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Migration and Selection Enforced Multiple Phenotypic and Genotypic Changes in the Population of *Phytophthora infestans* in Israel During the Last 36-Year Period

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Abstract. Late blight caused by the oomycete pathogen *Phytophthora infestans* is a devastating disease of potato and tomato worldwide, including Israel. The population structure of this pathogen was monitored in potato and tomato fields in Israel during a 36-year period of 1983-2019. Isolates of the pathogen were tested for sensitivity to phenylamide fungicides, mating type, race structure, and genotype. The phenotypic and genotypic structure of the population from potato have changed greatly from one year to another, from one season to the next, within a season and within a single field. Major changes also occurred in the population collected from tomato crops. The mechanisms driving these multiple changes and the heterogeneous nature of the population in Israel are shown to derive from multiple migration events of the pathogen via seed tubers from Europe and from fitness-driven selection processes.

Keywords: late blight; mating type; mefenoxam; metalaxyl; potato; SSR genotyping; race structure; tomato.

1. Introduction

Late blight incited by the oomycete *Phytophthora infestans* (Mont. De Bary) is one of the most devastating diseases of potato and tomato worldwide responsible for major crop losses (Anderson et al, 2004). It has a notorious history as the cause of the Irish Potato Famine in the 1800s (www.historyplace.com). The global economic burden of productivity loss and associated costs has been quantified at more than $6 billion per year (Haverkort et al., 2008).

P. infestans can easily overcome control strategies including host resistance and fungicides. Its lifestyle helps it to evade plant defenses, effectors that suppress host defenses and promote susceptibility, profuse sporulation with a short latent period that enables rapid dissemination, and a genome structure that promotes the adaptive evolution by fostering genetic diversity (Leesutthiphonchai et al., 2018).

This algal-like oomycete is a diploid heterothallic species, requiring mating of A1 and A2 mating types to form sexual oospores that might be an important source of primary inoculum (oospores) generating genetically diverse populations (Fry, 2016; Klarfeld et al., 2009; Martin et al., 2019). Some clonal lineages have been shown to be triploid, capable of producing offspring progenies when crossed with diploid or triploid isolates of opposite mating type (Hamed and Gisi, 2013). All progenies from sexual populations in nature are diploid, whereas nearly all dominant asexual lineages, including the most important pandemic lineages US-1 and 13_A2 are triploid. Such triploids possess significantly more allelic variation than diploids (Li et al 2017).

A series of epidemiologically successful clonal lineages that were distributed primarily by trade of seed tubers or young plants have dominated in many key production areas (Fry, 2016). The FAM-1 lineage was the primary wave of infection from Mexico to North America and Europe in the 19th century. It was displaced by the US-1 lineage, which dominated global populations for decades and remains prevalent in some regions. The major US-1 migration consisted of the A1 mating type. Subsequent migrations into Europe and USA from Mexico included both mating types and genetic diversity has increased. Clonal lineages such as US-8 and, more recently, US-22 and US-23 in North America and 13_A2 in Europe have emerged to become locally dominant (Fry, 2016; Martin et al., 2019).

The movement of *P. infestans* between continents adds to the complexity of controlling late blight. This has resulted in the introduction of strains with novel virulence, increased fitness, fungicide resistance and of the opposite mating type to the one already present (Njoroge et al 2019).

Isolate characterization may be studied by means of several phenotypic and genotypic markers. Common phenotypic markers are mating type, virulence spectrum, and metalaxyl resistance (Cohen, 2000). Genotypic markers include multilocus simple-sequence repeats (SSRs) that, due to their higher repeatability and resolution, have been selected as the standard genotyping strategy for *P. infestans*

(Cooke and Lees 2004; Lees et al., 2006). These SSR markers are embedded in the EuroBlight monitoring (www.euroblight.net) and USAblight (usablight.org) systems. SSRs and mitochondrial haplotypes are valuable tools for examining pathogen dispersal and the evolutionary history of *P. infestans* populations (Martin et al., 2019).

Diversity studies that consider both phenotypic and genotypic markers have enabled an understanding of population dynamics which has been useful for disease management. Knowing the population structure of *P. infestans* within a region helps to understand whether different strains have arisen and increased in frequency over time, hence providing a better insight into pathogen migrations. Disease management strategies can then be refined by knowledge of the phenotypic characteristics of the prevailing pathogen strains such as virulence profile or fungicide sensitivity. *P. infestans* populations can differ dramatically among countries and locations, and predictions concerning their phenotypic behavior need to be based on the correct population structure in a region (Njoroge et al 2019). For example, two migration cases from Europe to sub-Saharan Africa were recently reported: the EU_33_A2 lineage in Plateau State in Nigeria and the EU_13_A2 clone in potato crops in Senegal (Cooke et al. 2019).

 According to Fry (2016), migration followed by selection has led to rapid changes in populations of *P. infestans* over large regions. Because of such rapid population shifts, the characterization of a population in one year might not necessarily predict the population in the future. He stated that sexual recombination contributes little or nothing to the population diversity in a region even if both mating types are present (Fry, 2016).

Major changes in population structure of *P. infestans* were reported from Europe. The proportion of metalaxyl-resistant isolates fluctuates from year to year and within the season (Gisi and Cohen, 1996). Almost concurrent with the appearance of resistant isolates was the discovery of the A2 mating type of *P. infestans* in many European countries and in other parts of the world including Israel. No genetic correlation was found between resistance and mating type. Inheritance studies showed that resistance to metalaxyl is controlled by a single semi dominant gene. F1 isolates produced by mating of sensitive and resistant parental isolates of opposite mating types were partially resistant (Gisi and Cohen, 1996). Resistant isolates express equal or greater fitness than sensitive isolates. No correlation was detected between resistance and race structure (Gisi and Cohen, 1996).

In Spain, isolates of *P. infestans* collected during 2003-2005 belonged to the 2_A1 or 3_A2 lineage whereas those collected during 2011-2015 belonged to the 13_A2 lineage (Alor Romero et al. 2019). In Turkey, all isolates collected during 2013-4 were sensitive to metalaxyl, most of them were A1, haplotypes Ia, and belonged to lineage US-1 or US-6 (Gunacti, et al 2019) whereas isolates collected in 2015-17, were resistant to mefenoxam, A2 mating type and haplotype Ia, and belonged to lineage 13_A2

(Gore et al. 2019). In both reports, the authors speculated that the potato varieties imported in Turkey from other countries could be the reason for these changes.

According to Cooke et al (2019), the most obvious change in EU population in the years 2016-2018 was the decline in the combined frequency of the clones EU_13_A2, EU_6_A1 and EU_1_A1 from 60 to 40% of the population and the increase from 10 to 36% of the clones EU_36_A2, EU_37_A2 and EU_41_A2 in the sampled population.

  Major changes in population structure of *P. infestans* were reported from U.S. Most clonal lineages were resistant to mefenoxam from the mid-1990s to 2009. However, recent lineages have been largely sensitive to mefenoxam. Savile et al. (2015) reported that the US-8 and US-11 clonal lineages were insensitive to mefenoxam while the US-20 to US-24 clonal lineages were sensitive to mefenoxam. These lineages are currently incompatible with tomatoes carrying the *Ph2* and *Ph3* genes for resistance (Fry 2016). A recent study revealed that sensitivity to mefenoxam in USA isolates is associated with two SNPs (single nucleotide polymorphism) whereas mating type is associated with one SNP (Ayala-Usma et al, 2019).

Three major genetic changes occurred in the Israeli population of *P. infestans* during the 18 years period of 1983-2000 (Cohen, 2002). The first in 1983, when resistance to metalaxyl and the A2 mating type were introduced. The second in 1993, when the A2 population was replaced by the A1 mating type while isolates with intermediate resistance to metalaxyl first appeared and the third in 1999, when A1-sensitive population with extreme virulence variation and high aggressiveness to tomato dominated. It was then concluded that these shifts in population structure might partially reflect the changes that occurred in Europe from which potato tubers were annually imported, but also implicate a rapid local evolution of new genotypes.

More than 20 cultivars of potato are grown in Israel in two seasons, autumn and spring. In the autumn, sowing takes place in September (with locally produced seed tubers) and harvest in the following December-January. In the spring, sowing (with seed tubers imported from Europe) takes place in January and harvest in the following May. Potato crops in both seasons are exposed to winter rain (November-March) or sprinkling overhead irrigation, which facilitates late blight epidemics and enables oospore production in the bottom leaves (Cohen et al 2000). Tomato are grown in greenhouses all year around, and in the summer, also in the open field.

Multiple sources of primary inoculum may initiate epidemics of late blight in potato or tomato in Israel: infected (symptomatic or latent, imported or locally produced) potato seed tubers (Kadish et al. 1990; Kadish and Cohen,1992; Galperin et al 2018), volunteer potato plants from the previous season, solanaceous species such as *Petunia hybrida* and *Withania somnifera*, seed-borne oospores in tomato

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(Rubin et al 2001; Rubin and Cohen, 2004; 2006) and soil-borne oospores (Cohen et al 1997; 2000). During the season, landing of air borne sporangia from upwind fields of potato or tomato contribute to further enhancing the epidemics. Our data show that an infection event occurs when the following conditions prevail: RH of ≥ 90% at temperature of 10-21^{0}C for a period of ≥6h (Galperin et al 2018).

In the current study we followed the annual and seasonal changes in the population structure of *P, infestans* in Israel since the last monitoring survey. Isolates that were collected during 2001-2019 were examined for phenotypic traits. Isolates that were collected during 2004-2019 were also tested for their genotypic traits. The results confirmed the high importance of imported tubers from Europe as a source of primary inoculum but also revealed the importance of local conditions in survival of some but not all immigrants. Some preliminary data on these findings were published (Shamba et al 2017; Cohen et al 2019: Hermann et al. 2019).

2. Results

2.1 Potato isolates

2.1.1 Phenylamide sensitivity

Data in Fig. 1 show the frequency of sensitive (S), intermediately resistant (I), and resistant (R) isolates among 2024 isolates collected during the 36-year period of 1983-2019. Resistant isolates dominated the population for 9 years, from 1983, until the first I isolates appeared in 1993. The frequency of R isolates reached another high peak of 61% in 2010 (Fig.1), which was an unusual rainy year. In the last 5 years, 12-51% of the isolates were resistant. Except 1994 and 2013 in which no I isolates were detected, I isolates comprised 2-60% of the population (Fig.1). The frequency of S isolates reached highest levels during 1993-1995 and 2011-2013. In 2018 the frequency of S, I, and, R isolates was 10, 55 and 35% and in 2019- 40, 48 and 12%, respectively (Fig.1).
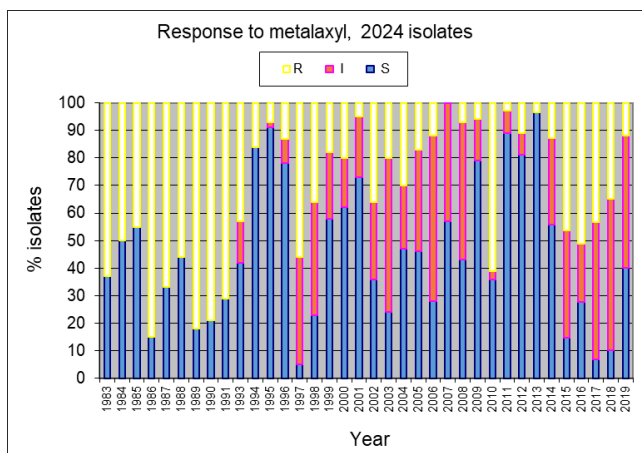
Figure 1. Frequency of phenylamide-sensitive, intermediate and resistant isolates of *Phytophthora infestans* in potato crops in Israel during the period of 1983-2019. Data of 1983-2000 were taken from Cohen (2002). Total number of isolates tested=2024. Detached potato leaves (*cvs*. Nicola or Mondial or tomato leaves *cv*. ZH), laid on a wet filter paper in Petri dished at 20^0C, were sprayed with 0, 1, 10 or 100 ppm ai of metalaxyl or mefenoxam and thereafter inoculated with sporangia of the test isolate. An isolate that sporulated on leaves treated with 0 and 1 ppm ai was considered sensitive; An isolate that sporulated on leaves treated with 0, 1 and 10 ppm ai was considered intermediate; An isolate that sporulated on leaves treated with 0, 1, 10 and 100 ppm ai was considered resistant.

2.1.2 Mating type

Data in Fig. 2 show the frequency of mating types among 1757 isolates collected during the 36 years period of 1983-2019. Not shown are 10 isolates that were homothallic and 42 isolates that were sterile. In the first nine years of the survey, 1983-1991, only A2 isolates occurred in the population (Fig. 2). As shown in Fig.1, these A2 isolates were R or S to metalaxyl. A1 isolates were first detected in 1993. They dominated the population for 17 years, from 1993 until 2009. Their frequency declined sharply in 2010 and remained low for additional 4 years. A1 has re-dominated the population during 2015-2019 (Fig.2). A unique mating type, A1A2, occurred at low frequency in potato fields. It was first detected in 2004 (Fig.2). An A1A2 isolate produces no oospores when inoculated singly onto potato or tomato leaves but produces them when mix-inoculated with either A1 or A2 reference isolate. Due to the heavy rains that prevailed in 2010, late blight epidemics were unusually severe. During that year, 29% of the samples collected from potato fields carried both A1 and A2 mating types (Fig.2).
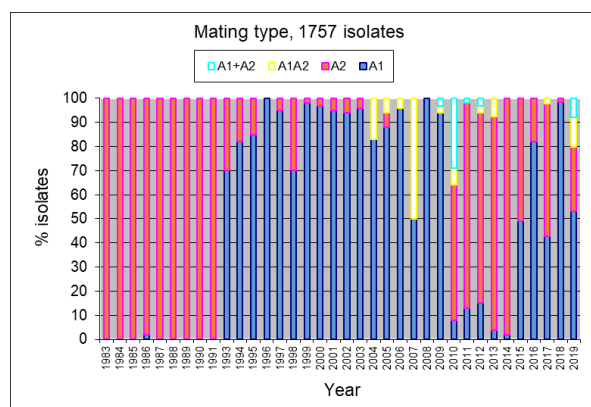

Figure 2. Frequency of mating types A1; A2; A1A2; A1+A2 among isolates of *Phytophthora infestans* collected from potato fields in Israel during the period of 1983-2019. Total number of isolates tested=1757. Data of 1983-2000 were taken from Cohen (2002). An A1 isolate produced oospore only when co-inoculated together with an A2 reference isolate onto detached leaves of potato or tomato. An A2 isolate produced oospores only when co-inoculated together with an A1 reference isolate onto detached leaves of potato or tomato. An A1A2 isolate failed to produce oospores when inoculated singly but did produce oospores when

co-inoculated together with either an A1 or an A2 reference isolate. An A1+A2 isolate is a mixture of A1 isolate (s) and A2 isolate (s) in a single lesion.

2.1.3 Genotype

 SSR genotype analysis was employed to 651 isolates that were collected from potato crops during a 16-year period of 2004 to 2019. Results given in Fig.3A show that four genotypes occurred in the country, US-7 like, 23_A1, 13_A2 and 36_A2 at a proportion of 9.21, 79.26, 10.75 and 0.77%, respectively. Genotype 23_A1 was major in the population every year but was temporarily over dominated by US-7 like during 2008-2009 and by 13_A2 in 2016 (Fig.3B). Genotype 13_A2 appeared in 2010, reached a peak in 2016 and 2017 and disappeared in 2018-2019 (Fig.3B). Genotype 36_A2 appeared in 2018 and again in 2019 (Fig.6 A, B).
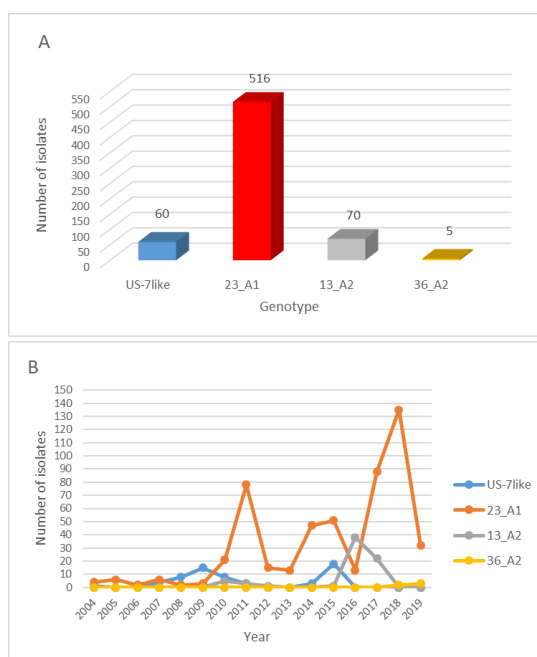

Figure 3. Frequency of genotypes among isolates of *Phytophthora infestans* collected from potato crops in Israel during the period of 2004-2019. A- Overall occurrence of the genotypes during 2004-2019. B- Yearly occurrence of the genotypes during 2004-2019. Total number of isolates tested=651.

2.1.4 Race structure

Race structure analysis was employed to 1165 isolates that were collected from potato fields in the autumn (Nov-Jan) and the spring (Feb-May) growing seasons during the 16-year period of 2004 to 2019. Among the many isolates collected in Nov and Dec (n=219 and 280, respectively), 61 and 63 race combinations were identified. In Jan and Feb, the number of isolates and race combinations declined. In

March, the number of isolates increased to 264; among them, a high number of 94 races were identified. In Apr, both figures declined, reaching in May 63 isolates with 34 race combinations (Fig. 4A).

Virulence factors 1, 3, 4, 7, 9 were predominant (Fig.4B). Thus, the mean frequency of virulence factors 1, 3, 4, 7 and 9 during 2004-2019 was 91, 85, 92, 91 and 85%, respectively (Fig 4B). The next frequent virulence factors were 2, 6, and 11, prevailing in 26-28% of the isolates. Factors 5, 8 and 10 were the rarest, occurring in only 18-20% of the isolates (Fig. 4B). Data in Fig.4 C show that isolates which carry five virulence factors were most frequent (19.3-55.0%) during Nov to April. In May 12.7, 22.2 and 14.3 % of the isolates carried 9, 10 and 11 virulence factors, respectively.
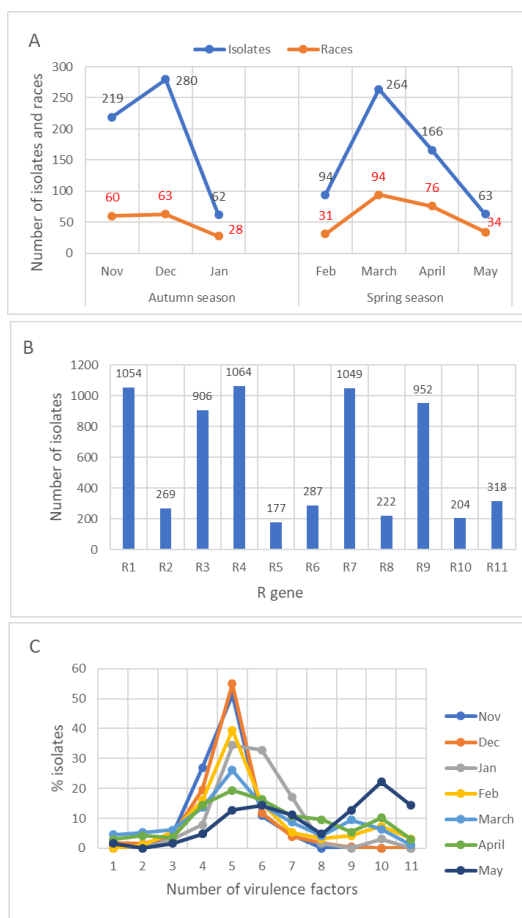

Figure 4. Race structure of 1165 isolates of *Phytophthora infestans* collected from potato crops in Israel during 2004-2019. A- Total number of isolates and the number of race combinations identified during Nov-May. B- The number of isolates which carry virulence factors 1-11 that overcome potato resistance genes *R1-R11*. C- Distribution of virulence factors among isolates during Nov –May. Note that isolates collected in May carry highest number of virulence factors.

Race structure in the 1983-2000 population (Cohen, 2002) was compared to that of 2004-2019 (Figs. 5 and 6). Fifty-six races were identified among 324 isolates in 1983-2000, as compared to 183 races identified among 1114 isolates in 2004-2019 (Fig.5).
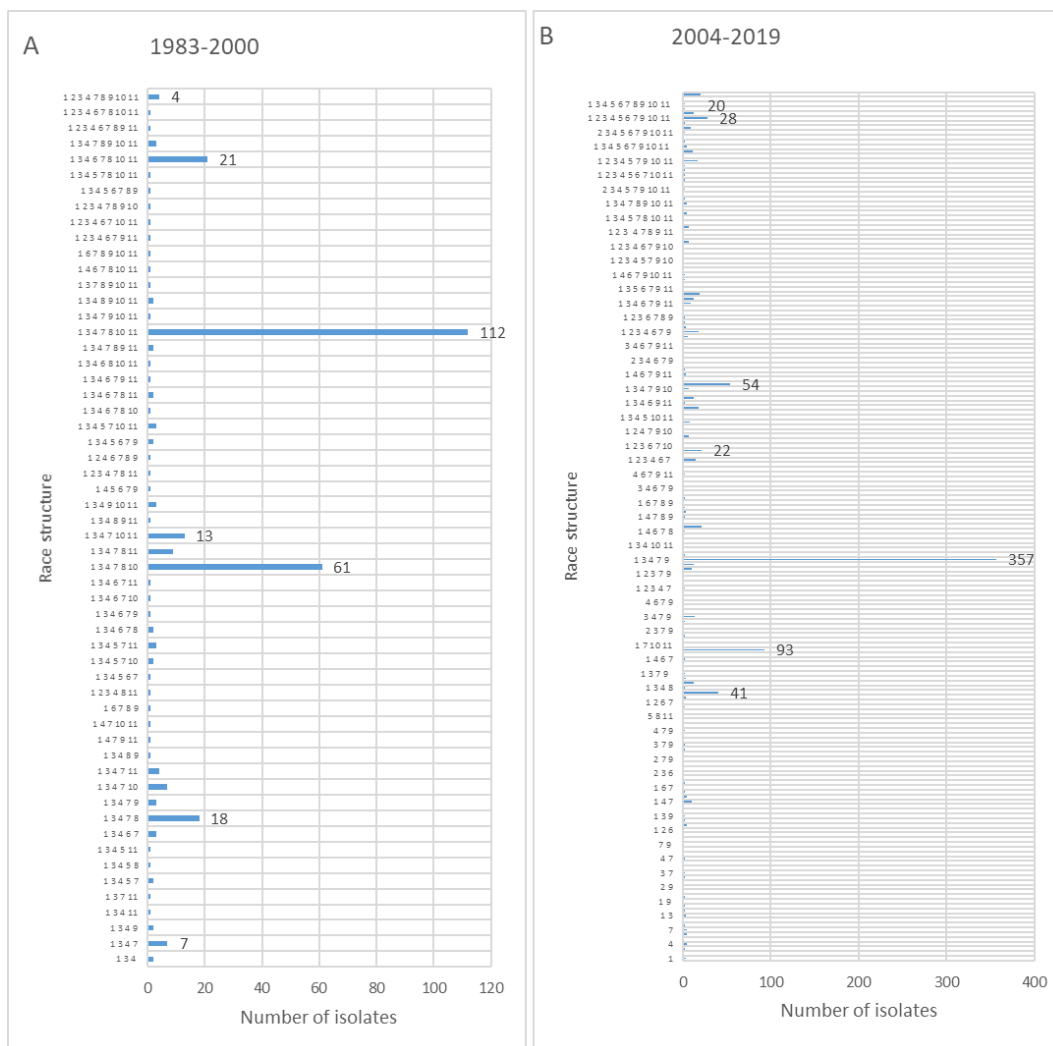
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Figure 5. Races of *Phytophthora infestans* that occurred in potato crops in Israel during two monitoring periods. A- 1983 to 2000 (234 isolates, 56 races, according to Cohen, 2002). B- 2004 to 2019 (1155 isolates, 183 races).

The most frequent races (≥ 2.0%) during 1983-2000 were 1 3 4 7 8 10 and 1 3 4 7 8 10 11, comprising 35% and 19% of the population, respectively (Fig. 6A) whereas race 1 3 4 7 9 was predominant during 2004-2019, comprising 31% of the population (Fig. 6B). No complex races (9-11 virulence factors) were detected in 1983-2000 (Fig. 6A), whereas 4% of the isolates in 2004-2019 were complex, carrying 10 or 11 virulence factors (Fig.6B). The frequency of factors 1-7 was very similar in the two populations. However, factor 8 declined from 78% in 1983-2000 to 9.4% in 2004-2019 whereas factor 9 increased from 11% to 81%. Smaller increments occurred with factor 10 (38%) and factor 11 (49%) (Fig. 6C).
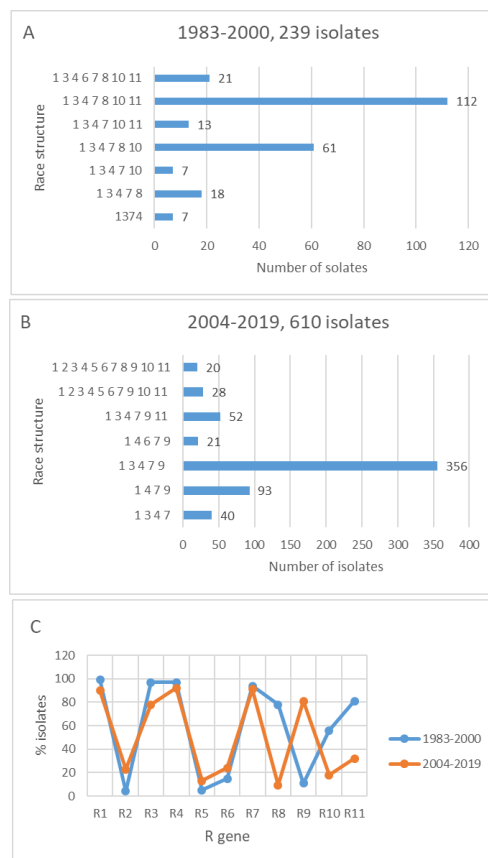
Figure 6. Number of the most common (≥ 2.0%) races of *Phytophthora infestans* that occurred in potato crops in Israel during two monitoring periods. A- 1983 to 2000 (according to Cohen, 2002). B- 2004 to 2019. C- Frequency of virulence factors that overcome resistance genes *R1-R11* in the two populations. Note the major changes in R8-R11.

2.1.5 Latent infection in imported seed tubers

On January 11, 2017, 36 days after planting, five plants with foliar symptom of late blight were detected. No blight symptoms were observed on the surface of the mother seed tuber when the blighted plants were uprooted from the soil but necrotic symptoms typical to late blight were observed on the below-ground emerging stems. Typical sporulation of *P. infestans* developed on tuber slices which were cut away from the symptom-less mother seed tubers after surface disinfection with hypochlorite. The isolates recovered from these tubers were 13_A2, R to MFX and A2 mating type. They carried 10 virulence factors 1 2 3 4 5 6 7 9 10 11.

2.1.6 Population structure within a single field

 Major genotypic and phenotypic changes occurred in a single organic plot within a month. In April, 42 and 58% of the isolates belonged to genotype 23_A1 and 13_A2, whereas in May, 77

and 23 % of the isolates belonged to genotype 23_A1 and 13_A2, respectively. Races became more complex in May compared to April. In April, only four isolates (11.8%) were complex carrying 10-11 virulence factors, whereas in May, 20 isolates (48.8%) carried that number of virulence factors. The mean number of virulence factors per isolate was 5.8±2.6 in April as against 7.3±2.4 in May.

3.1 Tomato isolates

3.1.1 Phenylamides sensitivity

During 1983-1991, the population comprised of R and S isolates (Fig.7A), as were the potato isolates during this period. The first I isolate appeared in 1993. During 1993-2016, S, I and R isolates comprised the population in various proportions (Fig.7A). The ratio between S: I: R during 2015-2016 was about 1:1:1.

3.1.2 Mating type

Only A2 isolates comprised the population during 1983-1991 (Fig.7B). First A1 isolates appeared in 1993. They dominated the population for 17 years until 2009. A2 isolates re-dominated the population during the next 5 years from 2010 to 2014 (Fig.7B). A1 re-dominated the population during 2015-2016. Interestingly, in 2005-2014, a few isolates were A1A2, namely producing oospores when mated with either A1 or A2 reference isolates. In 2010, a heavily rainy year, about one third of the isolates were a mixture of A1 and A2 mating types (Fig.7B).

3.1.3 Virulence

Tomato isolates carried 1-4 virulence factors during 2004-2014 (Fig. 7C). In 2015 and 2016, 19 and 14% of the isolates, respectively, carried all five virulence factors 1, 1.2, 1.3, 1.2.3 and 1.2.3. 2,3. (Fig. 7C). Such complex races can infect and sporulate on all five differential lines of tomato, including NC 45, which carry both *Ph2* and *Ph3*.

3.14. Genotype

The most prominent genotypes were US7-like and 23_A1. In 2010, genotype 13_A2 appeared for the first time. This genotype dominated the population in 2016 (Fig. 7D). In early March 2019, an unusual epidemic of late blight occurred in open-field tomato crops. Plants showed severe infection on basal stem and bottom leaves soon after planting, suspecting seed infection (Rubin and Cohen, 2004). All five isolates collected from these tomato plants belonged to genotype 23_A1.

Figure 7. Phenotypic characteristics of *Phytophthora infestans* isolates collected from tomato crops in Israel during 1983-2016. A- Sensitivity to metalaxyl (1983-2005) or mefenoxam (2006-2016). Total number of isolates = 1839. B- Mating type. Total number of isolates = 1608. C- Virulence of *Phytophthora infestans* isolates to tomato differential lines. Total number of isolates = 352. D- Genotypes of *Phytophthora infestans* isolates collected from tomato crops in Israel during 1983-2016, Total number of isolates=430.

3. Discussion

In a previous study (Cohen 2002), we reported that the Israeli population of *P. infestans* underwent three major genetic changes during 1983-2000. We then concluded that these shifts may partially reflect the changes that have occurred in Europe from which potato tubers are annually imported, but also implicate a rapid local evolution of new genotypes.

In the present study, we show that the population of *P. infestans* in Israel has continued to change during 2001-2019. Sensitivity to phenylamide (PA) fungicides (metalaxyl or mefenoxam) fluctuated, mating type replacement occurred frequently, new races displaced the old ones, and new genotypes showed up.

The frequency of S and R isolates showed opposite sinusoidal shapes. The frequency of resistant isolates had several peaks, of which the pick of 1985-1991 was highest (Fig. 4C). Isolates with intermediate resistance to PA fungicides appeared in 1993, parallel to the appearance of A1 isolates in the country, suggesting a possible role of oospores in their appearance. This corroborates with our earlier finding that hybrid isolates produced by mating of R and S isolates were intermediately resistant to PA fungicides (Table 2 in Gisi and Cohen, 1996). The proportion of I isolate reached a level of ≥50% in 2003, 2006 and 2017-9, a year or two after the S isolates reached their peak. Whether the I isolates in Israel have evolved locally or imported from Europe needs a further study.

The mating type picture for 1983-2019 shows four clear phases: 1983-1991, all isolates were A2; 1993-2009- most isolates were A1; 2010-2014- most isolates were A2; 2015-2019- most isolates were A1. The reasons for this back and forth behavior of the population is not clear. In 2004, a new mating type A1A2 comprised 17% of the population. It reached in 2007 a remarkable proportion of 50% of the population. This mating type is bisexual (not homothallic), capable of producing oospores when mated with either A1 or A2 reference isolates, but not when inoculated alone. In the rainy year of 2010, about 28% of the isolates were composed of both A1 and A2 isolates in the same lesion.

We identified four genotype of *P. infestans* in Israel: US7-like, 23_A1, 13_A2 and 36_A2 at a proportion of 9.21, 79.26, 10.75 and 0.77%, respectively. Genotype US7-like occurred from 2004 to 2015. It was absent in the last three years. Genotype 23_A1 was major, recorded from 2004 until 2019. It was the most frequent genotype in the population in 13 out 15 years of this survey. Genotype 13_A2 appeared in 2010, reached a high proportion in 2016, 2017, and disappeared in 2018, suggesting that 13_A2 was not as fit as 23_A1. Two isolates of genotype 36_A2 appeared in 2018 and 3 in 2019. Interestingly, 23_A1, prevailing in Israel since 2004, is now rarely sampled in Europe, indicating its high fitness and survivability under the local Israeli conditions. Indeed, the adaptation of isolates to various temperatures were recently shown to be genetically associated with a few SNPs (Ayala-Usma et al, 2019). The European genotypes 1_A1, 6_A1, 37_A2, and 41_A2 (www.euroblight.net) have not been sampled in Israel.

In our previous survey of 1983-2000, we identified 56 race combinations. The most frequent races were 1 3 4 7 8 10 (18.8% of the isolates, 6 virulence factors) and 1 3 4 7 8 10 11 (34.6% of the isolates, 7 virulence factors). Only 1.9% of the races were more complex, carrying nine virulence factors. No race was found to carry 10 or 11 virulence factors. During the current monitoring period of 2004-2019 we detected 183 race combinations of which, race 1 3 4 7 9 was most frequent (31.2% of the isolates, 5 virulence factors). However, 2.5 and 1.8 % of the races were complex, carrying 10 and 11 virulence

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factors, respectively, indicating on expanded cultivar range of the population in the last decade. Such complex races were frequent in May, at the end of the spring season, suggesting adaptation to higher temperatures.

There are several reasons that might be responsible for the extreme diversity of the pathogen population in Israel and the rapid changes between years, between seasons and within a season or a field:

  (i) Annual migration of *P. infestans* from Europe is probably the major reason for the shift in population structure between years in Israel. We found that 1% of healthy-looking imported seed tubers carried latent infection with *P. infestans*. Genotypes, such as 13_A2 and 36_A2, showed up in Israel a year or two after their emergence in Europe. Nevertheless, many genotypes that are prevalent in Europe did not appeared in Israel, probably due their low fitness in potato tubers or low fitness under Israeli conditions. Tuber trade was also responsible for the rapid shift in the population structure in Turkey (Gore, et at 2019; Gunacti, et al., 2019). On the other hand, the lack of potato tuber trade between China and India was shown to prevent gene flow of *P. infestans* between the two countries (Wang et al 2019). Similarly, in Peru the same four genotypes (EC-1, US-1, PE-3 and PE-7) persisted during the last two decades because import of seed potato from other continents is nonexistent and therefore, the risk of new lineages from overseas is low (Lindqvist-Kreuze et al, 2019)

  (ii) The shift in the population structure from one year to the next may also be related to the differential over-seasoning capacity of the isolates harbored in locally produced seed tubers, volunteer potato plants, or wild Solanaceae plants (Kadish and Cohen, 1992).

  (iii) The rapid changes in the population structure that happen within a season, even in a single field within a month, might be attributed to fitness parameters that drive the outcome of the competitions between isolates (Kadish and Cohen 1988b; Kadish and Cohen 1988c; Kadish et al., 1990). Genotype 23_A1 that have emerged in Europe persisted well in Israel but not in Europe probably because it is better adapted to higher temperatures. As indicated by Ayala-Usma et al (2019), isolates may carry specific SNPs that are associated with their ability to grow at 15, 20 or 25^0C.

  (iv) Israeli isolates of *P. infestans* recovered from potato were all infective to tomato and *vice versa*, suggesting a continuous cross talk between the two populations. The rapid changes in the tomato population, including the appearance of highly complex races which can attack *Ph2.Ph3* cultivars, seems to be driven by complex mechanisms. Tomatoes grown indoors in autumn and spring in the vicinity of potato crops may get infected by potato isolates growing outdoors. On the other hand, tomatoes which are prone to infection with oosporic variants of the pathogen arising from seed-borne oospores (Rubin et al., 2001; Rubin and Cohen, 2004; Cohen and Rubin, 2006) may transmit such variants to potato crops. During the summer season tomato isolates are exposed to fungicide and high temperature selection. They may infect potato crops in the following autumn.

(v) Soil-borne oospores (Cohen et al., 1997; 2000; Kiiker et al., 2018) or seed-borne oospores (Rubin et al., 2001; Rubin and Cohen, 2004; Cohen and Rubin, 2006) may lead to the emergence of new variants of the pathogen. SSRs analyses performed in this study did not provide positive evidence on the occurrence of recombinant isolates in the Israeli population of *P. infestans*. The role of oospores in the epidemiology of the disease in Israel needs further investigation.

About 25 European potato cultivars are imported to Israel every year. The European countries from which potato seed tubers are imported to Israel on a yearly basis (for sowing in spring season) are France, Holland, Germany and Scotland. They present a rich spectrum of susceptibility/partial resistance to late blight. The influence of host resistance on population structure of *P. infestans* in Israel is unknown. The fact that the population could shift strongly on one cultivar within a single season may indicate on a limited role of host genetics on the population structure.

4. Methods and Materials

4.1 Sample collection

This survey reports on samples collected during 2001-2019 from commercial potato fields distributed in the Western Negev (Fig.8), and samples collected during 2004-2016 from commercial tomato crops throughout the state of Israel.

Figure 8. Map of Israel showing the Western Negev region (green circle) where from late blight infected potato leaves were sampled for this study.

Blighted foliage of potato or tomato were collected by county agents and shipped overnight to the laboratory at Bar-Ilan University. Infected leaves were placed in moistened petri dishes at 15°C for 15h in the dark to enhance sporulation of the pathogen. Sporangia were collected from a single leaflet into ice-cold glass-distilled water and used for inoculation bioassays. Potato leaf samples were collected during the epidemic season, namely November through May, whereas tomato samples were collected throughout the year.

4.2 Bioassays

Sporangia from each sample (isolate) were used for three bioassays: sensitivity to phenylamide fungicides (PA, metalaxyl or mefenoxam, MFX), compatibility with differential cultivars (virulence phenotype) and mating type determination. Sensitivity to PA was conducted as described by Kadish and Cohen (1988a). Pathogenicity assays to determine virulence factors in the pathogen were done with the 12 standard potato differentials, *R0* to *R11* (Black et al 1953) obtained from Syngenta Crop Protection, Stein, Switzerland. Plants were clonally reproduced in vitro from stem segments, and then grown in 1-liter pots in the greenhouse. Leaflets were detached, placed in moistened petri dishes, lower side uppermost, and inoculated each with six 10-µl droplets (5,000 sporangia per ml), two leaflets per potato genotype. Plates were incubated in 18°C growth chambers with a 12h photoperiod for 10 days. Reaction to the pathogen was assessed as either compatible (expanded lesions with sporulation) or incompatible (hypersensitive response). Virulence factors of an isolate are given as a series of numbers representing the *R*-genes of the potato genotypes that produced compatible interaction with that isolate. In all tests, tomato leaflets (inbred ZH carrying no resistance genes) and potato Bintje (no resistance genes) were included. Mating type assays were conducted in vivo on detached leaflets of ZH tomato as described by Cohen et al. (1997). The occurrence of oospores in the test leaves was examined microscopically at 10 dpi.

4.3 Latent infection in imported potato seed tubers

Five hundred seed tubers *cv* Nicola which were imported from Holland in late 2016 were planted in a net house (no potato cultivation history) at BIU Farm on December 6, 2016. The developing plants were inspected daily (except Saturdays) for the appearances of late blight. On January 11, 2017 five plants with foliar symptoms of late blight were detected. They were uprooted, each placed in a sterile plastic bag and taken to the laboratory for further inspection.

4.4 Population structure within a single field

In spring 2017, we followed the changes in the population structure of *P. infestans* within a single potato field. Late blight infected leaves were collected from a 9-hactare potato field (cv.

Nicola, organic management) at Kibbutz Nirim, Western Negev; 38 samples were collected on 3.4.2017 and 46 samples at four weeks later, on 1.5.2017.

4.5 Tomato isolates

Isolates from late blight affected tomato crops were collected throughout the country during 1983-2016. They were tested for sensitivity to phenylamide fungicides (metalaxyl during 1983-2005 or MFX during 2006-2016) and mating type. The isolates which were collected during 1994-2016 were maintained at-80^{0}C on sporulating tomato leaves until used for SSR genotyping. Tomato isolates were tested for compatibility with five tomato genotypes that carry various resistance genes: ZH (own inbred, no genes for resistance, *Ph0*), New-Yorker (carrying *Ph1*), Pieraline (carrying *Ph2*), *Lycopersicon pimpenellifolium* L3707 (carrying *Ph3*) (Irzhansky and Cohen, 2006), and NC45 (carrying both *Ph2* and *Ph3,* obtained from R. Gardner, NC State University, USA). All isolates were compatible with *Ph-0*. Isolates which were compatible with *Ph1*; *Ph1+Ph2*; *Ph1+Ph3*; *Ph1+Ph2+Ph3*; or *Ph1+Ph2+Ph3+Ph2.Ph-3* were considered to belong to race 1; 1.2; 1.3; 1.2.3; or 1.2.3. 2.3, respectively.

4.5 Genotype determination

 For each sample of *P. infestans* 12 SSR loci (Pi02, Pi04, Pi4B, Pi63, Pi70, D13, G11, PinfSSR2, PinfSSR4, PinfSSR6, PinfSSR8 and PinfSSR11) were amplified according to a previously published multiplex protocol (Li et al., 2013). All 12 markers were run simultaneously in a 3730 capillary DNA analyzer according to the manufacturers default settings (Life Technologies, Grand Island, NY) and the alleles were edited and scored using GeneMapper 3.7 (Life Technologies) before being exported to a spreadsheet for comparison to other lineages (Martin et al., 2019).

5. Conclusions

The long-term data collected in Israel corroborate with studies from other parts of the world reaffirming the dynamic temporal behavior of *P. infestans* on potato and tomato crops (Fry, 2006; Martin et al., 2019; Njoroge et al., 2019). The unique practice in Israel of growing potato twice a year with seed tubers supplied locally in one season and imported from Europe in the other season, enhances the diversity of *P. infestans* via migration. Fitness and competition between variants of the pathogen further shape the structure of the population. It is indeed true that the characterization of a population in one year may not necessarily predict the population in the future (Fry, 2006).

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