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Mancozeb: essential tool for sustainable protection of potato against early blight (*Alternaria* spp.*)*

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SUMMARY

In recent years Applied Plant Research International, part of Wageningen University Research (Wageningen U.R.) and UPL Europe Ltd (UPL) have demonstrated that mancozeb is effective on the 13_A2, 6_A1 and 33_A2 genotypes of late blight (*Phytophthora infestans*). In 2015 research was extended to study the efficacy of mancozeb on early blight (*Alternaria* spp.) and in particular *Alternaria solani* with the F 129 mutation associated with strobilurin resistance.

Initial laboratory research was conducted by Wageningen U.R. to study the efficacy of the fungicides, azoxystrobin, boscalid +pyraclostrobin and mancozeb on the spore germination of 15 *A. solani* isolates collected between 2006 and 2014. The results indicated that there was no shift in sensitivity to mancozeb within the *A. solani* populations tested but it was shown that some of the *A. solani* isolates had reduced sensitivity to azoxystrobin and to a lesser extent to boscalid+pyraclostrobin.

The objective of the 2016 field research by Wageningen U.R. was to verify the efficacy of difenoconazole, boscalid+ pyraclostrobin and mancozeb in controlling different genotypes of *A. solani*. The trial area was broadcast with *Alternaria* infected wheat kernels, a mixture of 95% wildtype and 5% F129L type and disease assessments were carried out regularly. All treatments were effective until 9th September (15 days after the last spray). In order to assess the *Alternaria* genotype and the presence of the F129L mutation, leaves with *Alternaria* spp. lesions were collected on September 17th and genotyped. No significant shift of the *A. solani* genotype was found where mancozeb was used compared to the untreated control. Where boscalid+pyraclostrobin or the same followed by difenoconazole was sprayed, significantly more F129L types were found compared to the untreated control.

A field trial was conducted by UPL in potatoes in the Netherlands in 2015. The objective was to determine the efficacy of selected fungicides in controlling *Alternaria* spp. The fungicides tested were mancozeb, azoxystrobin, boscalid+pyraclostrobin and difenoconazole, Results demonstrated that mancozeb provided significantly better control than the reference boscalid+pyraclostrobin programme. No significant differences were observed between other treatments.

Mancozeb has been registered for more than 60 years and due to its multi-site mode of action, it has consistently maintained its efficacy against both *A. solani* and *P. infestans* and remains an important component of fungicide resistance management programmes.

KEYWORDS
A. solani, fungicide, resistance, mancozeb, genotype, sensitivity

INTRODUCTION

Late blight, caused by *P. infestans*, is the most important disease in potato production but early blight, *A. solani* is regularly found in potato fields in the second half of the season. Fungicide products commonly used for *P. infestans* control programmes are based on products that contain actives such as boscalid, pyraclostrobin, azoxystrobin, difenaconazole and mancozeb. It is recognised that some fungicide products used to control *P. infestans* also exhibit some control of *A. solani*, especially those that contain mancozeb.

Resistance of *A. solani* to azoxystrobin has been reported in the United States (Pasche and Gudmested, 2008). This resistance is associated with the F129L mutation. This mutation has also been reported in Germany (Leiminger et al., 2014), and one isolate with the F129L mutation has been found in the Netherlands (Evenhuis et al., 2013).

A. solani isolates were collected from field experiments in the Netherlands during the period 2006 to 2014 and stored in liquid nitrogen at Wageningen U.R. In 2015 at the request of UPL a laboratory experiment tested the efficacy of boscalid+pyraclostrobin, azoxystrobin and mancozeb to control 15 isolates of *A.solani*.

Following on from the 2015 laboratory study a field trial was carried out by Wageningen U.R. in 2016 to test the efficacy of selected fungicide programmes to control a mixture of *A. solani* isolates (wild genotype and F129 genotype) at the request of UPL.

A field experiment with different fungicide programmes was conducted in 2015 by UPL investigating the control of *Alternaria spp.*

LABORATORY RESEARCH (2015)

Testing the efficacy of fungicides on the spore germination of *A. solani,* research conducted by Wageningen UR.

MATERIALS AND METHODS

To establish EC_{50} values using selected fungicides a dose rate series was determined. The efficacy of boscalid 26.7% + pyraclostrobin 6.7% w/w and azoxystrobin 250 g/l was tested at 0.01, 0.1, 1, 10 and 100 ppm. Mancozeb (75% w/w) was tested at 0.1, 1, 10, 100 and 1000 ppm. The ppm values were adjusted to the dose rate of the active ingredients. In the case of boscalid+pyraclostrobin the dose rate was added up. The fungicides at the appropriate dose rate were added to cooling Water Agar and poured into Petri dishes. To boscalid+ pyraclostrobin and azoxystrobin 100 mg / l SHAM dissolved in methanol was added, regardless of the dose rate tested. SHAM is a known inhibitor of the alternative oxidase (AOX) pathway that has been

suggested as a possible mode of QoI resistance in vitro in other fungi, therefore it was added to the medium.

A selection of 15 *A. solani* isolates was taken from the isolates that had been collected between 2006 and 2014 by Wageningen UR.
The inoculum density was set at approximately 10,000 sporangia per ml and the spore suspension was sprayed on to the agar plates containing the fungicides. The plates were incubated for 6 hours at room temperature (20°C) under day light conditions. After warm incubation, the plates were transferred to a dark chamber held at 4°C until germination assessments were carried out.

The germination rate of *A. solani* was established by counting the number of germinated and non-germinated spores under a light microscope. The spores were considered germinated when the length of the spore tube was at least the same as the diameter of the spore. The percentage germination was calculated by division of the number of germinated spores with the total number of spores counted multiplied by 100.

The experiments were replicated two times and each replication consisted of one Petri dish containing spores of a known *A. solani* isolate. Fungicide sensitivity was measured as the concentration at which spore germination was inhibited by 50% relative to the untreated control (EC_{50} value) and was determined for each isolate. Analysis of variance on Log10 (EC_{50}) was made using GENSTAT 17th Edition.

RESULTS

This experiment was designed to establish the EC_{50} values of fungicides to control different *A. solani* isolates. The results are presented in Figures 1-7.

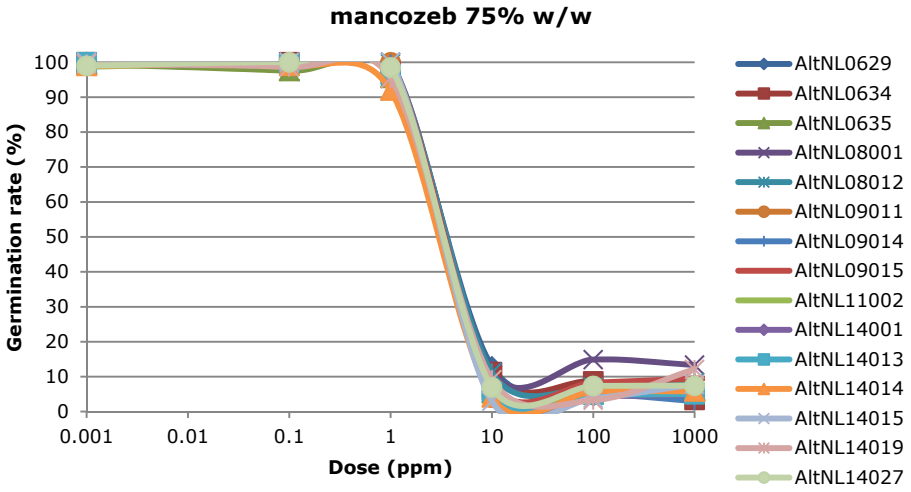

Figure 1. *Dose response effect of mancozeb to control 15 isolates of A. solani. The untreated control was placed at 0.001 ppm.*

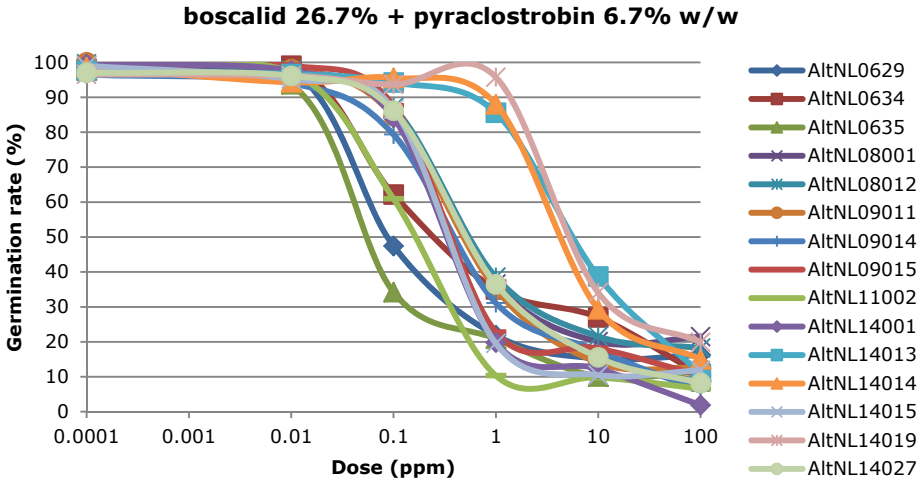

Figure 2. *Dose response effect of boscalid+pyraclostrobin to control 15 isolates of A. solani. The untreated control was placed at 0.0001 ppm.*

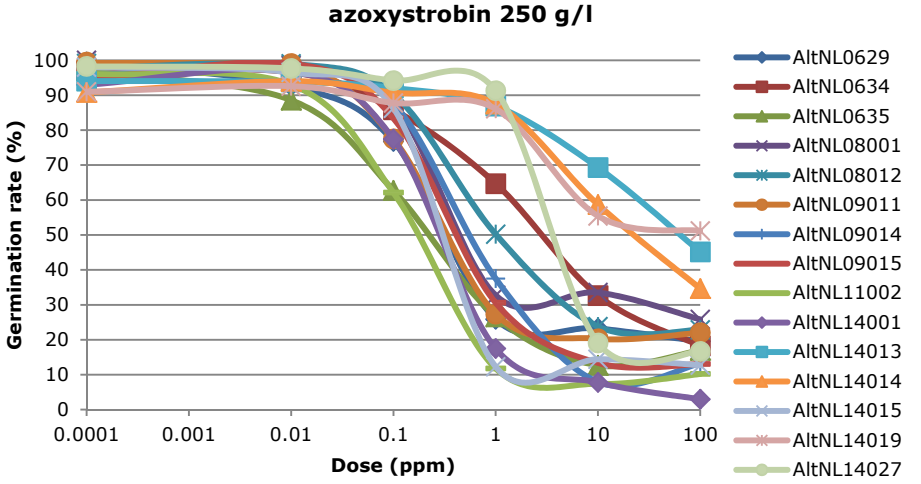

Figure 3. *Dose response effect of azoxystrobin to control 15 isolates of A. solani. The untreated control was placed at 0.0001 ppm.*

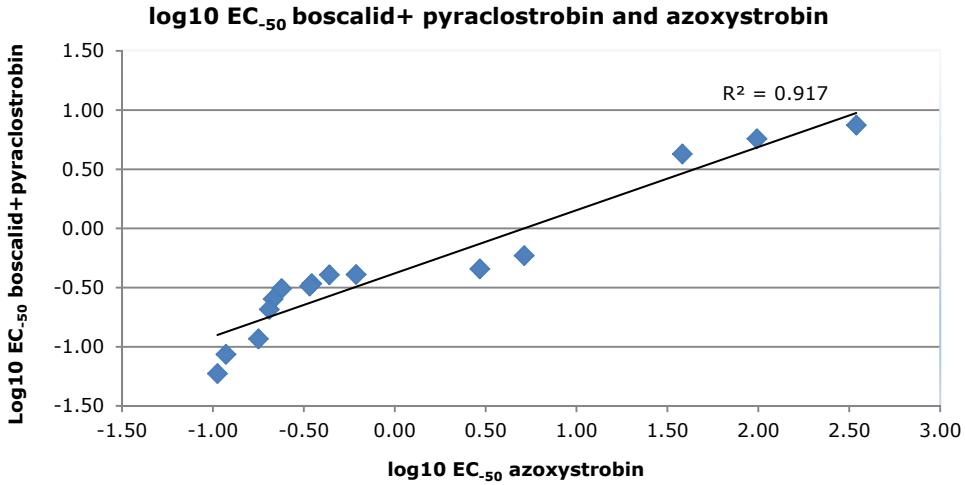

Figure 4. *Correlation between EC-50 values for spore germination of boscalid+pyraclostrobin and azoxystrobin.*

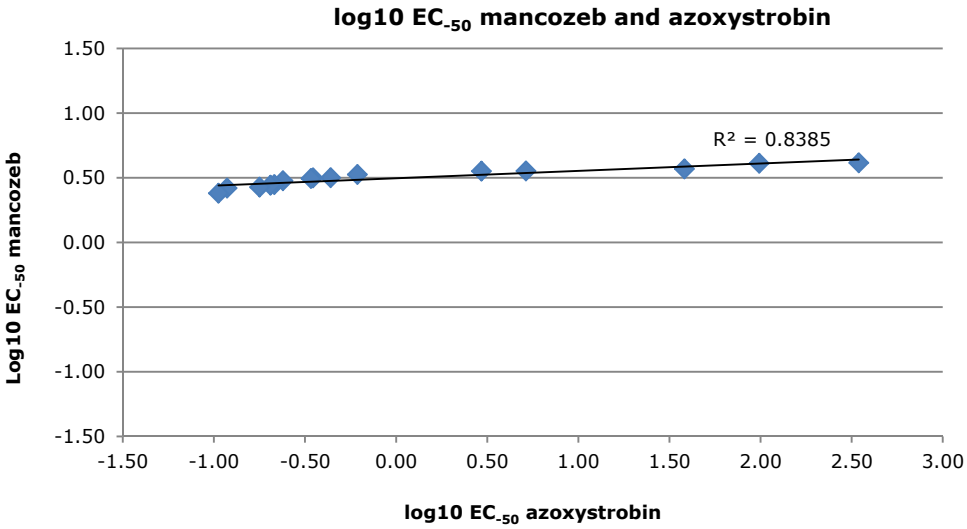

Figure 5. *Correlation between EC-50 values for spore germination of mancozeb and azoxystrobin.*

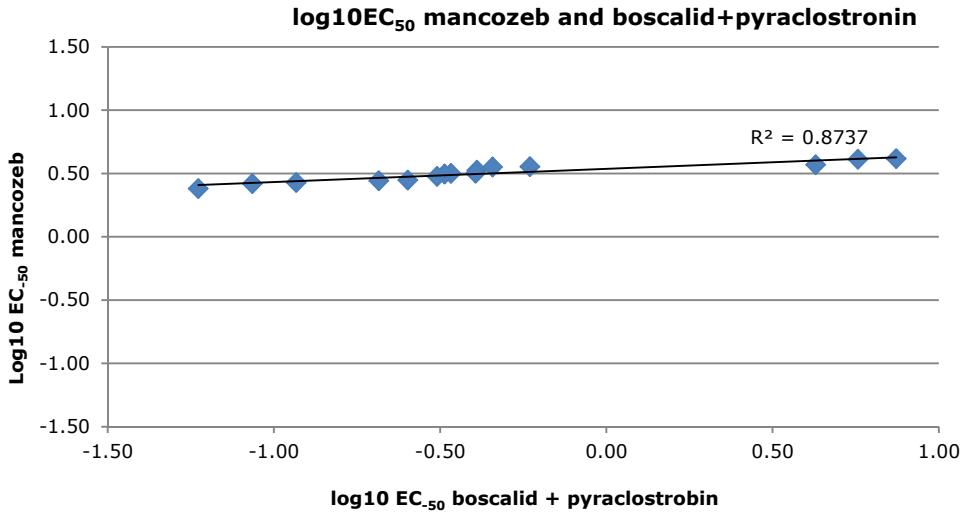

Figure 6. *Correlation between EC_{-50} values for spore germination of mancozeb and boscalid+pyraclostrobin*

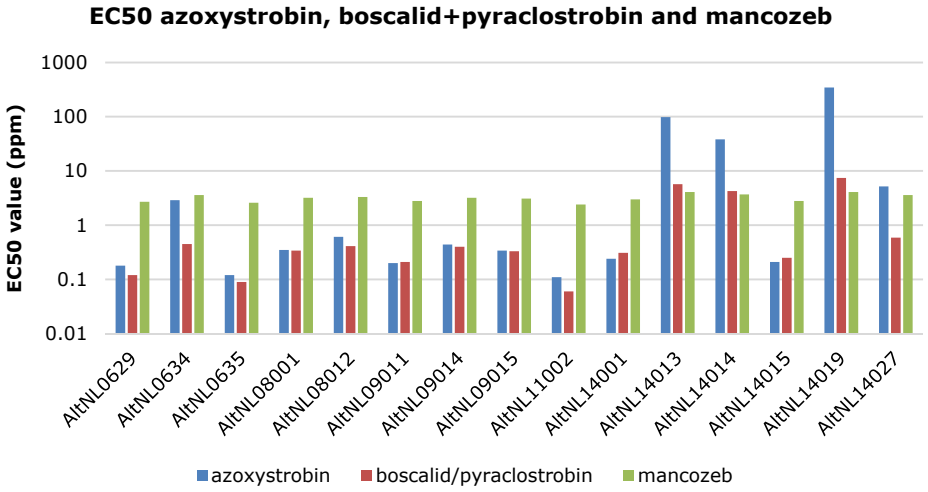

Figure 7. *EC_{50} Values (ppm) of the fungicides used, showing the 15 Isolates of A. solani, in order of sampling year*

DISCUSSION AND CONCLUSION

Within the *A. solani* isolates tested the sensitivity to mancozeb did not significantly differ, although the EC_{50} values for mancozeb in most cases were higher than for azoxystrobin and boscalid+pyraclostrobin. The EC_{50} values for mancozeb were very consistent this indicates that there was no shift in sensitivity to mancozeb within the *A. solani* population tested.
The results suggest that some of the *A. solani* isolates became less sensitive to azoxystrobin and to a lesser extent to boscalid+ pyraclostrobin particularly for the 2014 samples.

From the data there appears to be some correlation in the sensitivity of *A. solani* isolates to the different fungicides tested. This does not necessarily mean that this is caused by cross resistance, it may be caused by fitness of the *A. solani* isolate. You would expect an isolate that more readily germinates to do so under any circumstances compared to a less fit isolate. The slope of the line is much steeper when boscalid + pyraclostrobin and azoxystrobin are compared (Figure 4) than when the comparison is made with mancozeb (Figures 5 and 6). No significant difference in mancozeb sensitivity was found between the *A. solani* isolates thus cross resistance, between mancozeb on one hand and azoxystrobin or boscalid+pyraclostrobin on the other is not likely. For both boscalid+pyraclostrobin and azoxystrobin the EC_{50}-value differs significantly between isolates tested. In this case isolates less sensitive to boscalid+pyraclostrobin seemed to be also less sensitive to azoxystrobin, suggesting the possibility of cross resistance or sensitivity.

FIELD STUDY (2016)

Efficacy of fungicides to control different early blight genotypes, research conducted by Wageningen U.R.

MATERIALS AND METHODS

Fungicide applications were carried out using a trial site sprayer with Airmix 110.04 nozzles. Nozzles were hanging approximately 50 cm above the foliage. Sprayings were carried out based on 300 l/ha. Potato plants were sprayed for the first time when they reached a height of 20-30 cm when rapid growth started. Specific sprays to control *Alternaria spp.* commenced mid-July 2016. Haulm killing was carried out on 30 September 2016, despite natural senescence of the crop. In Table 1 the fungicides used and dose rates are presented. Treatment A is the untreated control and H the reference treatment chosen. Spray strategies are given in Table 2.

Table 1. *The fungicides used and the applied dose rates.*

Code	Active ingredient	Dose rate (L or kg/ha)
N	difenoconazole 250 g/l	0.5
S	boscalid 26.7% + pyraclostrobin 6.7% w/w	0.2
P	mancozeb 80% w/w	2.0

Table 2. *Spray strategies and date of application.*

Treatment	Product	12-7	19-7	26-7	2-8	9-8	16-8	23-8
A	UTC	-	-	-	-	-	-	-
B	S	S	-	S	-	S	-	S
C	P	P	P	P	P	P	P	P
D	P + S	P+S	P	P+S	P	P+S	P	P+S
E	N	N	-	N	-	N	-	N
H	S or N	S	-	S	-	N	-	N

For inoculation a selection of 2 *A. solani* isolates was made. AltNL03003 was isolated in 2003 and belongs to genotype 1 and is a wild type. AltNL15002 belongs to genotype 2 and possesses the F129L mutation. Both isolates were grown on wheat kernels separately. A mixture of 95% wildtype and 5% F129L type Infested kernels were broadcasted in the field on 15 July 2016.

To assess *Alternaria spp.* genotype and the presence of the F129L mutation 8 leaflets with *Alternaria* spp. lesions were sampled per plot. Samples were taken four times during the season, in the last week of August and in the first three weeks of September. Only the last sample was genotyped. The samples were air dried and stored in Petri dishes until further processing. At the laboratory monospore cultures were made on agar. Mycelium was scraped from the agar plate after incubation. DNA was extracted from the mycelium. The genotype involved was assessed by carrying out two PCR described by Pasche et al., 2004 and Lieminger et al., 2015. The PCR product was extracted from the gel and sequenced. The nucleotide order was assessed and the presence of the F129L mutation was established according to the publication of Lieminger et al., 2015.

Statistics: analysis of variance on the parameters was made using GENSTAT 18th Edition. The experiment was carried out with four replications in a randomised block design. Each replication consisted of a plot. Transformation of data was carried out when necessary.

RESULTS

Field assessments

On 22 July 2016 no *Alternaria* was found. A week later on 29 July *Alternaria* was present in all plots at a low disease severity rate of 0.001% (data not shown). From 5 August onwards the *Alternaria* epidemic started (Figure 8). After 17 September it was not possible to assess *Alternaria* because it was too much entangled with natural senescence to distinguish.

Disease severity of all spray strategies was significantly lower than the untreated control. Based on AUDPC, the efficacy of treatments D, E and H were significantly better than treatments B and C.

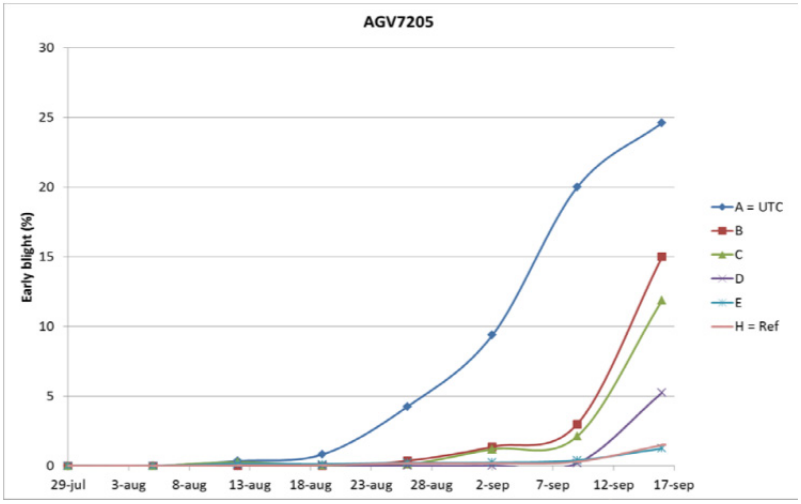

Figure 8. *Alternaria disease severity (%)*

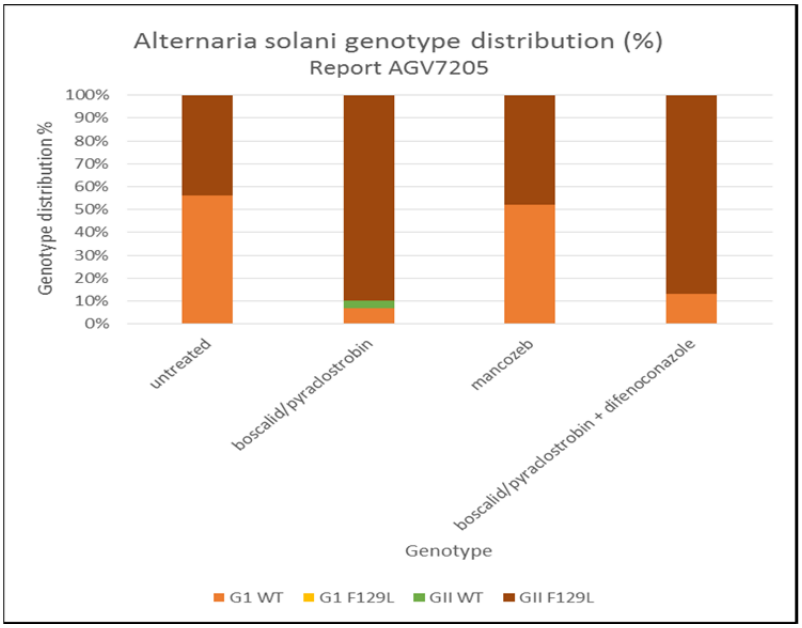

Figure 9. *A. solani genotype distribution (%)*

Genotyping

The results are presented in Figure 9 no significant shift of the *A. solani* genotype was found when mancoze*b* was used compared to the untreated control. When boscalid+pyraclostrobin (B) or boscalid+pyraclostrobin followed by difenoconazole (H) was sprayed significantly more F129L types were found compared to the untreated control.

DISCUSSION AND CONCLUSION

A field experiment was carried out in potato cultivar Agria. The *Alternaria* epidemic developed late despite artificial inoculation. The weather in June was conducive for *P. infestans* and not so much for *Alternaria*. *P. infestans* was controlled with cover sprays using fungicides which have no known efficacy to control *Alternaria*.

Alternaria severity in the untreated control was significantly higher than all other treatments indicating that the inoculation was successful. Furthermore all spray strategies effectively controlled *Alternaria*, although the efficacy varied between strategies.

Disease severity of treatment B and C increased more towards the end of the season compared to treatments D, E and H. The last spray application was 23 August and the increase was observed from 9 September onwards. Interestingly when mancozeb was added to boscaclid+pyraclostrobin (or v.v.) the efficacy to control Early Blight increased compared to the products used solo. However the efficacy to control *Alternaria* was still less than using difenoconazole or the combination boscaclid+pyraclostrobin followed by difenoconazole.

The field was inoculated with wheat kernels with 2 *Alternaria* genotypes. Genotype I (GI)) is wild type and Genotype II (GII) possesses the F129L mutation. Predominantly these two genotypes were found in the field (Figure 9). Only 1 isolate found was Genotype II, wild type. No Genotype I isolates were found possessing the F129L mutation. In the untreated control 57% of the isolates were Genotype I and wild type. The experiment was inoculated with 90% Genotype I wildtype. This could suggest that the F129L mutation was already present in the natural *A. solani* population at the location of the field experiment. Alternatively GII might be more aggressive than GI and therefore was found back more frequently.

Leaves were picked at four times during the season. *A. solani* was isolated only from the leaves picked at the last sampling date. Leaves of the other sampling dates were stored under dry conditions at room temperature and could be used for additional assessments.

When boscaclid+pyraclostrobin or boscaclid+pyraclostrobin followed by difenoconazole was sprayed, significantly more GII F129L *A. solani* types were found than in the untreated control and when mancozeb was sprayed. This suggests that selection towards GII F129L occurred under influence of spray strategies B and H. It is known that the F129L mutation causes a reduced sensitivity to QoI fungicides. One of the active ingredients, pyraclostrobin is a strobilurin, QoI fungicide.

FIELD STUDY (2015)

Control of *Alternaria spp*. in potatoes in the Netherlands, research conducted by UPL

MATERIALS AND METHODS

Fungicide applications were carried out using a AZO sprayer with Teejet 110.02 nozzles. Nozzles were hanging approximately 50 cm above the foliage. Sprayings were carried out based on 300 l/ha. Potato plants were sprayed for the first time at BBCH stage 67 against *Alternaria* at that point no *Alternaria* infestation was visible. In total 4 applications were made: 24 July, 31 July, 07 August and 19 August 2015. In Table 3 the fungicides used and dose rates are presented. Treatment 13 is the untreated control (untreated strips) were regularly situated within the trial.

Table 3. *Fungicide actives used and the applied dose rates.*

Code	Active ingredient	Dose (L or kg/ha)
1	mancozeb 75% WG	1.3
2	mancozeb 75% WG	1.6
3	mancozeb 75% WG	2.0
7	boscalid 26.7% + pyraclostrobin 6.7% WG	0.2
8	difenoconazole 250 g/l EC	0.5
9	azoxystrobin 250 g/l SC	0.25
13	Untreated	

Table 4. *Alternaria severity*

				7-Aug		19-Aug		28- Aug	
Trt No.	Treatment Name	Rate	Rate Unit						
1	mancozeb 75% w/w	1.3	kg/ha	0.001	a	0.02	a	2.35	bc
2	mancozeb 75% w/w	1.6	kg/ha	0.001	a	0.04	a	1.06	bc
3	mancozeb 75% w/w	2.0	kg/ha	0.000	a	0.02	a	0.28	c
7	boscalid + pyraclostrobin	0.2	kg/ha	0.033	a	0.35	a	3.95	b
8	difenoconazole	0.5	l/ha	0.000	a	0.22	a	1.30	bc
9	azoxystrobin	0.25	l/ha	0.000	a	0.20	a	2.00	bc
13	untreated strips			0.002	a	0.45	a	6.63	a

The infestation is this trial was natural. No laboratory determination of *Alternaria* was made, but the infestation symptoms appeared to be *A. solani*. After August 28th no further assessments were possible, due to natural senescence of the crop.
Results were evaluated using Agricultural Research Manager (ARM), version 2016. The experiment was carried out with four replications in a randomised block design. Each replication consisted of a block. No transformation of data was carried out.

DISCUSSION AND CONCLUSION

A field experiment was carried out in the potato cultivar Innovator. The *Alternaria* epidemic developed late and the severity in the untreated control stayed low. Potato late blight (*P. infestans*) was controlled with cover sprays using fungicides which have no known efficacy to control *Alternaria*.
All treatments showed a significantly lower infestation of *A. solani* compared to the untreated control. A dosage effect of mancozeb was clearly visible although this was not significant, the highest dosage of 2.0 kg/ha showing the lowest infestation of *A. solani*. The control with mancozeb 2.0 kg/ha was significantly better than the reference product boscalid+pyraclostrobin 0.2 kg/ha. No significant difference was obtained between other treatments.

CONCLUSION

Mancozeb has been found to be effective against all genotypes of *A. solani*. When mancozeb was used no shift in the *A. solani* genotypes was found compared to the untreated control as opposed to boscalid+pyraclostrobin and azoxytrobin where a difference was observed. It can be

concluded that mancozeb is an essential tool in managing fungicide resistance of populations of *A. solani* that have been identified in the past and present.

Studies in recent years have also demonstrated that mancozeb is efficient on all genotypes of *P. infestans*. As with *A. solani* mancozeb does not cause any change in the composition of the populations of the two pathogens thus maintaining the natural equilibrium between the two main potato diseases.

Due to its "multi-site" mode of action on foliar diseases, mancozeb remains a key active for sustainable protection of the potato crop and is essential in helping to prolong the efficacy of fungicides with single site mode of action.

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