Vegetative Incompatibility of *Phytophthora infestans*

M.I. Cherepennikova-Anikina[1], L.V. Savenkova[1], A.V. Dolgova[1], D.S. Shaw[2] & Yu.T. Dyakov[1]

[1]*Department of Mycology, Moscow State University, Vorob'evi gori, Moscow, Russia, 119899.*
E-mail: mianikina@yahoo.com; diakov@herba.msu.ru
Fax: (095)-939-39-70)

[2]*University of Wales, Bangor, School of Biological Sciences, Bangor, Gwynedd, LL57 2UW, UK.*

ABSTRACT

The article describes morphological characters manifestation of vegetative incompatibility between isolates of *Phytophthora infestans* and influence of nutrient media on manifestation of vegetative incompatibility was observed. It is demonstrated that isolates of *P. infestans* have the ability to change reaction of interaction as result of treatment by N-nitro-N-nitrosomethylurea solution or fungicide metalaxyl. Heterokaryon between vegetative incompatibility isolates was obtained. It was slowly grew and did not sporulate. His status was studied by the isolation of single-hyphal-tip cultures from the heterokaryon, DNA fingerprinting analysis, fluorescent cytophotometry.

Key words: *Phytophthora infestans,* vegetative incompatibility, heterokaryon, barrage.

Черепникова-Аникина М.И., Савенкова Л.В., Долгова А.В., Шоу Д.С. и Дьяков Ю.Т. Вегетативная несовместимость у *Phytophthora infestans*.

В статье описывает морфологические характеристики проявления вегетативной несовместимости между изолятами *Phytophthora infestans* и влияние питательных сред на проявление реакции вегетативной несовместимости. Продемонстрировано, что изоляты *P. infestans* способны к изменению реакции взаимодействия между изолятами в результате обработки N-нитро-N-нитрозометилмочевины или фунгицида металаксила. При сращивании несовместимых изолятов был получен медленно растущий и не способный к споруляции гетерокарион. Его статус изучали посредством изоляции культур из гифальных кончиков, анализа ДНК (фингерпринтинг) и флюоресцентной цитометрии.

INTRODUCTION

Phytophthora infestans (Mont.) De Bary is a phytopathogenic oomycete that causes a late blight of potato and tomato. The disease is widespread in North America, South America, Europe, and Asia (Spielman et al., 1991). To control the disease strategies can be developed utilizing knowledge on the biology of this pathogen and mechanisms involved into exchange of genetic material by *P. infestans*.

Fungi exchange genetic material via mutations, sexual, and parasexual recombinations. Parasexuality is the transfer of genetic material from one organism to another without meiosis or development of specialized sexual structures and it is important for phytopathogenic fungi, which often do not undergo a sexual cycle (Pontecorvo, 1956). The parasexual cycle is widespread among fungi. It involves hyphal anastomosis, heterokaryon formation, karyogamy and recombination resulting from haploidizating, chromosome disjunction and mitotic crossing-over. All of these stages of the parasexual cycle have been reported in *P. infestans* (Dyakov, Kuzovkina, 1973, 1974; Poedinok et al., 1982). For study cultivar specific pathogenicity heterokaryons of *Phytophthora* have been used (Kuhn, 1991). The possibility of stable heterokaryon formation in *P. infestans* is fully described by Dyakov and Kuzovkina (1973, 1974). The heterokaryon formations are studied by genetic and cytological methods. Hyphal anastomosis is formed between genetically different isolates. In *P. megasperma* heterokaryons are obtained between both auxotrophic mutants and mutants, which are resistant to antibiotics (Long & Keen, 1977; Layton & Kuhn, 1990). It has been pointed out that *P. infestans* formed heterokaryons on potato tubers, which in turn were infected by two mutant isolates (Kulishch et al., 1978). The heterokaryotic state is maintained only under some conditions, such as reproduction by part of the mycelium, or multinuclear spores (Caten, 1972). *P. infestans* reproduces by multinuclear sporangia and mononuclear nuclear spores (Dyakov, Kuzovkina, 1973; Shaw, 1990). Probability of multinuclear zoospores formation of *P. infestans* is approximately 4.65% (Dyakov, Kuzovkina, 1973). It has

been suggested that these spores maintain heterokaryotic state of fungus.

However, heterokaryon formation is restricted by the vegetative incompatibility system that prevents the vegetative hyphal fusion of genetically distinct individuals (Rayner et al., 1984). If fungal individuals undergo hyphal fusion but differ in allelic specificity at any of a number of vegetative incompatibility loci (*vic*; sometimes referred to as *het* for heterokaryon incompatibility), the hyphal fusion cells are compartmentalized and died. This can be seen as a demarcation zone with dead cells on the boundary of two colonies (barrage). Vegetative incompatibility as common occurrence as parasexual cycle among fungi (Kuhn, 1991) and it was described for all groups of fungi with all types of reproductive morphologies and behaviors (Dyakov, Dolgova, 1995; Leslie, 1993).

P. infestans is the first object of Oomycetes class, for which vegetative incompatibility is described (Poedinok, Dyakov at al., 1981; Gorbunova et al., 1989). The isolates of wild populations of *P. infestans* were divided into 2 vegetative incompatibility groups, referred to as *vcg*1 and *vcg*2 (Dyakov, Dolgova, 1995). The boundary zone between the isolates of different vegetative incompatibility groups was observed by microscopy, showing vacuolization, destruction and lysis of substrate or aerial hyphae. Five characteristics of interaction modes between the isolates are described for *P. infestans* grown on the oatmeal agar medium (Anikina et al., 1993). During the prolonged incubation of the pairing colonies changes were observed in the demarcation zone: a) the overgrowing of zone by aerial mycelium in the form of the separate beams of hyphae or mycelial roller; b) the expansion of the zone of lysis; c) the complete lysis of aerial mycelium of one of the isolates; d) to the appearance of lytic spots on the colony. These processes can be one- directional or bi-directional and reflect the ability of the mycelium of one isolate to penetrate to the territory of the other.

The isolates, which belong to the different groups of vegetative incompatibility, are not capable to the formation of heterokaryons during the fusion of protoplasts. The frequency of regeneration was 1.5 – 3 times lower than that of the control. During the protoplasts fusion from the isolates of one vegetative compatibility group, the frequency of heterologous fusion was 17.7% (Gorbunova et al., 1990). During inoculation the vegetative compatible and vegetative incompatible isolates zoospores mixture into liquid nutrient medium, mycelial mat was formed only when isolates had vegetative compatibility. If isolates were vegetative incompatible, then the visible mycelium was not formed. In some incompatible combinations the rare nonviable mycelium was formed, which did not renew growth after replacing to the agar media. The isolates, whether compatible or incompatible with testers of both vegetative incompatibility groups, were encountered in the collection of wild isolates and in oospore progeny from the crossing of the vegetative incompatible isolates, but such cases were rare (Anikina, 1994; Dyakov, Dolgova, 1995).

Despite the fact that the vegetative incompatibility leads to the death of substrate and aerial hyphae in the boundary zone, it does not interfere with the normal sexual process and the formation of oospores. Sexual reproduction occurs only in case where parasexual reproduction is impossible due to vegetative incompatibility, and the genetic systems of sexual incompatibility and vegetative incompatibility function in the opposite phase. In experiments with the self-fertile isolates was shown that these isolates are forming more oospores on the boundary with the vegetative incompatible partner than on the boundary with vegetative compatible (Anikina et al., 1997), i.e., vegetative incompatibility strengthens sexual production (similar data was obtained for *Ophiostoma ulmi* (Brasier, 1984).

The occurrence of vegetative incompatibility in *P. infestans* is well established (Dyakov, Dolgova, 1995). This paper reports the study on the nuclei migration between the mutually incompatible and compatible isolates; the formation of heterokaryons between them; spontaneous lysis; influence of metalaxyl and/or nitrosomethylurea on the vegetative incompatibility and the role of the different types of media in the vegetative incompatibility reactions. Part of these data was published earlier (Anikina et al., 1997).

MATERIALS AND METHODS

Fungus

Thirty-three isolates of *P. infestans* used in this study are listed in Table 1. The isolates were obtained from collection of Department Mycology & Algology, Moscow State University. The isolate 6033 was obtained from collection of Bangor University, UK. All isolates used in this study were derived from single propagules of either sporangia or zoospores. Single-hyphal-tip isolates were established from heterokaryon isolates (Fyfe & Shaw, 1992). Cultures were maintained in Petri dishes (9 cm) containing oatmeal agar at 18°C in the dark. For long-term storage, all cultures were placed under sterile mineral oil in test tube slants on oatmeal agar at 18°C in the dark. Following media were used in this study: oatmeal agar (130 g of oatmeal, 15 g of agar, 1 liter of distilled water); pea agar (300 g of frozen garden pears, 5 g of glucose 15 g of agar, 1 liter of distilled water); potato agar (Gaerthneur, 1959); rye A agar (60 g of rye grain, 20 of sucrose, 15 g of agar, 1 liter of distilled water (Caten & Jinks, 1968). The isolates 1S1 and B5 were used as tester isolates to detection of *vcg* and mating type of isolates.

Detection of vegetative incompatibility

To test for vegetative incompatibility groups, a 4-to 5-mm block of agar and mycelial plug from one of the testers was placed in a Petri dish containing oatmeal agar. A si-

TABLE 1

Host, area of isolation, mating type, vegetative incompatibility groups (*Vcg*), antibiotic or fungicide sensitivity of *P. infestans* used in this study

Isolate	Mating type	*vcg*	Host of origin	Area and year of isolation	Addition
1S1	A2	2	tomato	Moscow region, 1985	Streptomycin resistant
B5	A1	1	potato	Moscow region, 1985	Blastocitidin resistant
9	A1A2	1	potato		
1234	A1A2	2	potato	Moscow region, 1985	
182	A1A2	2	potato	Ukraina, 1984	
K29	A1	1	potato	Moscow region, 1992	
K16	A2	2	potato	Moscow region, 1992	
K22	A2	2	potato	Moscow region, 1992	Metalaxyl resistant
138	A1	1	potato	Moscow region, 1995	Metalaxyl resistant
1A4c	A1	1	potato	Moscow region., 1985	Acriflavin resistant
5A4c	A1	1	potato	Moscow region , 1985	Acriflavin resistant
52	A2	2	potato	Moscow region, 1994	Metalaxyl resistant
K1	A1A2	1	potato	Sverdlovsk region, 1985	
K102	A1	1	potato	Sverdlovsk region, 1985	
K103	A1	1	potato	Sverdlovsk region, 1985	
KT 19	A2	2	potato	Moscow region, 1993	
KT 16	A2	2	potato	Moscow region, 1993	
214E	A1	1	potato	Estonia, 1984	
6033	A1	1	potato	Bangor, UK, 1995	
303	A1	1	potato	Ukraina, 1984	
45	A2	2	potato	Sverdlovsk region	
10-VIR	A2	2	potato	Moscow region, 1989	
PrTpl6	A2	2	tomato	Moscow region, 1989	
K27	A2	2	potato	Nijnii Novgorod region	Metalaxyl resistant
T11P5	A1	1	tomato	Moscow region, 1989	
522	A2	2	potato	Bellorussia, 1986	It was showing vegetative incompatibility with both testers
550	A2	2	*Solanum sp*.	Mexico	
Cabul	A2	2	potato	Georgia, 1986	
Gatch-6	A1	1	potato	Ukraina, 1984	
154	A2	2	potato	Moscow region, 1989	
Krasnodar	A1	1	potato	Krasnodarskii krai, 1985	
Novosibir	A1	1	potato	Novosibirsk region, 1985	

x — vegetative incompatibility with both testers.

milar block of unknown isolate was placed adjacent to the testers and allowed to grow together, in two reiterations. The plates were incubated for 14 days in darkness at 18°C. If barrage was observed, the tested isolate was ascribed to the opposite vegetative compatibility group.

Determination of mating types

The isolates were sown in pairs with tester isolates in plates with oat agar, in two reiterations. The plates were incubated for 14 days in darkness at 18°C. If abundant oospores were revealed, the isolate tested was ascribed to the opposite mating type. The isolates were also examined for presence of oospores in the single culture. If oospores in the single culture were revealed, the isolate was ascribed to self-fertile isolates.

Zoospore production

To obtain a fungal zoospore suspension, zoosporangia were washed off the surface of the 8–10-days old culture with sterile distilled water. The suspension obtained was incubated at +4°C for 30 to 40 minutes, thus stimulating zoospore formation and release.

Mutagenesis

A 5 μg ml^{-1} N-nitro-N-nitrosomethylurea (NMU) was used as a mutagene. Zoospores were treated with the mutagene solution for 18 to 20 hours in darkness at 4°C. Effective mutagene concentrations were taken off by means of threefold centrifugation in sterile distilled water at 3000 revolutions per minute for 15 minutes. The supernatant was poured out, and sterile distilled water was added to the residue. The suspension of zoospores treated with the mutagene was sown out in oat agar with metalaxyl at a concentration of 600 μg ml^{-1}. Single zoospore colonies were isolated and their characteristics were studied.

Migration of nuclei

Isolates for the experiment were placed in pairs in the Petri dishes on the oatmeal agar medium. The boundary zone was defined as the point of contact between the two colonies. The air hyphae samples were taken by sterile needle at a distance of 0.5, 1.0, 1.5 and 2.0 cm on both sides of the boundary zone. These air hyphae samples were taken every day after contact of colonies (Fig. 1). The air hyphae samples were placed in oatmeal agar me-

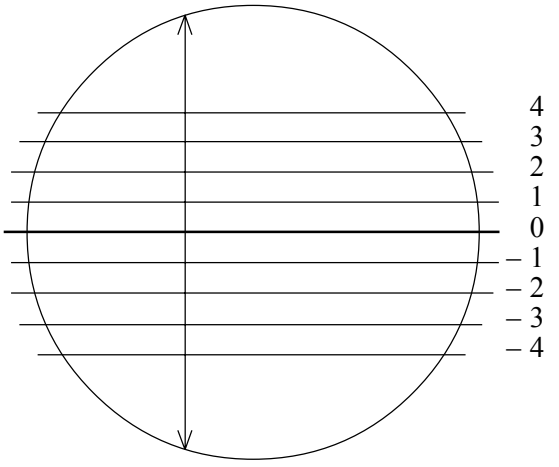

Fig. 1. Diagram of the study of the mutual migration of hyphae and nuclei during the inoculation of two isolates of *P. infestans*. 0 — the contact zone; lines 1 and –1, 2 and –2, 3 and –3, 4 and –4 are arranged / located at a distance of 0.5, 1.0, 1.5, 2.0 cm respectively along both sides from the contact zone; pointers showed the directions of the isolation of the mycelium in the point of intersection with lines 0, 1 ,2 ,3 ,4 or 1, –2, –3, –4 (Anikina et al., 1997).

dia were supplied with antibiotics. The tested isolates were resistant to these antibiotics (Table 1). A growth of the mycelium in the presence of two antibiotics demonstrates that heterokaryon was formed. The hyphae of the resistant isolate can be observed to grow into the territory of the isolate, which is sensitive to the antibiotic. Thus, the method used made it possible to investigate the speed with which the hyphae of one isolate spreads into the colony of another. This is accomplished by: 1) recording of the time of the appearance of resistant isolates in the colony of sensitive isolates at the different distances from the boundary; 2) the presence of heterokaryon and the speed of the migration of nuclei after the two isolates were combined (according to the capability of isolates from the resistant and sensitive colonies to grow on the medium with antibiotics). All experiments were carried out three times.

DNA fingerprinting

Cultures were grown in pea broth at 18°C in a dark incubator for 10 days. Mycelia were harvested, frozen at – 80°C and then lyophilized. DNA was extracted from lyophilized mycelium as previously described (Goodwin et al., 1992a). Gel electrophoresis, hybridization with the probe RG57 and autography using standard techniques were performed as previously described (Carter et al., 1991; Goodwin et al., 1992b).

Fluorescent cytophotometry to quantify of DNA content

Mycelia of *P. infestans* isolates were fixed in methanol, post-fixed in 6N HCl during 8 min. Nuclei were stained using the fluorescence staining method of Rosanov & Kudryavtsev (1967). DNA content is shown in arbitrary units (a. u.).

RESULTS AND DISCUSSION

Influence of nutrient media on the manifestation of the vegetative incompatibility of *P. infestans*

Crossings of isolates were carried out in pairs. Anikina et al. (1993) showed that the metabolites of the third isolate could influence the manifestation of the interaction reaction between two isolates. The effect may be either the removal of incompatibility reaction in the incompatible isolates, or its appearance in compatible ones (Fig. 2).

The vegetative incompatible and vegetative compatible isolates of *P. infestans* were pairing on the potato, rye, pea and oatmeal agar media. When the inoculation was between of the vegetative compatible or vegetative incompatible isolates of *P. infestans* on the potato, rye and oatmeal agar media, the interactions were identical and stable. Thirty-eight combinations of the pairing isolates were tested for the reaction of vegetative incompatibility on the oatmeal, rye A and potato agar media. Twenty-seven of 38 pairs of isolates demonstrated the reaction of vegetative compatibility and 11 of 38 pairs of isolates were vegetative incompatibility (width of barrage varied from 0.5 to 1.6 cm). The same combinations were tested thoroughly the pea agar medium. Each combination of pairing was duplicated and the experiment was repeated five times. Eighteen pairs of isolates demonstrated the reaction of vegetative compatibility and 20 pairs of isolates were vegetative incompatibility. Nine pairs changed the reaction of vegetative compatibility for the reaction of vegetative incompatibility. The reaction between those isolates revealed the reaction of vegetative incompatibility "thread" (Anikina et al., 1993). The width of barrage was 0.1 – 0.3 cm. The inoculation of the vegetative incompatible isolates on the pea agar medium resulted in the contraction of the boundary in contrast to remaining media (Table 2, 3). These data suggest that in certain cases the isolates, which are visibly vegetative compatible, are in fact vegetative incompatible. But in this case the formations of anastomosis did not occur. One can speculate that the chemical composition of pea medium can stimulate the formation of anastomosis and lysis of fused hyphae.

The influence of 1 % charcoal on the manifestation of the reaction of vegetative incompatibility showed that the width of the zone of the barrage between the vegetative incompatible isolates decreased at least twice in the presence of adsorbent (Table 4). The charcoal prevented the lysis of some isolates. Thus, isolate 45 was lysed during interaction with isolates B5. In the presence of 1 % charcoal the lysis of isolates 45 did not occur (Fig. 3).

It was shown that the addition of 1 % charcoal to the medium influences the interaction of isolates in other fungi. The charcoal adsorbs the fungus metabolites during the addition to the medium (Butler, Bolkan, 1973; Day, Anagnostakis, 1973).

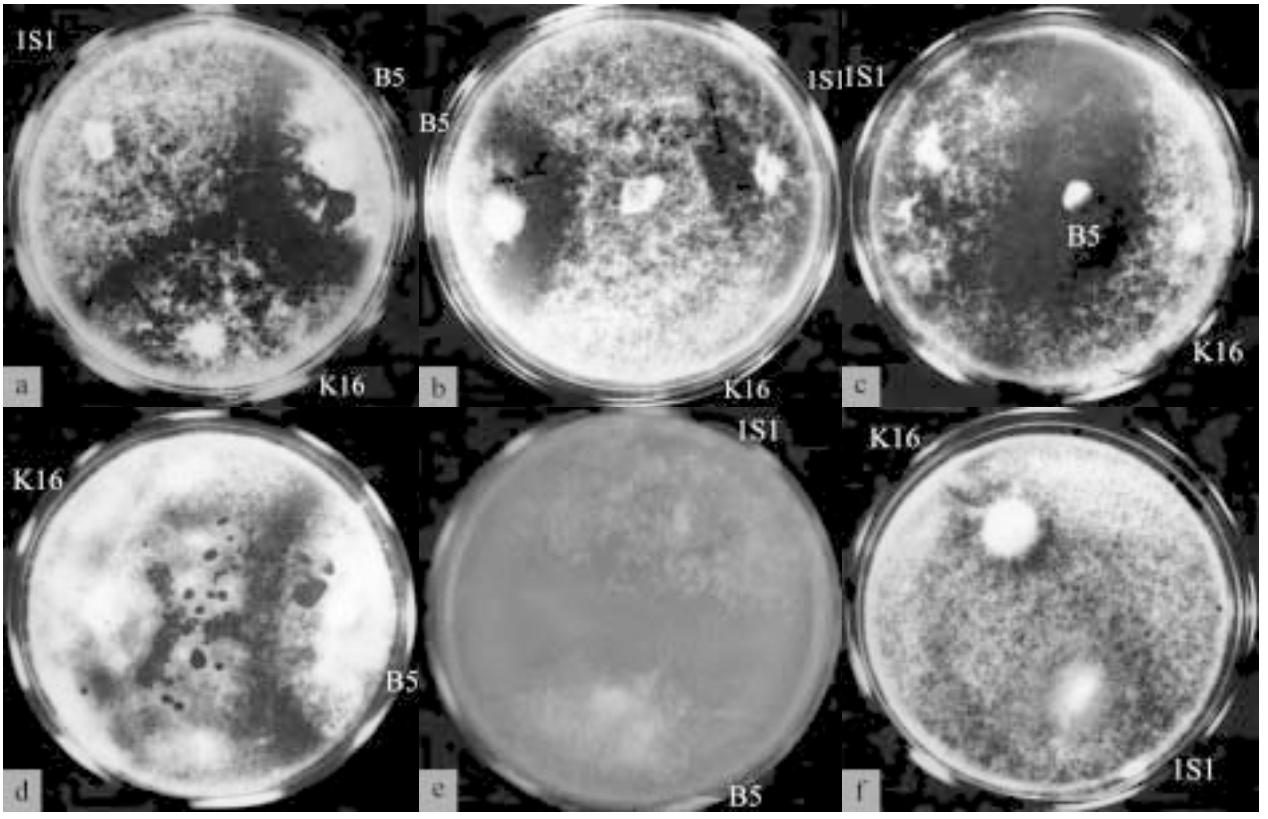

Fig. 2. Manifestation of interaction between the isolates of *Phytophthora infestans* depending on the method of the inoculation.
a — triangle 1S1 x B5 x K16; b — line B5 x K16 x 1S1; c — line 1S1 x B5 x K16; d — B5 x K16; e — 1S1 x B5; f — K16 x 1S1.

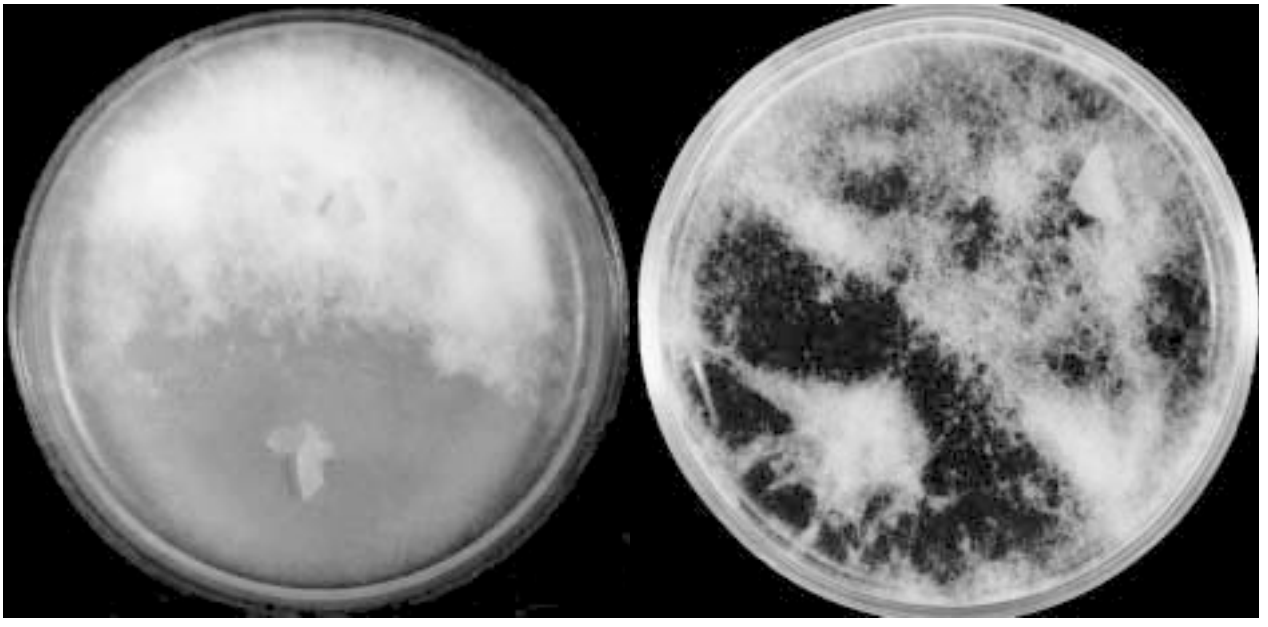

Fig. 3. Interaction between the vegetative incompatible isolates 45 x B5 of *Phytophthora infestans* on the media.
a — oatmeal agar medium; b — oatmeal agar medium with addition 1% charcoal.

Influence of the fungicide metalaxyl and N-nitro-N-nitrosomethylurea on the manifestation of the vegetative incompatibility of *P. infestans*

To study the influence of the fungicide metalaxyl and NMU on the vegetative incompatibility, the isolates with different characteristics were used. Metalaxyl was added to the agar medium at a concentration of 1 – 5 μg ml^{-1} for the metalaxyl sensitive isolates K29, K16 and 150 μg ml^{-1} for the metalaxyl resistant isolates K22, 138. The isolates were cultivated in Petri dishes with oatmeal agar conta-

TABLE 2

Interaction of isolates of *P. infestans* with isolate 1S1on oatmeal agar and pea agar media

Isolate	Interaction of isolate with isolate 1S1on						single culture	
	oatmeal agar			pea agar			lysis of isolate	oospores
	vegetative compatibility	lysis of isolate	oospores	vegetative compatibility	lysis of isolate	oospores		
1S1	*c*	no	no	*c*	no	no	no	no
K22	*c*		no			no	no	no
52	*c*	no	no	*c*	no	no	no	no
1A4C	*i* (0.7–1.1)*	lysis of both colonies	yes	*i* (0.5)	lysis of both colonies	yes	no	no
5A4C	*i* (0.8–1.0)	lysis of both colonies	yes	*i* (0.4)	lysis of both colonies	yes	no	no
10-VIR	*c*	10-VIR	no	*i* (0.2)	lysis of 10-VIR, litic spots on 1S1	no	no	no
PrTpl6	*c*	lysis of both colonies	no	*i* (0.2)	lysis of both colonies	no	no	no
182	*c*	litic spots at 182	yes	*i* (0.2–0.3)	182	yes	no	yes
1234	*c*	1234	yes	*c*	1234	yes	no	yes
KT19	*c*	no	no	c	no	no	no	no
K29	*i* (0.5–1.1)	no	yes	*i* (0.4)	lysis of both colonies	yes	no	no
K103	*i* (0.6–0.8)	lysis of both colonies	yes	*i* (0.5)	lysis of both colonies	yes	no	no
Krasnodar	*i* (0.8–1.1)	litic spots at Krasnodar	yes	*i* (0.5)	lysis of both colonies	yes	no	no
Novosibir	*i* (1.0–1.4)	no	yes	*i* (0.5)	no	yes	no	no
154	*c*	no	no	*c*	no	no	no	no
6033	*i* (0.6–0.8)	no	yes	*i* (0.4)	no	yes	no	no
Gatch-6	*i* (1.1–1.6)	1S1	yes	*i* (0.9)	1S1	yes	no	yes

* The width of barrage zone is given in brackets.

TABLE 3

Interaction of pairing isolates of *P. infestans* with isolate 1S1 on oatmeal agar and pea agar media in triangular inoculations

Isolate x isolate	Interaction of isolate with isolate 1S1 on					
	oatmeal agar			pea agar		
	vegetative compatibility	lysis of isolate	oospores	vegetative compatibility	lysis of isolate	oospores
52 x KT19	*c*	litic spots at KT19	no	*i* (0.3)	KT19	no
$T_{11}P_5$ x 138-2/400	*c*	138-2/400	no	*i* (0.2)	lysis of 138-2/400, litic spots on T11P5	no
$T_{11}P_5$ x 138	*c*	no	no	*c*	T11P5	no
10-VIR x cabul	*c*	cabul	no	*i* (0.3)	cabul	no
KT19 x cabul	*c*	KT19	no	*i* (0.2)	KT19	no
K22 x cabul	*c*	cabul	no	*i* (0.1)	lysis of both colonies	no
52 x 154	*c*	no	no	*c*	no	no
182 x cabul	*c*	no	yes	*c*	no	yes
52 x 45	*c*	no data	no	*i* (0.1)	no data	no
K29 x B5	*c*	no	no	*c*	no	no
K22 x K29	*i* (0.8–1.0)	no	yes	*i* (0.3–0.6)	K22	yes
K1 x K29	*c*	no	yes	*c*	no	yes
K1 x B5	*c*	litic spots at B5	yes	*c*	lysis of both colonies	yes
K22 x KT16	*c*	lysis of both colonies	no	*c*	lysis of both colonies	no
KT19 x 45	*c*	45	no	*c*	45	no
10-VIR x 138-2/400	*i* (0.8–1.2)	lysis of both colonies	yes	*i* (0.6)	lysis of both colonies	yes
138 x B5	*c*	no	no	*c*	no	no
138-2/400 x B5	*c*	no	no	*c*	138-2/400	no
KT16 x cabul	*c*	lysis of both colonies	no	*c*	lysis of both colonies	no
45 x 138	*i* (0.6–0.9)	no	yes	*i* (0.5)	lysis of both colonies	yes
Krasnodar x B5	*c*	litic spots at Krasnodar	no	*c*	litic spots at B5, lysis of krasnodar	no

ining metalaxyl and incubated for 30 days in the dark. After that aerial hyphal samples from the edge of colony were transferred into tubes with oatmeal agar slopes. The obtained isolates were placed in oatmeal agar media with increasing concentrations of metalaxyl (2; 10; 20 μg ml^{-1} for K29, K16 and 200; 250; 300 μg ml^{-1} for K22, 138), and analysis of single zoospore cultures was carried out. The chosen isolates were tested for the vegetative incompatibility with tester isolates 1S1 and B5. After treatment by metalaxyl only the isolate K29 (metalaxyl sensitive), which belongs to *vcg*1 group, a change of reaction of the vegetative compatibility was observed (Table 5), i. e., the reaction of vegetative compatibility with the tester B5 changed to incompatible. In the isolates belonging to *vcg*2 group, the change in the reaction of vegetative compatibility was not observed. The isolate K29-4 was obtained after treatment of the isolate K29 by metalaxyl in the concentration of 1.5 μg ml^{-1}. The isolate K29-4 showed the reaction of vegetative incompatibility with both testers after 5 passages. A width of barrage with 1S1 was 0.8 – 1.1 cm and a width of barrage with B5 was 0.5 – 0.7 cm. When analysis of single zoospores was performed, 61 of 65 single zoospore isolates K29-4 returned to the initial reaction of vegetative compatibility. Four isolates were kept reaction of vegetative incompatibility with B5. The single zoospore isolates from K29-4 were paired with themselves, and with the wild isolates. Each pairing was duplicated and the experiment was repeated three times. The isolates K29-4/2 and K29-4/39 showed the reaction of vegetative incompatibility with both testers, with all single zoospore isolates (Table 6), obtained from the isolate K29-4 (except the single zoospore isolate K29-4/32). It was shown with the wild isolates of the groups *vcg*1 and *vcg*2 (Table 7), except isolate Gatch-6 (*vcg*1). The single zoospore isolates K29-4/2 and K29-4/39 showed the reaction of vegetative compatibility with K29-4/32 and Gatch-6 (Table 5, 6).

The study on the vegetative incompatibility in single zoosporic isolates obtained from isolates K29, 138 (A1 *vcg*1) and K22, K16 (A2 *vcg*2) after treatment with 5 μg ml^{-1} NMU showed that the mutagene provoked change in the reaction of vegetative incompatibility (Table 5). Thirty-four isolates were obtained after the treatment of the isolate K29 with NMU. Two of 34 isolates became vegetative compatibility and 22 isolates became vegetative incompatibility with both tester isolates. Isolates, which changed the initial reaction of vegetative compatibility, were tested repeatedly. Ten of 24 isolates restored their initial reaction of vegetative compatibility. After treatment of the isolate K22 with 5 μg ml^{-1} NMU solution, only 19 of 38 isolates changed the reaction of vegetative incompatibility. Nineteen isolates became vegetative incompatibility with 1S1 and B5. Thirteen of 30 single zoospore isolates of 138 and 11 of 23 single zoospore isolates of K16 changed their reaction of vegetative incompatibility after treatment with 5 μg ml^{-1} NMU solution. All of them demonstrated reaction of vegetative incompatibility with 1S1 and B5. In the analysis of single zoospore isolates that changed reaction of vegetative incompatibility, all clones showed reaction of vegetative incompatibility with both tester isolates.

After treatment with 5 μg ml^{-1} NMU solution, the isolate K29 was inoculated in a oatmeal agar medium to which metalaxyl at a concentration of 600 μg ml^{-1} was added. Twenty colonies were obtained from this medium; 14 of them kept initial reaction of vegetative incompatibility with both tester isolates, 6 became vegetative incompatibility with 1S1 and B5. A width of barrage with 1S1 was 0.8 – 1.10 cm and a width of barrage with B5 was 0.3 – 1.2 cm.

TABLE 4

Influence of 1% charcoal on interaction of the colonies of strains of *Phytophthora infestans*

isolate	types of interactions			
	without charcoal		with charcoal	
	1S1	B5	1S1	B5
522	*i*	*i*	*c*	*i* (0.8)
1234	*c*	*i* (0.6)	*c*	*i* (0.3)
9	*i* (1.0)	*c*	*i* (0.5)	*c*
45	*c*	*i* (1.5)	*c*	*i* (0.7)
krasnodar	*i* (1.0)	*c*	*i* (0.4)	*c*
novisibirsk	*i* (1.0)	*c*	*i* (0.5)	*c*
303	*i* (0.9)	*c*	*i* (0.4)	*c*
Gatch-6	*i* (0.8)	*c*	*i* (0.3)	*c*

TABLE 5

Influence of metalaxyl or NMM on reaction of vegetative incompatibility between isolates of *P. infestans*

Isolate	Metalaxyl	Vegetative compatibility with testers		Metalaxyl				NMM			
				Number of single zoospore isolates		Vegetative compatibility with testers		Number of single zoospore isolates		Vegetative compatibility with testers	
		1S1	B5	Common	Single zoospore isolates which change reaction of vegetative compatibility	1S1	B5	Common	Single zoospore isolates which change reaction of vegetative compatibility	1S1	B5
K16	sensitive	*c*	*i*	43	0	*c*	*i*	23	11	*i*	*i*
K22	resistant	*c*	*i*	68	0	*c*	*i*	38	19	*i*	*i*
K29	sensitive	*i*	*c*	70	1	*i*	*i*	34	22	*i*	*i*
									2	*c*	*c*
K29-4	sensitive	*i*	*i*	65	61	*i*	*c*	no data	no data	no data	no data
138	resistant	*i*	*c*	76	0	*i*	*c*	30	13	*i*	*i*

TABLE 6

Vegetative compatibility of single-spore isolates K29-4/2 and K29-4/39 of *P. infestans* with tester's isolates (1S1 and B5) and other single-spore isolates of K29-4

Single-spore isolates	vegetative compatibility with testers		vegetative compatibility with mutans	
K29-4/#	1S1	B5	K29-4/2	K29-4/39
1	*i*	*c*	*i*	*i*
2	*i*	*i*	*c*	*i*
3	*i*	*c*	*i*	*i*
4	*i*	*c*	*i*	*i*
5	*i*	*c*	*i*	*i*
6	*i*	*c*	*i*	*i*
7	*i*	*c*	*i*	*i*
8	*i*	*c*	*i*	*i*
9	*i*	*c*	*i*	*i*
10	*i*	*c*	*i*	*i*
11	*i*	*c*	*i*	*i*
12	*i*	*c*	*i*	*i*
13	*i*	*c*	*i*	*i*
14	*i*	*c*	*i*	*i*
15	*i*	*c*	*i*	*i*
16	*i*	*c*	*i*	*i*
17	*i*	*c*	*i*	*i*
18	*i*	*c*	*i*	*i*
19	*i*	*c*	*i*	*i*
20	*i*	*i*	*i*	*i*
21	*i*	*c*	*i*	*i*
22	*i*	*c*	*i*	*i*
23	*i*	*c*	*i*	*i*
24	*i*	*c*	*i*	*i*
25	*i*	*c*	*i*	*c*
26	*i*	*c*	*i*	*i*
27	*i*	*c*	*i*	*c*
28	*i*	*i*	*i*	*i*
29	*i*	*c*	*i*	*i*
30	*i*	*c*	*i*	*i*
31	*i*	*c*	*i*	*i*
32	*i*	*c*	*c*	*c*
33	*i*	*c*	*i*	*i*
34	*i*	*c*	*i*	*i*
35	*i*	*c*	*i*	*i*
36	*i*	*c*	*i*	*i*
37	*i*	*c*	*i*	*i*
38	*i*	*c*	*i*	*i*
39	*i*	*i*	*i*	*c*
40	*i*	*c*	*i*	*i*
41	*i*	*c*	*i*	*i*
42	*i*	*c*	*i*	*i*
43	*i*	*c*	*i*	*i*
44	*i*	*c*	*i*	*i*
45	*i*	*c*	*i*	*i*
46	*i*	*c*	*i*	*i*
47	*i*	*c*	*i*	*i*
48	*i*	*c*	*i*	*i*

When analysis of single zoospore cultures of isolates, which changed the initial reaction of vegetative compatibility, was performed, 43 of 48 single zoospore cultures shown the initial reaction of vegetative compatibility. After treatment of the isolate K22 with 5 μg ml^{-1} NMU solution and its inoculation in oatmeal agar medium to which metalaxyl at a concentration of 600 μg ml^{-1} was added, 5 clones were isolated. They all had the initial reaction of vegetative incompatibility with 1S1 and B5 and kept it for following the analysis of single zoospore cultures.

TABLE 7

Vegetative compatibility of single-spore isolates K29-4/2 and K29-4/39 with tester's isolates (1S1 and B5) and wild isolates of *P. infestans*

isolate	vegetative compatibility with testers		vegetative compatibility with mutans	
	1S1	B5	K29-4/2	K29-4/39
9	*i*	*c*	*i*	*i*
K29	*i*	*c*	*i*	*i*
45	*c*	*i*	*i*	*i*
214E	*i*	*c*	*i*	*i*
Gatch-6	*i*	*c*	*c*	*c*
T11P5	*i*	*c*	*i*	*i*
cabul	*c*	*i*	*i*	*i*
154	*c*	*i*	*i*	*i*
182	*c*	*i*	*i*	*i*
10-VIR	*c*	*i*	*i*	*i*
K102	*i*	*c*	*i*	*i*
K103	*i*	*c*	*i*	*i*
138	*i*	*c*	*i*	*i*
K22	*c*	*i*	*i*	*i*
522	*c*	*i*	*i*	*i*
K27	*c*	*i*	*i*	*i*
1234	*c*	*i*	*i*	*i*
PrTpl6	*c*	*i*	*i*	*i*
5A4c	*i*	*c*	*i*	*i*
52	*c*	*i*	*i*	*i*
K16	*c*	*i*	*i*	*i*
K19	*c*	*i*	*i*	*i*
krasnodar	*i*	*c*	*i*	*i*
novisibirsk	*i*	*c*	*i*	*i*

Study of the mutual migration of hyphae and nuclei between the isolates of *P. infestans*

The vegetative incompatible and vegetative compatible isolates of *P. infestans* were paired on the oatmeal agar medium. The appearance of lytic spots in the colony of one isolate and complete lysis of one or both the colonies during the prolonged incubation of the crossed isolates were observed. These processes can be unidirectional or bidirectional (Fig. 4).

The reaction of vegetative incompatibility was observed by pairing isolates ISI x B5 (width of barrage zone was 1 cm) and 52 x B5. With the prolonged joint growth lytic spots were formed in the zone of the colony of isolate B5; and the colony of isolate B5 was lysed gradually. The aerial hyphal samples taken from the B5 side grew on oatmeal agar medium with blasticidin S added, while those taken from 1S1 side did not. The aerial hyphal samples taken from both sides grew on oatmeal agar medium with streptomycin added. The zone of aerial hyphal samples, resistant to streptomycin, gradually enlarged, which showed that hyphae of isolate ISI, grew to the colony of isolate B5. This penetration of the mycelium was accompanied by a growth in the frequency of anastomosis on the entire colony of isolate B5 and its gradual lysis.

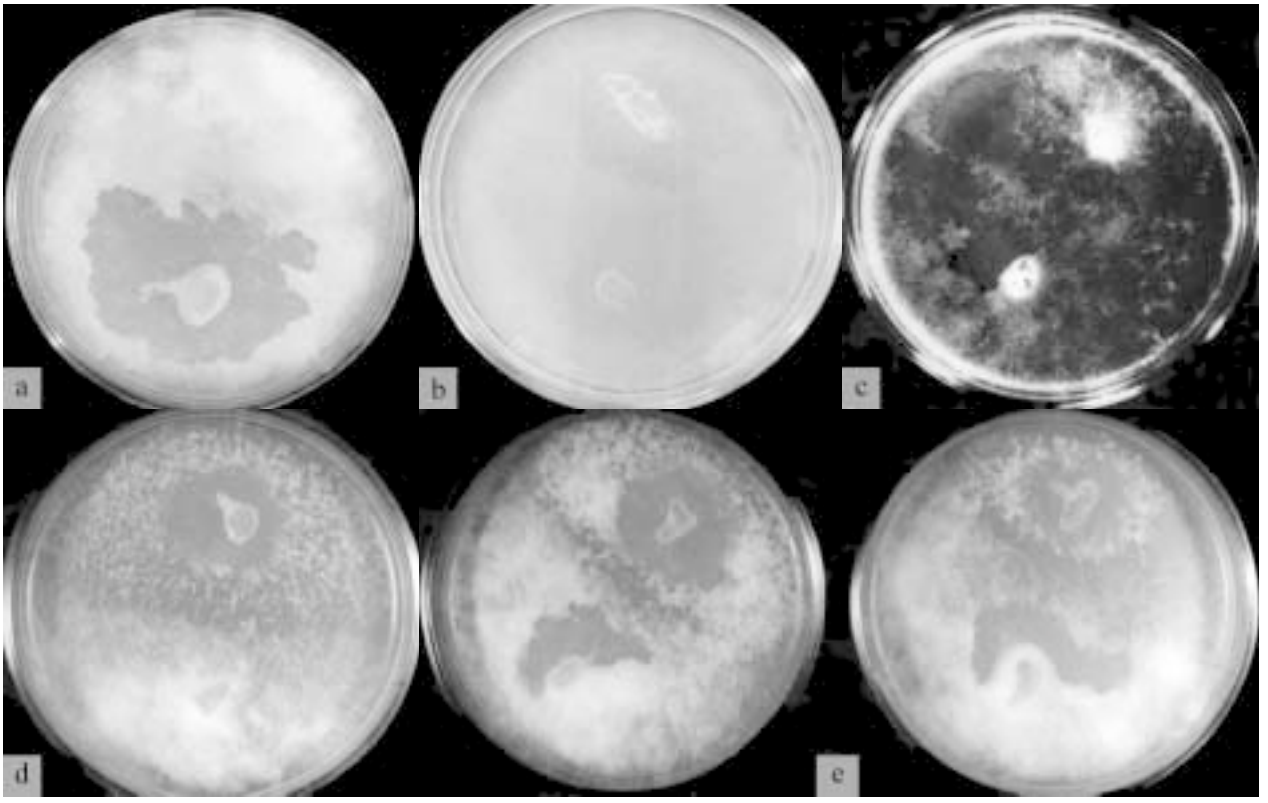

Fig. 4. Changes in the demarcation zone during the prolonged incubation of the pairing cologies.
I. Vegetative compatibility pairing: a — lysis of one isolate; b — lysis of both isolates; c — litic spots of both colonies.
II. Vegetative incompatibility pairing: d — litic spots on one colonie; e — litic spots of both colonies.

During the crossing of isolates 52 x B5 (width of barrage zone was 1 cm) interactions were similar to the foregoing combination: penetration of hyphae of isolate 52 to the territory of isolate B5 was observed (growth both on the medium with blasticidin S and on the medium with metalaxyl); the reciprocal growth of hyphae of isolate B5 into the territory of isolate 52 was not observed (the aerial hyphal samples grew only on the oatmeal agar medium, which was supplied with metalaxyl). Heterokaryons were not formed in any of the combinations.

The pairing isolates 52 x ISI (vegetative compatible) formed heterokaryons on both sides of boundary zone. Heterokaryons grew on the oatmeal agar medium, which was supplied with metalaxyl and streptomycin. Their formation in the side of isolate ISI began earlier than in the side of isolate 52. It's possibly, together with the reciprocal migration of nuclei, the heterokaryon sites of the colony were formed as a result of reciprocal migration and the fusion of hyphae.

The isolates, which grew on the medium with metalaxyl and acriflavine (heterokaryons), were collected from the pairing of isolates 52 x 1A4C or 52 x 5A4C (width of barrage zone was 0.5 cm). Growth of both 1A4C isolate, and 5A4C isolate in the presence of isolate 52 was suppressed; therefore the inoculation of medium with isolate 52 was carried out 1 week after inoculation with isolate 1A4C or 5A4C. Isolates 1A4C and 5A4C were independent mutants obtained from one initial isolate 4C; therefore their interactions to other isolates were similar.

The pairing isolates ISI x 1A4C and ISI x 5A4C were vegetative incompatible (width of barrage zone was 1.2 cm). Heterokaryons were not found with a joint growth in the isolates ISI x 1A4C and ISI x 5A4C. Slow unidirectional penetration of hyphae ISI into the colony of isolate 1A4C was observed. Similar data was obtained when pairing isolates ISI x 5A4C.

The pairing of isolates B5 x 1A4C and B5 x 5A4C were vegetative compatible. The gradually expanding lysis of isolate B5 began at 25 day after inoculation. The interpenetration of hyphae (to the territory of isolate B5 faster than to the territory of isolate 1A4C or isolate 5A4C) without the formation of heterokaryons and lysis was observed in case of the pairing of isolates B5 x 1A4C and B5 x 5A4C.

The pairing of isolates 138 x 1A4C and 138 x 5A4C were vegetative compatible. By 20 – 25 day lysis of both isolates began growth from inoculation points resulted in the interpenetration of hyphae (to the side of isolate 138 faster than to the side 1A4C or 5A4C). Heterokaryon was not found.

The pairing of isolates 138 x B5 was vegetative compatible. Hyphae of isolate B5 did not grow to the colony of isolate 138. Hyphae of isolate 138 slowly grew into the colony of isolate B5. In particular, the aerial hyphal samples of isolate B5, which grew on the oatmeal agar

medium with metalaxyl, up to the distance 0.5 cm from the boundary was observed on second day after the coalescence of the colonies of these isolates and on third day — at a distance 2 cm. Heterokaryon was not found.

During the pairing of isolates 138-2/400 x B5 (vegetative compatible) heterokaryons were not found. The interpenetration of hyphae (to the territory of isolate 138-2/400 faster than to the territory of isolate B5) was observed. The paired isolates 138-2/400 x B5 kept reaction of vegetative compatibility at oatmeal agar medium within 40 days. Total lysis of 138-2/400 began on pea agar medium by 24 days.

TABLE 8

Interaction modes of pairing colonies of *P. infestans*

mode	barrage	migration of hyphae	hetero-karyon	isolates
I	yes	one-directional	no	1S1 x B5; 52 x B5; ISI x 1A4C; 1S1 x 5A4C
II	no	one-directional	no	138 x B5
III	yes	reciprocal	yes	52 x 1A4C; 52 x 5A4C
IV	no	reciprocal	no	138 x 1A4C; 138 x 5A4C
V	no	reciprocal	no	B5 x 1A4C; B5 x 5A4C
VI	no	reciprocal	yes	52 x 1S1

TABLE 9

Ratio of frequent of anastomoses and reaction of incompatibility at different interaction modes of pairing isolates of *P. infestans*

Fusion of hyphae	Protoplasmatic incompatibility	Interaction modes
often	strong	I
rare	strong	II, IV
rare	weak	V
often	weak	VI

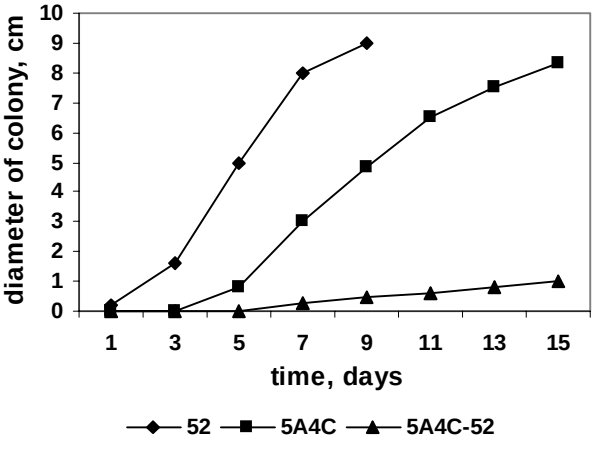

Fig. 5. Linear growth of heterokaryon 5A4C-52 and parental strains of *P. infestans*

Three types of lysis were observed in interaction of colonies.

1. Lysis began in the boundary zone, which was being expanded gradually. This is caused, apparently, by the frequent anastomosis of contacting hyphae.

2. Lysis in the boundary zone did not increase (or was absent), but it appeared in the form of the expanding lytic spots on one (during the unidirectional migration of hyphae) or both (during the reciprocal migration of hyphae) colonies. Apparently, this is caused by anastomosis with penetrating hyphae in the colony of another isolate and by death of the fusing parts of the mycelium. The growth of hyphae of one isolate on another when they were vegetative incompatible was described for the agent of the Dutch disease of the elms *Ophiostoma ulmi* (Brasier, 1984).

3. Lysis of donor isolate, which begins not from the boundary zone, but from the center of colony (isolate ISI by the joint growth with 1A4C), implied the contribution of hyphae metabolites, which migrate from points of their contact.

The interaction modes of paired isolates in this study are listed in Table 8. Thus, all interaction modes depend on the ratio of two events — frequency of the hyphae fusion and intensity of the reaction of protoplasmic incompatibility and can be arranged into the continuous scale from the type I to the type VI (Table 9). The absence of heterokaryons in the combinations with the visible reaction of vegetative compatibility is probably caused by rare anastomosis in the absence (type V) or presence (types II, IV) of the reaction of vegetative incompatibility. The thin band of barrage appeared on the boundary between colonies of isolates with the reactions of types II and IV when using a pea agar medium instead of oatmeal agar medium. Apparently, the substances, which are present in pea, influence the composition of cellular wall and the frequency of anastomosis.

Type III stands apart: in this case heterokaryons were obtained in protoplasmic incompatibility. Since the appearance of heterokaryons between the vegetative incompatible isolates is an extremely rare phenomenon, we conducted additional experiments.

Heterokaryon between the vegetative incompatible isolates

Heterokaryotic isolates were obtained during the inoculation of isolate 52 (A2, *vcg*2, met^r) with the isolates 1A4C and 5A4C (A1, *vcg*1, acr^r). Heterokaryon 1A4C-52 proved to be unstable and after the passage through the medium without the antibiotics lost capability for growth on the medium with the antibiotics. In the second combination (5A4C x 52) two of 50 inocula grew on the medium with both antibiotics. The rate of growth in heterokaryons (5A4C-52) was 2.0–2.5 cm / month (Fig. 5). Heterokaryons, obtained during the inoculation of the compatible isolates, had a rate of growth higher than parents (data did not show).

Heterokaryon 5A4C-52 of *P. infestans* was joined with the parental and with the tester isolates of two vegeta-

tive incompatibility groups (ISI and B5). The pairings of heterokaryon 5A4C-52 with isolates 52 and ISI resulted in the wide zone of barrage 1.2 – 1.5 cm. Heterokaryon 5A4C-52 formed oospores with isolates 1S1 and 52. The pairings of heterokaryon 5A4C-52 with isolates 5A4C and B5 resulted in the narrow zone of barrage (0.3 cm). Heterokaryon 5A4C-52 did not form oospores with isolates B5 and 5A4C (Table 10). Consequently, heterokaryon 5A4C-52 had a mating type A1 (as parental isolate 5A4C), but it was vegetative incompatible with the isolates of both vegetative incompatibility groups (vcg1 and *vcg*2).

Heterokaryon 5A4C-52 did not sporulate. The induction of heterokaryon sporulation was made by cholesterol and lecithin. Oatmeal and pea agar media were supplied with cholesterol (in concentrations 0.5, 1, 2, 5, 10 and 20 μg ml^{-1}), lecithin (in the same concentrations) and cholesterol with the lecithin (0.5 + 20, 20 + 0.5, 5 + 5 μg ml^{-1}). However, sporulation was not observed on one of the media. The heterokaryons, which were heteroallelic on 1-2 *het*- genes, were obtained between the incompatible isolates of *Aspergillus nidulans*. These heterokaryons also slowly grew and had weak sporulate (Jinks et al., 1966; Dales et al., 1993).

We failed to establish their status (heterokaryons or heterozygous diploids) by cloning single zoospore cultures. To clarify their genetic nature 3 methods were used: the isolation of single-hyphal-tip cultures from the heterokaryon, DNA fingerprinting analysis, fluorescent cytophotometry. It was impossible isolated cultures from the single-hyphal-tips. The death of the significant sections of the damaged hyphae occurred because of the coenocytic, multinuclear mycelium.

Profile DNA fingerprinting was examined by digesting DNA with the *Eco*RI restriction enzyme for heterokaryon 5A4C-52, parent isolates 5A4C and 52 (Fig. 6). Bands were formed as a result of the hybridization of the digested of DNA with the fluorescent marker. Probe RG57 revealed more than 25 different bands for this restriction enzyme in *P. infestans*. The hybridization of the digested DNA showed that the profile DNA fingerprinting of parental isolates 52 and 5A4C differed by one band. The profile DNA fingerprinting of isolate 52 lacked the RG57 band 1, diagnostic of *P. infestans* phenotype. Loss of band 1 could have been due to deletion. The profile DNA fingerprinting of heterokaryon 5A4C-52 had band 1 and corresponded to the profile of isolate 5A4C (Fig. 6). Thus, the using of this method did not make it possible to determine status of the isolate 5A4C-52.

The method of fluorescent cytophotometry was used to quantify the DNA content in the nuclei of heterokaryon and parental isolates 52 and 5A4C. The results of measurements were shown in the Table 11. Standard Mexican isolate M-550 has diploid nuclei. The obtained data suggest that isolates 52 and 5A4C were triploids. Significant variability in the DNA content was due to the presence of nuclei in the different stages of cellular cycle. As can be seen from table, the average DNA content in the nuclei of heterokaryon 5A4C-52 was intermediate between the content in the initial isolates 52 and 5A4C; therefore, this was the actual heterokaryon, whose properties were due to the presence of the nuclei of two types. At the same time, the highest variability and greatest maximum nuclear size can indicate that the heterozygous polyploid nuclei can be located together with the nuclei of parents in this isolate.

The attempts to obtain the heterokaryotic isolates between heterokaryon 5A4C-52 and isolates ISI and B5 (on the growth on the media with metalaxyl, acriflavine, streptomycin and metalaxyl, acriflavine, blasticidin S) proved unsuccessful.

TABLE 10

Vegetative compatibility and oospore formation of heterokaryon 5A4C-52 of *P. infestans* with parent strains (52 and 5A4C) and tester isolates (1S1 and B5)

pairing	oospore formation	vegetative compatibility
5A4C-52 x 52	yes	*i* (barrage 1–1.5 cm)
5A4C-52 x 5A4C	no	*i* (barrage 0.3 cm)
5A4C-52 x 1S1	yes	*i* (barrage 1.3 cm)
5A4C-52 x B5	no	*i* (barrage 0.3 cm)

i — incompatibility

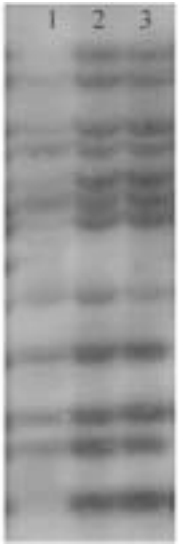

Fig. 6. DNA fingerprinting from heterokaryon 5A4C-52 (3), parent isolates 5A4C (2) and 52 (1) of *P. infestans* probed with RG57.Each lane contains approximately 2 μg of total genomic DNA digested with *Eco*RI.

TABLE 11

Nuclear DNA content (arbitrary units, a.u.) of *P. infestans*

isolate	Number of measurements	DNA content	The standard deviation (a.u.)	min	max
5A4C	65	4.19	1.24	1.80	7.12
52	52	3.64	1.71	1.12	7.74
5A4C+52	56	3.70	1.87	1.26	9.92
550	55	2.89	1.1	1.00	5.78

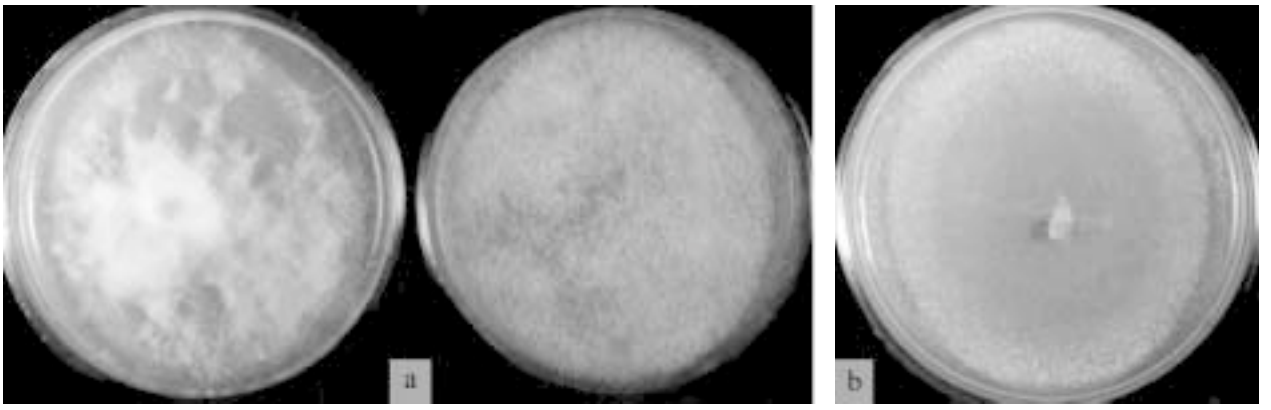

Fig. 7. Morphology of isolates of *P. infestans*.
a — normal morphology; b — spontonous litic isolate PrTplC

Spontaneous lysis

The data of this study show that lysis of colonies can occur in both compatible and incompatible combinations. These suggest that in this case there occurs the later formation anastomosis and the lysis of heterokaryotic hyphae However, the lysis of cultures can be caused by different factors (aging cultures, the presence of virus infection, etc.). The aging is most frequently manifested in the fungi with the frequent reinoculations or with the continuous illumination. It is proved that aging in fungi is controlled by nucleus and nonnuclear genetic elements. Thus, it is caused by the presence of an extrachromosomal element, a mitochondrial plasmid (pl DNA) (Böckelmann & Esser, 1986; Griffith, 1992) and double-stranded RNA (ds RNA) for (Koltin, 1977; Osierwasz et al., 1989). The spontaneous lysis has not been reported before for *P. infestans* (Fig. 7). Anikina (1994) showed that the part of single oospore isolates in F1 generation was inclined to lysis as parental isolate. Analysis of single zoospore cultures of isolate PrTpl4 was performed. Fifty-five of 59 single zoospore cultures shown spontaneous lysis. Spontaneous lysis of isolates was observed on the oatmeal, oatmeal with addition 1 % charcoal, rye A, pea and potato agar media. Lysis began 20 days after inoculation in the center of colony. It began as lytic spot of the indeterminate form. Isolate was lysed completely by 25–30 day after inoculation. To determinate the influence of the exhaustion of nutrient medium on the lysis the following experiment was set up. The sterile cellophane (diameter of 9 mm) was placed in the Petri dishes with the oatmeal agar medium. The isolate was inoculated to the center of Petri dish. The rate of growth was measured every day. The cellophane was removed on 8 day after inoculation and a five-day inoculum was placed in the Petri dish. The rate of growth was measured every day after sowing. A control (without the cellophane) of the sowing isolate was placed on oatmeal agar medium on 8 day after the flood of medium. Studies showed that there were no changes in the nature of the curve of a growth in the fungus. Lytic spots appeared 20 days after inoculation. Temperature and illumination did not have an effect on the spontaneous lysis. Isolate PrTpl4 was grown on the oatmeal agar medium for 30 days at temperatures 14 °C, 18 °C, and 25 °C. The diameters of colonies were measured every day. Fungus was slowly grown and slowly lysed at temperatures 14 °C and 25 °C. The complete lysis of isolate PrTpl4 was on 29 day after inoculation at temperatures 14 °C and 25 °C. The isolate PrTpl4 more actively grew and more rapidly lysed at an optimum temperature 18 °C. The complete lysis of isolate PrTpl4 was on 25 day after inoculation. It was not clear what causes spontaneous lysis.

DISCUSSION

Obtained data make it possible to separate out 2 types of the mechanism of the vegetative incompatibility in *P. infestans*: partial incompatibility and protoplasmic incompatibility.

Partial vegetative incompatibility refers to frequency of the appearance of heterokaryons between the mutants, obtained from the different isolates; it is reliably lower than between the mutants of one isolate. The mixture of spores of genetically marked isolates was inoculated in the Petri dish with oatmeal agar medium. The grown mycelium was transferred to the selective medium. Heterokaryotic growth will be detected only in the single slugs (Poedinok et al., 1981). If two marked isolates are pairing at a certain distance from each other, the colonies grow together without the demarcation line (on the pea agar medium sometimes thin line between the colonies is observed). The reinoculation of the mycelium from the boundary zone and from the center of colonies to the selective media showed that hyphae of one isolate penetrates to the territory of another without the fusion and forming of heterokaryons. Therefore it is assumed that the absence of interactions between the cell walls of different isolates is the basis of partial vegetative incompatibility.

Protoplasmic vegetative incompatibility is expressed as appearance zone of barrage with a width of 2 – 20 mm on the boundary of two mycelia. This zone is characterized by absence of aerial mycelium and by destroyed substrate hyphae (Gorbunova et al., 1989). During the prolonged incubation of colonies changes in the demarcation zone are frequently observed. These processes can be uni- and bi-directional depending on the relative «mycelial force» of the interacting isolates. The latter resemble synnemata lines (Brasier, 1984), which are formed in *Ophiostoma ulmi* during interaction of completely incompatible isolates reactions (wide or narrow), analogous to reactions 1 and 2 in *P. infestans* (Anikina et al., 1993), and are the beams of pigmented dead hyphae. They can be observed at a different distance from the edge of colonies and showed effect of introductions, i. e. the ability of the mycelium of one isolate to penetrate to the territory of another. Capability for introduction is different in various isolates. It is possible to draw a parallel between the beams of dead pigmented hyphae, characteristic for *Ophiostoma ulmi*, and by lytic spots in *P. infestans*. In both cases the bi-directional penetration (with the identical or different mycelial force) and uni-directional penetration was observed, depending on the partners. We observed that the colonies of the mutually incompatible isolates ISI and B5 grow separately approximately at an identical rate; however, upon joint growth isolate B5 formed the small colony, divided from the normally growing isolate ISI by the broad band of lysed hyphae. The isolated mycelium from the boundary zone and from the center of colonies during the inoculation of ISI x B5 to the selective media showed that hyphae of isolate ISI penetrate to the territory B5. Apparently, during the inoculation of isolates ISI and B5, the unidirectional death of hyphae of isolate B5 occurs similar to the unidirectional death of the cells *Podospora anserine* during the inoculation *R/v* isolates (Bernet, 1992). The mixture of zoospores of isolates 1S1 and B5 was inoculated in the Petri dish with oatmeal agar medium. Analysis shown that only the mycelium of isolate ISI grew.

We assume on the basis of these data that the types of changes depend on the frequency of anastomosis of hyphae of the interacting isolates. The expansion of the zone of lysis is observed with the frequent anastomosis and the death of the fusing cells, with the rare local lytic spots on the colonies. Lytic spots appear in the places, where mycelium penetrates in the zone of the partner of the incompatible isolate. Thus, the second type of vegetative incompatibility is caused by the death of the fusing parts of the mycelium; therefore heterokaryons between the incompatible isolates are formed not only after interaction of hyphae, but also after interaction of protoplasts on the medium with polyethylene glycol. Consequently in *P. infestans* a number of small of VCG groups and two super-groups can be discriminated by the mechanism of vegetative incompatibility.

LITERATURE CITED

Anikina, M. I. (1994) A vegetative incompatibility of *Phytophthora infestans* (Mont.) De Bary. *Ph. D. thesis,* Moscow State University, Moscow (in Russian).

Anikina, M.I., Dolgova, A.V. & Dyakov, Yu.T. (1993) Variations in expression of vegetative incompatibility in plant pathogen *Phytophthora infestans* (Mont.) De Bary. *Moscow Univ. Sci. Bull. ser. Biology* **2:** 61–69 (in Russian).

Anikina, M.I., Savenkova, L.V. & Dyakov, Yu.T. (1997) The interactions between isolates of *Phytophthora infestans* (Mont.) De Bary. *Mikologiya i Fitopatologiya* **31:** 33–38 (in Russian).

Bernet, J. (1992) In *Podospora anserina*, protoplasmic incompatibility genes are involved in cell death control via multiple gene interactions. *Heredity* **68:** 79–87.

Böckelmann, B. & Esser, K. (1986) Plasmids of mitochondrial origin in senescent mycelia of *Podospora anserina*. *Curr. Genet*. **10:** 803–810.

Brasier, C. M. (1984) Inter-mycelial recognition systems in *Ceratocystis ulmi*: their physiological and ecological importance. In: *Ecology and Physiology of the Fungal Mycelium.* H. H. Jennings & A. D. M. Rayner (Eds.) Cambridge Univ. Press. P: 451–497.

Butler, E.E. & Bolkan, H. (1973) A medium for heterokaryon formation in *Rhizoctonia solani. Phytopathology* **63:** 542–543.

Carter, D. A., Archer, S. A., Buck, K. W., Shaw, D. S. & Shattock, R. C. (1991) DNA polymorphisms in *Phytophthora infestans*: the UK experience. In: *Phytophthora.* J. A. Lukas et al. (Eds.) Cambrige Univ. Press. P: 272–294.

Caten, C.E. (1972) Vegetative incompatibility and cytoplasmic infection fungi. *J. Gen. Microbiol.* **72:** 221–229.

Caten, C. E. & Jinks, J. L. (1968) Spontaneous variability of single isolates of *Phytophthora infestans.* I. Cultural variation. *Canad. J. Bot.* **46:** 330–348.

Dales, R.B.G., Moorhause, J. & Croft, J.H. (1993) Evidence for multi-allelic heterokaryon incompatibility (Het) locus detected by hybridization among 3 heterokaryon compatibility (h-c) groups of *Aspergillus nidulans*. *Heredity* **70:** 537–543.

Day, P.R. & Anagnostakis, S.L. (1973) The killer system in *Ustilago maydis*: heterokaryon transfer and loss of determinants. *Phytopathology* **63:** 1017–1018.

Dyakov, Yu.T. & Dolgova, A.V. (1995) Vegetative incompatibility in plant phytopathogenic fungi. Moscow State University Press, Moscow (in Russian).

Dyakov, Yu. T. & Kuzovkina, T. A. (1973) Heterokaryons of *Phytophthora infestans* (Mont.) De Bary: I Cytological investigations. *Mikologiya i Fitopatologiya* **7:** 479–485 (in Russian).

Dyakov, Yu. T. & Kuzovkina, T. A. (1974) Heterokaryons of *Phytophthora infestans* (Mont.) De Bary: II Genetic investigations. *Mikologiya i Fitopatologiya* **8:** 81–89 (in Russian).

Fyfe, A.M & Shaw, D.S. (1992) An analysis of sell-fertility in field isolates of *Phytophthora infestans. Mycol. Res*. **96:** 390–394.

Gaerthneur, A. (1959) Versuche sur kunstlichen kultur von *Phytophthora infestans. Archiv. fur Microbiol*. **32:** 261–269.

Goodwin, S.B., Drenth, A. & Fry, W.E. (1992a) Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Curr. Genet*. **22:** 107–115.

Goodwin, S.B., Spielman, L.J., Matuszak, J.M., Bergeron, S.N. & Fry, W.E. (1992b) Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. *Phytopathology* **82:** 995–961.

Gorbunova, E.V., Bagirova, S.F., Dolgova, A.V. & Dyakov, Yu. T. (1989) A vegetative incompatibility in plant phytopathogenic fungus *Phytophthora infestans* (Mont.) De Bary. *Dokladi AN USSR.* **304:** 1245–1248 (in Russian).

Gorbunova, E. V., Dolgova, A. V. & Dyakov, Yu. T. (1990) Investigation of vegetative incompatibility in *Phytophthora infestans* (Mont.) De Bary by the protoplast fusion technique. *Mikologiya i Fitopatologiya*. **24:** 499–503 (in Russian).

Griffith, A. J. F. (1992) Fungal senescence. *Ann. Rev. Genet.* **26:** 351–372.

Jinks, J.L., Caten, C.E., Simchen, J.H. & Croft, J.H. (1966) Heterokaryon incompatibility and variation in wild populations of *Aspergillus nidulans*. *Heredity* **21:** 227–239.

Koltin, J. (1977) Virus — like particles in *Ustilago maydis* with partial genomes. *Genetics* **86:** 527–534.

Kuhn, D. N. (1991) Parasexuality in *Phytophthora*. In: *Phytophthora.* J. A. Lukas et al. (Eds.) Cambrige Univ. Press. P: 242–255.

Kulish, V. B., Dyakov, Yu. T. & Erokhina, S. A. (1978) Heterokaryosis of *Phytophthora infestans* (Mont.) De Bary. III Phytopathological investigations. *Mikologiya i Fitopatologiya* **12:** 406–409 (in Russian).

Layton, A. C. & Kuhn, D. N. (1990) In planta formation of heterokaryons of *Phytophthora megasperma* f. sp. *glycinea. Phytopathology* **80:** 602–606.

Leslie, J. F. (1993) Fungal vegetative incompatibility. *Ann. Rev. Phytopathol*. **31:** 127–150.

Long, M. & Keen, N.T. (1977) Evidence for heterokaryosis in *Phytophthora megasperma* f. sp. *sojae. Phytopathology* **67:** 670–674.

Osiewasz, A.C., Kim, W.K. & Barr, D.J.S. (1989) Presence of double – stranded RNA in fungi. *Mycologia* **79:** 657–661.

Poedinok, N. L., Dolgova, A. V. & Dyakov, Yu. T. (1981) Discovering of vegetative incompatibility in plant phytopathogenic fungus *Phytophthora infestans* (Mont.) De Bary. *Mikologiya i Fitopatologiya* **15:** 275–279 (in Russian).

Poedinok, N. L., Dolgova, A. V. & Dyakov, Yu. T. (1982) Parasexual cycle in phytopathogenic fungus *Phytophthora infestans* (Mont.) dBy. *Genetika* **18:** 1423–1428 (in Russian).

Pontecorvo, G. (1956) The parasexual cycle in fungi. *Ann. Rev. Microbiol.* **10:** 393–400.

Rayner, A.D.M., Coats, D., Ainsworth, A.M., Adams, T.J.H., Williams, E.N.D. & Todd, N.K. (1984) The biological consequences of the individualistic mycelium. In: *Ecology and Physiology of the Fungal Mycelium.* H.H. Jennings & A. D. M. Rayner (Eds.) *Cambridge Univ. Press*. P 509–540.

Rosanov, Yu. M. & Kudryavtsev, B. N. (1967) A method of fluorescent cytophotometry for the purposes of a quantitative determination of DNA. *Citologiya* **3:** 361–368 (in Russian).

Shaw, D.S. (1990) The comparative genetics of *Phytophthora. Bull. OEPP* **20:** 67–76.

Spielman, L.D., Drenth, A., Davidse, L.C., Sujkowski, L.J., Gu, W.K., Tooley, P.W. & Fry, W.E. (1991) A second word — wide migration and population displacement of *Phytophthora infestans*? *Plant Pathol.* **40:** 422–430.