds and the development of the disease. Therefore temperature and air humidity were logged. (Appendix 3)

2.3 Artificial inoculation of *B. Lactuca*

To ensure an adequate and homogeneous level of disease all over the trial, spores of *B.* were sprayed on to the crop (37,5 X 10^3 spores/m^2). There was no artificial inoculation in the out-of-trial rows.

Before inoculation water was sprayed to increase the air humidity and to get the crop wet. The weather was cloudy.
In the inoculum also spores of *Botrytis* were present but in a very low concentration. Symptoms of both diseases are well distinguishable.

2.4 Experimental set-up

The experiment was in randomised block design and consists at five treatments in four blocks. Net plot size was 28 plants (appendix 1, 2). A survey of the treatments is given in Table 1.

Table 1. Treatments

code			active ingredients	dosage ml/ha	frequency	number of sprayings
A	**untreated**		-	-	-	-
B	**Tanos**	**DPX 301**	cymoxanil + famoxadone	0.6 kg/ha (=0.06 g/m^2)	weekly	6
C	**Acrobat**	**BAS 551 00 F**	dimethomorf + mancozeb	2 kg/ha (= 0.2 g/m^2)	weekly	6
D	**Flint**	**AC 2112**	trifloxystrobine	0.5 kg/ha (=0.05 g/m^2)	weekly	6
E	**Previcur**		propamocarb + hydrochloride	1.5 l/ha (=0.15 ml/m^2)	weekly	6

Because the fungicides have a preventative effect artificial inoculation of the fungus was carried out one day after the first application of the treatments. Further sprayings were carried out weekly after the assessment.

Table 2. Survey of treatment and assessment dates

week number	assessment	treatment
1.	-	06-01 (07-01 inoculation fungus)
2.	14-01	15-01
3.	21-01	22-01
4.	28-01	29-01
5.	04-02	05-02
6.	11-02	12-02
7.	18-02	
8.	25-02	
9.	06-03	
10.	11-03	
11.	18-03 / 22-03 (final assessment)	

Weekly all plants were examined for the presence of symptoms of *B. lactuca.* During the trial only infected and uninfected plants were assessed. Every plant with symptoms of *B. lactuca* was marked on a map. As final assessment also the percentage area infected per diseased leaf was examined. The final assessment was carried out when the plants achieve a mean plant weight of circa 300 g and took place one month after the last application of the treatments (Appendix 4).

2.5 Statistical analysis

Data were incorporated statistically by using the program GenStat release 6.1 (PC/ Windows NT).

Analysed was: % area infected per plant, number infected plants per assessment date, mean weight of the plants / plot.

3 Results and discussion

The first infected plants were found three weeks after inoculation. Weekly the number of infected plants increased, but very slowly. In spite of optimisation of the climate settings, the development of the disease in the untreated plots did not increase as expected. Infection of *Bremia* was seen as small areas on the old leaves. The number of infected plants was scored. Table 3a and 3b show the results of the statistical analysis. There was no significant effect between the treatments.

This is probably due to a bad development of the fungus in the trial. This could be a concurrence of circumstances. The trial was a few months delayed so it was carried out in a sub optimal period of the year. Artificial inoculation of the fungus and creating of a climate with high air humidity should have handled this. But, in this very time of the year there were even for the Netherlands exceptional weather conditions (sunny and freeze). Also in practice growers did not have problems with this fungus in their crops. A few weeks later when the weather was cloudy and rainy again horticultural growers have had problems with *Bremia* in their crops.

Table 3a. Development of the number infected plants during the trial

treatment	28-01-03	04-02-03	11-02-03	18-02-03	25-02-03	06-03-03	11-03-03
A (untreated)	0.50	1.25	3.00	3.00	3.25	4.25	6.50 (23%)
B	0.00	0.25	1.00	2.50	3.00	4.00	8.00 (29%)
C	0.25	0.75	2.50	3.25	3.75	5.25	10.75 (38%)
D	0.00	0.75	1.50	2.25	3.00	4.00	6.75 (24%)
E (Previcur)	0.00	0.00	0.00	0.50	1.00	1.25	6.25 (22%)
LSD (one sided)	ns	ns	ns	ns	ns	ns	ns

ns= not significant (p=0.05)

Table 3b. Percentage infected area at the final assessment (18-03-03) and yield.

treatment	% infected area / plant (teruggetransformeerde waarden na logtransformatie)	mean weight/plant
A (untreated)	1.47	255.1
B	1.75	284.7
C	1.80	290.8
D	2.67	271.1
E (Previcur)	1.80	273.3
LSD (one sided)	ns	ns

ns= not significant (p=0.05)

None of the tested products showed fytotoxical reactions. Visible residue was found in treatment C.

4 Conclusions

In this trial no statistically significant effects were found between the treatments. The fungus did not develop as expected probably due to the extreme sunny and dry weather conditions during the experiment.

None of the tested products showed any fytotoxicity to lettuce.

Treatment C gave visible residue.

It is recommended to repeat this trial in autumn, when the circumstances for *B. lactuca* are optimal.

Appendix 1: Trial form

Project leader	:	M. van der Staaij
Experiment leader	:	M.A. Haaring-Schepman
Project number	:	41201630
Working title	:	Efficacy evaluation of three fungicides against *Bremia lactuca* in lettuce.
Location	:	PPO-Naaldwijk, compartment 303-04
Experiment type	:	Greenhouse
Duration	:	December- March 2003
EPPO guidelines	:	PP 1/65(3). Guideline for the efficacy evaluation of fungicides; Downy mildews of lettuce and other vegetables.
Number of plants	:	2640
-per plot	:	54 (6 * 9)
-per net plot	:	28 (5 * 7)
Water supply	:	overhead
Numbers buffer rows	:	2
Plant variety	:	Lettuce (*Lactuca sativa) cv.* Wynona
Replicates	:	4
Disease	:	*Bremia lactuca fysio* (virulent for the used lettuce variety)

Treatments: :

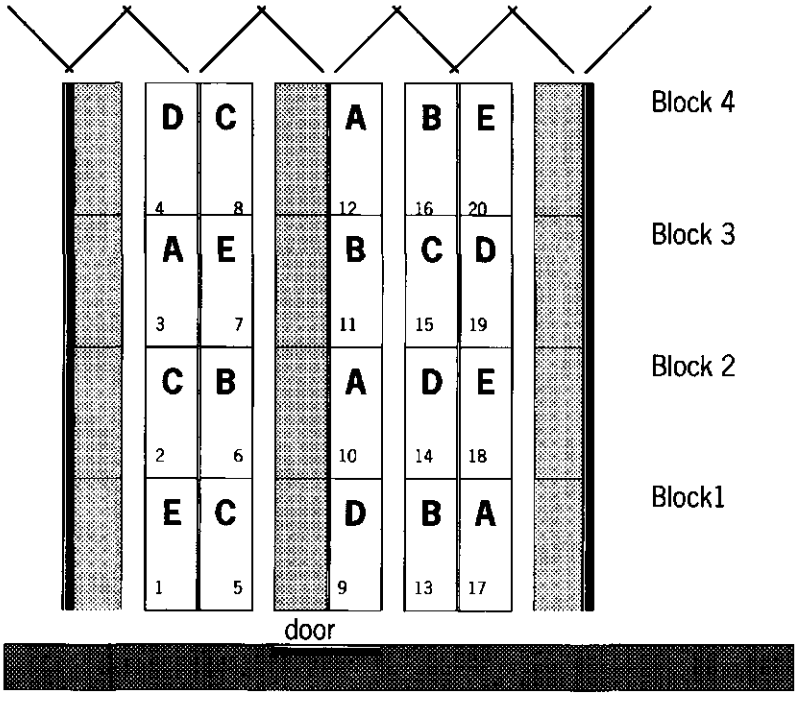
code			a.i.	dosage ml/ha	freq.	needed per treatment (12 m²)
A	**untreated**					
B	**Tanos**	DPX 301	cymoxanil + famoxadone	0.6 kg/ha(=0.06 g/m²)	5	0.72 g
C	**Acrobat**	BAS 551 00 F	dimethomorf + mancozeb	2 kg/ha (= 0.2 g/m²)	5	7.20 g
D	**Flint**	AC 2112	trifloxystrobine	0.5 kg/ha (=0.05 g/m²)	5	0.60 g
E	**Previcur**		propamocarb + hydrochloride	1.5 l/ha (=0.15 ml/m²)	5	1.8 ml

Preparation pesticide solution	:	fungicide solution prepared per treatment
Starting date	:	06-01-2003
Numbers of applications	:	5
Spray equipment	:	Mesto, Ferrum 3560 pulverisateur
Spray volume (per ha)	:	1000 l/ha * 4 hh=1000 ml
Spray volume (per plot)	:	ca 250 ml
Spray pressure	:	3 bar
Nozzle	:	nozzle with hollow cone, spray angle 65°
Application rate	:	1.24 litre per minute (3 bar)

Assessment

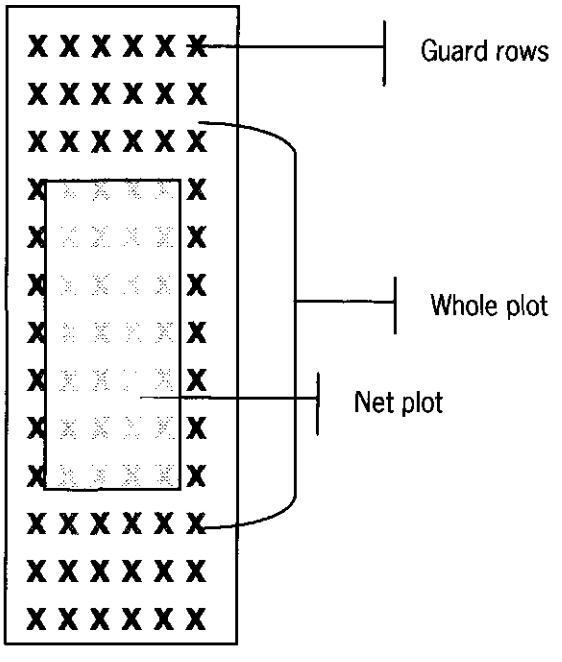
- during the trial	:	assessment of infected or uninfected plants (28 plants/plot); other diseases (if present make notes)
- phytotoxicity	:	if present, make notes and describe symptoms
- residue	:	if present, make notes
- final assessment	:	number of infected leaves per plant and % area infected per diseased plant (28 plants)
-climate	:	registration by climate computer (temperature, air humidity)

Appendix 2: Design and layout of the trial



	out of trial
1 to 20	field no
A to E	treatment codes

Overview of a plot



Appendix 3: Climate data

Figure 1 shows the mean temperature and relative air humidity during the experiment. At 22-01-03 climate settings were changed to create a better climate for the developing of the fungus. The next weeks were extremely sunny. It was hard to keep the relative air humidity high enough. At the end of February there were a few more cloudy weeks, but in the beginning of March it was very sunny again. In those days there was water sprayed all over the lettuce, but this seemed to have no lasting effect on air humidity.

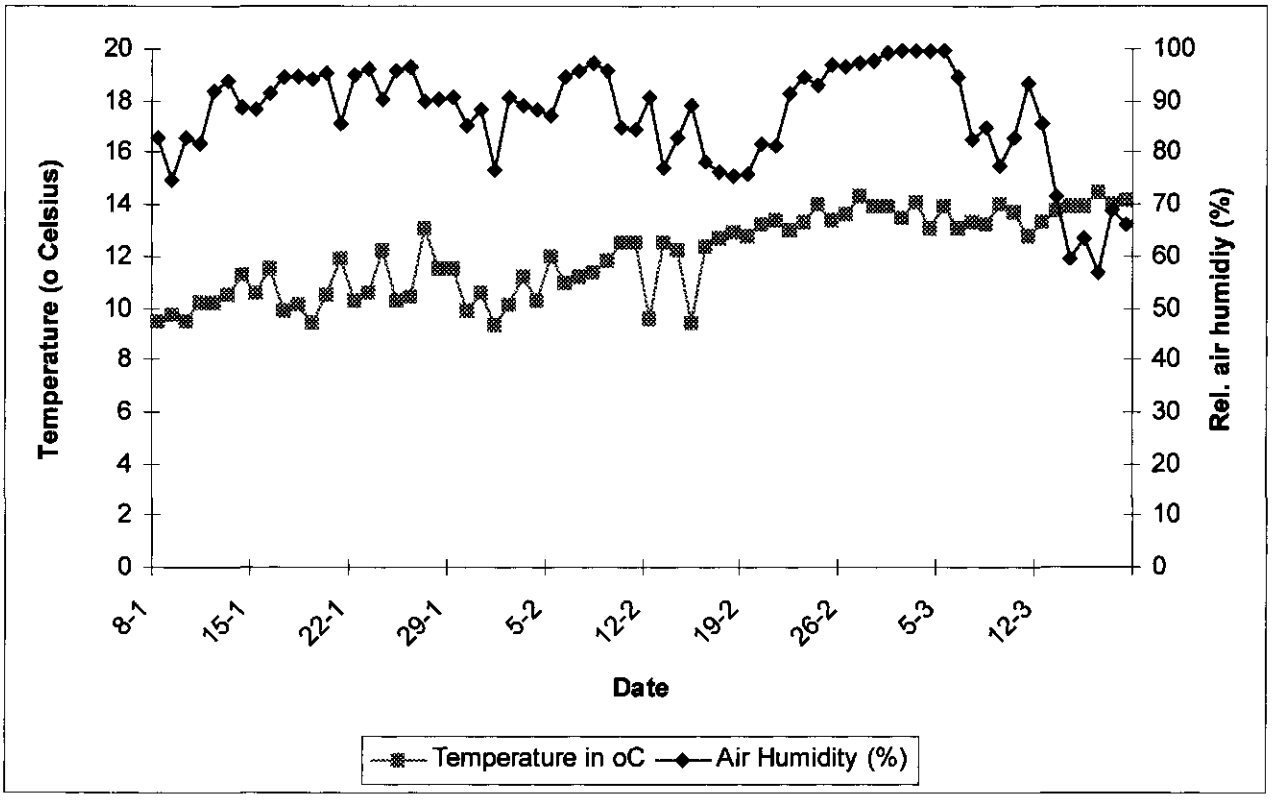


Figure 1. Mean temperature and relative air humidity during the trial in the glasshouse.

Appendix 4: Raw data

field	block	treatment	n030128	n030204	n030211	n030218	n030225	n030306	n030311	pbremia	weight
1	1	E	0	0	0	1	2	3	9	2.10	*
2	2	C	0	1	3	3	4	5	10	1.65	310.82
3	3	A	1	3	7	7	8	10	14	0.92	269.54
4	4	D	0	2	4	5	6	7	12	7.55	254.25
5	1	C	1	1	4	5	5	5	14	3.25	*
6	2	B	0	0	2	3	3	5	9	1.55	313.18
7	3	E	0	0	0	1	2	2	11	1.53	286.57
8	4	C	0	0	0	1	1	5	8	2.03	266.68
9	1	D	0	0	1	2	4	5	7	1.79	*
10	2	A	0	0	1	1	1	2	3	1.65	267.96
11	3	B	0	0	0	2	4	5	8	1.28	274.39
12	4	A	1	1	2	2	2	3	7	5.41	227.75
13	1	B	0	1	2	5	5	5	9	1.38	*
14	2	D	0	1	1	2	2	4	5	1.94	292.32
15	3	C	0	1	3	4	5	6	11	1.68	294.79
16	4	B	0	0	0	0	0	1	6	6.18	266.57
17	1	A	0	1	2	2	2	2	2	1.04	*
18	2	E	0	0	0	0	0	0	0	2.17	297.39
19	3	D	0	0	0	0	0	0	3	1.86	266.86
20	4	E	0	0	0	0	0	0	5	4.00	236.04

field	field number 1 to 20
block	block number 1,2,3,4
treatment	treatment A,B,C,D,E
n030128	number of diseased plants on yy-mm-dd (cumulative)
pbremia	% area infected per leaf (mean all leaves/plant/ 28 plants per field)
weight	mean plant weight of 28 plants/field (block numbers 2,3,4)