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Role of oospores in the overwintering and year-on-year development of the late blight pathogen on tomato en potato

M.A. KUZNETSOVA, T.I. ULANOVA, A.N. ROGOZHIN,
T.I. SMETANINA AND A.V. FILIPPOV

All-Russian Research Institute of Phytopathology, Bolshie Vyazemy,
Moscow Region, 143050 Russia; e-mail: kuznetsova@vniif.ru

SUMMARY
The role of oospores as a source for the regular late blight renewal has been studied in a long-term experiment using tomato strains of *P. infestans* and two tomato cultivars. The obtained results showed that oospores, generated after the mating of strains of different mating types (A1 and A2), maintained their infection ability after their overwintering in the soil. The presence of the infectious agent in the soil causes the spreading of the late blight to the above ground parts of plants, manifesting itself on the bottom and other parts of the stem. Therefore, at least in the course of the next vegetation season, *P. infestans* oospores can be an important source of infection, causing the appearance of first late blight lesions on plants.

KEY WORDS
potato, tomato, late blight, overwintering of oospores

INTRODUCTION
Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) dBy, is the most harmful disease of potato and tomato plants. During epiphytoties, potato production often drops in 1.5-2 times, and tomato crops can be fully lost. In Russia the main place of the pathogen overwintering and, therefore, the main source of the year-to-year infection renewal, is infected seed potato in the storage facilities. After the planting of infected tubers, *P. infestans* zoosporangia are formed on their surface, which then generate zoospores, infecting the underground parts of a plant and, as a result of a zoospore migration through soil capillars, bottom leaves, which contact with the ground (Boguslavskaya and Filippov, 1976). It was considered that before 1980 the primary manifestation of the disease on tomato plants was caused by pathogen spores, generating on potato fields.
Oospores represent another structure able to the overwintering. *P. infestans* is a heterothallic species with two mating types, A1 and A2. The contact of strains with two different mating types results in an oospore generation (Miller, 2001).
The formation of *P. infestans* oospores on potato was firstly revealed by Gallegy and Galindo in 1957. This study was carried out in Toluca valley (Central Mexico), which is considered to be a place of origin of this pathogen (Fry, Spielman, 1991). Until this time, the *P. infestans* population outside Central Mexico was represented by a single clonal line (A1). This line had only vegetative reproduction. The first non-Mexican A2 strains were revealed on potato field of Switzerland (Hohl,

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Iselin, 1984), and then in the Great Britain on potato tubers, imported from Egypt (Shaw *et al.*, 1985). A sexual reproduction occurs when two different types of mycelium, A1 and A2, contact with each other; in this case they form male and female reproductive organs, oogonium and antheridium. Each mating type can form both types of reproductive organs, though certain genotypes play rather male or female role (Judelson 1997). The meiosis takes place within reproductive organs, and, after their fusion, the fertilization can occur, which results in the oospore formation.

The sexual reproduction not necessarily results in an outcrossing; some of the offspring can represent a selfing product (Knapova *et al.*, 2002), other can be identical to the one of the parents (Carter 1999). Besides, many genotypes can be self-fertile, i.e. they are able to generate oospores at the absence of the opposite mating type (Smart *et al.*, 2000).

The first information about the revealing of the A2 mating type of *P. infestans* on potato in Russia was published in 1993 (Vorobyeva and Gridnev, 1993). Oospores, differing in their origin, were revealed in the Moscow region in 1998-1999 (Smirnov, 1998; Smirnov *et al.*, 1999). Meanwhile, in 1967 P.A. Kvartskhava and A.I. Maglakelidze revealed both mating types and the generation of *P. infestans* oospores on kangaroo apple (*Solanum laciniatum*) in Western Georgia (Maglakelidze, 1971).

Many scientists consider that the A2-containing "new population" was imported from Central Mexico to Europe and other continents in 1970 with the infected seed potato. However, the simultaneous revealing of A2 strains in Europe, Asia, and America allows us to consider that such global change in the structure of *P. infestans* population could be caused by any other reasons. The appearance of the A2 type coincided with significant changes of the population structure of *P. infestans* and the increase in its aggressiveness. The pathogen became less dependent on the temperature and humidity, and the first disease symptoms are often manifested too early for many places (Flier, 2001). It is considered that this is caused by a sexual recombination and the influence of an additional infection source, i.e. overwintered oospores (Andersson *et al.*, 1998; Hannukkala *et al.*, 2006).

The role of oospores as the primary source of infection was investigated under natural conditions and in the course of special laboratory and field experiments. According to the data, obtained by Turkensteen and Flier, in Netherlands oospores remain viable for 4 and 3 years in the sandy and clay soil, respectively (Turkensteen, Flier, 2000). There was also a report that in Finland they remain viable at least within one winter (Lehtinen *et al.*, 2002). It was also showed that on the fields, where potato was cultivated for several seasons, the late blight appeared by 9 days earlier on average, than on the fields with other advanced crops (Bødker *et al.*, 2006). We observed the cases, when the late blight initially appeared on the planted tomato seedlings and then spread on the adjacent potato plots. In such case we can suppose that overwintered oospores were the primary source of infection. Some authors consider that the presence of both mating types in a primary nidus of infection, the variety of genotypes, isolated from such nidus, and the dominating infection of bottom leaves, contacting with a soil, are typical signs of the fact that this nidus originated from overwintered oospores (Lehtinen, Hannukkala, 2004; Widmark *et al.*, 2007). According to Evenhuis and others (Evenhuis *et al.*, 2004), infected seed tubers represent the later late blight source than overwintered oospores. They also consider that all cases of the early disease manifestation (within first week after the appearance of shoots) in Netherlands were caused by oospores.

At the same time, other authors consider that oospores play a minor role as the source of a primary infection (Cook *et al.*, 2007). In many regions oospores are not generated at all or generated in a small number due to the domination of a monoclonal *P. infestans* population, represented by only one mating type. For example, such situation is typical for the most part of Siberia, where only A1 type was found (Elansly *et al.*, 2001) and several countries of Western Europe, where the earlier genotypes were almost fully replaced with the 13A2 genotype (Lees *et al.*, 2009).

The aim of this study was to obtain direct evidences of the significance of oospores as a source for the regular late blight renewal in Russia.

MATERIALS AND METHODS

The study was carried out using tomato strains of *P. infestans* and two tomato cultivars. We did not include potato into this study, since the possible hidden infection of tubers could influence on the results of our experiments.

Six pairs of monozoosporic *P. infestans* isolates of A1 and A2 types were examined for their ability to the oospore formation. The isolates were cultured on Petri dishes using rye medium (according to Caten and Jinks, 1968) and also were used for the inoculation of detached tomato leaves (cv. Otradniy). To determine the presence of oospores, infected leaves were boiled in 96% alcohol for 2-3 min to remove chlorophyll, then bleached in 10% solution of a chlor-containing substance ("Belizna") and microscoped in 50 fields of vision (1 mm^2 each). The oospore frequency below 51, from 51 to 250, and above 250 oospores per field of vision was evaluated as rare, moderate, and frequent, respectively (Amatkhanova *et al.*, 2004). Among six examined pairs, only two demonstrated an abundant oospore formation on Petri dishes and detached leaves. In our further experiments we used one of these two pairs, 14a (A1) and 36b (A2).

In 2007-2009 we completed the following series of experiments:

I. Study of the overwintering of ***P. infestans*** *oospores under field conditions.*

90 tomato plants (cv. Bomax) were grown in a greenhouse and in autumn of 2007 were infected with three variants of inoculum: 1) 14a (A1); 2) 36b (A2); 3) 14a (A1) + 36b (A2). In the third variant, the A1:A2 ratio was 1:1. The inoculum of the examined isolates was cultivated on rye medium. The used dose was 6000 zoosporangia per 1 m^2. Each variant included 10 plants.

After the first disease manifestation, detached leaves were analyzed for the oospore presence.

The oospore formation was observed only for plants, inoculated with the A1+A2 mix (Fig. 1); in other two variants, where plants were inoculated with only A1 or A2 type isolates, we did not reveal any oospores.

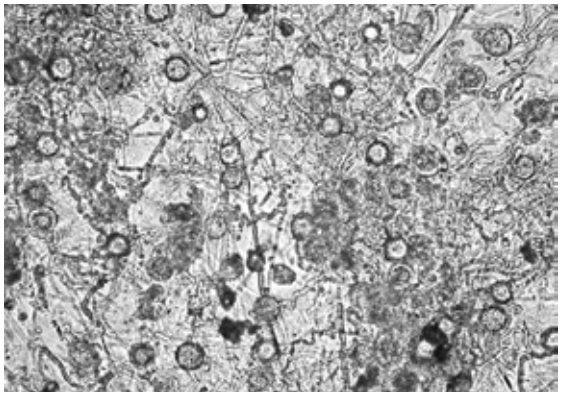

Fig. 1. *Oospores of P. infestans.*

In all three variants the infected plants were taken out of a greenhouse and buried into improved sod-podzol heavy soil at a depth of 20 cm. Plants of each variant were buried on a separate isolated place. Next spring we took soil samples, containing overrotten debris of infected plants, from each plot. These samples were used to prepare a suspension for the inoculation of potato tuber slices (cv. Sante) and detached leaves of tomato plants (cv. Otradniy), grown in a greenhouse. The suspension was prepared in the following way: 1 g of the soil was diluted in 10 ml H_2O. The suspension was dropped on the surface of leaves (18 leaves per variant) and fresh tuber slices (20 slices per variant).

Inoculated leaves and tuber slices were placed into wet chambers (trays covered with polyethylene film). After 5-day incubation we started everyday observation of the pathogen development (Fig. 2 A, B).

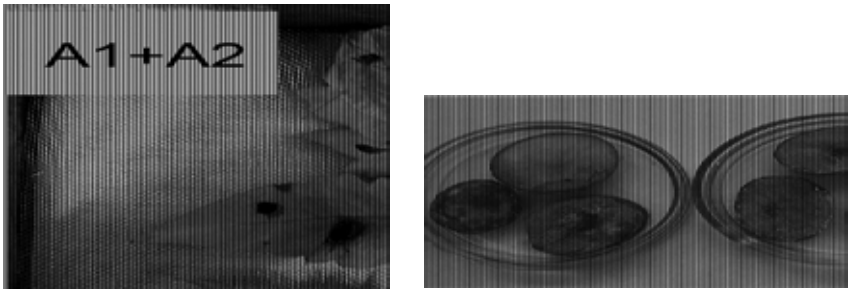

Fig. 2. *Late blight manifestation on tomato leaves (A) and potato tuber slices (B), inoculated with soil samples, containing overwintered P. infestans oospores (A1+A2 variant).*

In 2008-2009 we expanded our program of investigations. In spring 2009 we grew the seedlings of tomato plants (cv. Otradniy) in a greenhouse and then planted it on an experimental field (Fig. 3) in several different variants:
During a planting, we added 1 kg of soil, containing overwintered plant debris, infected with the A1 type isolate, into each planting hole;
During a planting, we added 1 kg of soil, containing overwintered plant debris, infected with the A2 type isolate, into each planting hole;
During a planting, we added 1 kg of soil, containing overwintered plant debris, infected with the A1+A2 mix of isolates, into each planting hole;
During a planting, we did not add any soil samples into planting holes (control).
Each plot was isolated from others by spring wheat. We provided the thrice repeatability of each variant. After the planting, we observed the late blight development each 5-7 days.

Fig. 3. *Plots with tomato plants, surrounded by spring wheat.*

II. Study of the way of transmission of the infectious agent from soil to the overground parts of a plant
The experiment was carried out in a greenhouse using tomato plants (cv. Bomax). After the development of 4-5 leaves, plants were taken out of the soil, and their roots were immersed into the suspension of *P. infestans* zoosporangia (A1 and A2 types, 6000 zoosporangia/ml) for 5 min.

Then the contaminated seedlings were planted into 5-liter soil-containing flowerpots. Plants were grown under daylight. The soil humidity was maintained at the level of 60% of the total soil water capacity. Ten days after planting we started our everyday examinations of each plant to determine the moment of a disease manifestation and the character of possible lesions. Plants, which roots were immersed into water, were used as a control. Each tested variant included 30 tomato plants.

RESULTS AND DISCUSSION

I. Overwintering of P. infestans oospores under field conditions

During both years of our study the inoculation of tomato leaves and tuber slices with the examined soil samples was successful only in the case when these samples contained overrotten debris of plants, infected with the A1+A2 mix of isolates (Table 1). In 2008 and 2009 we registered the late blight lesions with the fruiting on 27% and 17% of detached leaves and 20% and 25% of tuber slices, respectively.

Table 1 *Infectiousness of Phytophthora infestans after the overwintering in the soil depending on the type of inoculum (ARRIP, 2008-2009)*

Inoculum type	Presence of oospores	Late blight manifestation on leaves/tuber slices, %
A1 type isolate	No	0/0
A2 type isolate	No	0/0
1+2 mix (1:1)	Yes	22,0/22,5

In two other variants (soil samples with debris of plants, inoculated with A1 or A2 strains) we did not reveal any disease manifestations on both leaves and tuber slices. The obtained results show that the oospores, generated after the mating of 14 (1) and 36b (2) strains, maintained their infection ability after the overwintering.

This fact was also confirmed by the observations on the late blight development on tomato plants, grown in the field. The first disease manifestation on tomato plants, grown on the plot with plant debris, infected with A1+A2 variant, was registered two weeks after the planting (June 24). Late blight lesions were observed on 17% of plants; they were located mainly on the bottom leaves and the bottom part of the stem. In the case of two other variants and the control, we observed first lesions only 25 days after the planting (July 17; Table 2). However, the fact that the infection was simultaneously manifested in control plants, makes it possible to suppose that in this case its development was caused by any outer sources of infection. In this period the late blight symptoms were registered in the nearest potato and tomato fields. Therefore, our results can be considered as a direct evidence of the fact that oospores, generated after the mating of A1 and A2 type strains, can overwinter in the soil and cause the renewal of the late blight in the next season.

Table 2. *Late blight development after the pathogen overwintering in the soil depending on the inoculum type (ARRIP, 2009)*

Inoculum type	Presence of oospores	Late blight manifestation on tomato plants in the field
Control	No	17.07.09
A1 type	No	17.07.09
A2 type	No	17.07.09
1+2 mix (1:1)	Yes	24.06.09

II. Way of transmission of the infectious agent from soil to the overground parts of a plant

Two weeks after the root inoculation, we observed the first visible late blight symptoms on the bottom part of several stems. After two more weeks, we observed isolated late blight lesions on other

plants; these lesions were located at the different height of the stem (up to 15 cm). Such character of lesions allowed us to suggest the development of a pathogen mycelium took place within the stem and was asymptomatic for a long period. The lesions appeared mainly near internodes. A longitudinal cut of such stems demonstrated the necrotization of their tissues. The placement of stem pieces into a wet chamber, caused the generation of zoosporangia on necrotizing tissues and tissues adjacent to vessels. In our experiment we observed such type of lesion in 23% of samples (Fig. 4).

Fig. 4. *Late blight manifestation on tomato plants, grown on the oospore-containing soil.*

Thus, basing on these experiments, we can conclude that, in the case of a presence of the infectious agent in the soil, the disease can spread to the above ground parts of plants, manifesting itself on the bottom and other parts of the stem.

The obtained data show that *P. infestans* oospores can overwinter in the soil and, at least in the course of the next season, be a source of infection, causing the appearance of first late blight lesions on plants.

The modern *P. infestans* populations from many Russian regions include now both mating types, and A1:A2 ratio is often close to 1:1. In these cases this source of infection can play an important role, especially in private gardens, where usually there is no crop rotation. We can also suppose that, in the case of any other A1:A2 ratio in a population, the role of oospores as the source of primary infection becomes less important.

Unlike zoosporangia, oospores are generated within plant tissues. According to our observations, they acquire the germination ability only after the decay of surrounding plant tissues. It is known that the rate of decay and mineralization of plant debris and, therefore, the oospore's acquisition of the ability to infect plants, significantly depends on the temperature, moisture, and microbiological activity of the soil. This process seems to be slower for the northern latitudes. In the case of a prolonged soil moistening, oospores either directly infect underground parts of plants, or generate zoosporangia with the subsequent release of zoospores. After the germination of an oospore into a zoosporangium, plants are infected with zoospores, which move to the surface of the soil using soil capillaries and infect stems and leaves, which contact with the soil. In our opinion, it is not possible to distinguish late blight infection focuses, caused by oospores, from those caused by contaminated seed material. We also can not agree with the hypothesis that the typical feature of the oospore infection is the earlier disease manifestation, comparing to the infection, caused by a seed contamination. We consider that the period of the disease manifestation depends mainly from both the number of infected seed tubers and the level of contamination of the soil with oospores.

Basing on the results of our studies, we can conclude that in many regions of Russia oospores,

generating by the mating of A1 and A2 types of *P. infestans* strains, can overwinter in the soil and cause the late blight development in the next vegetation season.

CONCLUSION

Thus, basing on these experiments, we can conclude that, in the case of a presence of the infectious agent in the soil, the disease can spread to the above ground parts of plants, manifesting itself on the bottom and other parts of the stem.

The obtained data show that *P. infestans* oospores can overwinter in the soil and, at least in the course of the next season, be a source of infection, causing the appearance of first late blight lesions on plants.

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