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Alexei N. Smirnov
S. A. Kuznetsov

Russian Agrarian University — Moscow Timiryazev Agricultural Academy,
Department of Phytopathology, Lystvennichnaya alley 2, building 12,
Moscow, 127550 Russia

REPRODUCTIVE STRATEGIES OF FIELD *PHYTOPHTHORA INFESTANS* POPULATIONS ON POTATO AND TOMATO IN SOME REGIONS OF RUSSIA

ABSTRACT: The occurrence of zoosporangia and oospores is analyzed for 88 field populations of *Phytophthora infestans* (Mont.) de Bary in Moscow region and other regions of Russia from 1997 to 2006. For estimation of zoosporangia and oospore frequencies in the populations, the indexes IZ (for zoosporangia) and IO (for oospores) are suggested. The combinations of these indexes enabled a reveal of 4 strategies for *P. infestans* reproduction, which were found in the field populations of the pathogen. These strategies were more numerous in *P. infestans* field populations on tomato than on potato (4 versus 2) in spite of the fact that potato populations in Moscow region strongly predominated. It was connected with rareness or absence of oospores in potato and their presence in tomato, especially in fruit of many populations.

The knowledge about strategies of reproduction can be applied for verification of efficiency of new methods for crop protection against late blight, and for investigations of *P. infestans* populations.

KEY WORDS: *Phytophthora infestans*, late blight, oospores, zoosporangia, field estimation

INTRODUCTION

Phytophthora infestans is a dangerous parasite of potato and tomato. The losses of these crops caused by late blight are very high. Much is known about biology, ecology and genetics of *P. infestans* (Brasier, 1983; Shaw, 1987; Shattock et al., 1987; Dyakov, 1992). Effective methods are used (Kadish & Cohen, 1988) for estimation of fitness and aggressiveness of *P. infestans* isolates under the laboratory conditions. The approaches for estimation of late blight in the field are also numerous (Popkova, 1972; Dorozhkin et al., 1976).

The current situation with *P. infestans* investigation is that the laboratory and field studies of this parasite are mostly separated from each other. For instance, in Russia and some other countries, the occurrence of oospores (source of primary infection), as well as the intensity of formation of zoosporangia (source of secondary infection), are often excluded from field estimations at crop protection measures. On the other hand, the direct results of field observation on late blight severity are generally not closely connected with the investigations of biology of its agent.

Our purpose is to suggest the approach which enables a reveal of the ratio of structures for asexual and sexual reproduction (zoosporangia and oospores) in the field *P. infestans* populations. Thus, we would be able to clearly understand the strategies of reproduction of certain *P. infestans* populations and use adequate methods of protection against them.

MATERIAL

From 1997 to 2006, the samples from 88 field populations from different regions of Russia (Moscow; Moscow, Leningrad, Novgorod, Yaroslavl, Tula, Bryansk, Kaluga, Tambov, Ryazan, and Tomsk regions; as well as from Mordovia, Northern Caucasus, Stavropol Territory) were studied. The samples were blighted leaflets of Russian potato cultivars Nevsky, Lugovskoy, Lorkh, Syneglazka (blue-eyed potatoes), and Dutch cultivar Sante, as well as some other cultivars and their mixtures of undetermined status; the blighted leaflets and fruits of tomato cultivars Ottawa, Yamal, Beliy Naliv, etc. The number of samples per population varied from 10 to 50 depending on the size of a plot.

METHODS

Determination of presence of oospores and zoosporangia in the blighted samples. The blighted leaflets were placed in moist chambers (Petri dishes with moist filter paper). After one day of incubation the freshly formed zoosporangia were transferred to Petri dishes with oatmeal agar for collection of *P. infestans* isolates. Then, the leaflets were boiled in 96% ethyl alcohol for 3 min, for chlorophyll removal and exposed to 10% water solution of bleaching chlorine for 1 hour. The blighted tomato fruits were also put in moist chambers and the *P. infestans* isolates were collected from them in the same way. After this the fruits were incubated in the moist chambers until their rotting. Decolorized samples were investigated under the light microscope, and oospores and empty zoosporangia (as their content was destroyed; Fig. 1, 2) were looked for.

If the presence of oospores or zoosporangia was not more than 50 per field of vision (1 mm^2), from 51 to 250, and more than 250, it was assessed as rare, moderate, and frequent, respectively.

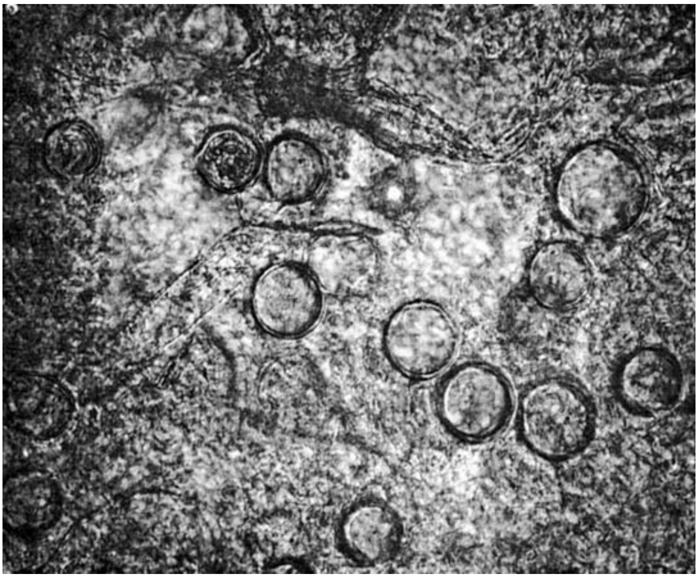

Fig. 1 — Numerous oospores of *P. infestans* in tomato fruits

Fig. 2 — Empty zoosporangia of *P. infestans* in potato leaf after boiling in ethanol solution and exposure to solution of bleaching chlorine.

The indexes of presence of oospores (IO) and zoosporangia (IZ) were calculated as follows:

$10 = 0.05\ .\ RO + 0.5\ .\ MO + FO$, where:
RO — presence of samples with rare oospores, %
MO — presence of samples with moderate oospores, %
FO — presence of samples with frequent oospores, %;

IZ = 0.05 . RS + 0.5 . MS + FS, where:
RS — presence of samples with rare zoosporangia, %
MS — presence of samples with moderate zoosporangia, %
FS — presence of samples with frequent zoosporangia, %.

Thus, this method allows offers qualitative estimation of occurrence of oospores and zoosporangia in the same blighted samples.

Determination reproduction strategies. Different distribution of indexes of presence of oospores and zoosporangia gave an outline of four possible strategies of *P. infestans* reproduction. Four strategies and 20 substrategies were finally revealed (Table 1).

Tab. 1 — Strategies and substrategies of reproduction of field *P. infestans* populations

Strategy	Substrategy	Occurrence of zoosporangia		Occurrence of oospores	
		IZ	Characteristic	IO	Characteristic
W (Weak populations)		**0.1—20.0**	**Rare**	**0—10.0**	**Limited**
	W1			0	absent
	W2			0.1—3.0	negligible
	W3			3.1—10.0	slight
A (Asexual populations)		**20.1—60.0**	**Quite frequent**	**0—10.0**	**Limited**
	A1	20.1—40.0	moderate	0	absent
	A2			0.1—3.0	negligible
	A3			3.1—10.0	slight
	A4	40.1—60.0	frequent	0	absent
	A5			0.1—3.0	negligible
	A6			3.1—10.0	slight
	A7	60.1—100	very frequent	0	absent
	A8			0.1—3.0	negligible
	A9			3.1—10.0	slight
S (Sexual populations)		**0.1—20.0**	**Rare**	**10.1—100**	**Quite frequent**
	S1			10.1—25.0	moderate
	S2			10.1—100	frequent
B (Both asexual and sexual populations)		**20.1—60.0**	**Quite frequent**	**10.1—100**	**Quite frequent**
	B1	20.1—40.0	moderate	10.1—25.0	moderate
	B2			10.1—100	frequent
	B3	40.1—60.0	frequent	10.1—25.0	moderate
	B4			10.1—100	frequent
	B5	60.1—100	very frequent	10.1—25.0	moderate
	B6			10.1—100	frequent

A certain strategy reflects the ratio of oospore and zoosporangia presence in the field *P. infestans* population and its fitness. The higher is the number of zoosporangia in population, the higher is its fitness.

RESULTS

Strategies of reproduction of *P. infestans* on potato and tomato in Moscow region. The analysis of 88 *P. infestans* populations revealed all four possible strategies for their reproduction (Table 2).

Tab. 2 — Number of *P. infestans* Russian populations with a certain strategy/substrategy of reproduction on potato leaves, as well as on leaves and fruits of tomato from 1997 to 2006

Strategy of reproduction	Substrategy of reproduction	Number of *P. infestans* populations on		
		potato leaves	tomato leaves	tomato fruits
W (Weak populations)		**9**	**2**	**2**
	W1	2	0	0
	W2	5	2	1
	W3	2	0	1
A (Asexual populations)		**46**	**12**	**10**
	A1	8	4	1
	A2	8	3	0
	A3	0	0	6
	A4	9	4	1
	A5	8	0	1
	A6	3	0	0
	A7	5	1	1
	A8	4	0	0
	A9	1	0	0
S (Sexual populations)		**0**	**0**	**2**
	S1	0	0	0
	S2	0	0	2
B (Both asexual and sexual populations)		**0**	**2**	**3**
	B1	0	1	2
	B2	0	0	0
	B3	0	0	1
	B4	0	0	0
	B5	0	1	0
	B6	0	0	0
General number of populations		55	16	17
General number of strategies		**2**	**3**	**4**
General number of substrategies		11	7	10
Index of Shannon (for substrategies)		3.23	1.85	2.94

Strategy A (asexual type of reproduction) predominated in populations with different origination. Strategy W (weak type of population producing just a few zoosporangia and/or oospores) was also quite common in different populations, but occurred much more rarely. However, strategies S (sexual type) and B (both sexual and asexual types of reproduction in population) were revealed only in several *P. infestans* populations isolated from tomato fruits (Table 2).

The calculation of general number of reproduction substrategies and corresponding Index of Shannon in all host plants has indicated that the diversity of reproduction substrategies was higher in *P. infestans* populations from tomato fruits and potato leaves than in *P. infestans* populations from tomato leaves (Table 2).

The testing determination of reproduction strategies. In 1997 for 20 field populations, the average means of oospore number per sample were calculated (Smirnov, Elansky, 1999). For these populations, IO was also determined and correlation between average means of oospore presence and IO means were calculated. The mean of correlation coefficient was 0.93 ± 0.03. Thus, the correlation between them approaches identity and IO values reflect oospore presence in field *P. infestans* populations very well.

DISCUSSION

In the previous investigations (Smirnov & Elansky, 1999; Smirnov & Kuznetsov, 2001; Smirnov et al., 2008) it was demonstrated that the field *P. infestans* populations may be very different from each other in occurrence of oospores and zoosporangia, so the idea about different strategies of reproduction was suggested. However, no clear quantitative assessment was made. Our current method of determination of strategies for reproduction provided principally new reliable results. Their authenticity was supported by the cluster and correlation analyses.

Previously, the ranked approach was applied by Flier et al. (2001) for the estimation of oospore formation in the Toluca Valley, Mexico.

The method used for detection and calculation of oospores and zoosporangia in the samples does not imply very high level of exactness. It provides true qualitative estimation. Majority of detected oospores were formed under the field conditions. For zoosporangia, our observations are not able to reveal all dynamics of their formation *in vivo* (and we did not investigate it in our study); it is only possible to assess the potential of their formation under the favorable conditions of moist chambers. IZ value reflects status of *P. infestans* mycelium in lesion very well.

The novelty of our method lies the joint analysis of oospore and zoosporangia occurrence with each of these features expressed by one figure. The count of oospore number per certain lesion area used in many previous investigations was always somewhat distorted by deviation from the average value. This complicates analysis of the obtained results. Per cent of samples with oospores is an unambiguous value, yet it does not guaranty a precise estimation because of different oospore occurrence in the samples. Our method excludes all aforementioned problems.

The suggested equations for the calculation of IO and IZ are analogous to the equation of intensity of disease development (Dorozhkin et al., 1976).

In the tomato populations, more strategies were found than in the potato populations. A few populations were similar to Mexican population on potato in Toluca Valley — so called ’small Mexico’ (Smirnov, Kuznetsov, 2001; Smirnov et al., 2008). It is probably not connected with genotypic differences between the populations on potato and tomato, since on these crops both the same and different strains can occur (Elansky et al., 1999). The formation of oospores in fruits and seeds of tomato was previously proved in Israel (Rubin et al., 2001). Under conditions of Moscow region, the tomato (especially fruits) is a suitable substrate for *P. infestans* suitable for formation of both oospores and zoosporangia, especially in the depressive seasons when such formation is difficult in potato. It is highly probable that tomato populations with S and B strategies can increase the pathogenicity (Goodwin et al., 1995), and under the conditions of Moscow region it might be dangerous for both primary (strategies S and B) and secondary (strategies A and B) infection of potato, though the infection dissemination from tomato to potato is not rapid. In potato, the populations with such strategies are rare or absent.

Interconnection between the occurrence of oospores and zoosporangia was not detected. This indicates that their formation is regulated by different factors.

Very important practical aspect in plant pathology is the estimation of fitness (aggressiveness) of *P. infestans* strains. For its estimation, it was necessary to isolate the strains as pure cultures, inoculate leaflets or tuber discs, and determine the infection frequency (IF), lesion area (LA) and sporulation capacity (SC). The compositive fitness index CFI = IF x LA x SC allows estimation of the fitness (aggressiveness) of a certain *P. infestans* strain (Kadish & Cohen, 1988). Thus, our approach can be adapted to this.

The aforementioned method was not applied in the field as it was difficult to estimate the sporulation capacity. The application of IZ solves this problem. The spread of disease (S) corresponds with the infection frequency (IF), intensiveness of disease (I) — sporulation capacity (SC). Thus, by combining new and well known approaches, it is possible to obtain the estimation of aggressiveness of the field *P. infestans* population. Laboratory estimation of aggressiveness of isolates collected from the latter would not provide reliable result as many properties of *P. infestans* can be essentially changed after cultivation on the agar media and re-isolation.

P. infestans fitness would be expressed differently under laboratory and field conditions. Under the laboratory (as a rule optimal) conditions all components (LA, SC, and IF) can essentially influence CFI, but under field conditions it is not always true. IZ better indicates fitness of the field *P. infestans* population and its alterations than S and I. In the laboratory experiments such effects do not play an important role.

The knowledge of strategy distribution will help understand the biology of *P. infestans* much better and increase the efficiency of protective measures against late blight. In ecology, sexual and asexual reproductions represent opposite strategies of survival (Brasier, 1983; Dyakov, 1992; Cohen et al., 2000). Hence, our approach can be used for development of models connected with DSS (Decision Support Systems) directed at proper application of protective measures against late blight.

Also, it is possible to apply our method to the issues which are usually investigated by means of traditional approaches to population biology. First of all, it is the comparison of *P. infestans* strains on potato and tomato, as well as the interconnection between occurrence of zoosporangia, oospores, and mating types.

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РЕПРОДУКТИВНЕ СТРАТЕГИЈЕ ПОПУЛАЦИЈЕ *PHYTOPHTHORA INFESTANS* КОД ПРОИЗВОДЊЕ КРОМПИРА И ПАРАДАЈЗА НА ОТВОРЕНОМ У НЕКИМ РЕГИОНИМА РУСИЈЕ

А. Н. Смирнов, С. А. Кузњецов

Руски пољопривредни факултет — Московска пољопривредна
академија Тимирјазев, Одељење фитопатологије, ул. Лиственичнаја 2,
зграда 12, Москва, 127550 Русија

Резиме

Испитивано је присуство зооспорангија и ооспора у 88 популација *Phytophthora infestans* (Mont.) de Bary у призводњи на отвореном у московском региону као и у другим регионима Русије у периоду од 1997. до 2006. За процену учесталости зооспорангија и ооспора у популацији предложени су индекси ИЗ (за зооспорангије) и ИО (за ооспоре). Комбинацијом ових индекса откривено је постојање 4 репродуктивне стратегије у њивским популацијама овог патогена. Ове стратегије су биле заступљеније у њивским популацијама *P. infestans* на парадајзу (4 према 2) упркос чињеници да је популација била доминантна на кромпиру у региону Москве. Ово је највероватније последица одсуства или незнатног присуства ооспора у кромпиру и њиховог присуства у парадајзу, нарочито плоду, код великог броја популација.

Познавање репродуктивне стратегије може се користити за утврђивање ефикасности нових метода заштите против пламењаче, као и за испитивање популације *P. infestans*.