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Resistance to metalaxyl in Phytophthora infestans in the Netherlands

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Abstract

Two hundred and twenty two isolates of *Phytophthora infestans* from infected potato foliage or tubers, obtained in 1981 from different potato fields in various regions of the Netherlands, were tested for their ability to infect potato leaf discs floating on solutions of metalaxyl of various concentrations. Isolates were designated as resistant to metalaxyl when sporulation occurred at a concentration of 1 μg ml^{-1} or higher.

Forty-one isolates appeared to be resistant. Thirty-five of the resistant isolates and 29 sensitive ones originated from three areas involving circa 15% of the total Dutch potato acreage. In these areas 28% of the fields sampled were metalaxyl-treated and 78% of these fields yielded resistant isolates. Only one of the three areas had a history of metalaxyl resistance in 1980. Six metalaxyl-resistant and 152 sensitive isolates were found in potato fields in other areas including those with major problems of resistance in 1980. All of four old potato cull piles sampled in the latter areas yielded metalaxyl-resistant isolates and two of these sites had possibly been inoculum sources for nearby potato fields. Only 9% of the fields sampled in these areas were metalaxyl-treated and none of these yielded a metalaxyl-resistant isolate.

The data are compatible with the idea that infected seed potatoes were the major source of the incoculum that initiated the early and severe late blight epidemic in 1981 in the Netherlands. The severity has been enhanced by late-blight-conducive weather conditions during the emergence of the crop and its early stages of development, when fungicides had not yet been applied. Old potato cull piles are assumed to have been less important in starting the 1981 epidemic.

Apparently, infected seed potatoes carried predominantly metalaxyl-sensitive strains, which explains the prevalence of metalaxyl-sensitive strains in most of the fields. In 1980, the seed potato crop had been harvested before the problem of metalaxyl-resistance surfaced. Hence, it might be assumed that infection of seed potatoes, favoured by late-blight-conducive weather in 1980, had been caused by metalaxyl-sensitive strains. Evidence that seed potatoes could also carry metalaxyl-resistant strains was obtained in two cases. It might explain their early occurrence in fields located in areas without a history of metalaxyl resistance in 1980 and the rapid build-up of a resistant population in a number of metalaxyl-treated fields.

Development of resistance in potato fields, which were under heavy disease pressure after spraying with a mixture of metalaxyl and mancozeb, could not be proven definitely. In one experimental field the seed tubers very likely carried metalaxyl-resistant strains, which initiated an epidemic, and in a second field the influx of resistant strains from elsewhere could not be completely ruled out. In the latter field spraying the mixture at a two-week interval could not effectively control the disease. In this field a considerable degree of tuber infection by metalaxyl-resistant strains was noticed.

No correlation existed between the behaviour of a particular strain towards metalaxyl and the genes for virulence present. Among a total of 79 isolates, the race of which has been identified, 23 different races were found. Race 1.3.4.7.10.11 was most frequently found, followed by race 1.3.4.7.11. Among 37 resistant isolates 10 different races could be identified.

Additional keywords: fungicide resistance, monitoring, fungicide mixtures, development of resistance, mancozeb, race spectrum, late blight epidemiology.

Introduction

In the Netherlands in 1980 a severe late blight epidemic caused by metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary resulted in considerable crop losses (Davidse et al., 1981). As a consequence the registration of metalaxyl, formulated either exclusively or in a mixture with mancozeb to control late blight, was cancelled in 1981, after the manufacturer had already decided to withdraw the product from the Dutch fungicide market.

In the potato growing season of 1981 nation-wide monotoring for the presence of metalaxyl-resistant strains was carried out to determine their proportion in the population, in order to evaluate a possible future use of mixtures of metalaxyl and conventional fungicides.

In addition in two field experiments a possible shift of a metalaxyl-sensitive population to resistance has been studied after application of a mixture of metalaxyl and mancozeb to the crop.

Race identifications have been carried out to determine if any relationship existed between the presence of metalaxyl resistance and genes for virulence.

Material and methods

Isolation of P. infestans and maintenance of the isolates. Infected foliage and blighted tubers werd collected from fields, old potato cull piles and volunteer plants by extension officers, specialists of the agrochemical industry, contracters, farmers or by the authors in various parts of the country. Isolations were made from infected tissue by placing the tissue underneath 0.5-0.7 cm thick slides of healthy potato tuber tissue (cv. Bintje) in 9-cm Petri dishes. These slices had been cut with a sterile knife from washed tubers which had been sterilized by immersion in a hypochlorite solution (0.75% active chlorine) for 10 min and 70% ethanol for 3 min. The dishes were kept at 15 °C until sporulating mycelium developed on the slices. Sporangia were washed off with sterile distilled water and used in the test for metalaxyl resistance. Isolates were maintained on tuber slices at 15 °C and trasferred to fresh slices at weekly intervals or stored in whole potato tubers at 4 °C. For storage surface-sterilized tubers were almost cut in half and a piece of a tuber slice culture of the fungus was placed between the two halves. The inoculated tubers were placed in paper bags at 4 °C. Reisolation of the fungus proved to be fairly successful up to 6 months after inoculation.

For prolonged storage sporangia were kep frozen in liquid N_2. To this end, detached potato leaflets were inoculated and kept in a moist environment at 15 °C and 16 h light per day. After sporulation occurred, the sporangia were washed off with an aqueous solution of skimmilk powder and glycerol (8.5% w/v and 10% v/v, respectively), concentrated by centrifugation and resuspended to a concentration of at least 5.10^4 sp ml^{-1}. The suspensions were pipetted in ampoules which were heat-sealed and slowly frozen at circa 1 °C per minute from ambient temperature to −40 °C, followed by quick immersion into liquid nitrogen (H. Dahmen and T.H. Staub, pers. comm.). The sporangia survived this procedure as has been tested in infection tests on detached potato leaflets with sporangia washed free from the cryoprotective solution by centrifugation and resuspension in distilled water.

Test for metalaxyl resistance. The sensitivity of the isolates to metalaxyl was determined in a leaf disc test at metalaxyl concentrations ranging from 100 μg to 0.01 μg a.i. ml^{-1} in five 10-fold dilutions (Davidse et al., 1981). Isolates were designated as resistant when sporulation occurred at a concentration of 1 μg ml^{-1} or higher. Isolates which did nog sporulate at 1 μg ml^{-1} were considered to be sensitive.

Race identification test. Race identifications of a number of isolates were carried out using the standard set of potato differentials of the Research Institute for Plant Protection according to standard techniques (L.J. Turkensteen and D. Looijen, pers. comm.). The differentials carried the following genes for late blight resistance R_1, R_2, R_3, R_4, R_5, R_7, R_8, R_{10}, R_{11}, R_1R_2, R_1R_3, R_1R_4, R_2R_3, R_2R_4 and $R_1R_2R_3$. Detached potato leaflets of the various genotypes were placed with their petioles in Oasis® , a water holding material, in boxes and inoculated with sporangial suspensions (circa 10^3 sp ml^{-1}). The leaves were kept in the closed boxes at 15 °C under 16 light and 8 h darkness until readings werd made after 6 to 7 days.

Field experiments. Two experiments were made to study a possible change in a metalaxyl-sensitive population towards resistance under field conditions and after application of a mixture of metalaxyl and mancozeb to a potato crop. Experiment 1 was made in a field of circa 5 ha planted with the cv. Bintje located in the newly reclaimed South Flevoland polder. In alle directions the field was at least 8 km away from the nearest potato field. The crop was grown according to common Dutch potato-growing practices. A contractor was hired for all operations. Parts of the field at four different locations covering in total 1.08 ha were initially left untreated, 0.24 ha was treated with mancozeb (1600 g ha^{-1}) and 3.6 ha was treated with a mixture of metalaxyl and mancozeb (200 + 1600 g ha^{-1}). The fungicides formulated as wettable powders were sprayed with a tractor-mounted sprayer initially at a 14-day interval on June 10 and 23, and on July 8. This spraying programme was changed on July 16 for reasons mentioned under results. That day the entire field was treated with a mixture of a full dose of a commercially available maneb/fentin acetate combination (850 g maneb + 288 g fentin acetate ha^{-1}) and half a dose of the metalaxyl/mancozeb mixture (100 + 800 g ha^{-1}). In addition to these mixtures the previously untreated parts received a full dose of the metalaxyl/mancozeb mixture (200 + 1600 g ha^{-1}). Additional applications of maneb/fentin acetate in combination with half a dose of the metalaxyl/mancozeb mixture were made to the whole field on July 22 and 28 and August 4, 12, 17 and 24. Haulm killing took place on September 4 with dinoseb and harvest was on September 26.

Plants at several sites along two rows in three of the four untreated parts of the field were inoculated in the evening of June 25 and July 2. Inoculations were intentionally not made in one untreated part in order to be able to determine the disease pressure generated by the inoculated sites. Race 4, a metalaxyl-sensitive strain, was used for the inoculation and sporangial suspensions (10^4 sp ml^{-1}) were sprayed on the abaxial sides of the leaves using a hand-operated pressure sprayer.

The crop was inspected for the presence of infected plants for the first time on June 10, after it had emerged on May 20, and at regular intervals thereafter. During early stages of the epidemic leaf infection percentages were calculated from the number of lesions and the average lesion size per unit area surveyed and the leaf area index. Infection percentages of individual plants in foci were estimated using key no. 3.1.1 of James (1971). During the later stages of the epidemic key no. 3.1.2 has also been used to estimate the percentages of leaf infection.

Data on temperature, rainfall and leaf wetness period were recorded on the experimental site. Rainfall was also recorded at a distance of about 5 km.

After the disease had been observed intensive sampling and testing for resistance has been carried out.

Experiment 2 was made in a 0.23-ha field planted with cv. Bintje, adjacent to a 1.6-ha field of the Foundation for Agricultural Plant Breeding at Heelsum where wild *Solanum* spp. and new clones and cultivars of potato were tested for resistance to late blight. Part of the experimental field (1820 m^2) was treated with the metalaxyl/mancozeb mixture on June 12 and 25, July 8, 17 and 29, August 10 and 21 and September 4 with a tractor-mounted sprayer. Two parts of the field (440 m^2 in total) on both the north and the south side of the treated area were left untreated. The entire selection field west of and adjacent to the experimental field was inoculated in the evening of June 25 with a mixture of the races 1.2.3, 1.3.4, 1.4.10 and 1.3.4.7.10.11 all of which were metalaxyl-sensitive. A zoospore suspension (50 sp ml^{-1}) containing equal ratios of zoospores of the races mentioned above was sprayed on the plants with a tractor-mounted sprayer. After inoculation had been finished an overhead sprinkler irrigation system was turned on to provide optimal conditions for infection. The irrigation system covering the experimental site was operated each night during the periode June 25 - August 17 to ensure a continuous leaf wetness period.

Disease progress was assessed during the season and samples were taken weekly after the disease had been observed in the treated area. Weather data were recorded at the experimental site.

Results

The late blight epidemic in 1981. The 1981 potato growing season in the Netherlands started with an early and heavy attack of late blight. Already on June 1 the first cases of infections were reported by farmers and within the next two weeks a severe epidemic swept the potato fields in all parts of the country.

Prevailing late-blight-conducive weather during the second half of May when the crop emerged, and early June, when blight fungicides had not yet been applied, has probably been the major factor that determined the severity of the epidemic. Although warnings predicting late-blight-conducive weather and advices to potato growers to spray their crop were broadcasted already on May 27, the growers reacted slowly. The unusual earliness of the advices and also the fact that in several parts of the country the land was too wet for using tractor-mounted sprayers made that treatments with blight fungicides were delayed, thus enhancing the severity of the disease. By June 12 already five warnings had been given but even then not alle potato fields had received the first treatment.

When farmers realized the severity of the situation, they tried to stop the spread of the disease by spraying – sometimes twice a week – with maneb, maneb/fentin acetate mixtures and cymoxanil-containing products, but they also used, especially in the south-west part of the country, mixtures of metalaxyl and the non-systemic fungicides. The latter mixtures were used merely on a nothing-chanced-nothing-gained basis. Since metalaxyl was no longer commercially available, farmers used up their own stocks or imported metalaxyl-containing fungicide mixtures from neighbouring countries.

The efficacy of the treatment with metalaxyl will be discussed in detail in the following sections.

The intensive fungicide applications had their effect and by July 10 the situation was largely under control. Dry and sunny weather during August undoubtedly has also contributed to the relatively low ultimate percentages of blighted tubers in the crop.

Number of resistant isolates and factors relevant to their occurrence. Two hundred and thirty five leaf samples with typical late blight lesions and 20 blighted tubers, each obtained from a different field in the period June 1 – November 13, 1981, yielded 222 isolates of *P. infestans*. The origin of the isolates and the numbers of metalaxyl-sensitive and resistant isolates are given in Table 1. Whether or not problems with metalaxyl resistance were encountered in 1980 has also been indicated.

As is evident from this table the situation in 1981 was completely different from that in 1980. In het northern part of the country (Groningen, Friesland, Veenkoloniën, Drente and IJsselmeerpolders), for instance, the population was predominantly resistant in 1980 (Ciba Geigy, unpublished; Davidse et al., 1981) but in 1981 in this region hardly any resistant isolate has been collected from fields. On the other hand, infected foliage of four old potato cull piles at different locations in these areas yielded mainly resistant isolates (Table 1). Both resistant isolates from fields in North Groningen/Friesland and one from a field in the IJsselmeelpolders were found nearby two of these old potato cull piles. It suggests that the latter had served as inoculum sources, indicating once more their importance in the life cycle of the late blight fungus.

In general, old potato cull piles might have hardly contributed to the early occurrence of the disease in the fields. If the epidemic had started with inoculum from old potato cull piles, than more resistant isolates would have been encountered in the fields than were found actually since on these sites probably a predominantly metalaxyl-resistant population was present, as has been demonstrated with the four old potato cull piles sampled. The prevalence of resistant strains fully agreed with the abundant occurrence of resistant strains in that part of the country in 1980 and probably is representative for all old potato cull piles in that area.

Infected seed potatoes are more likely candidates to have started the disease in the potato fields. The observation that the disease spread from foci which lay randomly scattered in the field and that heavily diseased plants had sometimes a rotten seed tuber whereas seed tubers from healthy plants were still intact, supports this idea. In

Table 1. Metalaxyl sensitivity and origin of isolates of *Phytophthora infestans* collected in 1981 in different regions of the Netherlands.

Region	Occurrence of metalaxyl resistance in 1980[1]	Number of metalaxyl-sensitive (S) and metalaxyl-resistant (R) isolates from: fields		old potato cull piles or volunteer plants	
		S	R	S	R
North Groningen/Friesland	yes	22	2	1[2]	3[3]
Veenkoloniën/Drente	yes	27	–	–	1[2]
IJsselmeerpolders	yes	37	4	4[2],[4]	10[4]
Gelderland	yes	2	–	–	–
North Holland	yes	2	–	1	–
Central South Holland	yes?	3	–	–	–
Hoekse Waard	yes?	4	13	–	–
Goeree Overflakkee	no	13	–	–	–
Zeeland	no	30	–	–	–
West Brabant	no	10	13	–	–
East Brabant/North Limburg	yes	15	9	1[2]	–
Middle and South Limburg	yes	16	–	–	–
Total		181	41	7	14

[1] Ciba Geigy, unpublished; Davidse et al., 1981.
[2] One isolate from volunteer plant.
[3] Two cull piles at different sites; one yielded one, and the other two resistant isolates.
[4] Two cull piles at different sites; one yielded one resistant isolate and the other three sensitive and nine resistant isolates.

Tabel 1. Aantal metalaxyl-gevoelige en -resistente isolaten van Phytophthora infestans en hun herkomst in 1981 in Nederland.

1980 prolonged periods of blight-conducive weather prevailed during the growing period of the seed potato crop, but it had been harvested before the problem of metalaxyl-resistance surfaced in August 1980. Hence, if tuber infection had taken place it might be assumed that the greater part of the infected seed potatoes carried metalaxyl-sensitive strains. This would explain the occurrence of metalaxyl-sensitive strains in most of the fields in 1981. But in two cases evidence has been obtained that infected seed tubers could also carry resistant strains. In the first one, involving a field in West Brabant, the seed tuber from a severely diseased plant sampled on June 9 yielded a resistant isolate, as did the infected foliage. A seed tuber from the same lot that had not been planted also yielded a resistant isolate. In the second case involving the South Flevoland experimental field (Experiment 1), an epidemic of resistant

Table 2. Use of metalaxyl on fields sampled in different regions of the Netherlands and the frequency of occurrence of resistant strains.

Region	Fields where metalaxyl has been used (% of total fields sampled per region)	Resistant isolates (% of total isolates obtained per region)
Hoekse Waard	39 (n[1] = 18)	76 (n[1] = 17)
West Brabant	31 (n = 36)	57 (n = 23)
East Brabant/North Limburg	17 (n = 29)	38 (n = 24)
other	9 (n = 191)	4 (n = 158)

[1] n = total number of fields or isolates per region.

Table 2. Toepassing van metalaxyl op bemonsterde percelen in verschillende gedeelten van Nederland en de frequentie in het voorkomen van metalaxyl-resistente isolaten.

strains undoubtedly was initiated by seed-borne inoculum. This case will be discussed in one of the following sections.

Although disease development from blight-infected seed potato tubers can fully explain the early and severe outbreak of the epidemic, it does not explain why in several parts of the country metalaxyl-resistant strains predominated. A greater proportion of resistant strains in the initial inoculum in those areas seems unlikely, since seed potato tubers, which are mainly grown in the IJsselmeerpolders and the northern part of the country, are almost randomly distributed throughout the whole country each year.

A more likely explanation for the prevalence of resistant strains in several areas is the greater use of blight fungicide mixtures containing metalaxyl. Because of the severity of the epidemic such mixtures were more frequently used by farmers in the Hoekse Waard and West Brabant than by farmers in other parts of the country (Table 2). As is evident from this table a correlation exists between the frequency of resistant strains and the use of metalaxyl.

Due to the selection pressure of metalaxyl the proportion of resistant strains in the population that initially has probably been low, increased considerably and as a result samples yielded predominantly resistant strains. The data in Table 3 illustrate the selection pressure of metalaxyl. Fourteen samples, each of a different field, out of 32 metalaxyl-treated fields yielded metalaxyl-resistant strains whereas only 13 samples out of 108 fields not treated with metalaxyl yielded a resistant isolate. Eleven from the latter 13 resistant isolates originated from fields located in one of the areas, where metalaxyl usage had been relatively high and as a result the population might have shifted towards resistance. Fields with unknown spraying history which yielded resistant isolates were also mainly located in those areas.

Since, before sampling, only one or at most two applications of metalaxyl in combination with a conventional fungicide had been made, the high frequency of resistant isolates indicates the high rate at which the population shifted towards resistance under the in 1981 prevailing circumstances.

The data also illustrate that when resistant isolates of *P. infestans* are detected in

Table 3. Numbers of metalaxyl-sensitive (S) and resistant (R) isolates in relation to the fungicide treatment of the crop.

Fungicides used	Number of isolates[1]		
	S	R	total
metalaxyl combined with others	18	14	32[2]
others	95	13[3]	108
unknown	68	14[4]	82
total	181	41	222

[1] One isolate per field.
[2] Eighteen fields where metalaxyl has been used were located in either the Hoekse Waard, West Brabant or East Brabant/North Limburg and 14 of these (78%) yielded resistant isolates.
[3] Eleven resistant isolates were obtained from fields located in either the Hoekse Waard, West Brabant or East Brabant/North Limburg.
[4] Ten resistant isolates were obtained from fields located in either the Hoekse Waard, West Brabant or East Brabant/North Limburg.

Tabel 3. Aantallen metalaxyl-gevoelige en -resistente isolaten in relatie tot het gebruikte fungicide.

an intensive monitoring programme, only a few sprayings are required to increase the frequency of resistant strains in the population from almost nil to 100%, a phenomenon that could also be predicted from theoretical models (Kable and Jeffery, 1980; Skylakakis, 1982).

Therefore, if large-scale use of metalaxyl and metalaxyl-containing fungicide mixtures had been continued in the Netherlands into 1981, the entire population would undoubtedly have shifted rapidly towards metalaxyl resistance. The decision, therefore, of the regulatory authorities to cancel the registration of metalaxyl-containing products for use against late blight is fully justified.

Origin of isolates according to variety, and race identification. Isolates were obtained from a number of potato cultivars differing in late blight susceptibility (Table 4). Most metalaxyl-resistant isolates were obtained from the cultivar Bintje. Since 'Bintje' was the most popular cultivar in the Hoekse Waard, West Brabant and East Brabant/North Limburg and was grown on 69, 89 and 75%, respectively, of the potato acreage in these areas, it is obvious that 'Bintje' yielded most of the resistant isolates.

The intensive growing of the highly susceptible cv. Bintje certainly has contributed to te spread of the fungus, but irrespective whether a sensitive or a resistant strain was involved. Otherwise in Zeeland where 'Bintje' was grown on 90% of the potato accreage also resistant strains would have been found.

The cvs Element and Estima, which both have a fair degree of race-nonspecific resistance against late blight, also yielded resistant isolates indicating that the occurrence of resistant strains was not restricted to highly susceptible cultivars.

Six different races were present among 14 resistant isolates and 15 different ones

Table 4. Numbers of metalaxyl-sensitive (S) and resistant (R) isolates in relation to the potato cultivar from which they were obtained.

Cultivar	Degree of resistance to *P. infestans*[1]	Number of isolates		
		total	S	R
Eersteling	3	5	5	0
Bintje	3	96	70	26
Parel	4	1	1	0
Primura	4	2	2	0
Sirtema	4	3	3	0
Krostar	R/4	3	3	0
Mentor	R/4	1	1	0
Alpha	5	2	2	0
Eigenheimer	5	4	3	1
Mirka	5	2	2	0
Nicola	5	3	3	0
Ehud	R/5	9	9	0
Prominent	R/5	3	3	0
Désirée	6	2	2	0
Spunta	6	2	2	0
Ajax	R/6	1	1	0
Astarte	R/6	4	4	0
Cardinal	R/6	3	3	0
Element	R/6	4	3	1
Estima	R/6	2	1	1
Irene	7	1	1	0
Mara	7	1	1	0
unknown		68	56	12
total		222	181	41

[1] Scale according to the Dutch List of Cultivars of Agricultural Crops. A higher value indicates higher degree of resistance. R indicates the presence of genes for resistance in the cultivar concerned. In this case the value gives the degree of resistance against a virulent race.

Tabel 4. Aantallen metalaxyl-gevoelige en -resistente isolaten in relatie tot het aardappelras waarvan zij werden geïsoleerd.

among 28 sensitives isolates (Table 5). Race 4 has been found five times associated with metalaxyl resistance, but, since four of these isolates were found in West Brabant it only indicates that there the population had a simple race spectrum. In the other areas where resistant strains predominated (East Brabant/North Limburg and the Hoekse Waard) four different races, viz. 4, 1.3.7.10, 1.3.4.7.11 and 1.3.4.7.10.11, were found among five resistant isolates of which the genotype had been determined.

Among the sensitive isolates the complex race 1.3.4.7.10.11 was the most frequent one. It has been found in four different parts of the country.

In Table 6 the frequency of virulence genes among sensitive isolates is compared with that among resistant isolates. It can be concluded that isolates carrying different genes for virulence have an equal propensity to develop resistance towards metalaxyl.

Table 5. Race spectra of metalaxyl-sensitive and resistant isolates of *Phytophthora infestans.*

Race	Prevalence of race among:		
	sensitive isolates	resistant isolates	all isolates
4	0	5	5
1.4	1	0	1
4.7	2	0	2
1.2.4	1	0	1
1.4.7.	1	0	1
1.4.10	1	0	1
3.4.7	1	0	1
3.10.11	1	0	1
1.2.4.7	1	0	1
1.3.4.7	0	2	2
1.3.4.11	0	1	1
1.3.7.10	0	1	1
1.4.10.11	2	0	2
1.4.7.11	1	0	1
4.7.10.11	1	0	1
1.3.4.7.11	4	3	7
3.4.7.10.11	2	0	2
1.3.4.7.10.11	7	2	9
1.3.4.7.8.10.11	2	0	2
total	28	14	42

Tabel 5. Fysiospectrum van metalaxyl-gevoelige en-resistente isolaten van Phytophthora infestans.

Table 6. Frequencies of virulence genes among metalaxyl-sensitive (S) and resistant isolates (R) of *Phytophthora infestans.*

Character of	Prevalence of virulence gene number							
	1	2	3	4	7	8	10	11
S	21	2	17	27	22	2	16	20
R	9	0	9	13	8	0	3	6

Tabel 6. Frequenties van virulentiegenen aangetroffen in metalaxyl-gevoelige en -resistente isolaten van Phytophthora infestans.

Field experiments. Experiment 1. Late blight was first observed in the South Flevoland experimental field in one of the untreated parts on July 1 (Table 7). A focus was found in which plants showed 5 to 10-cm-long lesions just above the soil surface and lesions on the lower leaves close to the infected stem indicating that the fungus had spread from the stems to the leaves. One of the plants had a very small and com-

Table 7. Disease ratings and sampling results in field experiment 1 until July 14.

Date	Untreated part (1.08 ha)			Metalaxyl/mancozeb-treated[1] part (3.6 ha)			Mancozeb-treated[1] part (0.24 ha)		
	disease rating[2]	sampling results[2,3]		disease rating	sampling results[3]		disease rating	sampling results[3]	
		S	R		S	R		S	R
June 10	0	–	–	0	–	–	0	–	–
June 24	0	–	–	0	–	–	0	–	–
July 1, 2	2 foci	1	1[4]	0	–	–	0	–	–
July 7	5 foci	0	3[5]	0	–	–	0	–	–
July 14	0.01-0.1% in foci 10%	0	14	12 lesions 2 foci	0	14[6]	0	–	–

[1] Dates of application: June 10 and 23 and July 8.
[2] Disease ratings and samplings were made outside inoculated sites.
[3] Number of samples taken at different sites yielding either metalaxyl-sensitive (S) or metalaxyl-resistant (R) isolates; – indicates that no samples were or could be taken.
[4] Race 4.
[5] Races 1.3.4.7 and 4.7. (2x).
[6] The race of three isolates has been determined: races 4 and 1.3.4.7.10.11 (2x).

Tabel 7. Ziektewaarnemingen en bemonstering in het proefveld in Zuid Flevoland tot 14 juli.

pletely necrotic shoot. The tuber of this plant was partly rotten. The seed tuber of a plant next to this plant was also rotten although to a lesser extent. Seed tubers from plants in other areas of the field were not rotten at that time. The lesions in this focus were clearly further developed than those on the inoculated plants, which just started to show symptoms and which grew at a different site, and, therefore, the lesions could not have been initiated by the inoculation of June 25. The appearance of the symptoms exactly matched those as described by Boyd (1980) for development of late blight from infected seed tubers. Each of three infected stems from this site yielded a sensitive isolate (Table 7). On July 2 and 7 more foci with lesions on the stems and on the lower leaves were found. Two of these foci were at least 90 m away from the inoculated plants. All four samples yielded a resistant isolate.

The occurrence in the foci of well-developed stem lesions just above the soil surface supported the idea that initial inoculum originated from infected seed tubers. Stem infections must have been initiated before June 25 since the lesions were further developed than those on plants which were inoculated that day. The location of the field at a distance of at least 8 km away from the nearest potato field made it unlikely that infections took place by sporangia originating from other fields. Moreover, if influx of inoculum from other areas had occurred this should have happened on either June 1, 9, 12 or 17 when rainfall was recorded in the area. In that period the crop was still in its initial stage of development after most plants had emerged in the week of May 20-27. Additional evidence that the resistant inoculum which started the epidemic was seed-borne might be obtained from the fact that eight samples collected during August on fields at distances between 8-15 km all yielded sensitive isolates.

Because resistant strains were already abundantly present on July 14, it was decided to abandon the original set up of the experiment and on July 16 the whole field was sprayed with a combination of a commercial product, containing maneb and fentin acetate, and half a dose of the metalaxyl/mancozeb mixture. The previously untreated parts received additionally a full dose of the metalaxyl/mancozeb mixture. The spraying was repeated at weekly intervals.

Although the early occurrence of resistant strains had jeopardized the original aim of the experiment, the observations made in the field were of great value because they contributed considerably to out knowledge of the late blight epidemic of 1981. The metalaxyl/mancozeb mixture completely controlled the disease up to July 14 when the first infections were found. The mancozeb treatment was equally effective. On the other hand, in the untreated and uninoculated part of the field the disease had spread already considerably, and since all samples taken there yielded metalaxyl-resistant isolates, the late blight population at that time must have been predominantly resistant. Apparently, the sensitive strain used for inoculation had not spread to such an extent that it could be found everywhere in the field. Hence, the disease-controlling effect of the mixture must have been due to mancozeb alone, as could also be observed in the mancozeb-treated part. Apparently, under the prevailing conditions mancozeb, although applied at a rather low dosage and a 14-day spray interval, was still able to withstand the disease pressure caused by soil-borne as well as by air-borne inoculum. The time of the spray application may have considerably contributed to the final effect since all three applications were accidentally made just before a period of late blight-conducive weather.

The race determinations showed that among seven isolates tested four different races were present, viz. races 4, 4.7, 1.3.4.7 and 1.3.4.7.10.11, indicating that if all races had developed from blighted tubers the 1980 crop of which the seed potatoes originated must have been infected with several races.

After the spraying of July 16, no further development of the disease was observed (Table 8). Late blight lesions on plants on parts of the field where inoculations were made and which were treated with metalaxyl at a dose of 300 g ha^{-1} showed a pronounced response 4-5 days after the treatment. A small dark-brown borderline was present between the dead tissue and the healthy part of the leaf. The tissue adjacent to the lesion did not have the light green colour as is characteristic for developing lesions of late blight. Lesions on plants on the non-inoculated part where previously all samples yielded resistant isolates were not affected at all by metalaxyl at this dosage. An experienced observer could easily recognize the differential lesion appearance and thus could tell whether a metalaxyl-sensitive or resistant strain was involved. Metalaxyl-sensitive strains were still viable, however, since on July 27 three out of five leaf samples yielded sensitive strains. Even on September 1 when the field

Table 8. Disease ratings and sampling results in field experiment 1 after July 14.

Date	Previously untreated part[1] (1.08 ha)			Previously metalaxyl/mancozeb-treated part[1] (3.6 ha)		
	disease rating[2]	sampling results[2,3]		disease rating	sampling results[3]	
		S	R		S	R
July 20	0.1-5%; in foci 75%	–	–	0	–	–
July 27	0.1-5%; in foci 100%	3	2	5 lesions/0.16 ha	–	–
August 4	n.d.	–	–	3 lesions/0.16 ha	0	1
August 10	n.d.	–	–	4 lesions/0.16 ha	0	1
August 25	n.d.	–	–	2 lesions/0.16 ha	0	1
September 1	1%; in foci 75-100%	2[4]	15[5]	5 lesions/0.60 ha	0	3[6]

[1] On July 16 the whole field was sprayed with maneb + fentin acetate + metalaxyl + mancozeb (850 + 228 + 100 + 800 g ha^{-1}). In addition the untreated part received metalaxyl + mancozeb (200 + 1600 g ha^{-1}). Additional applications with maneb + fentin acetate + metalaxyl + mancozeb were made on July 22 and 28 and August 4, 12, 17 and 24. Haulm killing was on September 4.

[2] Including the inoculated sites.

[3] Number of samples taken at different sites in the field yielding either metalaxyl-sensitive (S) or metalaxyl-resistant (R) isolates; – indicates that no samples were or could be taken.

[4] One isolate from blighted tuber, race 1.3.4.7.10.11 (2x).

[5] The race of one isolate has been determined: race 4.

[6] The race of two isolates has been determined: races 1.3.4 and 1.3.4.7.

Tabel 8. Ziektewaarnemingen en bemonstering in het proefveld in Zuid-Flevoland vanaf 14 juli.

had been repeatedly treated with the fungicide mixture including metalaxyl, a sensitive isolate could be isolated from an infected leaf.

Although the infection percentages in the initially untreated parts of the field did not increase during August due to intensive sprayings and late-blight-unfavourable weather, the epidemic had built up to such a level that tuber infections took place. One blighted tuber sampled yielded a sensitive isolate. Blighted tubers carrying resistant strains were also present since after storage of the crop for 4½ months, a resistant isolate could be obtained from a rotten tuber. Rotten tubers, however, were present at a rather low indidence (<0.1%) and did not interfere with the salability of the crop.

Disease incidence in the initially metalaxyl/mancozeb-treated part of the field remained at an extremely low level. During August only a few blighted leaves were found all of which yielded a resistant isolate.

Race identification showed that the three resistant isolates tested belonged to three different races, viz. races 4, 1.3.4. and 1.3.4.7. Race 1.3.4. had not been found before in the experimental field. Both sensitive isolates that were obtained belonged to race 1.3.4.7.10.11. The strain that has been used for inoculation (race 4, metalaxyl-sensitive) was not recovered.

Experiment 2. Late blight symptoms appeared on the inoculated plants seven to ten days after inoculation. The disease rapidly spread through the field and on July 27 about 50% of the foliage of uninoculated plants on the untreated part of the experimental field appeared to be destroyed (Table 9). In the metalaxyl/mancozeb-treated part the first lesions were found on August 5. Two of the four isolates obtained were metalaxyl-resistant. After that date new infections occurred but lesions yielded only metalaxyl-sensitive isolates at two subsequent sampling dates. On August 25 the disease incidence had passed the level at which haulm killing is considered to be necessary to prevent tuber infections. All samples yielded resistant strains. On September 3 many foci were present and again each sample (one per focus) yielded a resistant isolate. On September 16 the foliage was severely attacked and intensive sampling yielded again only resistant isolates. Tuber samples taken on September 21 showed that tuber infections in the treated part were not significantly fewer than in the untreated part although a marked difference had existed in foliar blight severity during the greater part of the summer. Infected tubers collected in both parts yielded metalaxyl-resistant isolates. It incicates that mancozeb, at the dosage and spray interval used, although it significantly retarded the development of foliar blight caused by metalaxyl-resistant strains, was far from effective against tuber blight.

The infection of tubers in the untreated part of the field has probably mainly taken place during the last half of August and throughout September by inoculum originating from the treated part of the field. If tuber infections in the untreated part would have occurred in July at the time sporulating lesions were still present on the foliage then mainly sensitive strains must have been obtained. At that time treatment with the metalaxyl/mancozeb mixture was still highly effective indicating that the population must have been predominantly sensitive.

The results of the race determinations made on isolates sampled in Experiment 2 are given in Table 10. Seven different races could be identified among 12 resistant isolates. Each of the seven sensitive isolates tested had the genotype 1.3.4.7.10.11, the

Table 9. Disease ratings and sampling results in field experiment 2.

Date	Untreated part (440 m²)			Metalaxyl/mancozeb-treated part[1] (1820 m²)		
	disease rating (%)	sampling results[2]		disease rating (%)	sampling results[2]	
		S	R		S	R
July 27	50	–	–	0	–	–
August 5	50	–	–	0.1	2[3]	2[3]
August 10	plants dead	–	–	0.5	4[3]	0
August 18	plants dead	–	–	0.5	5[3]	0
August 25	plants dead	–	–	1-5	0	4[3]
August 31	plants dead	–	–	1-5	0	5[3]
September 3	plants dead	–	–	1-5; in foci 10-75%	0	24[3]
September 16	plants dead	–	–	50-75; in foci 95-100%	0	23
September 21	2.2[4]	1[5]	4[5]	1.5[4]	1[5]	6[5]
September 23	n.d.	–	–	n.d.	0	15

[1] Dates of application: June 12 and 25, July 17 and 29, August 10 and 21 and September 4.
[2] Number of samples taken at different sites in the field yielding either metalaxyl-sensitive (S) or metalaxyl-resistant (R) isolates; – indicates that no samples were or could be taken.
[3] The race of some of the isolates has been identified, see Table 10.
[4] Percentage infected tubers. Plants (23 in the untreated part and 30 in the treated part) at randomly chosen sites were dug and the total number and the number of blighted tubers were determined.
[5] Tuber samples.

Tabel 9. Ziektewaarnemingen en bemonstering in het proefveld te Heelsum.

genotype of one of the races that had been used for inoculation. Not any of the three other races used for the inoculation was recovered. It is worth mentioning that in 1980 race 1.3.4.7.10.11 was frequently found in the northern part of the country (Davidse et al., 1981). As can be seen in Table 5 in 1981 it was again the most frequently encountered genotype among the isolates tested. Apparently, this race is highly virulent. Among the resistant isolates race 1.3.4.7.10.11 was not found until September 3 and then appeared to be the most frequent one. The first resistant isolates found carried the virulence genes 1.3.4.7 and 4.7., respectively, and each next sampling yielded additional different ones.

The important question of the origin of the resistant strains cannot be definitely answered. The possibility that resistant strains were seed-borne is unlikely since the size of the experimental field made it possible to inspect each plant and during July no infections have been seen in the treated part. Infections caused by resistant strains from other fields cannot be completely ruled out, although potato farming was not very intensive in the area around the site of the experimental field. In fields near

Table 10. Races collected in field experiment 2.

Date	Races	
	metalaxyl-sensitive	metalaxyl-resistant
August 5	1.3.4.7.10.11 (2×)[1]	4.7
		1.3.4.7
August 10	1.3.4.7.10.11 (2×)	
August 18	1.3.4.7.10.11 (3×)	
August 25		1.3.7
August 31		1.3.7
		1.3.7.11
September 3		4
		1.3.4.7
		1.3.4.7.11
		1.3.4.7.10.11 (4×)

[1] Number of times the race indicated has been collected.

Tabel 10. Overzicht van de in het proefveld te Heelsum aangetroffen fysio's.

Wageningen at a distance of 5 km south-west and 6.5 km west of the experimental field two sensitive isolates were found on August 5 and September 9, which were identified as race 1.4.10.11 and race 1.4.10, respectively. In a field 8 km south-west of the experiment one sensitive isolate (race 1.4.10.11) and three resistant ones, of which two were identified as race 1.3.4.7.10.11, were collected on July 14. Because of its agressiveness and high virulence a metalaxyl-resistant strain with the latter genotype had been used there to inoculate various potato cultivars in a field test for late blight resistance. Sporangia originating from the latter field may have reached the experimental field during conditions favouring late blight which prevailed on August 17 and 18. The fact, however, that various other races were found among the resistant, isolates obtained from the metalaxyl/mancozeb-treated area rather supports the idea that the resistant strains originated in the field itself.

Whether resistant sporangia came from elsewhere or were produced in the field itself, in both cases their frequency in the population must have been very low initially. Due to the efficacy of metalaxyl towards the sensitive strains and the inadequate effect of mancozeb at the rates and spraying intervals used, a resistant population could build up rapidly enough to cause infection beyond tolerable levels. The resistant population must have developed for the greater part in the metalaxyl/mancozeb-treated area because the foliage in the untreated part of the field and that of susceptible genotypes in the selection field was completely destroyed on August 10.

The failure of mancozeb to control foliar blight at the rates and frequency applied as observed in Experiment 2 contrast with its good efficacy in Experiment 1 during June and July. Yet the initial size of the resistant population in Experiment 2 might be assumed to have been much smaller than that in Experiment 1. Hence, the difference in performance of mancozeb in the two fields must be ascribed to other factors. Such a factor might have been the amount of the mancozeb deposit on the leaves being relatively rapidly reduced in Experiment 2 because of the overhead sprinkler irrigation. In Experment 1 the mancozeb deposit was only subject to natural weathering. Under the prevailing conditions its amount apparently did not decrease below an effective level. It stresses the importance of environmental factors on the performance of metalaxyl/mancozeb mixtures in preventing or delaying development of resistance.

Discussion

The survey, intended to estimate the proportion of metalaxyl-resistant strains in the population, unintentionally provided information about the source of the inoculum that initiated the early and severe late blight epidemic in 1981 in the Netherlands. Infected seed potatoes have probably been more important than old potato cull piles. Since the greater part of the infected seed potatoes carried metalaxyl-sensitive strains a predominantly metalaxyl-sensitive population developed in most parts of the country, including those areas, where the occurrence of metalaxyl-resistant strains had caused serious problems in 1980. The presence of metalaxyl-resistant strains in the seed tubers, presumably at a much lower frequency than sensitive strains, created an extremely labile situation. The data obtained from the survey in West Brabant and the Hoekse Waard indicate that if under such conditions the population is allowed to increase for two or three generations, a single curative application of metalaxyl, either exclusively or in combination with conventional fungicides, readily selects resistant strains to an easily detectable level in a monitoring programme. Therefore, monitoring for metalaxyl-resistance in late blight can only predict to a limited extent the benefit of a single spray of the fungicide, because of the rapid shift towards resistance that can occur. On the other hand, the high efficacy of the compounds against sensitive strains and the absence of any effect on resistant strains makes that the latter are easily recognized. Hence, each farmer can judge himself the efficacy of a metalaxyl treatment.

Experiment 2 provided evidence that a resistant late blight population can build up in one single season if metalaxyl is used in combination with mancozeb at a 14-day spray interval and if favourable conditions for development of the disease exist. It indicates that, although mancozeb or any other conventional fungicide under such conditions may delay the development of resistance, the use of a mixture does not guarantee that crop losses will no occur.

Tuber blight incidence might be relatively high when resistance develops in a situation where a fair control of foliar blight is achieved by the partner of metalaxyl in the mixture, because its rate and frequency of application is not sufficient to prevent tuber infections (Cooke, 1981). Strategies for late blight control with metalaxyl, either applied in a mixture or in alternation with conventional fungicides, should therefore not ignore weather conditions favouring disease development. When blight-conducive weather is forecasted a spray either with the mixture or a conventional fungicide exclusively should be considered depending on the residual activity of both fungicides left in the crop after the last spray and the disease pressure. It might mean that sometimes a spray of a conventional fungicide will be necessary between two sprayings with the mixture. Such an approach minimizes the size of a late blight population that is exposed to metalaxyl, thereby reducing the chance that resistance develops. On the other hand, it ensures that if resistance might develop tuber blight incidence will never exceed a level higher than tolerated by conventional fungicides only.

The widespread occurrence of resistance among races of the fungus and of races on cultivars without R-type genes for resistance minimize the chance that cultivars carrying particular genes for late blight resistance will escape attack by resistant strains once they have developed. In addition resistant strains may as easily mutate towards virulence as a sensitive strain. Theoretically, development of resistance in a *P. infestans* population present on cultivars with a relatively high degree of horizontal resistance to late blight should be less rapid than in populations on highly susceptible cultivars because of the lower apparent infection rates (Skylakakis, 1982). If cultivars of both types are grown together in the same area and exposed to metalaxyl treatment the population on the more resistant cultivars may shift as rapidly to resistance as that on the highly susceptible cultivars.

The experience with metalaxyl resistance in the Netherlands has learned how dangerous it can be to apply blight fungicides, to which readily resistance develops, in potato seed production as has been practized with metalaxyl in 1980. Fortunately, blighted seed tubers appeared to carry predominantly metalaxyl-sensitive strains which resulted in the outbreak of an epidemic of sensitive strains in 1981. If seed potatoes had contained resistant strains they still would have predominated in the population in 1981 and spread to many other areas as well.

The introduction of new highly specific fungicides for late blight control may evoke its own problems as has been described here for metalaxyl. Therefore, before introducing such a fungicide, the changes of development of resistance should be evaluated as thoroughly as possible and intensive efforts should be made te develop an application schedule based on present knowledge of potato late blight epidemiology and of factors affecting the rate of selection of fungicide-resistant genotypes and on experiences with late-blight-forecasting systems. In the long run this approach will optimally profit both growers and manufacturers of fungicides.

Note added in proof

During the summer of 1982 the *P. infestans* population was again monitored for the presence of metalaxyl-resistant strains. A total number of 37 isolates were tested in the leaf-disc assay. Three isolates obtained on August 21 from an experimental field. in Zeeland that had been treated with a 50WP formulation of ofurace at 440 g a.i. ha^{-1} on June 23, July 2, 16 and 30 and August 13 proved to be resistant. The remaining isolates (34) were metalaxyl-sentitive and originated from farmers fields in the Veenkoloniën (29), East Brabant (4) and South Limburg (1). Two of these fields had been sprayed with a mixture of metalaxyl and a conventional fungicide. The data indicate that resistant strains are still present in the *P. infestans* population in the Netherlands.

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Samenvatting

Resistentie tegen metalaxyl in Phytophthora infestans in Nederland

Gedurende de zomer en najaar van 1981 werden 222 veldisolaten van *Phytophthora infestans* met behulp van een drijftoets getoetst op resistentie tegen metalaxyl. Per perceel werd meestal één isolaat, hetzij afkomstig van een door de aardappelziekte aangetast blad, hetzij van een zieke knol, getoetst.

Eén en veertig isolaten bleken resistent te zijn. Van deze 41 waren er 35 afkomstig uit West Brabant, de Hoekse Waard en Oost Brabant/Noord Limburg, waar ongeveer 15% van het totale aardappelareaal in Nederland is gelegen. Daarnaast werden uit deze gebieden 29 gevoelige isolaten verkregen. Van de bemonsterde percelen uit de drie genoemde gebieden was 29% één- of tweemaal met metalaxyl behandeld, meestal in combinatie met andere middelen. Uit 78% van de aldus behandelde percelen werd een resistant isolaat verkregen. In de genoemde gebieden werden in 1980 alleen in Oost-Brabant/Noord Limburg problemen ondervonden van metalaxylresistentie.

In percelen in overige gedeelten van Nederland, waar in 1980 algemeen metalaxylresistentie werd waargenomen, werden in totaal slechts zes metalaxyl-resistente en 152 gevoelige isolaten aangetroffen. Geen enkel van de met metalaxyl bespoten percelen (9% van het aantal bemonsterde percelen) leverde hier een resistent isolaat op. Uit een viertal aardappelafvalhopen in deze gebieden waarop de aardappelziekte voorkwam, werden metalaxyl-resistente stammen verkregen en twee ervan hadden waarschijnlijk een naburig perceel besmet.

De resultaten van het onderzoek en de waarnemingen in de percelen wijzen erop dat de vroege en hevige epidemie van de aardappelziekte in 1981 is veroorzaakt door besmet pootgoed waaruit secundair zieke planten zijn ontstaan. Daarnaast werd het uitbreken van de epidemie vooral begunstig door de voor *Phytophthora* gunstige weersomstandigheden gedurende de periode eind mei tot half juni en het feit dat de eerste bespuiting te laat werd uitgevoerd, hetzij door overmacht, hetzij door het te traag reageren op de waarschuwingen via de radio.

In 1980 hebben voornamelijk metalaxyl-gevoelige stammen het pootgoed kunnen besmetten, hetgeen verklaarbaar is uit het feit dat in dat jaar de problemen met metalaxylresistentie pas werden waargenomen nadat het loof van het pootgoed was vernietigd. In een tweetal gevallen zijn evenwel aanwijzingen verkregen dat pootgoed ook besmet kon zijn met resistente stammen. Een dergelijke besmetting is waarschijnlijk ook de verklaring voor het op grote schaal voorkomen van resistente stammen in West Brabant en de Hoekse Waard na een eenmalige toepassing van metalaxyl.

In een tweetal proefvelden, waarvan er één was gelegen in Zuid-Flevoland en het andere te Heelsum, is het ontstaan van een resistante populatie bestudeerd bij een twee-wekelijkse toepassing van een mengsel van metalaxyl en mancozeb. Pootgoed,

gebruikt in het op praktijkschaal uitgevoerde experiment in Zuid-Flevoland, bleek waarschijnlijk besmet te zijn met metalaxyl-resistente stammen, zoals op 1 juli werd vastgesteld in een onbehandeld gedeelte van het perceel. Op het behandelde gedeelte bleek het mengsel de ontwikkeling van de ziekte aanzienlijk te vertragen; evenwel werd na 16 juli een wekelijkse bespuiting met een maneb/fentin acetaat-bevattend produkt uitgevoerd voor een zo goed mogelijk ziektebestrijding.

Op het in Heelsum gelegen proefveld werd op 5 augustus de ziekte het eerst waargenomen in het behandelde gedeelte, nadat de planten in het onbehandelde gedeelte, ten gevolgen van een doelbewuste inoculatie met gevoelige stammen op een naast gelegen aardappel-selectieveld, reeds voor meer dan 50% waren aangetast. Uit aangetaste bladeren van het behandelde gedeelte konden metalaxyl-resistente stammen worden geïsoleerd. In de loop van augustus bleek het metalaxyl/mancozeb-mengsel niet in staat verdere uitbreiding van de ziekte tegen te gaan en trad knolinfectie op. Begin september werden uitsluitend resistente stammen aangetroffen.

Geen enkel verband kon worden vastgesteld tussen het optreden van metalaxyl-resistentie en het voorkomen van een bepaald fysio van de schimmel. Op een totaal van 79 isolaten konden 23 verschillende fysio's worden geïdentificeerd. Het fysio 1.3.4.7.10.11 werd de meeste keren gevonden, gevolgd door fysio 1.3.4.7.11. Tien verschillende fysio's werden aangetroffen onder de 37 getoetste metalaxyl-resistente isolaten.

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