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Distribution of Mating Types and Resistance to Metalaxyl of *Phytophthora infestans* in Germany

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SUMMARY
In 2010 a monitoring was carried out to characterise several German populations of the pathogen *Phytophthora infestans* (Mont.) de Bary based on mating type and sensitivity to metalaxyl. All tests were done by laboratory experiments. Mating types were determined in a pairing test. By using a floating leaf disc method the sensitivity to metalaxyl was evaluated. After first detecting mating type A2 in 1984 in Europe many trials were done to investigate the changes in the populations. Latest studies showed a high presence of A2 in European countries (Gisi *et al.*, 2011). The result of this work agrees with this observation. The proportion of A2, as well as the level of resistance, was proved to be still high in Germany. The frequency of mixed populations, found in the fields, was conspicuously high. It can be assumed that sexual reproduction of *P. infestans* takes place in many German production areas which causes high genetical variability. The analyses of a linkage between mating type and resistance to metalaxyl revealed no relationship between both traits.

KEYWORDS
Phytophthora infestans, late blight, mating type, metalaxyl resistance, monitoring

INTRODUCTION

Phytophthora infestans is a heterothallic species with two compatible mating types designated as A1 and A2. If both mating types come into physical contact it results in the formation of oospores (Drenth, 1994). Until the early 1980s the European populations were dominated by mating type A1. The epidemic spread was affected of asexual sporangia (Schulte, 2011). The exchange of crossing partners with same mating types is not possible. Thus, the pathogen is limited adaptable. Hohl and Iselin (1984) reported first data on A2 mating type occurrence in Switzerland. Two years later first observations of this type followed in Germany (Schöber and Rullich, 1986). In 1987, the detection of oospores in the field succeeded (Götz, 1991). By sexual recombination the late blight gains genetic variability and adaptability (Hausladen, 2007). Increasing aggressiveness (Krauthausen and Flier, 2005) and foreshortening of latency periods were reported for example of other implications (Hausladen, 2007). After detecting in 1986, several studies were carried out in Germany on the population structure of *P. infestans*. During 1986 and 1999 the proportion of A2 was in a range of 0% to 40%. In 2000 the ratio significantly changed to a proportion of about 70% of A2 (Bangemann,

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2009). Subsequently, investigations were reduced and only carried out sporadically, but analysed samples from neighbouring European countries from 2006 to 2007 also showed a high presence of A2 up to 90% (Gisi *et al.*, 2011). Furthermore a simultaneous increase of metalaxyl resistance with the appearance of A2 was observed in many countries (Schöber-Butin, 2001).
The aim of this study was to resume the research and to get a current survey about the population structure of *P. infestans* in Germany. Therefore a monitoring was carried out in 2010 to collect isolates for characterisation by mating type determination and evaluation of their resistance to metalaxyl. Furthermore, it was studied if the two traits may be genetically linked.

MATERIAL AND METHODS

Origin of P. infestans isolates
37 isolates were collected from 14 locations out of six federal states of Germany with different potato cultivars (Table 1). The isolates were sampled at various dates during 2010. Up to five isolates were taken per monitoring field. At five locations only one isolate could be educed. The single-lesion isolates were maintained in-vitro as axenic cultures on pea agar medium and incubated at 15°C in darkness.

Table 1: Origin of German isolates collected in 2010

Location	Number of isolates	Potato cultivar	Date of sampling
Hamm	5	Magda	15. Jun.
Guntersblum	1	Magda	16. Jun.
Hechtsheim 1	1	n.s.	21. Jun.
Gimbsheim	5	Pirol	21. Jun.
Bretzenheim	3	Agria	22. Jun.
Hechtsheim 2	1	n.s.	29. Jun.
Schwalmtal	3	n.s.	11. Aug.
Oberlichtenau	3	Talent	11. Aug.
Nemt	2	Ditta	12. Aug.
Müncheberg	1	Ballerina	14. Aug.
Neusustrum	1	Euronova	18. Aug.
Lathen	4	Euronova	18. Aug.
Puch	2	Agria	18. Aug.
Straßmoos	5	Agria	18. Aug.

Mating type determination
The experiment was done following the protocol of Bakonyi and Cooke (2004). The mating type of each unknown isolate was determined by individually pairing them with known A1 and A2 tester strains (Plant Research International, Wageningen, NL). The isolates were placed in a distance of 3 cm apart in Petri dishes (4.5 cm in diameter) containing pea agar. The plates were incubated in the climatic chamber at 15°C and darkness. After ten days of incubation the combinations were microscopically observed. The presence

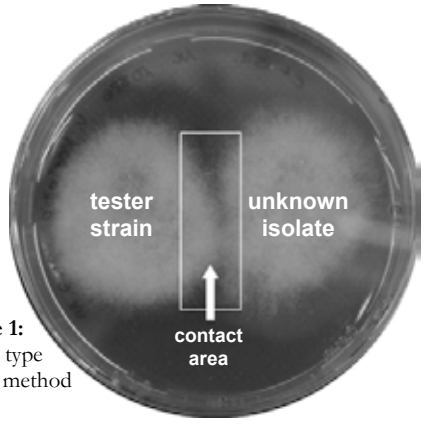

Figure 1: mating type testing method

or absence of oospores was recorded in the "contact area" where the mycelia of both mating partners crossed (Figure 1). If the contact area showed oospores, the unknown isolate was assigned to the complementary mating type of the tester strain's type. The result was confirmed by an observation of exclusive sporangia formation in the other plate.

Metalaxyl sensitivity test
The sensitivity to metalaxyl of the isolates was assessed by using a floating leaf disc method (Sozzi *et al.*, 1992). It was analysed on potato leaf discs from six week old plants of the cultivar Laura, grown under glass. Petri dishes (9.5 cm in diameter) were filled with 15 ml fungicide solution (Fonganil Neu, Novartis Agro AG) at four concentrations (0.01, 0.1, 1.0 and 10.0 ppm) plus an untreated control consisting of distilled water. Ten leaf discs (10 mm in diameter) per dish were placed with the abaxial side upwards on the solution. For each isolate a suspension of sporangia (105/ml) was prepared by flooding two week old cultures with 6 ml sterile water (½ distilled water, ½ tap water). Sporangia colonies were gently scrapped off the surface and solutions were filtrated through cotton wool to separate mycelia. Leaf discs were inoculated by a 10 µl droplet of sporangia suspension centred on each disc. The test was incubated for ten days at 15°C and with 12-hour photoperiod. Leaf discs were examined for sporulation and diseased discs were counted in each Petri dish. Tested isolates were rated as sensitive when sporulation proceeded at 0 to 0.01 ppm, as intermediate when sporulation proceeded at 0.1 to 1.0 ppm and as resistant when the pathogen sporulated at 10.0 ppm. Incubation period and latency time were detected in the untreated control in 24-hour intervals.

RESULTS

Mating types
With a proportion of 64.9% the majority of all field isolates was determined as mating type A2. With 21.4%, a mixed population was found on every fifth field. By excluding the locations with only one extracted isolate per monitoring site the proportion of fields with a mixed population amounts to more than 33%.

Sensitivity to metalaxyl
In the untreated controls the isolates showed incubation periods (time between inoculation and appearance of first symptoms) less than 24 hours. Latency times (time between inoculation and appearance of first sporangiophores) were between 3-4 days. Eight isolates sporulated until the third day. Except the field of Guntersblum at least one isolate per location sporulated until the fourth day. With 51.6% the isolates (n=31) were mostly resistant to metalaxyl. Only 3.2% stopped sporulation

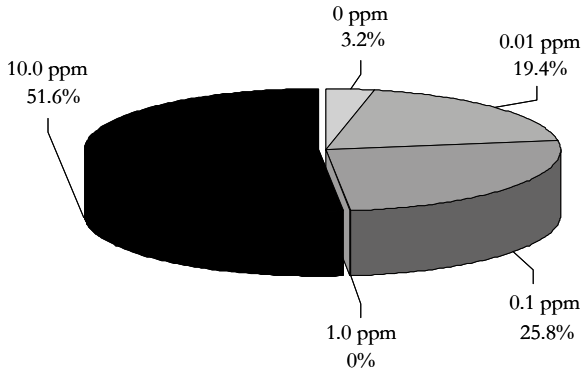

Figure 2:
Proportion of isolates with sporulation per concentration level

at 0.01 ppm and 19.4% stopped at 0.1 ppm. Thus, a proportion of 22.6% responded sensitive. A reduced effectiveness of metalaxyl was recorded at 25.8% of the isolates (Figure 2). All isolates of Gimbsheim and one isolate of Oberlichtenau showed no sporulation in all variants and were not evaluated. No site with a sample size of more than one showed only sensitive or intermediate isolates. Both sensitivity levels occurred always in common. Oberlichtenau was the only site where sensitivity occurred next to resistance.

Climatic conditions in 2010 were first favourable to late blight. Due to cool and humid weather, infections of *P. infestans* were found in June. High temperatures in July repressed late blight and sampling was not continued until August. By examining the two sampling periods a difference in the level of resistance becomes clear. Samples collected later in the season showed a higher level of resistance compared to samples collected early in the season. While 45.5% of the early sampled isolates responded sensitive to metalaxyl and as many isolates were intermediate, the isolates sampled in August were sensitive in only 10% and intermediate in 15% of the cases. Though, the resistance level increased from 9% in June to 75% in August (Figure 3).

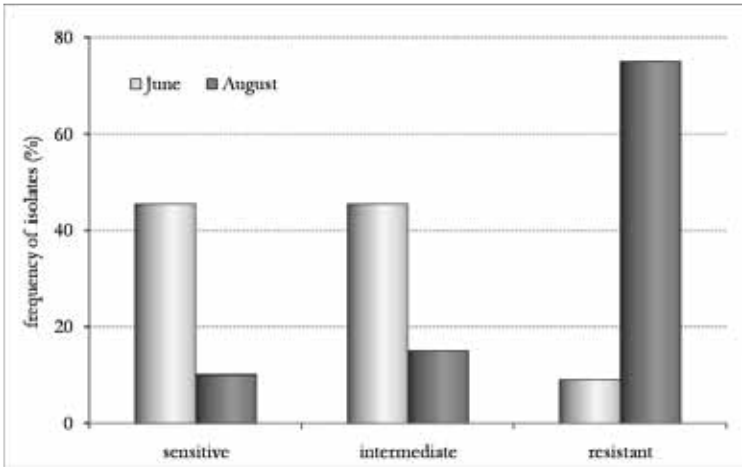

Figure 3:
Level of resistance depending on sampling time

The observation of mating types in relation to their sensitivity to metalaxyl showed that mating type A2 occurred preferably in conjunction with resistance. With 87.5% most of the resistant isolates were assigned to A2. Mating type A1 was resistant in 20% of the cases. Sensitive and intermediate A1 isolates were both found in 40% of the cases. Isolates with mating type A2 showed resistance in about 67% of the cases. Full efficacy of metalaxyl was observed at only 14% of the A2 isolates (Figure 4).
The geographical distribution did not show a regional concentration, neither regarding mating types, nor regarding sensitivity to metalaxyl.

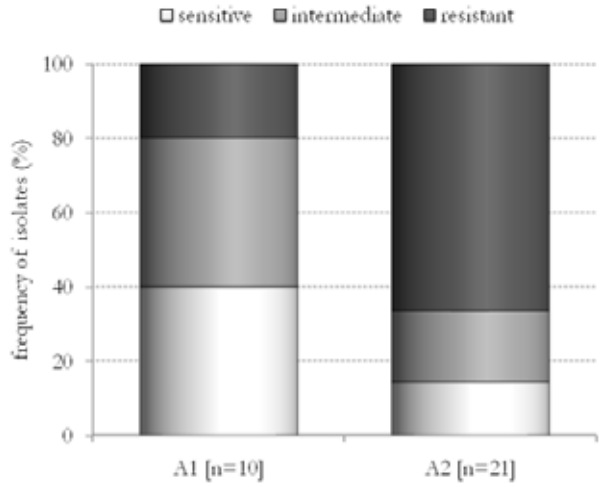

Figure 4. Level of resistance in conjunction with mating types.

DISCUSSION

After first detecting mating type A2 in 1984 in Europe, many trials were done to investigate the changes in the populations. Latest studies showed a high presence of A2 in European countries. From 2006 to 2007 the proportion of A2 was up to 90% in the neighbouring countries (Gisi *et al.*, 2011). The result of this work agrees with this observation. With 65%, the proportion of mating type A2 in the population proved to be high in Germany. Despite the low numbers of isolates per monitoring field (max. 5) mixed populations were found. The mating types occurred in a ration of 1:1 which leads to a significant chance of sexual reproduction in German potato production areas. The possibility of an occurrence of both mating types in a different relation cannot be ruled out in the remaining fields, especially not in those where only one isolate was won.

Leaf disc test showed latency periods between 3-4 days. Latency times of the old populations are indicated with 5-7 days (Hausladen, 2007). It can be assumed that the tested isolates belong to the new population. The level of resistance was high in 2010. However, the validity of this result strongly depends on the fungicide strategies which are used by the seed producers.

Caused by the fact, that *P. infestans*-isolates were collected in two different sampling periods, it was possible to observe an increasing level of resistance. Bangemann (2008) obtained a similar result in his investigations. Late samples showed barely a sensitive reaction, while the majority of isolates collected early in the season represented a high efficacy of metalaxyl. The variations in sensitivity during the season can be explained by a reduced hibernation ability of the resistant strains. Due to their higher vitality, the mycelium continues growing at low temperatures (in the storage) in the tissue of the tubers. By reaching and infecting the eyes, the tuber is prevented from germination in the following spring. Though, first infections of late blight are mostly initiated by sensitive strains. Increasing selection pressure during the season results the occurrence of resistant strains.

Mating type A2 mostly appeared in conjunction with resistance properties. However, the hypothesis of a dependency between mating type and resistance was disproved. Sensitive, intermediate and resistant isolates were observed in both mating types. Same observations were made by Gisi *et al.* (2011). Consequently the two traits are genetically unlinked.

CONCLUSIONS AND OUTLOOK

The results of monitoring the distribution of mating types and resistance to metalaxyl showed that mating type A2 occurred not only prevalent in German acreages, but also in combination with its mating partner A1. Added to the formation of sporangia, *P. infestans* most likely propagates by sexual reproduction. Despite the fact that more than half of all isolates reacted resistant to metalaxyl, the fungicide is still applicable in consideration of a strict resistance management strategy. The level of resistance increases significantly during the season. To prevent the formation of resistant strains, metalaxyl can be used prophylactic at the beginning of the season. Subsequently, other active substances should be applicated to avoid the use of phenylamids.

To further observe the level of resistance and the development of the ratio of A1 to A2, the monitoring was continued in 2011. The data are currently in evaluation.

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Distribution of Mating Types and Resistance to Metalaxyl of *Phytophthora infestans* in Germany

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Introduction

P. infestans (Mont.) de Bary is a heterothallic organism with two mating types designated as **A1** and **A2**. The interaction of both mating types results in formation of oospores.

Until the early 1980s the European populations were dominated by mating type A1. In 1984 mating type A2 was first discovered in Switzerland. In 1986 first observations in Germany followed. Ever since, the proportion of A2 steadily increased in Europe. Simultaneously a rise in resistance to fungicides with the active ingredient metalaxyl was reported from many countries.

In 2010 a monitoring was carried out to determine the occurrence of mating type A2 and resistance to metalaxyl in Germany. The genetic linkage between the two traits was analyzed.

Materials and Methods

Figure 1: mating type testing method

Mating type determination

37 isolates were collected from 14 plots in 2010.

For mating type determination every isolate was paired seperately with an A1 and an A2 tester strain on pea agar (Figure 1).

After ten days of incubation at 15°C in the dark, the zone of contact was checked for oospore production. If the contact zone showed oospores, the unknown isolate was assigned to the complementary mating type of the tester strain's type.

Metalaxyl sensitivity test

The sensitivity to metalaxyl was analysed by potato leaf discs. Petri dishes were filled with 15 ml fungicide solution at four concentrations (0.01, 0.1, 1, 10 ppm) plus an untreated control consisting of destilled water. Ten leaf discs per dish were placed upside down on the solution and inoculated by a 10µl droplet of sporangia suspension (10^5/ml). The test was incubated for ten days at 15°C with 12-hour photoperiod.

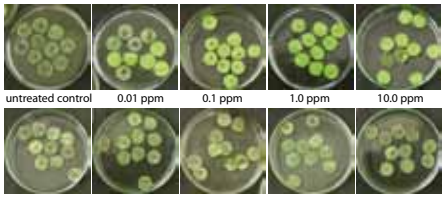

Figure 2: sensitive isolate (top) and resistant isolate (bottom) in leaf-disc test

Leaf discs were examined for sporulation and diseased discs were counted in each dish. Tested isolates were grouped into **sensitive** (sporulation at 0-0.01 ppm), **intermediate** (sporulation to 0.1-1 ppm) and **resistant** strains (sporulation to 10 ppm). Incubation period and latency time were detected in the untreated control in 24-hour intervals.

Results

Mating type

With a proportion of **64.9 %** the majority of all field isolates was determined as mating type A2. In **21.4 %** of the fields a mixed population was found.

Metalaxyl sensitivity

The isolates showed incubation periods less than 24 hours. Latency time was between 3-4 days. **51.6 %** of all isolates showed resistance to metalaxyl. Only **22.6 %** responded sensitive and **25.8 %** were rated intermediate (Figure 3). Mating type A2 occurred preferably in conjunction with resistance. **87.5 %** of the resistant isolates were assigned to A2.

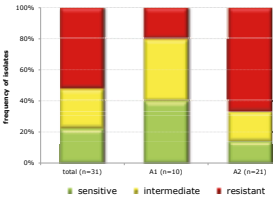

Figure 3: Resistance level of all isolates, A1- and A2-isolates

Samples collected later in the season showed a higher level of resistance than samples collected earlier in the season. (Figure 4).

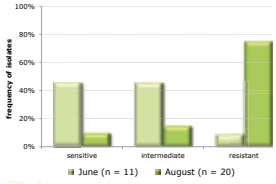

Figure 4: Sensitivity to metalaxyl depending on sampling time

The geographical distribution did not show a regional concentration, neither regarding mating types, nor regarding sensitivity to metalaxyl.

- resistant
- intermediate
- sensitive
- not evaluable

Figure 5: Distribution of mating types and resistance to metalaxyl

Conclusion

The proportion of A2 in the population proved to be high in Germany. Despite the low number of isolates per field (max. 5), mixed populations were found. The mating types occurred in a **ratio of 1:1**, which leads to a significant chance of sexual reproduction. The possibility of an occurrence of both mating types in a different relation cannot be ruled out on the remaining fields.
Variations in sensitivity during the saison can be explained by increasing selection pressure and a reduced hibernation ability of the resistant strains.
The hypothesis of a dependency between mating type and resistance was disproved. Sensitive and resistant isolates were observed in both mating types. Consequently the two traits are genetically unlinked.