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Potato late blight forecasting and initial inoculum sources in Norway

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SUMMARY
Initial sources of inoculum of *Phytophthora infestans* were investigated in ten potato fields with early outbreaks of potato late blight. Infected plant samples and isolates from these fields were examined with respect to mating type prevalence, fungicide resistance and genotypes based on microsatellites A high proportion (91 %) of the isolates recovered were of mating type A1. However, both mating types were found in 3 of 9 fields with more than one isolate recovered, and sometimes both mating types were found on the same plant. Most of the isolates recovered from fields treated with metalaxyl-M prior to sampling had reduced sensitivity or were resistant to metalaxyl-M, and most of the isolates recovered form fields without metalaxyl treatment were sensitive. The isolates recovered from fields treated with propamocarb prior to sampling had a higher frequency of reduced sensitivity to propamocarb than isolates from fields without propamocarb treatment. We found that most plants contained more than one *P. infestans* SSR-genotype. Clustering analysis of the infected samples revealed that most samples clustered together according to fields. By combining information from *P. infestans* isolates and DNA extracts from the leaf lesions we found examples of both mating type A1 and A2 having the same multilocus genotype. This result indicates that both of these genotypes have a common ancestor, hence the inoculum originates from oospores. Although this a minor study of only 10 fields with a limited amount of isolates and plant samples, the results indicate oospores in the soil is an inoculum source. Hence the forecasting model to predict outbreaks of potato late blight should be modified to include this.

KEYWORDS
Phytophthora infestans, mating type, fungicide resistance, Simple-sequence repeat (SSR), tuber blight, inoculum source.

INTRODUCTION

Potato late blight, caused by *Phytophthora infestans*, is the most important potato disease in Norway. To control the disease multiple fungicide applications are necessary. In average 8

fungicide applications per field were used to control potato late blight disease in Norway in 2012 (based on the amount on fungicides sold in 2012 and the total potato production area). The relatively high number of applications in 2012 was probably caused by the humid weather with an early onset of the potato late blight epidemics in both 2011 and 2012. Advisors and farmers can use the decision support tools, freely available in VIPS (www.vips-landbruk.no), to time their fungicide applications. In VIPS the Negative prognosis model (Ullrich & Schrödter, 1966) is used to predict the onset of the potato late blight epidemics. Normally the accumulated risk values form this model excides the threshold (150) before the first appearance of late blight in potato fields, but the last years the model has failed. In order to improve the forecast for risk of early onset of potato late blight, it is important to have knowledge of the initial inoculum sources. Therefore we have sampled and genetically characterized early outbreaks of potato late blight in a systematic way to study the relative importance of infected seed tubers versus oospores in the soil. Another aspect of importance to disease control is the presence of fungicide resistance in the late blight population; hence isolates were tested for resistance to both metalaxyl and propamocarb.

MATERIALS AND METHODS

Samples were collected form early outbreaks of potato late blight in 10 potato fields distributed in three districts in Norway, in July 2012. In each field five potential inoculum source plants were sampled. The potential inoculum source was defined as the plant in center of the disease foci, with more late blight than the surrounding plants and one or more stem lesions. From each plant one stem containing minimum three leaflets with single lesions and one stem lesion were sampled. These lesions were excised and put into separate bags. The mother tuber from each plant was collected in a separate bag with a piece of the stem of interest left on, while the other stems were pulled away, to indicate which area of the mother tuber to sample from. However some of the mother tubers were completely decomposed and not possible to sample. In one field (S) mainly single lesion plants were detected, and consequently more plants were sampled.

In the lab, the area of the mother tubers close to the stem of interest were cut into thin slices to look for symptoms and for DNA extraction. From each leaf lesion one half was used for isolation of *P. infestans* and the other half was used for DNA extraction. The stem lesions were only used for DNA extraction. DNA was extracted according to Cullen *et al.* (2001) and purified using a spin column filled with polyvinylpolypyrrolidone. Six polymorphic SSR regions were amplified using PCR with primers developed previously: Pi02, Pi04, Pi26, Pi33 (Lees *et al.*, 2006); 4B and G11 (Knapova & Gisi 2002). The fluorescently labeled PCR products were analyzed by using an automated ABI 3730 DNA analyzer as described by Brurberg *et al.* (2011).

P. infestans was isolated from the leaf lesions by using potato tuber slices of cultivar Bintje as selective "growth media" before transferred to pea agar as described by Lehtinen *et al.* (2008). The mating type of the isolates were determined by the presence or absence of oospores after pairing them with standard isolates on pea and rye B mixed agar as described by Hermansen *et al.,* 2000. The sensitivity to metalaxyl-M and propamocarb were determined by the isolates ability to grow and sporulate on leaf discs floating on water with different fungicide concentrations as described by Lehtinen *et al.* (2008). The effects of the fungicides were calculated as the % inhibition of growth and sporulation in comparison to growth on leaves floating in water without fungicides.

RESULTS AND DISCUSSION

All the 10 potato fields sampled had outbreaks of late blight earlier than forecasted with the Negativeprognose model at the respective locations. Most of the fields had low level of late blight at the time of sampling (Table. 1). From 148 leaf samples with lesions, 73 *P. infestans* isolates were recovered. The recovery rate was relatively low, which is probably because the samples were collected from fungicide treated fields. Surprisingly most (91%) of the recovered isolates were of mating type A1 (Fig. 1). In previous studies, where we have sampled fewer isolates per field and later in the epidemic, we have found a more even distribution of mating types (Hermansen *et al.*, 2000; Lenhtinen *et al.*, 2008). In 3 of 9 fields, with more than one isolate recovered, both mating types were found. In the two fields with both mating types recovered and more than one isolates recovered per plant (fields L and V), we found both mating type on the same plant.

Table 1. *Origin and background of potato / P. infestans samples. Ridomil contains metalaxyl-M and mancozeb, Tyfon contains propamocarb and fenamidone, Revus contains mandipropamid and Ranman contains cyazofamid*

District	Field	Cultivar	% late blight at sampling in the haulm	Fungicide treatments before sampling	Number of plants sampled	Sample size		
						Leafs	Stems	Tubers
Vestfold	S	Folva	0.01	Revus, Ridomil, Tyfon	12	14	2	1
Vestfold	M	Berber	0.5		6	16	5	6
Vestfold	L	Folva	0.1	Ridomil, Ranman, Tyfon	5	15	5	5
Buskerud	P	Asterix	10	Ridomil, Revus, Tyfon	5	15	5	5
Buskerud	G	Kerrs Pink	0.1		5	15	5	5
Buskerud	O	Pimpernel	0.5		5	15	5	5
Hedmark	K	Beate	0.2		5	15	5	2
Hedmark	N	Folva	0.1		5	15	5	1
Hedmark	H	Asterix	0.2	Ridomil	5	15	5	5
Hedmark	V	Asterix	0.2		5	15	5	5

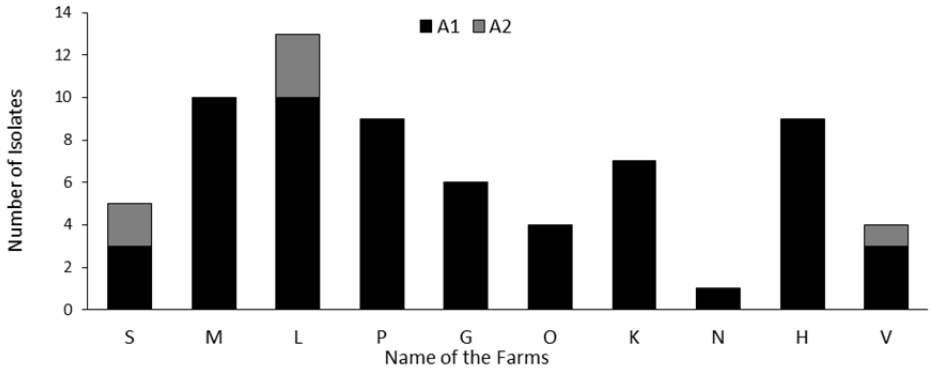

Figure 1. *Number of isolates recovered from leaves from different fields and their mating type*

Many of the isolates recovered from fields treated with metalaxyl-M prior to sampling, were resistant (19 of 39) or had reduced sensitivity (16 of 39) to metalaxyl-M. Only one of the isolates recovered from fields not treated with metalaxyl containing fungicide prior to sampling was resistant, and two had reduced sensitivity (Fig. 2). This indicates that application of metalaxyl-M exerts a strong selection pressure. It is only allowed to apply metalaxyl once per season in Norway. The results show that it is advisable to continue to limit the number of metalaxyl applications to one treatment per season to prevent metalaxyl resistance problems.

Two of the 70 isolates were resistant to propamocarb; they grew and developed spores on the highest concentration of propamocarb (Fig. 3). They were recovered from a field treated with propamocarb containing fungicide prior to sampling. Some isolates (14 of 70), had reduced sensitivity to propamocarb, they were able to grow and sporulate on some of the leaf discs at 100 ppm propamocarb. Most of these isolates (10 of 14) came from fields treated with propamocarb prior to sampling. Only 19 of 70 isolates were fully inhibited of the lowest propamocarb concentration, 10 ppm. They came from both types of fields. The difference between fungicide resistance in isolates sampled form fields with or without treatments with fungicide containing propamocarb prior to sampling was not so clear cut as for metalaxyl, but it was a tendency of higher tolerance to propamocarb among the isolates recovered form treated fields.

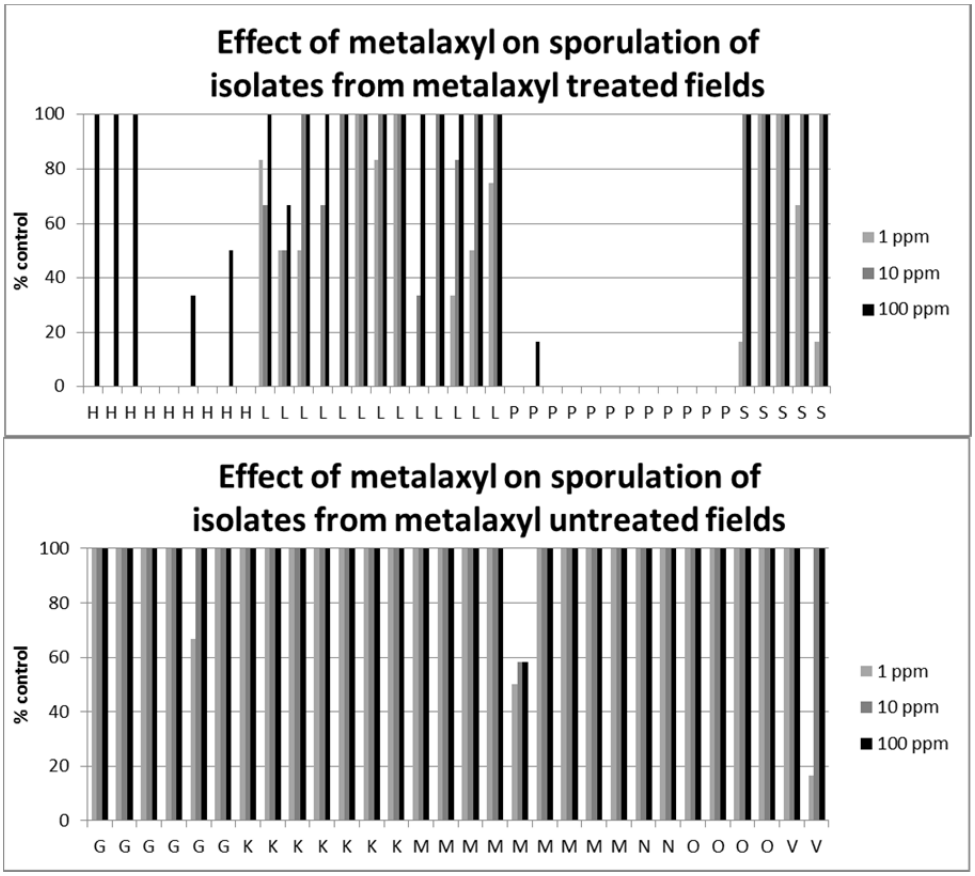

Figure 2. *The ability of metalaxyl-M to control potato late blight, calculated as the inhibiting effect on sporulation of P. infestans isolates growing on potato leaf disks floating on water with different concentrations of metalaxyl in comparison to sporulation on potato leaf disks floating on water without fungicides. The top graph presents the effect of metalaxyl on isolates recovered form fields treated with fungicides containing metalaxyl before sampling. The lower graph presents the effect of metalaxyl on isolates recovered form fields not treated with fungicides containing metalaxyl before sampling. The letters on the x-axis represent the field the isolates come from*

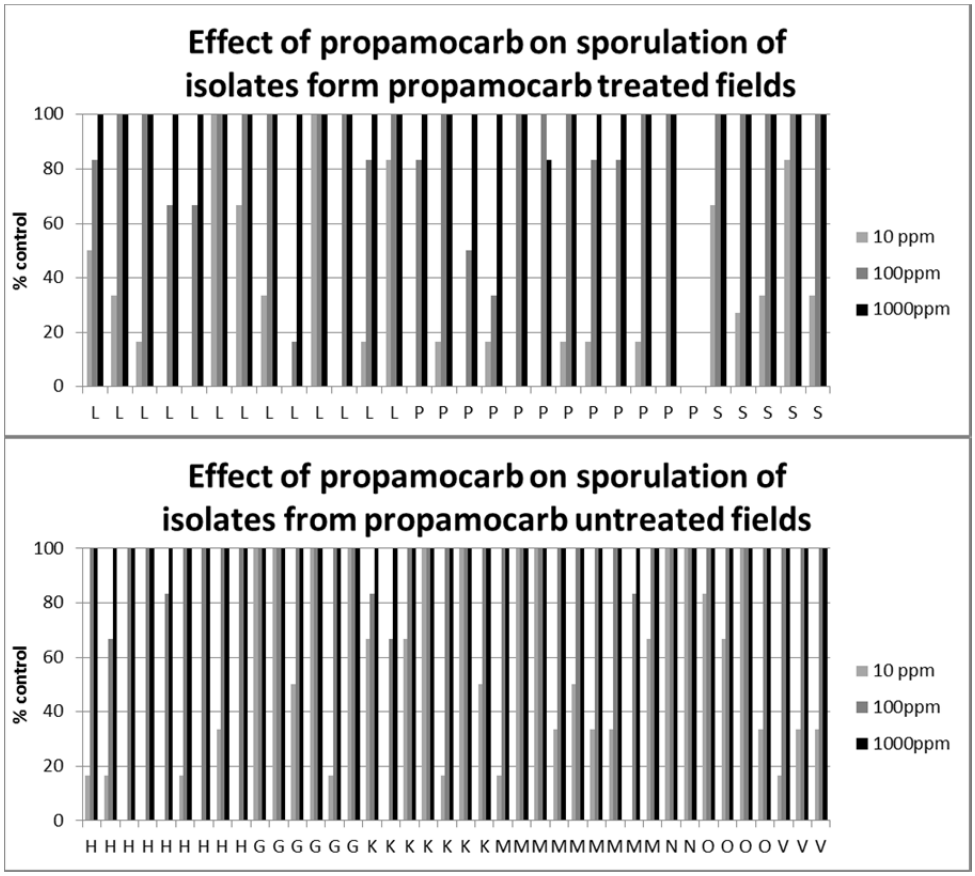

Figure 3. *The ability of propamocarb-HCl to control potato late blight, calculated as the inhibiting effect on sporulation of P. infestans isolates growing on potato leaf disks floating on water with different concentrations of metalaxyl in comparison to sporulation on potato leaf disks floating on water without fungicide. The top graph presents the effect of propamocarb-HCl on isolates recovered form fields treated with fungicides containing propamocarb before sampling. The lower graph presents the effect of propamocarb -HCl on isolates recovered form fields not treated with fungicides containing propamocarb before sampling. The letters on the x-axis represent the field the isolates come from*

DNA was isolated from all plant samples and genotyped using SSR primers. 187 of the 197 leaf and stem samples were successfully fingerprinted with all six SSR primers. None of the tubers had typical late blight symptoms. DNA was nevertheless extracted, but failed to give PCR products with most of the SSR primers. In total 32 alleles were detected at six SSR loci (Table 4). An allele not previously detected in Norway (146 of PiG11), was found in 26 samples originating from different fields. Several of the samples produced more than two alleles per loci, a phenomenon that also has been reported previously (Lees et al 2006; Brurberg et al 2011). We found that most plants contained more than one genotype, and the few tuber samples that gave SSR products had mainly different alleles than the corresponding stem and leaf samples. Clustering analysis (UPGMA) of leaf and stem samples revealed that most samples clustered

together according to fields (Fig. 4). By combining information from *P. infestans* isolates and DNA extracts from the leaf lesions we found examples of both mating type A1 and A2 having the same SSR multilocus genotype. This result indicates that both of these genotypes have a common ancestor, hence the inoculums originate from oospores.

Table 2. *Allele frequencies for SSR markers in 187 P. infestans infected samples from leaves and stems*

SSR locus	Allele	Allele frequency	SSR locus	Allele	Allele frequency
PiG11	142	0.50	Pi04	160	0.04
	146	0.14		166	0.82
	148	0.04		168	0.10
	152	0.20		170	0.90
	154	0.34	Pi26	173	0.14
	156	0.40		177	0.42
	158	0.11		179	0.36
	160	0.25		181	0.72
	162	0.30		183	0.14
	166	0.01		185	0.11
Pi02	152	0.28		187	0.13
	158	0.61	Pi4b	205	0.44
	160	0.58		213	0.63
	162	0.76		217	0.60
	164	0.09	Pi33	203	0.99
				206	0.31

CONCLUSIONS

We found indications of significant genetic variation of *P. infestans* within fields, even though we only sampled 5 plants per field. There seems to be a strong shift in the *P. infestans* population in the field after treatments with fungicide (in particular metalaxyl-M), increasing the frequency of resistant clones. We found *P. infestans* in some of the seed tubers, but we found no clear indications of tuber inoculum leading to outbreaks of late blight. However, we found indications of both mating types having the same SSR multilocus genotype in one field and most fields seemed to have their own family of *P. infestans*. Although this a minor study of only 10 fields with a limited number of isolates and plant samples, the results indicate that oospores in the soil could be an important inoculum source. Hence the forecasting model to predict outbreaks of potato late blight should be modified to include this.

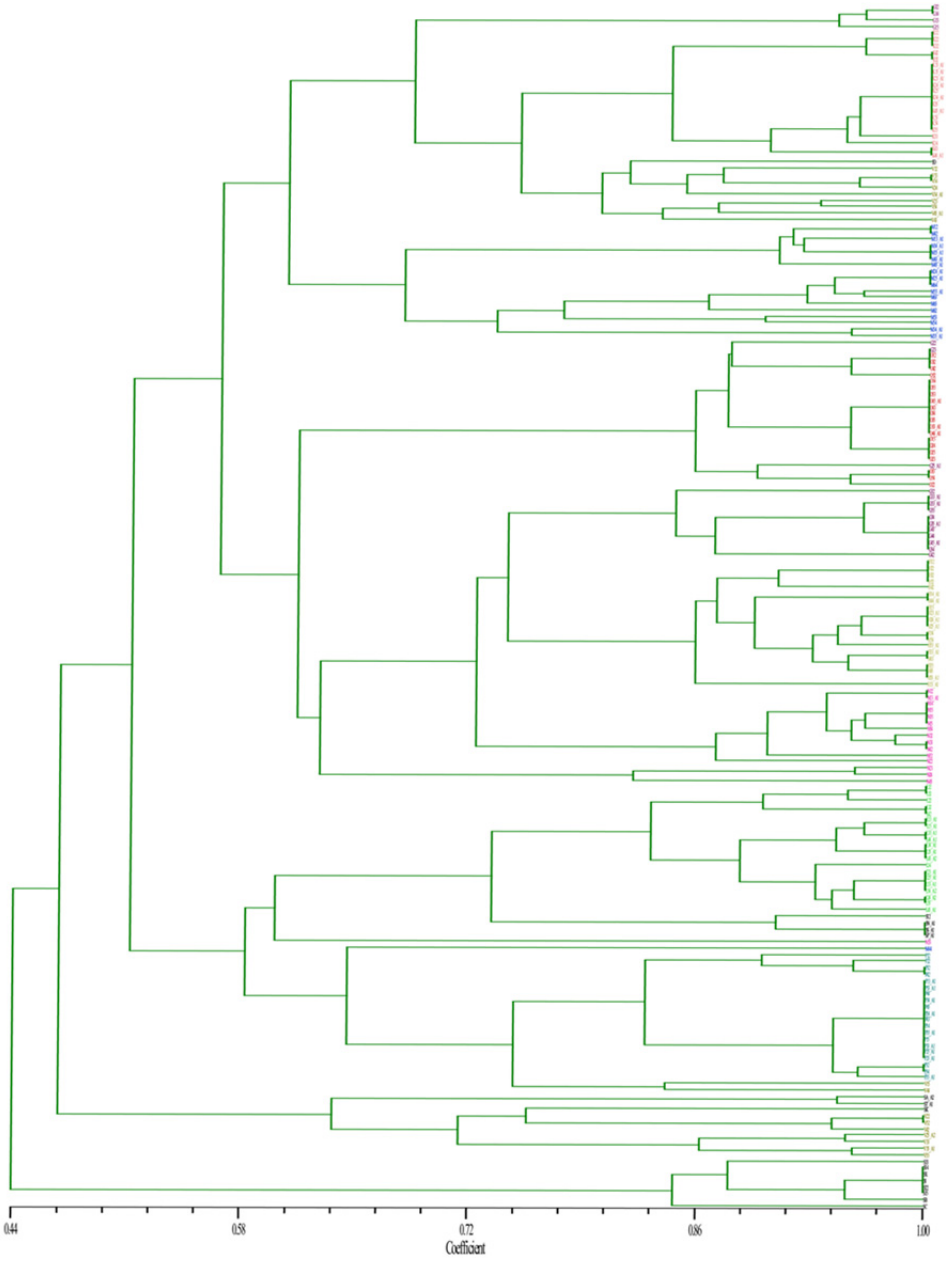

Figure 4. *Dendrogram of 187 P. infestans infected samples from 10 fields generated form matrices of similarity based on SSR cluster analysis*

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