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FULL RESEARCH PAPER

AFLP analysis of Russian *Alternaria tenuissima* populations from wheat kernels and other hosts

Philipp B. Gannibal · Sonja S. Klemsdal · Mark M. Levitin

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Abstract *Alternaria tenuissima* is a common pathogen on a number of plants described in several geographic regions of the world. Genetic variation within and between Russian Far East, North West and Caucasus populations of *A. tenuissima* from wheat was examined. In addition, genetic differences between isolates from various hosts were estimated. In total, 101 isolates of *A. tenuissima* were studied using amplified fragment length polymorphism (AFLP) with four primer combinations. Wright's fixation index (F_{st}), gene flow (N_m) and gene diversity (H_s) were calculated. AFLP banding patterns indicated significant genetic distance and at the same time a low level of gene flow between the Far East and the two other groups of isolates originating from the European part of country. The degree of similarity between the North West and Caucasus populations was very high, as was the migration rate. Isolates analysed by UPGMA-based cluster analysis were grouped according to location of origin but irrespective of plant host. Based on the F_{st} value, the group of isolates originating from wheat and barley were not found to differ significantly from each other.

Keywords Genetic distance · Host specialization · Population structure

Introduction

Species of the genus *Alternaria* are widespread pathogens of wheat and other cereals. They are known to be a cause of wheat leaf blight, black point disease and as a source of food contamination by toxins (Rotem 1994). Previously *A. alternata* was frequently reported in papers dealing with cereal diseases. This species was suggested to be the most ubiquitous in the genus and included almost all small-spored *Alternaria* taxa (conidial length up to 60 μm in culture). *Alternaria alternata* occurs on a huge number of various substrata, sometimes as a parasite, but usually as a saprotroph. Sometimes, however, researchers proposed heterogeneity in this taxon (Neergaard 1945). During the last two decades, thanks to Simmons, *Alternaria* systematics has been thoroughly revised. Introduction of the new taxonomic criteria (Simmons and Roberts 1993) facilitated the accurate characterisation of small-spored species.

In particular, criteria of the species *A. tenuissima sensu* Simmons have become clearer and more

P. B. Gannibal (✉) · M. M. Levitin
All-Russian Institute of Plant Protection, Podbelskogo shosse 3, St. Petersburg, Pushkin 196608, Russia
e-mail: phbgannibal@yandex.ru

S. S. Klemsdal
Plant Health and Plant Protection Division, Bioforsk – Norwegian Institute of Agricultural and Environmental Research, Høgskoleveien 7, Aas 1432, Norway

comprehensible. Distribution and ecology of this species were defined more precisely. *Alternaria tenuissima* was found to be able to infect various parts of plants belonging to different families. Many researchers have found this fungus to be a common pathogen on a number of plants in different parts of the world. It can, for example, induce late blight of pistachio in the USA (Pryor and Michailides 2002), and was established as a major cause of apple dry core rot in South Africa (Serdani et al. 2002).

Alternaria tenuissima can infect a high percentage of cereal grains (Andersen et al. 1996; Gannibal 2004) producing some toxins dangerous for plant, animal and human health, e.g., alternariol, alternariol monomethyl ether, tenuazonic acid, altertoxin I and other metabolites (Andersen et al. 2002). Those toxins have been detected in high concentrations in affected wheat grains in Europe (Logrieco et al. 1990), Australia (Webley et al. 1997) and North America (Webley and Jackson 1998).

Simmons (1990, 1995) found some strains with sporulation patterns similar to those of *A. tenuissima*. Consequently, a few new species were described as additional members of the *A. tenuissima* species-group. Also some small-spored species that produce host-specific toxins (e.g., *A. mali* and *A. longipes*) have morphological characters very similar to *A. tenuissima* (Simmons 1999). The results of RAPD-PCR (Roberts et al. 2000) showed heterogeneity among groups of strains having a sporulation pattern similar to *A. tenuissima.* In general, this subdivision correlated with the host plant. Theoretically these observations can reflect the existence of several *A. tenuissima*-like species or specialized forms of *A. tenuissima*.

In spite of the fact that *A. tenuissima* is an important ubiquitous pathogen, its intraspecific genetic diversity, population structure and host specialization remain indistinct. This knowledge is required by plant pathologists to understand fungus distribution and evolutionary potential, and to design epidemiological predictive models. Careful species identification based on sporulation habits in combination with sensitive molecular methods like AFLP will make it possible to study the population biology of small-spored *Alternaria* species. The objectives of our study were to examine genetic differentiation within and between *A. tenuissima* populations present on Russian wheat, and also to test the hypothesis that *A. tenuissima* has no host specialization.

Materials and methods

Isolates

In total, 101 single spore isolates of *A. tenuissima* were analysed (Table 1). Seventy-seven isolates were recovered from various cultivars of wheat from three regions of Russia. For the population comparison, two regions were chosen in the European part of the country. Twenty-six isolates were collected in Leningrad region (Leningradskaya oblast) and 19 in Krasnodar region (Krasnodarskiy kray) (Fig. 1). The third group of 32 isolates was sampled in the Russian Far East, namely in Primorsky region (Primorskiy kray). These three regions are geographically separated by thousands of kilometres. To avoid the influence of possible host specialization on the population genetic study, only isolates from wheat were included when analysing the genetic diversity of the populations.

A majority of the cultures was isolated from grains. Twelve randomly chosen seedlots were harvested during 2002–2003 from neighbouring fields in the three geographic regions mentioned above and labelled by special numbers (the first 3 digits in the isolate ID). One hundred seeds were arbitrarily selected from each seedlot. Kernels were surface-sterilized by shaking in 0.1% silver nitrate for 1 min and rinsed in sterile water three times for 30 s. Kernels were then plated on Petri dishes of potato carrot agar and incubated at 25°C in the light. *Alternaria* isolates were identified according to Simmons (1990, 1995) giving heed mostly to three-dimensional sporulation patterns. All *A. tenuissima* isolates obtained in this way were included in our study. We also tested a few isolates obtained from necrotic spots on wheat leaves in the same regions. To test for host specificity we included a group of 12 strains of *A. tenuissima* from barley from Leningrad region and 12 isolates representing different hosts and locations. Those cultures were isolated as described above. We also added to our study E. G. Simmons's representative isolate of *A. tenuissima* (EGS 34-015), which was used as the standard of this species. Representative isolates of

Table 1 Isolates of *A. tenuissima* studied, also included are one isolate of *A. alternata* and one isolate of *A. infectoria* used as outgroups

ID of isolate or isolate group	Number of isolates	Host species	Host part	Origin	Year
A. tenuissima					
414[a]	1	*Triticum aestivum*	Kernels	Primorsky region	2002
480, 481, 482	31	*Triticum aestivum*	Kernels	Primorsky region	2003
341, 503	8	*Triticum aestivum*	Kernels	Leningrad region	2002
452, 455, 504, 507	17	*Triticum aestivum*	Kernels	Leningrad region	2003
448	1	*Triticum aestivum*	Leaves	Leningrad region	2003
266, 329, 348	5	*Triticum aestivum*	Leaves	Krasnodar region	2002
478, 505	14	*Triticum aestivum*	Kernels	Krasnodar region	2003
356	2	*Triticum aestivum*	Leaves	China, Harbin	2002
362	12	*Hordeum distichon*	Kernels	Leningrad region	2002
006, 086	2	*Cirsium arvense*	Leaves	China, Harbin	2002
099, 011	2	*Cirsium arvense*	Leaves	Stavropol region	2002
131	1	*Cirsium arvense*	Leaves	Republic of North Ossetia—Alaniya	2002
127	2	*Helianthus annuus*	Leaves	Belgorod region	2001
016	1	*Phoenix canariensis*	Inflorescence	Krasnodar region	2001
008	1	*Quercus incana*	Old leaves	Krasnodar region	2001
009	1	*Sonchus* sp.	Leaves	Leningrad region	2002
EGS[b] 34-015 (IMI[c] 255532)		*Dianthus* sp.		UK	1981
A. infectoria—EGS 27-193		*Triticum* sp.		UK	1969
A. alternata—EGS 34-016					
(IMI 254138)		*Arachis hypogaea*		India	

[a] Numbers without acronym mean isolates from collection of Ph. B. Gannibal (PHBG), All-Russian Institute of Plant Protection, St. Petersburg, Russia

[b] EGS—E. G. Simmons, Crawfordsville, IN, USA

[c] IMI—CABI Bioscience, Genetic Resources Collection, Surrey, UK

Fig. 1 Location of regions where populations of *A. tenuissima* were sampled

A. alternata (EGS 34-016) and *A. infectoria* (EGS 27-193) were included as outgroup species.

DNA extraction and AFLP

Alternaria tenuissima isolates were grown in Petri dishes containing potato dextrose agar in darkness at 23°C for 1 week. Mycelium was harvested with a sterile scalpel, collected into 2-ml tubes with one 3-mm tungsten-carbide bead and ground using a TissueLyser (Retsch MM301). DNA was isolated with the Puregene® DNA Isolation Kit D-600A (Gentra System, MN), according to the instructions from the manufacturer, with some modifications (Bonants et al. 2000).

The methods used were based on Vos et al. (1995), Bonants et al. (2000) and Eikemo et al. (2004). DNA (approx. 250 ng) was digested in a 40-μl reaction volume with *Eco*RI (5 U) and the *Mse*I isoschizomer *Tru*I (5 U) in RL buffer (10 mM Tris–HAc, pH 7.5, 10 mM MgAc, 50 mM KAc, 5 mM dithiothreitol, 50 ng μl^{-1} bovine serum albumine) for 2 h at 37°C followed by 2 h at 65°C. *Eco*RI (5 pmol) and *Mse*I (50 pmol) adapters (Bonants et al. 2000) were ligated overnight at *ca* 20°C in a 40-μl reaction volume

containing 0.33 µl of ligase (3 U μl^{-1}; Promega), 5 nmol ATP and 30 µl of digested DNA. The cold amplification (pre-amplification) with the 0- (non-selective) primers was performed with 5 µl of 5-times diluted ligation product added to 2.5 µl of 10× PCR buffer (500 mM KCl, 100 mM Tris–HCl pH 8.3, 15 mM $MgCl_2$), 1 U of Taq DNA polymerase (Hoffmann-La Roche, Basel), 0.24 µl of 2.5 mM dNTPs, 2.5 µl of each of the 0-primers (50 ng μl^{-1}) in a 25-µl reaction volume. The amplifications were performed in a Gene Amp 9700 thermal cycler (Applied Biosystems, Foster City, CA) with the following programme: denature 2 min at 94°C, then 45 cycles, where each cycle consisted of 30 s at 94°C, 30 s at 56°C and 90 s at 72°C with final extension for 10 min at 72°C and cooling to 4°C. Products of the pre-amplification were separated on a 1% agarose gel stained with ethidium bromide and visualised using UV illumination. The rest of the pre-amplification products were diluted 20 times and stored at −20°C until used in the selective PCR.

The selective amplification and polyacrylamide electrophoresis were carried out as described by Eikemo et al. (2004). *Mse*I (5′-GATGAGTCCTGA GTAANN) and *Eco*RI (5′-GACTGCGTACCAATTC NN) primers contained two selective nucleotides at the 3′ ends. Primer combinations used for specific amplification were: E12 + M15; E13 + M16; E13 + M17; E14 + M16. For primer E12 the two selective nucleotides were AC, E13—AG, E14—AT, M15—CA, M16—CC and M17—CG. The *Eco*RI primer was labelled with [γ^{33}P] ATP. After electrophoresis the gel was dried and exposed against γ-ray film (Kodak Biomax MR) at −80°C for 2–3 days.

Autoradiographs were examined visually and only unambiguous bands were scored. They were assumed to be independent and those of identical size were assumed to have identical sequence. Bands obtained from all four primer sets were recorded in a 0/1 (absent/present) combined binary data matrix. All primer combinations were run twice, with new selective amplification reactions. Twenty of 101 isolates were included in the second selective PCR.

Statistical analysis

The degree of population subdivision between regions was measured by Wright's fixation index (F_{st}) (Wright 1943), using the software Arlequin version 2.000 (Schneider et al. 2000). The measure of Wright's index is based on the comparison of frequencies of identical alleles within and between groups. It varies from 0 (no isolation) to 1 (complete isolation) but in the case of bias to polymorphic loci, lower values (e.g., 0.5) may indicate complete isolation between populations (Taylor et al. 1999). The null distribution of pairwise F_{st} values under the hypothesis of no difference between the populations is obtained by permuting haplotypes between populations. The *P*-value of the test is the proportion of permutations leading to F_{st} value larger or equal to the observed one (Schneider et al. 2000). Gene flow (N_m) was calculated from the extent of population subdivision (G_{st}) (Slatkin and Barton 1989), using the Popgene 1.32 programme (Yeh et al. 1999). In order to analyze genetic variability between isolates within locations, we calculated gene diversity (H_s) (Nei 1973) using the Popgene software. Gene diversity depends on the number and frequencies of alleles at a locus. Theoretically it can range from 0 to 0.5 for haplotypic data.

Dendrograms were obtained by cluster analysis of all strains using the unweighted pair group method with arithmetic means (UPGMA) based on the Nei and Li (1979) similarity coefficient. The Treecon 3.1b programme was employed for this analysis (Van de Peer and De Wachter 1994). Confidence in specific clusters of the resulting topology was estimated by bootstrap analysis with 1000 replicates. Ordination was carried out by principal coordinate analysis (PCA), based on Nei's genetic distance and computed by the GenAlEx 5.1 programme (Peakall and Smouse 2001). PCA was done with all three populations from wheat and a group of strains collected from barley in Leningrad region.

Results

Amplification of *Alternaria* spp. DNA by each AFLP primer combination produced around 200 bands. Most of them were reproducible with the exception of a few weak ones. A total of 149 bands for all species, obtained from 4 primer sets, were scored, of which 130 were present in *A. tenuissima*. Within *A. tenuissima*, 117 loci (90.0%) were polymorphic (Table 2). There were three groups of 2–3 strains from wheat that had identical AFLP patterns. Four isolates from

Table 2 Information summary for populations of *A. tenuissima* from wheat

Population	No. of isolates	% Polymorphic loci	$H_s^a \pm SD^b$
Primorsky	32	64.6	0.16 ± 0.17
Leningrad	26	56.2	0.14 ± 0.18
Krasnodar	19	51.5	0.15 ± 0.18
Total	101	90.0	0.16 ± 0.18

[a] H_s—Gene diversity

[b] SD—Standard deviation

Leningrad region shared two haplotypes. The third recurrent haplotype was found in two isolates from Leningrad region and one from Krasnodar region. Several groups of isolates had haplotypes that differed only at a few loci.

Calculation of Wright's F_{st} indicated isolation between the Primorsky population and the groups from the European part of the country (Table 3). The F_{st} value for combined groups of isolates from the European part and from the Far East was significantly different from 0 (0.272, $P = 0.05$). F_{st} showed significant, but very small, differences between the Krasnodar and Leningrad populations. Gene flow in general was in accordance with Wright's index (Table 3). The highest measure of N_m was observed between Leningrad and Krasnodar regions.

Gene diversity ranged from 0.14 to 0.16 (Table 2). The Primorsky population was the most diverse one. But the non-considerable difference between sample groups does not allow any conclusion about difference in population age, size of populations or selection intensity.

Dendrograms constructed from each individual AFLP primer combination using UPGMA were in general concordant (data not shown). Analysis of the combined data matrix showed that isolates generally clustered by origin. All *A. tenuissima* isolates were divided into two major groups and four distinct isolates (Fig. 2). But the bootstrap support for major clusters was not high. The first cluster consisted of 64 isolates from Krasnodar and Leningrad regions and from other places in the European part of Russia. Only two wheat isolates from Primorsky region were included in this group. The second cluster included 34 strains: 28 of 32 from the Russian Far East, all four strains from North-East China, one 'barley' strain from Leningrad region and the representative isolate of *A. tenuissima*. Representative isolates of *A. alternata* and *A. infectoria* were clustered separately from *A. tenuissima* at distances of 0.42 and 0.83, respectively.

In contrast to the UPGMA cluster analysis, the two-dimensional plot resulting from PCA showed that the Krasnodar and Leningrad populations tended to form different clusters, with, however, a partial overlap (Fig. 3). Barley isolates from Leningrad region were mixed with both Leningrad and Krasnodar wheat samples. The Primorsky population was completely separated from all the others.

All isolates on the dendrogram (Fig. 2) were mixed independently of the host plant and host organ (seed or leaf). Being collected from different hosts, several pairs of isolates had almost the same haplotypes. For instance, isolate 362-011 from barley and 016-011 from date palm differed at the distances level 0.03. The F_{st} value for Leningrad wheat and Leningrad barley groups of isolates was very low (0.010) and did not significantly differ from 0 ($P = 0.05$). Moreover, comparison between Krasnodar wheat and Leningrad barley populations gave an even smaller value of Wright's index ($F_{st} = 0.001$).

Table 3 Degree of migration and population differentiation

	Primorsky	Leningrad	Krasnodar
Primorsky	–	2.27	2.41
Leningrad	0.276	–	11.49
Krasnodar	0.283	0.061	–

All F_{ST} values significantly differ from 0 at $P = 0.05$ (significance was calculated for F_{st} only)

Gene flow N_m (above diagonal) and Wright's fixation index F_{st} (below diagonal)

Discussion

As previously mentioned, *A. tenuissima* was found on many plant species in various parts of the world. It can infect pistachio leaves in the USA (Pryor and Michailides 2002), apple fruits in South Africa (Serdani et al. 2002), cereal grains in North Europe (Andersen et al 1996; Kosiak et al. 2004) and Russia (Gannibal 2004), English walnut and hazelnut (Belisario et al. 2004), strawberry fruit in Korea (Lee and Kim 2001) and broad bean leaves in Japan (Honda et al. 2001). We have observed *A. tenuissima*

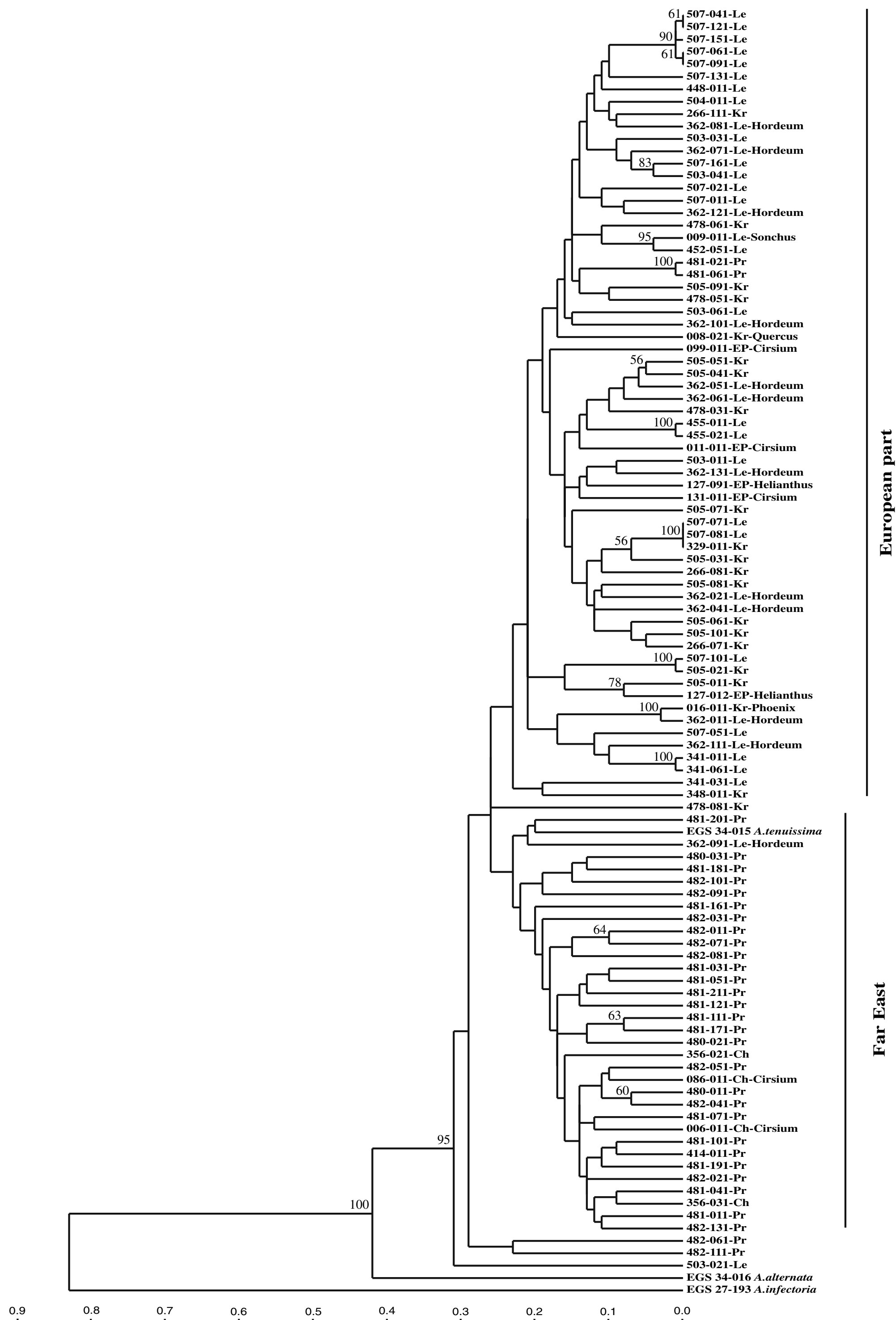

Fig. 2 UPGMA analysis of genetic distance between isolates of *A. tenuissima*. Detailed isolate information (origin and host) is located next to isolate number on the tree. Regions: Kr, Krasnodar; Le, Leningrad; Pr, Primorsky; EP, other locations in the European part of Russia; Ch, China. Absence of the host name means wheat. Numbers above the branches indicate bootstrap values of 1000 replicates. Only bootstrap values >50% are shown

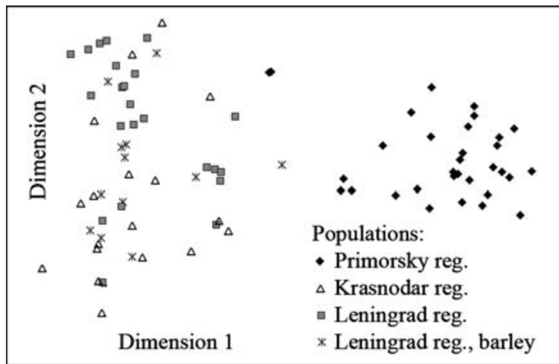

Fig. 3 Two-dimensional display of principal co-ordinate analysis of four groups of *A. tenuissima* isolates

as a common pathogen of leaves of thistle, saw thistle (Ph. B. Gannibal and A. O. Berestetskiy, All-Russian Institute of Plant Protection, Russia unpubl.), apple and many other plants in different regions of Russia (data not shown). Thus, the 'morphological' species *A. tenuissima* does not have substratum specialization. The AFLP results confirmed this conclusion, showing no molecular evidence for the presence of specialized types of *A. tenuissima*. We did not find any strains that were sufficiently different from the type strain to be recognized as a separate species. More likely, this species is the most widespread one in the genus. In a certain sense, this species covers the biggest part of an aggregate of small-spored Alternarias called *A. alternata sensu lato*, while *A. alternata sensu* Simmons is a rather rare species (Simmons 1993).

Whereas the AFLP patterns of the *A. tenuissima* strains revealed no correlation with the species of the host plant, they could be grouped according to their geographic origin. The statistical analyses performed clearly showed the difference between Far Eastern (Primorsky) and European (Krasnodar and Leningrad) populations of *A. tenuissima*. No strains were found with identical or closely related haplotypes representing both geographic areas. This indicates that the populations were separated a relatively long time ago and have had an independent history of development. As a consequence of separation, populations may have different intraregional structure and pathological properties, i.e., aggressiveness or toxin profiles.

The population differentiation result is in complete accordance with the gene flow index showing that migration between the different parts of the country was very rare. We found, however, several examples in which a pair of strains from different regions in European Russia had identical or very similar AFLP patterns. Some isolates collected in the Leningrad region were clustered by two-dimensional component analysis in the Krasnodar group and vice versa. This observation suggests regular bidirectional migration between regions, a hypothesis further supported by the calculated value of the gene flow. Further studies are needed in order to investigate whether *A. tenuissima* isolates originating from the rest of the European territory belongs to the same genetic cluster as those from European Russia.

The genetic difference between populations was correlated with the geographic distance between them. The distance between Primorsky region and Leningrad or Krasnodar region is more than 6,500 km. The latter two regions are less than 2,000 km apart. Conidia can be transported by wind or by human activity. Hundreds of conidia from *Alternaria* spp. have been found per m^3 of air sampled 15 m above ground in Europe, (Angulo-Romero et al. 1999), USA (Dixit et al. 2000) and Australia (Mitakakis and McGee 2000). However, for *Puccinia recondita* it was shown that the general directions of air mass movement prevented exchange of races between the European and Asian parts of Russia (Pavlova and Mikhailova 1997). A high economic activity in the European part of the country can aid migration of the fungus. The direction of *A. tenuissima* migration coincides with the routes of intensive movement of people and goods. In all cases the absence of substratum specificity will encourage migration of clones, since the clone can easily infect almost any plant on its route of migration.

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References

Andersen, B., Thrane, U., Svendsen, A., & Rasmussen, I. A. (1996). Associated field mycobiota on malt barley. *Canadian Journal of Botany, 74*, 854–858.

Andersen, B., Krøger, E., & Roberts, R. G. (2002). Chemical and morphological segregation of *Alternaria arborescens*,

A. infectoria and *A. tenuissima* species-group. *Mycological Research, 106*, 170–182.

Angulo-Romero, J., Mediavilla-Molina, A., & Dominguez-Vilches, E. (1999). Conidia of *Alternaria* in the atmosphere of the city of Cordoba, Spain in relation to meteorological parameters. *International Journal of Biometeorology, 43*, 45–49.

Belisario, A., Maccaroni, M., Coramusi, A., & Corazza, L. (2004). First report of *Alternaria* species groups involved in disease complexes of hazelnut and walnut fruits. *Plant Disease, 88*, 426.

Bonants, P. J. M., Hagenaar-de Weerdt, M., Man in't Veld, W. A., & Baayen, R. P. (2000). Molecular characterization of natural hybrids of *Phytophthora nicotianae* and *P. cactorum*. *Phytopathology, 90*, 867–874.

Dixit, A., Lewis, W., Baty, J., Crozier, W., & Wedner, J. (2000). Deuteromycete aerobiology and skinreactivity patterns. A two year concurrent study in Corpus Christi, Texas, USA. *Grana, 39*, 209–218.

Eikemo, H., Klemsdal, S. S., Riisberg, I., Bonants, P., Stensvand, A., & Tronsmo, A. M. (2004). Genetic variation between *Phytophthora cactorum* isolates differing in their ability to cause crown rot in strawberry. *Mycological Research, 108*, 317–324.

Gannibal, Ph. B. (2004). Small-spored species of the genus *Alternaria* on grasses. *Micologiya i Fitopatologiya, 38*, 19–28 (In Russian).

Honda, Y., Rahman, M. Z., Islam, S. Z., & Muroguchi, N. (2001). Leaf spot disease of broad bean caused by *Alternaria tenuissima* in Japan. *Plant Disease, 85*, 95.

Kosiak, B., Torp, M., Skjerve, E., & Andersen, B. (2004). *Alternaria* and *Fusarium* in Norwegian grains of reduced quality—a matched pair sample study. *International Journal of Food Microbiology, 93*, 51–62.

Lee, H. B., & Kim, C. -J. (2001). First report of strawberry fruit rot caused by *Alternaria tenuissima* in Korea. *Plant Disease, 85*, 563.

Logrieco, A., Bottalico, A., Solfrizzo, M., & Müle, G. (1990). Incidence of *Alternaria* species in grains from Mediterranean countries and their ability to produce mycotoxins. *Mycologia, 82*, 501–505.

Mitakakis, T. Z., & McGee, P. A. (2000). Reliability of measures of spores of *Alternaria* and pollen concentrations in air over two towns in rural Australia. Multiple sites for Burkard sampling. *Grana, 39*, 141–145.

Neergaard, P. (1945). *Danish species of Alternaria and Stemphylium. Taxonomy, parasitism, economical significance*. Copenhagen: Munksgaard, Oxford University Press, 560 pp.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. In *Proceedings of the National Academy of Sciences USA*, 70, 3321–3323.

Nei, M., & Li, W. -H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. In *Proceedings of the National Academy of Sciences USA*, 76, 5269–5273.

Pavlova, T. V., & Mikhailova, L. A. (1997). Role of *Puccinia recondita* Rob. *ex* Desm. f. sp. *tritici* spores migration in fungal population development and spread of epiphytotics. *Micologiya i Fitopatologiya, 31*, 60–66 (In Russian).

Peakall, R., & Smouse, P. E. (2001). *GenAlEx V5: Genetic analysis in Excel. Population genetic software for teaching and research*. Canberra, Australia: Australian National University.

Pryor, B. M., & Michailides, T. J. (2002). Morphological, pathogenic, and molecular characterization of *Alternaria* isolates associated with *Alternaria* late blight of pistachio. *Phytopathology, 92*, 406–416.

Roberts, R. G., Reymond, S. T., & Andersen, B. (2000). RAPD fragment pattern analysis and morphological segregation of small-spored *Alternaria* species and species groups. *Mycological Research, 104*, 151–160.

Rotem, J. (1994). *The genus Alternaria. Biology, epidemiology and pathogenicity*. St. Paul, Minnesota: APS Press, 326 pp.

Schneider, S., Roessli, D., & Excoffier, L. (2000). *Arlequin ver. 2.000. A Software for population genetic data analysis*. Geneva, Switzerland: Genetics and Biometry Laboratory, University of Geneva.

Serdani, M., Kang, J.-Ch., Andersen, B., & Crous, P. W. (2002). Characterisation of *Alternaria* species-groups associated with core rot of apples in South Africa. *Mycological Research, 106*, 561–569.

Simmons, E. G. (1990). *Alternaria* themes and variations (27–53). *Mycotaxon, 22*, 79–119.

Simmons, E. G. (1993). *Alternaria* themes and variations (63–72). *Mycotaxon, 48*, 91–107.

Simmons, E. G. (1995). *Alternaria* themes and variations (112–144). *Mycotaxon, 55*, 55–163.

Simmons, E. G. (1999). *Alternaria* themes and variations (236–243). Host-specific toxin producers. *Mycotaxon, 70*, 325–369.

Simmons, E. G., & Roberts, R. G. (1993). *Alternaria* themes and variations (73). *Mycotaxon, 58*, 109–140.

Slatkin, M., & Barton, N. H. (1989). A comparison of three indirect methods for estimating average levels of gene flow. *Evolution, 43*, 1349–1368.

Taylor, J. W., Geiser, D. M., Burt, A., & Koufopanou, V. (1999). The evolutionary biology and population genetics underlying fungal strain typing. *Clinical Microbiology Reviews, 12*, 126–146.

Van de Peer, Y., & De Wachter, R. (1994). Treecon for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Computer Applications in Bioscience, 10*, 569–570.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., & Zabeau, M. (1995). AFLP—a new technique for DNA-fingerprinting. *Nucleic Acids Research, 23*, 4407–4414.

Webley, D. J., & Jackson, K. L. (1998). Mycotoxins in cereals—a comparison between North America, Europe and Australia. *Australian Postharvest Technical Conference*, 63–66.

Webley, D. J., Jackson, K. L., Mullins, J. D., Hocking, A. D., & Pitt, J. I. (1997). *Alternaria* toxins in weather-damaged wheat and sorghum in the 1995–1996 Australian harvest. *Australian Journal of Agricultural Research, 48*, 1249–1255.

Wright, S. (1943). Isolation by distance. *Genetics, 28*, 114–138.

Yeh, F. C., Yang, R., & Boyle, T. (1999). *Popgene version 1.31. Microsoft Windows-based freeware for population genetic analysis*. Canada: University of Alberta.