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# Fungicide evaluation to rate the efficacy to control early blight for the EuroBlight table

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# Summary

Early blight caused by *Alternaria solani* is the second most important foliar disease in the cultivation of potatoes after potato late blight. The potato crop needs to be protected against *A. solani* by spraying fungicides regularly during the second half of the growing season. It is important to use fungicides that effectively protect leaves against this disease.

Up until now (July 2017) the ratings of the fungicides to control early blight were based upon expert judgement, both from agrochemical companies and independent researchers.

To evaluate the effectiveness of fungicides harmonised protocols were discussed at Brasov. It was proposed that ratings of fungicides for the EuroBlight-table are calculated when field experiments are carried out over 2 years in 3 European countries in concordance with the potato late blight rating. In 2015 the first three experiments were carried out in Germany, Denmark and the Netherlands. In 2016 four experiments were carried out. In fact 6 field experiments were set up to compare the effectiveness of fungicides against early blight by measuring the protection of leaves in a standard 14 or 7-day spray schedule.

Based on the average StAUDPC (mstAUDPC), ratings for the effectiveness of the fungicides to control early blight were calculated, according to formula 0 and presented in Table 0.

$$ER_k = 5 \frac{\text{MAX}(y) - y_k}{\text{MAX}(y)}, \quad (0)$$

$ER_k$  = efficacy rating of the fungicide  $k$  to control potato early blight during the whole growing season.

$y$  = mstAUDPC

$\text{MAX}(y)$  = mstAUDPC of the fungicide with the highest mstAUDPC determined in the series of experiments.

**Table 0.** Efficacy of fungicides for the control of early blight caused by *Alternaria spp.* expressed as a decimal rating (0-5), published on the EuroBlight web-site.

Product	Efficacy rating <sup>1,2</sup>		
	14 day interval	Strategy	7 day interval
<b>Spray interval 14 days</b>			
mancozeb 2.0	1.7	-	-
<b>Spray strategy<sup>3</sup></b>			
(zoxamide + mancozeb) <sup>a</sup> 1.8 + azoxystrobin <sup>b</sup> 0.5 <sup>4</sup>	-	3.7	-
(zoxamide + mancozeb) <sup>a</sup> 1.8 + difenoconazole <sup>b,5</sup> 0.5	-	3.9	-
<b>Spray interval 7 days</b>			
mancozeb 2.0	-	-	2.5
(zoxamide + mancozeb) 1.8	-	-	2.8
(fenamidone + propamocarb) 2.0	-	-	2.2
(fluazinam + azoxystrobin <sup>5</sup> ) 0.5	-	-	3.1
(dimethomorph + mancozeb) 2.0	-	-	2.9

<sup>1</sup> : Ratings for *Alternaria* are based on results from EuroBlight field trials during 2015-2016, and only compounds included in these trials are rated for *Alternaria*. The scale for *Alternaria* is a 0-5 scale (see technical report to be uploaded soon).

<sup>2</sup> : The ratings are intended as a guide only and will be amended in future if new information becomes available.

<sup>3</sup> : The active ingredients were sprayed in a spray strategy with a 7 day interval (<sup>a</sup>) or a 14 day interval (<sup>b</sup>)

<sup>4</sup> : azoxystrobin was sprayed at label rate which is 0.5 for DK and DE, and 0.25 for NL.

<sup>5</sup> : *Alternaria solani* isolates that are less sensitive to QoI-fungicides have been isolated from potato plants in Europe. Therefore resistance management strategies should be implemented (see FRAC web site for details). Rating will be lower where fungicide insensitive strains are present.

This report describes the analysis of the efficacy of fungicides to control early blight during the second half of the season.

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# 1 Introduction

Early blight caused by *Alternaria solani* is the second most important foliar disease in the cultivation of potatoes after potato Late Blight. The potato crop needs to be protected from *A. solani* by spraying fungicides regularly during the second half of the growing season. It is important to use fungicides that effectively protect leaves against this disease.

Some fungicides are registered specifically to control early blight. Some fungicides used to control potato Late Blight also (partly) control early blight. Up until now (July 2017) the ratings of the fungicides to control early blight were based upon expert judgement, both from agrochemical companies and independent researchers.

To evaluate the effectiveness of fungicides harmonised protocols were discussed at Brasov. It was proposed that ratings of fungicides for the EuroBlight-table are calculated when field experiments are carried out over 2 years in 3 European countries in concordance with the potato Late Blight rating. In 2015 the first three experiments were carried out in Germany, Denmark and the Netherlands. In 2016 four experiments were carried out. In fact 6 field experiments were set up to compare the effectiveness of fungicides against early blight by measuring the protection of leaves in a standard 14 or 7-day spray schedule (this standard spray schedule is not necessarily related to the label recommendations). This protection originates from the protectant and/or curative properties of the active ingredients. Dose rates were the highest preventative doses registered in Europe. The results of the trials will be used to re-evaluate the effectiveness of fungicides to control early blight. This report describes the analysis of the efficacy of fungicides to control early blight during the second half of the season.

## 2 Materials and methods

### 2.1 Trial set up

Experiments were conducted in Denmark, Germany and the Netherlands. Full details are contained in the individual trial reports. Experiments were carried out for the first time in 2015. The experiments were carried out according to the harmonised protocol as discussed during the Workshops of the "European network on Potato Late Blight" in Brasov (2015) and the early blight subgroup meeting in Munich (2014). The protocol can be found on the EuroBlight website (<http://www.euroblight.net/EuroBlight.asp>) and is given in Appendix 1.

In general the trials conformed to local good agricultural practice, only the fungicide sprayings against *A. solani* were carried out more or less weekly or with a 14-day frequency. The experiments were carried out in accordance with GEP.

### 2.2 Fungicides

In the Netherlands fungicide applications were carried out using a CDH-sprayer with Teejet XR110.04 nozzles approximately 50 cm above the foliage. Sprayings were carried out with a spray volume of 250 l/ha.

In Denmark Hardi flat fan (ISO) LD 025 was used. The fungicides were sprayed with pressure of 3.0 bar, at 4.0 km/h and with 300 l water / ha.

In Germany the plots were sprayed by Technik TUM.

Potato plants were sprayed for the first time at approximately 6 to 8 weeks after emergence in each experiment. Fungicides were sprayed weekly or in a fourteen day interval, according to the protocol. Fungicides evaluated are listed in Table 1. The crop was sprayed full field with fungicides to control potato Late Blight. The Late Blight fungicides chosen had no efficacy to control early blight.

**Table 1. Fungicides sprayed in the experiments.**

Fungicide	Active ingredient	Dose rate	Spray interval
		L or kg /ha	days
Unikat Pro (Electis) 7	(zoxamide + mancozeb)	1.8	7
Unikat Pro 7 + Amistar 14	(zoxamide + mancozeb) + azoxystrobin	1.8 + 0.5 <sup>2</sup>	7 / 14
Unikat Pro 7 + Narita 14	(zoxamide + mancozeb) + azoxystrobin	1.8 + 0.5	7 / 14
Consento 7	fenamidone + propamocab	2.0	7
Vendetta 7	(fluazinam+azoxystrobin)	0.5	7
Acrobat DF (Invader) 7	(dimethomorph+mancozeb)	2.0	7
mancozeb 14	mancozeb	1.5 <sup>1</sup>	14
mancozeb 7	mancozeb	1.5 <sup>1</sup>	7
UTC	-	-	-

<sup>1</sup>: dose rate of the active ingredient mancozeb; various products may have been used.

<sup>2</sup>: in the Netherlands 0.25 l/ha was used



## 2.3 Experimental conditions

The experimental conditions are presented in Tables 2 and 3. Artificial inoculation was not necessary in Germany in 2015 and 2106. In Denmark and the Netherlands the field experiments were inoculated by distributing *Alternaria solani* infested grain kernels between the rows. Inoculation was carried out with genotype I, wild type.

Cover sprays with fungicides, which have no efficacy to control early blight, controlled potato Late Blight, i.e. Revus, Infinito and Ranman Top were used. Details are described in individual trial reports.

**Table 2. Experimental conditions at the different locations 2015.**

Experimental conditions	Denmark	Germany	the Netherlands
Location	Flakkebjerg	Kirchheim	Valthermond
Soil	Sandy clay loam	Pararendzina	Reclaimed peat
Tillage	CONTIL	CONTIL	CONTIL
Previous crop	Wheat	Triticale	Wheat
Variety	Kardal	Maxilla	Festien
Planting	25 April	13 April	8 May
Crop emergence	1 June	11 May	4 June
Inoculation	24 June	-	27 July
Specific sprayings July	13 <sup>1</sup> , 20, 28	10 <sup>1</sup> , 17, 24	30 <sup>1</sup>
Specific sprayings August	4, 11	3, 10	6, 13, 20 & 26
Specific sprayings September	-	-	3, 10, 18
Haulm kill			15 October

<sup>1</sup>: first specific *Alternaria* spray application. Depending on the treatment, the spray interval was 7 or 14 days.

**Table 3. Experimental conditions at the different locations 2016.**

Experimental conditions	Denmark	Germany	the Netherlands
Location	Flakkebjerg	Kirchheim	Valthermond
Soil	Sandy clay loam	Pararendzina	Reclaimed peat
Tillage	CONTIL	CONTIL	CONTIL
Previous crop	Wheat	Triticale	Spring wheat
Variety	Kuras	Maxilla	Festien
Planting	6 May	4 April	10 May
Crop emergence	1 June	15 May	7 June
Inoculation	1 July	-	19 July
Specific sprayings July	4 <sup>1</sup> , 11, 18, 25		21 <sup>1</sup> , 27
Specific sprayings August	1, 8, 17		4, 11, 18, 25
Specific sprayings September	-	-	1
Haulm kill	-		-

<sup>1</sup>: first specific *Alternaria* spray application. Depending on the treatment, the spray interval was 7 or 14 days.

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## 2.4 Disease observations

During the growing season, the percentage foliar infection caused by *Alternaria spp.* was assessed at weekly intervals. To evaluate the epidemic, the Area under the Disease Progress Curve (AUDPC) was determined. StAUDPC values were calculated by dividing the AUDPC value by the number of days between the first and last disease observation. Obviously, for each fungicide within an experiment the same number of days was used. The number of days from the first to the last disease observation varied for each experiment. The StAUDPC provides an indicator for the efficacy of the fungicides during the whole growing season. Appendix 2 lists StAUDPC values for fungicides tested in each experiment, for each replicate separately.

## 2.5 Statistical analyses

Six experiments were carried out. Each experiment was laid out as a randomised complete block design with one treatment factor, the fungicides being tested, and four replicates. A mixed model analysis (REML) was performed on StAUDPC measured per experimental plot. REML analysis was used because not every fungicide was present in all six experiments. A mixed model consists of fixed treatment terms (here fungicide) and random block terms (here experiment, block and plot; formula 1):

$$stAUDPC_{ijkp} = \mu + E_i + B_{ij} + \beta_k + P_{ijp}, \quad (1)$$

where

$\mu$  = overall mean

$E_i$  = effect of experiment  $i \sim N(0, \sigma_E^2)$

$B_{ij}$  = effect of block  $j$  within experiment  $i \sim N(0, \sigma_B^2)$

$P_{ijp}$  = effect of plot  $p$  within block  $B_{ij} \sim N(0, \sigma_P^2)$

$\beta_k$  = effect of fungicide  $k$

StAUDPC was analysed instead of AUDPC because the assessment period was not equal in all trials. StAUDPC equals the AUDPC divided by the number of days between first and final disease assessments. The code of the Genstat 18<sup>th</sup> ed. (Payne et al., 2009) used for the statistical analysis and the essential output are presented (Appendix 3).

Plots with high residuals were identified to establish non – consistent performance of fungicides. The stability of fungicide effectiveness between experiments was evaluated. The mean StAUDPC per fungicide (mstAUDPC) is reported in Appendix 2.

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Based on the average StAUDPC (mstAUDPC), ratings for the effectiveness of the fungicides to control Early blight were calculated, according to formula 2.

$$ER_k = 5 \frac{MAX(y) - y_k}{MAX(y)}, \quad (2)$$

$ER_k$  = efficacy rating of the fungicide  $k$  to control potato early blight during the whole growing season.

$y$  = mstAUDPC

$MAX(y)$  = mstAUDPC of the fungicide with the highest mstAUDPC determined in the series of experiments.

Formula 2 calculates a rating of 0-5 with the untreated control as a reference fixed at 0.0

The experiments were conducted in three countries during two seasons. Disease pressure varied with each experiment. The REML directive takes the specific conditions of the experiment into account. Assume that fungicide A was tested in experiments with a relatively high disease pressure and fungicide B in experiments with a relatively low disease pressure. Then the arrhythmic mean of mSTAUDPC of fungicide A would be adjusted with a decrease and fungicide B would be adjusted with a rise of mSTAUDPC. By doing so the disease pressure for all the fungicides is adjusted to the same level, making a fair comparison between fungicides in different experiments possible.

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## 3 Results and discussion

### 3.1 Early blight epidemics

Table 4 gives the first *Alternaria* lesions in the untreated control and in the treated plots per year and location.

**Table 4. First observation of *Alternaria* infected foliage in the untreated control and in treated plots, during the experiments.**

Year	Untreated			Treated		
	DK	DE	NL	DK	DE	NL
2015	14-7	15-6	19-8	14-7	15-6	26-8
2016	< 12-7	< 1-7	9-8	< 12-7	< 1-7	9-8

#### 3.1.1 2015

In Denmark the first *Alternaria* lesions were found mid-July, approximately 3 weeks after inoculation. The exponential phase of the epidemic started end of August in the untreated control and in the second week of September for most of the treatments.

In Germany the first *Alternaria* lesions were found already on 15 June, both in the untreated control and in some of the treated plots. However the early blight epidemic was stopped due to extremely high temperatures between 24 June and 8 July. This was followed by extremely hot and dry weather from 15 July onwards. The lower part of the crop senesced due to warmth and drought. From 17 August onwards disease observations were made in the first 1/3 of the potato plant. Until 10 August no differentiation was found in the early blight severity. As a consequence the StAUDPC was calculated from 17 August until 19 September based on disease assessments on the upperpart of the potato plants.

In the Netherlands early blight was observed in the spreader rows on 19 August, approximately 3.5 weeks after inoculation. The first *Alternaria* lesions in the plots were found on 26 August. The exponential phase in the untreated control started mid-September.

The effectiveness of fungicides to control the early blight epidemic for each experiment separately is given in Figures 1-3.

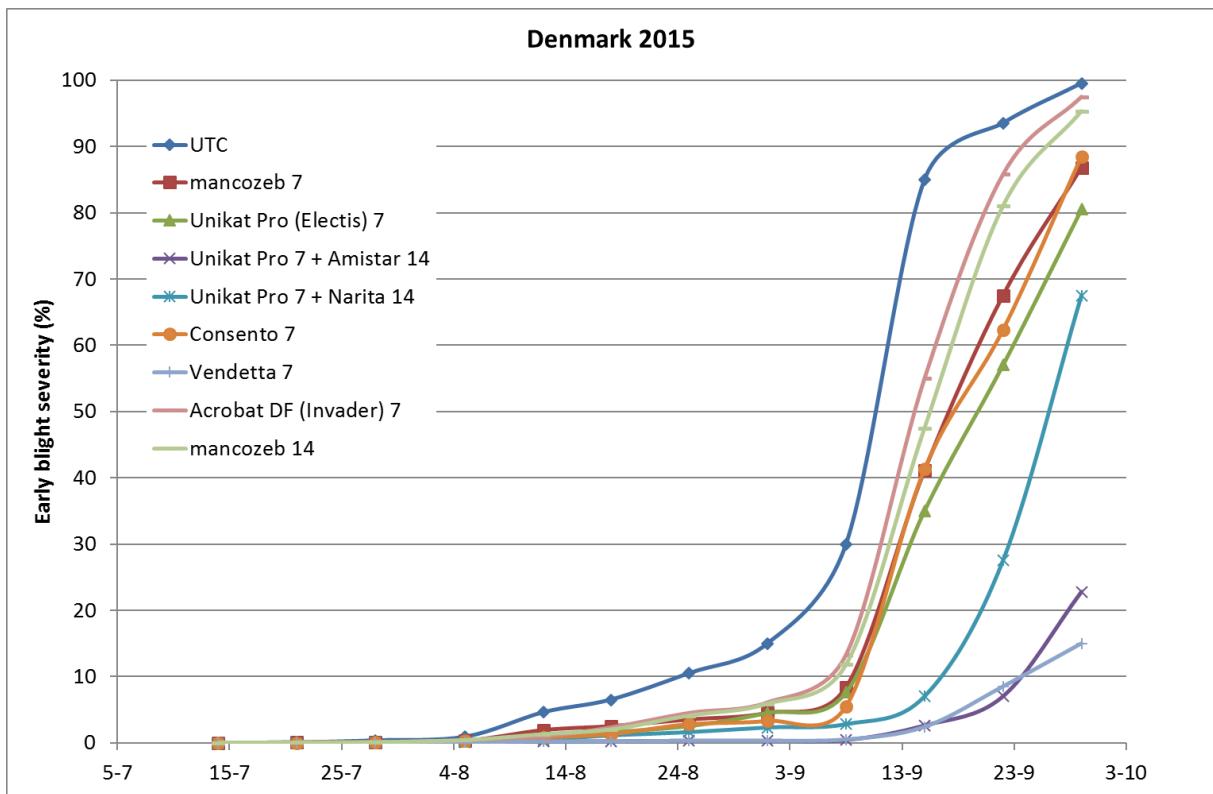


Figure 1 Early blight epidemic in Denmark 2015, the digit gives the spray interval.

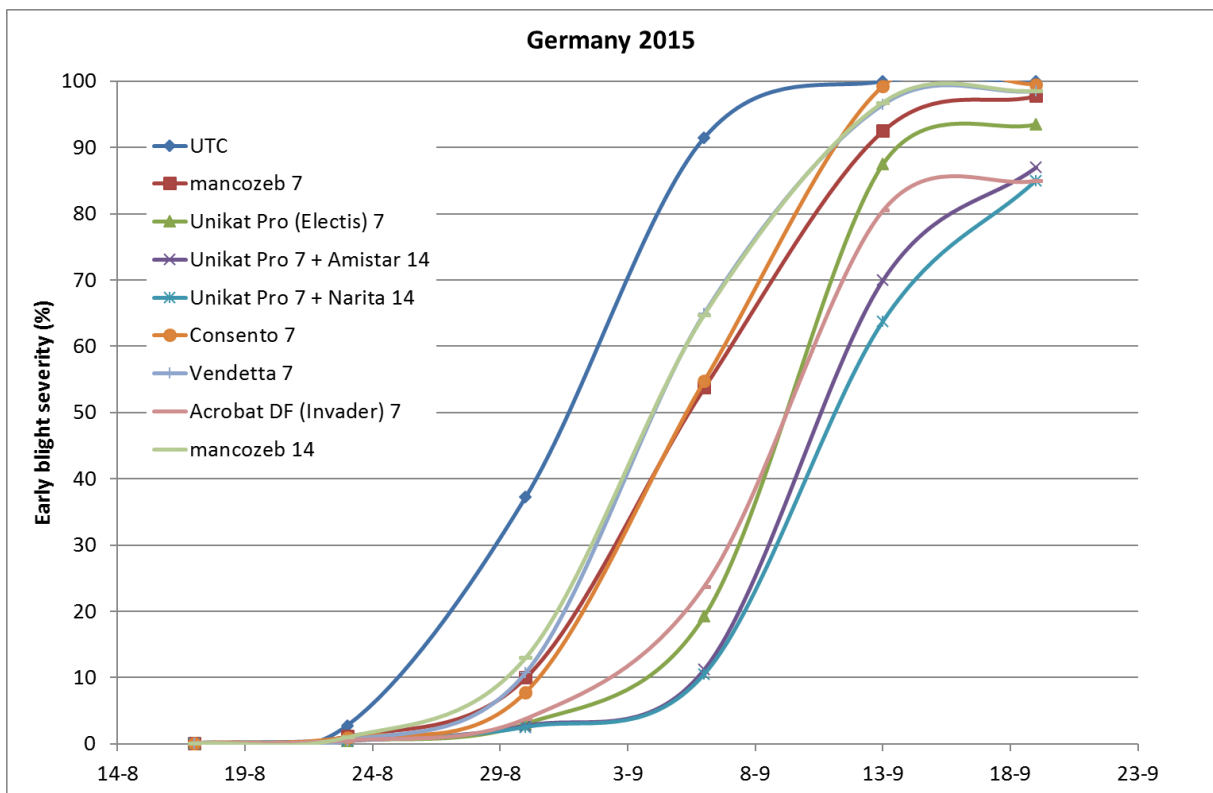


Figure 2 Early blight epidemic in Germany 2015 the digit gives the spray interval.

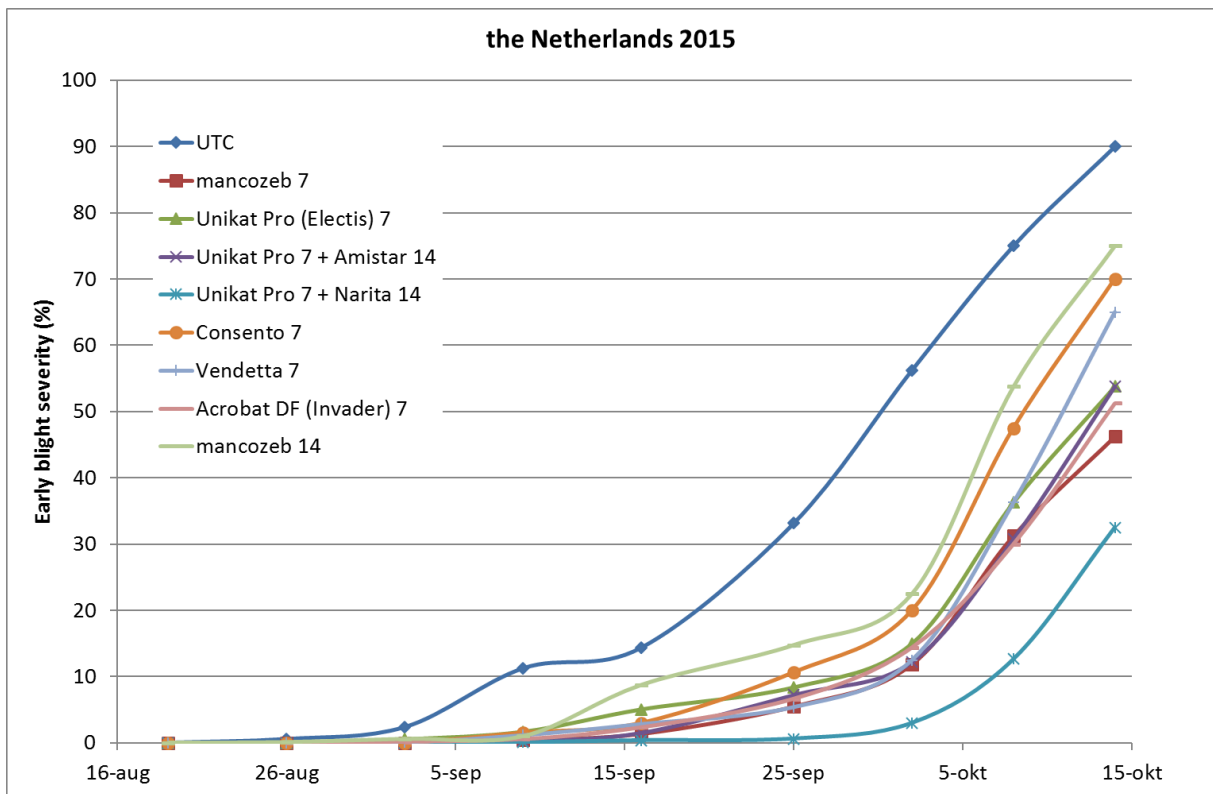


Figure 3 Early blight epidemic in the Netherlands 2015 the digit gives the spray interval.

### 3.1.2 2016

In Denmark the first *Alternaria* lesions were found mid-July, approximately 3 weeks after inoculation. The exponential phase of the epidemic started mid-August in the untreated control and end August for most of the treatments.

In Germany the first *Alternaria* lesions were found before 1 July, both in the untreated control and in some of the treated plots. In the first week of August the exponential phase of the epidemic started and a week later in the treated plots.

In the Netherlands (Valthermond) early blight was observed in the spreader rows on 9 August, approximately 3.5 weeks after inoculation. The first *Alternaria* lesions in most plots were found on 16 August. The exponential phase in the untreated control started the first week of September. In the treated plots this was in the second or third week.

The effectiveness of fungicides to control the early blight epidemic for each experiment in 2016 separately is given in Figures 4-6.

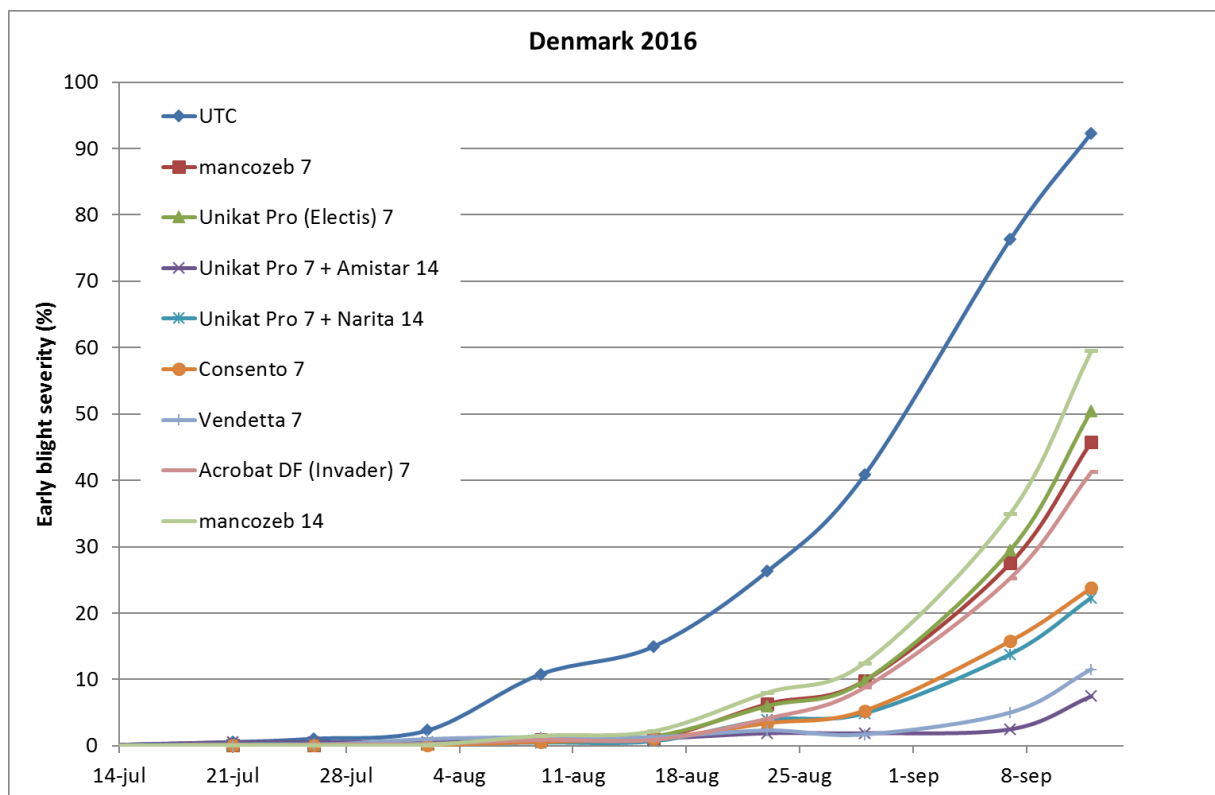
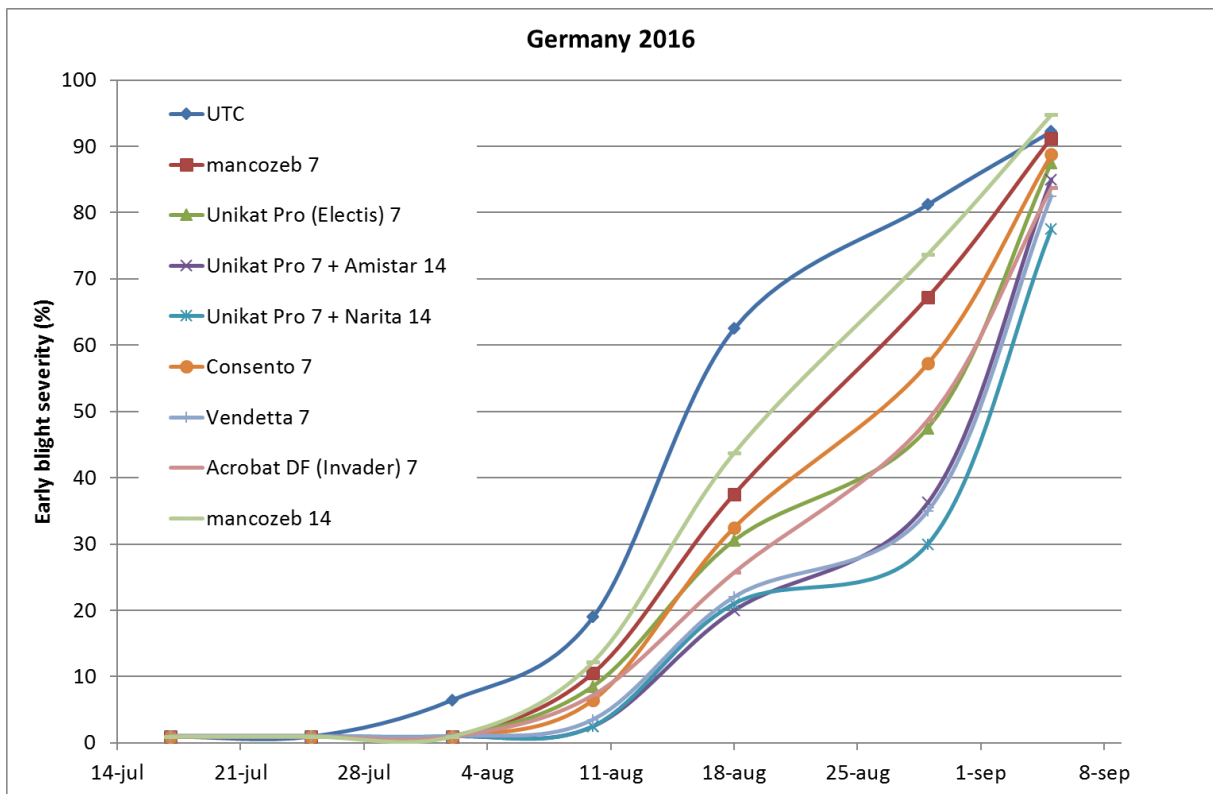
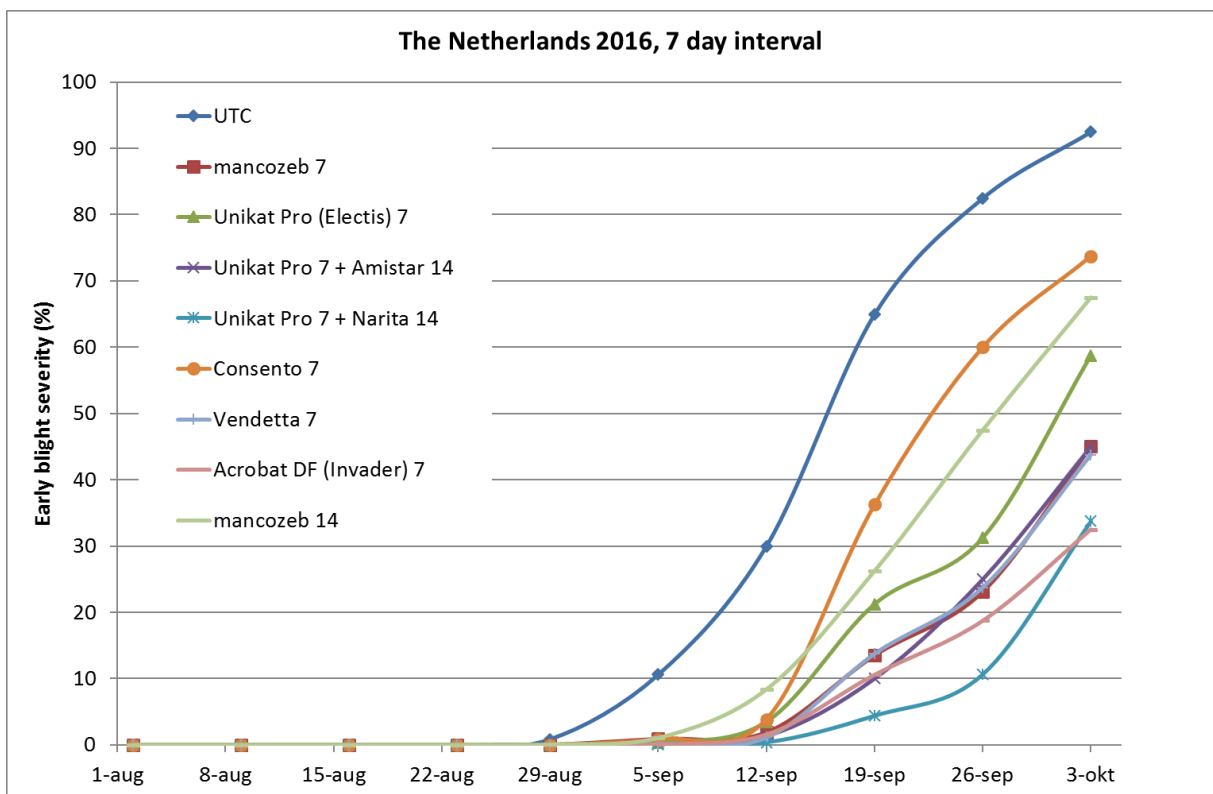


Figure 4 Early blight epidemic in Denmark 2016 the digit gives the spray interval.



**Figure 5 Early blight epidemic in Germany 2016 the digit gives the spray interval.**



**Figure 6 Early blight epidemic in the Netherlands 2016 the digit gives the spray interval.**



## 3.2 StAUDPC

The AUDPC or the StAUDPC can be used as a measure for the severity of the early blight epidemic. Control of early blight by fungicides will decrease the rate of the epidemic, and therefore reduce the AUDPC and StAUDPC value.

Disease severity differed between the six trials and the range of observed StAUDPC's was higher in trials with high disease pressure. Evaluation of the power transformation according to Box and Cox (Montgomery and Peck, 1982) showed the log transformation stabilized the variance. However we did not use this transformation because with it trials with low and high disease incidence have more or less equal weight. Without transformation results of trials with high disease incidence will have greater influence on the arithmetical means for each fungicide. Another argument not to use the logarithmic transformation was that the interaction between trial and fungicide was only partly reduced by the logarithmic transformation.

Scaling the StAUDPC to values between 0 and 5 for each trial separately, transforms absolute differences to relative differences. Also in that case trials with low disease intensity get equal weight relative to trials with higher disease intensity. The interaction between trial and fungicide remained significant. Therefore we propose to analyse the StAUDPC without transformation and use the arithmetical means for scaling to a score between 0 and 5. By default the untreated control is scaled at 0 and is used as a reference in all experiments.

## 3.3 Effectiveness of the fungicides based on StAUDPC

As a reference for the performance of the fungicides an untreated control and mancozeb sprayed in a 7 and 14 day interval were introduced in the field experiments. **Table 5** lists StAUDPC values after spraying fungicides during the second part of the season. In general it can be assumed that the efficacy of the fungicide is higher when the StAUDPC value is lower.

**Table 5. Early blight disease severity expressed as StAUDPC as a result of fungicides sprayed**

Fungicide	2015				2016			
	D	DK	NL	Average	D	DK	NL	average
Unikat Pro (Electis)	33.3	13.6	10.7	19.2	22.2	7.5	10.9	13.5
Unikat Pro 7 + Amistar 14	27.2	2.1	8.9	12.7	17.1	1.4	7.5	8.7
Unikat Pro 7 + Narita 14	25.4	7.0	3.6	12.0	15.6	3.7	4.1	7.8
Consento 7	45.1	14.7	13.3	24.4	24.1	3.9	17.6	15.2
Vendetta 7	47.1	1.8	10.1	19.7	17.2	1.9	7.7	8.9
Acrobat DF (Invader) 7	32.0	19.7	9.0	20.3	21.1	6.3	6.0	11.1
mancozeb 7	43.8	15.7	8.2	22.6	27.7	7.1	7.9	14.2
mancozeb 14	47.7	18.3	16.0	27.3	30.6	9.2	14.9	14.2
UTC	59.7	26.9	27.9	38.2	37.3	23.4	29.9	23.9

### 3.4 Rating of fungicides to control early blight

A new rating system became necessary since fungicides were introduced on the market with better control properties than existing fungicides. At the Munich subgroup *Alternaria* meeting and at the Brasov EuroBlight meeting it was decided to re-evaluate the fungicide ratings. A protocol for evaluating the efficacy of fungicides during the second part of the season was agreed upon and is given in Appendix 1.

In Aarhus it was decided that the reference for the early blight trials was to be the untreated control and not mancozeb either in a 7 or 14 day interval. As a consequence fungicides will be rated according to formula 2 in which the StAUDPC (Table 6) was converted into a decimal rating and the untreated control is fixed at 0.0. The ratings are published on the EuroBlight website (Table 7).

It was decided to put the decimal ratings in the EuroBlight fungicide table and not round the values up or down to the nearest whole number. The decimal rating reflects the efficacy of a fungicide more accurately than the rounded up or down value.

Discrimination between specific and non-specific fungicides to control early blight was made. The specific fungicides were sprayed in a 14 day interval and target on *Alternaria* spp. specifically. The non-specific fungicides target mainly on potato Late Blight with a side effect on *Alternaria* spp. These fungicides were sprayed in a 7 day interval.

**Table 6. Effectiveness of fungicides to control early blight based on StAUDPC; the proposed ratings are compared to the untreated control (rating = 0) as a reference.**

Fungicide + spray interval	Dose rate Kg or L /ha	StAUDPC <sup>1</sup>	Decimal Rating <sup>2,3,5</sup>
Unikat Pro (Electis) <sup>4</sup>	1.8	13.3	2.8
Unikat Pro 7 + Amistar 14	1.8+0.5	7.6	3.7
Unikat Pro 7 + Narita 14	1.8+0.5	6.8	3.9
Consento 7	2.0	16.7	2.2
Vendetta 7	0.5	11.2	3.1
Acrobat DF (Invader) 7	2.0	12.6	2.9
mancozeb 7	1.5	15.3	2.5
mancozeb 14	1.5	19.8	1.7
UTC	-	30.0	0.0

<sup>1</sup> : Value established by REML Analysis

<sup>2</sup> : Decimal ratings based on a minimum of 6 experiments. Currently 6 experiments have been carried out: D 2; DK 2 and NL 2.

<sup>3</sup> : The ratings are intended as a guide only and will be amended in future if new information becomes available.

<sup>4</sup> : Fungicides were not tested in each experiment; for details see Materials & Method and Appendix 2.

<sup>5</sup> : Rating based on the untreated control as reference set at 0.0

**Table 7 Efficacy of fungicides for the control of early blight caused by *Alternaria spp* expressed as a decimal rating (0-5), published on the EuroBlight web-site**

Product	Efficacy rating <sup>1,2</sup>		
	14 day interval	Strategy	7 day interval
<b>Spray interval 14 days</b>			
mancozeb 2.0	1.7	-	-
<b>Spray strategy<sup>3</sup></b>			
(zoxamide + mancozeb) <sup>a</sup> 1.8 + azoxystrobin <sup>b</sup> 0.5 <sup>4</sup>	-	3.7	-
(zoxamide + mancozeb) <sup>a</sup> 1.8 + difenoconazole <sup>b,5</sup> 0.5	-	3.9	-
<b>Spray interval 7 days</b>			
mancozeb 2.0	-	-	2.5
(zoxamide + mancozeb) 1.8	-	-	2.8
(fenamidone + propamocarb) 2.0	-	-	2.2
(fluazinam + azoxystrobin <sup>5</sup> ) 0.5	-	-	3.1
(dimethomorph + mancozeb) 2.0	-	-	2.9

<sup>1</sup> : Ratings for *Alternaria* are based on results from EuroBlight field trials during 2015-2016, and only compounds included in these trials are rated for *Alternaria*. The scale for *Alternaria* is a 0-5 scale (see technical report to be uploaded soon).

<sup>2</sup> : The ratings are intended as a guide only and will be amended in future if new information becomes available.

<sup>3</sup> : The active ingredients were sprayed in a spray strategy with a 7 day interval (<sup>a</sup>) or a 14 day interval (<sup>b</sup>)

<sup>4</sup> : azoxystrobin was sprayed at label rate which is 0.5 for DK and DE, and 0.25 for NL.

<sup>5</sup> : *Alternaria solani* isolates that are less sensitive to QoI-fungicides have been isolated from potato plants in Europe. Therefore resistance management strategies should be implemented (see FRAC web site for details). Rating will be lower where fungicide insensitive strains are present.

**Disclaimer:** Whilst every effort has been made to ensure that the information is accurate, no liability can be accepted for any error or omission in the content of the table or for any loss, damage or other accident arising from the use of the fungicides listed herein. Omission of a fungicide does not necessarily mean that it is not approved for use within one or more EU countries. The ratings are based on the label recommendation for a particular product. Where the disease pressure is low, intervals between spray applications may be extended and, in some countries, fungicide applications are made in response to nationally issued spray warnings and/or Decision Support Systems. It is essential therefore to follow the instructions given on the approved label of a particular early blight fungicide appropriate to the country of use before handling, storing or using any early blight fungicide or other crop protection product.

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## 3.5 Conclusions

In the Alternaria subgroup meeting in Munich (March 2014) and the EuroBlight meeting in Brasov (May 2015) a more dynamic ratings system for fungicide efficacy in controlling early blight was presented. The ratings are based on non-transformed StAUDPC values. The main advantage is that ratings are determined using a system that is more objective than that used to produce table ratings up until the Brasov meeting in 2015. Another advantage is that there is scope for future, more effective fungicides to be rated higher than 4 plusses, the current maximum. Furthermore ratings once given are not fixed, thus relative changes in the effectiveness of fungicides can be made apparent. It was agreed at the Brasov meeting that as soon as new ratings are calculated from trials and are approved the fungicide table on the EuroBlight website will be updated.

At Aarhus it was decided that the untreated control will be the reference. The ratings proposed will be exclusively based on the results of the trials described in this report. The future ratings will be based on fungicides tested in the highest dose rate registered in Europe. In agricultural practise lower dose rates are and will be used. The ratings do not reflect the efficacy of the fungicide when lower dose rates are used or when different spray intervals are carried out.

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# References

- Montgomery, D.C. and Peck, E.A., (1982). *Introduction to Linear Regression Analysis*. John Wiley & Sons. New York.
- Payne, R.W., Harding, S.A., Murray, D.A., Soutar, D.M., Baird, D.B., Glaser, A.I., Channing, I.C., Welham, S.J., Gilmour, A.R., Thompson, R., Webster, R. (2009). *The Guide to GenStat Release 12, Part 2: Statistics*. VSN International, Hemel Hempstead

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# Appendix 1. Protocol for testing effectiveness to control early blight

Adapted protocol for the evaluation of the efficacy of fungicides to control early blight

The Protocol for testing effectiveness to control early blight was prepared by Huub Schepers, Jürgen Leiminger, Bent Nielsen, Hans Hausladen, Jan Spoelder, Jozefa Kapsa, Pieter Vanhaverbeke, Dani Shtienberg and Bert Evenhuis and is published below.

## Purpose/aim of trials

To compare the "Effectiveness to early blight" by measuring the protection of leaves against infection by early blight resulting from the application of a fungicide according to this requirements. This spray schedule is not necessarily related to the label recommendations. This protection originates from the protectant and/or curative properties of the active ingredients.

EPPO guideline PP 1/2 (3) (revised in 1996) describes the standard requirements of the field trial.

Specific additional requirements:

- A susceptible local ware or starch potato variety. The growth habit of the cultivar should be recorded i.e. determinate or indeterminate growth.
- Potato late blight is controlled in a weekly scheme using fungicides with no efficacy to control early blight. For instance start with mandipropamid and end the spray schedule using cyazofamid.
- Preferably the experiment is carried out with natural infection. However if conditions are less suitable inoculation may be carried out with *A. solani* infested grain kernels on the soil within the plot. The artificial inoculation is carried out 3-days before the first spray until 7-days after the first spray. When the inoculation is not successful it will be repeated.
- Misting is permissible, when conditions are exceptionally dry.
- Each treatment consists of applications of the fungicide to be tested regardless of the limited application numbers on the label.
- First spray depends on local conditions, but needs to be applied before the start of the epidemic and should be timed approximately at 7-8 weeks after crop emergence.
- Crop growth stage should be recorded on the days that the trial is sprayed. The BBCH key should be used.
- Untreated plots are part of the field experiment. In 2017 the untreated control was accepted as a reference which also applied to the earlier experiments.
- A reference treatment (two variants) is part of the field experiment i.e. 1500 g a.i. mancozeb. Sprayed in a 14-day interval and in a 7-day interval. From 2017, onwards the mancozeb references are not necessarily part of the experiments, an untreated control is mandatory.
- Spray frequency is every 7-days (+/- 1 day) or every 14-days (+/- 1 day), to be chosen by the participants. A spray strategy with more than 1 fungicide is allowed, even if this means that 1 fungicide is sprayed in a 7-day interval and the other in a 14-day interval. The time frame of the spray applications should be the same for all treatments.
- The efficacy of the Early blight fungicide(s) was to be compared to one of the two reference treatments accordingly. However, from 2017 onwards the efficacy is compared to the untreated control, allowing also spray strategies to be included in the trials.
- The number of sprays depend on the early blight epidemic and the spray interval chosen.
- Dose rate is highest dose registered in Europe
- Assessment: every week (or more frequently when necessary) in plots by rating the % infected leaf area. To assess early blight we recommend using the assessment key in the EPPO-guideline PP 1/263 (1).
- Desiccation: timing and method according to GAP.
- It is not strictly necessary to harvest the trial.
- A method for determining the rating for the "EuroBlight Fungicide Table" will be proposed when 6 successful trials (2 seasons x 3 trials) have been carried out by independent research institutes in at least 3 different growing regions/countries in Europe. The proposed methodology will be agreed by independent researchers and the agrochemical manufacturers and where possible will be used to

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analyse data from registration trials, in which the relevant standard products are included. In this way a robust dataset will form the basis of the rating given for the "Effectiveness against early blight".

N.B. A successful trial is one that is strictly carried out according to this protocol and early blight is observed in the plots (>10% foliar infection in the worst treatment). The rating is set by determination and comparison of the AUDPC's of the 6 successful trials. A validation of this method will have to be carried out with existing trial data to find out whether a linear, a logarithmic or another transformation has to be carried out on the data.

## Appendix 2. Raw data

Plot data of early blight StAUDPC from each experiment in 2015 and 2016, fungicides sprayed in a 7 day interval.

fungicide	D 15	Dk 15	NL 15	D 16	Dk 16	NL 16
<b>UTC</b>	<b>59.7</b>	<b>26.9</b>	<b>27.9</b>	<b>37.3</b>	<b>23.4</b>	<b>29.9</b>
1	57.9	24.7	20.3	36.3	28.9	29.1
2	60.3	23.2	28.5	37.5	28.3	34.2
3	59.8	29.5	27.1	37.7	19.8	29.0
4	60.9	30.1	35.9	37.6	16.4	27.5
<b>Unikat Pro (Electis) 7</b>	<b>33.3</b>	<b>13.6</b>	<b>10.7</b>	<b>22.2</b>	<b>7.5</b>	<b>10.5</b>
1	33.4	5.7	4.0	23.1	10.6	10.9
2	32.9	9.2	15.6	22.3	8.6	11.9
3	32.4	18.8	7.2	20.9	8.5	12.6
4	34.6	20.6	16.0	22.7	2.2	7.5
<b>Consento 7</b>	<b>45.1</b>	<b>14.7</b>	<b>13.3</b>	<b>24.1</b>	<b>3.9</b>	<b>11.6</b>
1	44.1	7.5	9.5	25.3	7.5	17.6
2	45.1	7.8	20.4	23.6	3.4	19.7
3	46.8	20.9	8.6	23.3	3.3	17.6
4	44.3	22.6	14.8	24.3	1.5	17.0
<b>Vendetta 7</b>	<b>47.1</b>	<b>1.8</b>	<b>10.1</b>	<b>17.2</b>	<b>1.9</b>	<b>17.9</b>
1	48.7	1.3	7.5	20.9	2.0	7.7
2	47.7	1.1	17.8	16.6	2.9	14.1
3	46.5	2.0	5.2	15.7	1.9	4.8
4	45.5	2.9	10.1	15.7	0.7	5.5
<b>Acrobat DF (Invader) 7</b>	<b>32.0</b>	<b>19.7</b>	<b>9.0</b>	<b>21.1</b>	<b>6.3</b>	<b>7.1</b>
1	34.2	16.0	7.5	20.9	8.9	6.0
2	31.2	15.8	12.9	22.3	9.8	7.1
3	30.3	25.3	6.7	21.0	4.0	4.5
4	32.5	21.8	8.9	20.1	2.4	6.8
<b>mancozeb 7</b>	<b>43.8</b>	<b>15.7</b>	<b>8.2</b>	<b>27.7</b>	<b>7.1</b>	<b>7.9</b>
1	42.1	7.5	3.1	29.2	11.4	5.5
2	45.1	13.0	6.9	27.0	8.7	10.0
3	42.1	22.6	9.0	27.2	5.5	4.3
4	45.7	19.8	14.0	27.4	2.7	11.7



Plot data of early blight StAUDPC from each experiment in 2015 and 2016, fungicides sprayed in a 7 and 14 day interval.

fungicide	D 15	Dk 15	NL 15	D 16	Dk 16	NL 16
<b>UTC</b>	<b>59.7</b>	<b>26.9</b>	<b>27.9</b>	<b>37.3</b>	<b>23.4</b>	<b>29.9</b>
1	57.9	24.7	20.3	36.3	28.9	29.1
2	60.3	23.2	28.5	37.5	28.3	34.2
3	59.8	29.5	27.1	37.7	19.8	29.0
4	60.9	30.1	35.9	37.6	16.4	27.5
<b>Unikat Pro 7 + Amistar 14</b>	<b>27.2</b>	<b>2.1</b>	<b>8.9</b>	<b>17.1</b>	<b>1.4</b>	<b>7.5</b>
1	25.1	0.8	2.7	18.6	1.1	5.8
2	31.8	3.6	10.6	15.9	1.2	8.0
3	24.9	1.3	10.7	17.8	1.1	10.7
4	26.7	2.5	11.6	16.0	2.3	5.5
<b>Unikat Pro 7 + Narita 14</b>	<b>25.4</b>	<b>7.0</b>	<b>3.6</b>	<b>15.6</b>	<b>3.7</b>	<b>4.1</b>
1	24.6	4.9	2.4	15.3	7.2	3.2
2	26.1	4.7	4.5	15.3	3.9	3.2
3	24.4	7.7	4.1	15.9	2.3	3.6
4	26.5	10.6	3.3	15.9	1.3	6.5
<b>mancozeb 14</b>	<b>47.7</b>	<b>18.3</b>	<b>16.0</b>	<b>30.6</b>	<b>9.2</b>	<b>14.9</b>
1	46.1	14.8	20.3	30.7	13.1	16.6
2	48.4	14.4	9.8	29.5	9.2	11.2
3	49.6	17.7	15.9	32.4	9.8	12.2
4	46.6	26.4	17.9	29.8	4.5	19.6

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## Appendix 3. REML to establish fungicide rating

```
IMPORT '~\Evenhuis\EU Tabel Alternarial\EU Tabel Alternaria 2015-2016.xlsx'; \
  ISAVE = isave; SHEET = 'genstat'

DHIST AUDPC

VARI [ VAL = 1 ... 468 ] nr
REST nr, AUDPC; AUDPC .EQ. MAX ( AUDPC )
PRIN nr, AUDPC
REST nr, AUDPC

TABU [ CLASS = jaar; COUNTS = counts ]
PRIN counts; F = 5
DELE [ REDE = yes ] counts

TABU [ CLASS = plot, Exp; COUNTS = counts ]
PRIN counts; F = 5
DELE [ REDE = yes ] counts

TABU [ CLASS = fungicide; PRIN = mean ] weight

SUBSET [ weight .GT. 0; yes ] isave[]

TABU [ CLASS = jaar, Exp; PRIN = c ]
TABU [ CLASS = jaar, country; PRIN = c ]

TABU [ CLASS = Exp, herhaling; PRIN = c ]

GETA [ ATTR = label ] fungicide; SAVE = save
TEXT [ VAL = #save[] ] label

BLOC Exp / herhaling
TREA expr * fungicide

TABU [ CLASS = jaar; PRIN = mean; IP = as ] AUDPC, stAUDPC, rAUDPC

FOR [ INDEX = i ] y = AUDPC, stAUDPC, rAUDPC; \
  m = mAUDPC, mstAUDPC, mrAUDPC; \
  mSq = SqAUDPC, SqstAUDPC, SqrAUDPC

VCOM [ FIXED = fungicide ] Exp / herhaling
REML [ PRIN = * ] SQRT(y); RESI = resi; FITT = fitt
VKEE Exp . herhaling; COMP = comp
IF comp .GT. 0
  VCOM [ FIXED = fungicide ] Exp / herhaling
  REML [ PRIN = #, mean ] SQRT(y); RESI = resi; FITT = fitt
ELSE
  VCOM [ FIXED = fungicide ] Exp
  REML [ PRIN = #, mean ] SQRT(y); RESI = resi; FITT = fitt
ENDIF
VKEE fungicide; MEAN = MEAN
```

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VARI [ VAL = #MEAN ] mSq  
GRAP [ NR = 21; NC = 51 ] resi; fitt

VCOM [ FIXED = fungicide ] Exp / herhaling  
REML [ PRIN = \* ] y; RESI = resi; FITT = fitt  
VKEE Exp . herhaling; COMP = comp  
IF comp .GT. 0  
    VCOM [ FIXED = fungicide ] Exp / herhaling  
    REML [ PRIN = #, mean ] y; RESI = resi; FITT = fitt  
ELSE  
    VCOM [ FIXED = fungicide ] Exp  
    REML [ PRIN = #, mean ] y; RESI = resi; FITT = fitt  
ENDIF  
VKEE fungicide; MEAN = MEAN  
VARI [ VAL = #MEAN ] m  
GRAP [ NR = 21; NC = 51 ] resi; fitt

ENDFOR

PRIN label, mAUDPC, mstAUDPC, mrAUDPC, SqAUDPC, SqstAUDPC, SqrAUDPC; F = 10  
DSCA      mAUDPC, mstAUDPC, mrAUDPC, SqAUDPC, SqstAUDPC, SqrAUDPC

FOR [ INDEX = i ] y = mAUDPC, mstAUDPC, mrAUDPC, SqAUDPC, SqstAUDPC, SqrAUDPC

IF i .IN. !(1, 2, 3)  
    CALC y = 5 \* ( MAX ( y ) - y ) / MAX ( y )  
ELSE  
    CALC y = 5 \* ( MAX ( y )\*\*2 - y\*\*2 ) / MAX ( y )\*\*2  
ENDIF

ENDFOR

CAPTION '0 - 5'; META

PRIN label, mAUDPC, mstAUDPC, mrAUDPC, SqAUDPC, SqstAUDPC, SqrAUDPC; F = 10

CALC mAUDPC, mstAUDPC, mrAUDPC, SqAUDPC, SqstAUDPC, SqrAUDPC = \  
    mAUDPC, mstAUDPC, mrAUDPC, SqAUDPC, SqstAUDPC, SqrAUDPC / 4 \* 9

CAPTION '4.5 - 9'; META

PRIN label, mAUDPC, mstAUDPC, mrAUDPC, SqAUDPC, SqstAUDPC, SqrAUDPC; F = 10

DSCA      mAUDPC, mstAUDPC, mrAUDPC, SqAUDPC, SqstAUDPC, SqrAUDPC

STOP

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