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Characterization of strains of *Pytophthora infestans* isolated from Western Algeria: Pathogenecity and virulence

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Key words: *Phytophthora infestans*, Aggressiveness, Pathogenicity, Sporulation, Virulence.

Abstract

The present work at a level that potato late blight attacks by *P. infestans* in western Algeria may be at the foliage level when infection is early and weather conditions are favorable. Thus, the impacts, severities and frequencies recorded in this region have remarkably increased the 2015-2016 crop year. Characterization of 40 isolates from harvested potato from different Wilaya revealed high variability in the population of *P. infestans* collected. This variability lies essentially in the coexistence of the two sex types A1 and A2 in the same plot or in separate plots. Thus, the isolates collected from the potato, those that come from the region of Mascara, Mostaganem, Ain Defla and chlef features biological and epidemiological significance than other isolates in mycelial growth, in vitro sporulation capacity, direct and indirect sporocyst germination pathogenicity and. Aggressiveness This study of the aggressiveness of isolates conducted on the leaves of different elements of a background that the potato varieties have levels of sensitivity to the pathogen. This great sensitivity noticed in the varieties tested suggests the presence in the Algerian field of a new population of the very aggressive pathogen.

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Introduction

The potato is one of the main vegetable crops in Algeria100 000 ha are reserved annually for the production of the potato, or 27% of the total area devoted to market garden crops spread over several production areas, the most important are : the plains of Ain Defla, Mostaganem and the highlands of Mascara. The improvement of potato production in Algeria is part of a dynamic of agricultural diversification and food security (Anonymous 2015). While the number of varieties grown in Algeria remains limited, however, the total area of this crop has declined during some of the agricultural campaigns, mainly because of drought. In Algerian fields, the potato can be attacked by several microorganisms, fungal diseases such as early blight, gray mold and downy mildew (Fry *et al.,* 1992, Zwankhuien, 1998)., Zwankhuien, 1998)

The latter is one of the most widely distributed diseases geographically when climatic conditions are favorable (Nicolat 2016). It is caused by oomycete *Phytophthora infestans*, a heterothallic species with two sex types A1 and A2 (Hammi 2003). These last coexist for a long time. Potato yield losses due to late blight vary from 20 to 50% in developed countries and can lead to the total loss of the crop in severe attacks of susceptible varieties (Andrivon, 1995; Andrivon 1996, Andrivon *et al.,* 1997). In Algeria, the disease affects the potato harvest in several regions and production area. The survey carried out, showed the existence of severe epidemics in the western Algerian zone and in the central Ain Defla and Metidja, the latter covers 40% of the national consumption. Given the progression and repercussions of the disease in the world demonstrated mainly by the rapid evolution of the causative agent populations, it is important to study the characteristics of the current population of the pathogen in Algeria.

This work aims at the estimation of the importance of the disease in western Algeria and a characterization of the local population using several parameters such as the pathogenicity, the virulence power.

Materials and methods

We selected 20 potato plots located in each of the wilayas of the western Algerian (Oran-Mascara-Ghelizane-Sidi Bel Abbes-Mostaganem Ain Defla and Chlef) in the form of 4 plots per wilaya collected during the months March, April, May and June during the 2015-2016 crop year After isolation and cultivation, isolates were characterized by their coupling types (A1 or A2), Isolation of *Phytophthora infestans* According to the method (Bakonyi *et al.*, 2002). Leaves, stems or infected are washed with tap water. Fragments are removed from the implementation of the forehead lesion. These transplants are superficially disinfected by soaking in a sodium hypochlorite solution at 1% for 2 to 3 minutes, then washed with sterile distilled water two to three times and dried for one or two minutes. They are then placed in petri dishes containing the small pea culture medium and incubated at 20°C in darkness. Observation is carried out daily until the appearance of the characteristic of the mycelial pathogen agent. It is subcultured several times until purification of the isolate

Evaluation of the pathogenicity of isolates (Hammi 2003)

The pathogenicity makes it possible to determine the degree of aggressiveness of the population studied. Two tests are used namely artificial inoculations of whole plants or detached plant tissues such as leaves or tubers. The test carried out on the detached sheets has the advantage of requiring a reduced space and thus makes it possible to simultaneously test a large number of isolates. The leaves of 4 potato varieties are used to test the pathogenicity of isolates collected from different localities (Désirée, Nicola, Spunta and Kondor). Healthy leaves are washed, disinfected with 1% sodium hypochlorite and then washed with sterile distilled water. After drying, the leaflets are incubated in Petri dishes containing moistened filter paper, the lower face being placed upwards.

Pathogenicity is assessed for 40 isolates. A preliminary test is carried out on potato tubers of the Désirée variety to ascertain the pathogenicity of the

isolates. After 6-7 days of incubation, the fungus is isolated and cultured on the pea-based culture medium. The test is then conducted with newly isolated strains. Sporangial suspension is prepared from 10-day old mycelial cultures for each isolate. The concentration is adjusted to 5.104 sporocysts/ml. Five leaves per isolate and variety are inoculated.

The evaluation criteria for the aggressiveness and the virulence of the isolates tested against the leaves of the varieties are: Latency, Dimension of the lesion, Sporulation (Bertrand 2015). Inoculated foliar tissue is monitored every 3 hours. For each isolated isolate x show, the time taken by the pathogen to manifest the infection is determined in hours. Hemmi 2003. After 7 days of incubation, the borders of the lesion are transferred onto a transparent paper and the marked surface is measured in mm 2 by a planimeter. After 7 days of incubation, the inoculated leaves are incubated for 2400h at 20°C and in the dark. They are then carefully placed in sterile Petri dishes and washed with 10ml of sterile distilled water using a sterile syringe. From the suspensions obtained, the concentration of sporocysts is determined using the THOMA cell. Ten measurements are made for each isolate x variety interaction (Nicolat 2016)

Results

Importance of the disease during the surveys carried out

During the surveys carried out during the year 2015-2016 in the various localities and Wilayas where the potato is grown: (Oran, Mostaganem, Ain Temouchent, Rélizane, Sidi Bel Abbes, Chlef, Mascara Ain Defla) and Since the beginning of the surveys, the average incidences are noted in the localities of Oran and Sidi Bel Abbes the highest severities are recorded in the wilayas of Mostaghanem, Mascar, Ain Defla on the other hand in the other zones (Ain Témouchent), On n noticed the appearance of the disease but with a low frequency, This formidable fungus which seriously compromises the production, and it caused considerable damage in 2015 has developed in favor of the climatic conditions (high humidity: 90% and temperature between 18°C to 21°C) (Anonyme 2015).

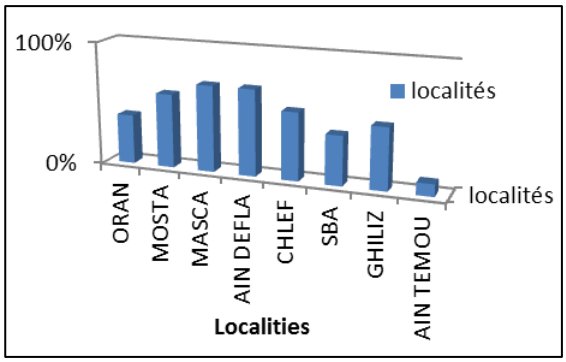


Fig. 1. Frequency of the disease in the different wilayas of the west during the 2015-2016 agricultural

Isolation

On 20 infected and prospected plots, we managed to isolate the pathogen from plants from only 12 plots. Successive transplants allowed us to purify *P. infestans* and obtain 40 isolates Pathogen isolation success rates from the different analyzed potato tissues are shown in Table 1. The latter shows that the isolation of *P. infestans* from the infected foliage of the potato is more successful than that made from the stems. This can be essentially explained by the presence of a fruiting of the more important pathogen.

Table1. Denomination and origin of *P infestans* isolates.

Origin of the strain	Location of isolation	Isolates	Date of isolation
Mascara (5 localités)	Leaves	PMA1 PMA2 PMA3, PMA4, PMA5, PMA6, PMA7, PMA8, PMA9	Mars 2015, Février 2016
Oran (03 localités)	Leaves	POR1, POR2, POR3, POR4,	Avril 2016
Ain Defla(5 localités)	Leaves	PAD1, PAD2, PAD3, PAD4, PAD5, PAD6	Mars 2016, Mai 2016
Chlef (03 localités)	Leaves	PCH1, PCH2, PCH3, PCH4, PCH5	AVRIL 2015, Mars 2016
Mostaghanem(7 localités)	Leaves + Stems	PMO1, PMO2, PMO3, PMO4, PMO5, PMO6, PMO7, PMO8,	Février 2015.Avril 2016 et Mai 2016
Ain Témouchent (02 localités)	Feuilles	PT1	Mars 2016
Rélizane(05 localités)	Leaves + Stems	PR1, PR2, PR3, PR4, PR5, PR6,	Février 2015, Mai 2015
Sidi bel Abbes (02 localités)	Leaves + Stems	PB1	Mars 2016

Preliminary pathogenicity

All isolates were pathogenic. In fact, the leaflets of the Spunta potato variety inoculated with sporangial suspensions all developed typical lesions of late blight 2-5 days after incubation (Fig. 2) .By contrast, tuber behavior is variable. Some of them did not quickly show the symptoms of the disease, others showed degrees rot variables, dark spots on the surface and brown on the inside (Fig. 3) characteristic of *Pytophtora infestans* as described by Montarry 2007.

Fig. 2. Pathogenicity test of PMO1 strain on Spunta tubers after 10 days of incubation.

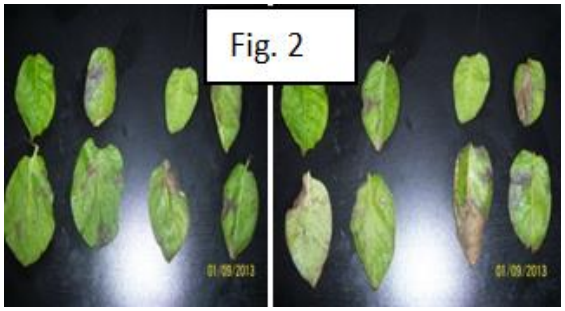


Fig. 3. Pathogenicity test of the PMO1 strain on Spunta leaflets after 10 days of incubation

Aggressiveness of isolates

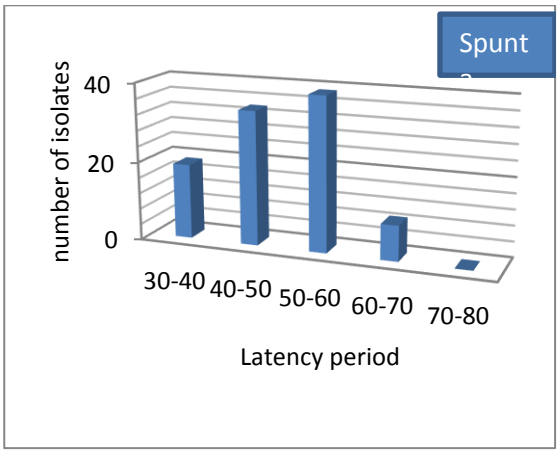
Latency period

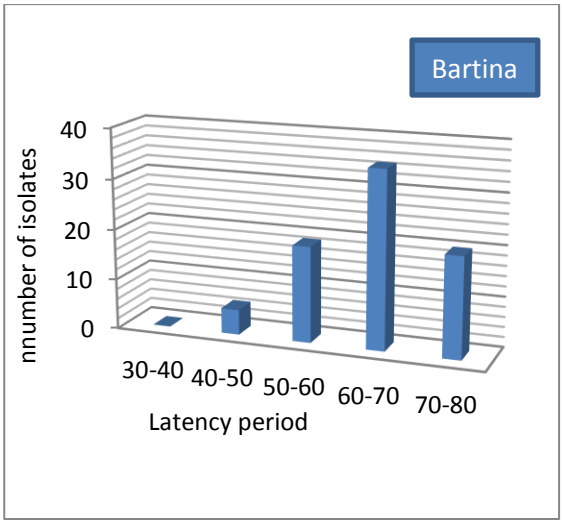
After 2 to 5 days of inoculation of the leaflets incubated at 20°C and a photoperiod of 16h, all isolates have practically developed lesions characteristic of the pathogen on the different varieties tested. These symptoms occur on inoculated foliage tissues after a lag time that differs among isolates. It has been observed that the average latency times for potato varieties are remarkably shorter by 50, 58, 55 and 60h respectively on the Spunta, Desiree, Bartina and Kondor varieties. Fig. 2 shows the distribution of isolates according to their latency determined on each variety tested. This distribution confirms the difference that exists in the behavior of isolates on the same variety or on different varieties. In light of this heterogeneous distribution and considering the average latency observed on the 4 varieties tested, the isolates can be classified in four groups.

Group A consists of isolates that cause infections more rapidly than other isolates. Indeed, the latency time in this group is between 30 and 40 hours, in Spunta varieties and desire:

- Group B includes isolates with mean latency that vary between 40 and 50 hours in Spunta and desire varieties and at least in condor and Bartina
- Group C with latency that oscillates between 50 and 60 hours or we find the majority of strains and more precisely Spunta and desire

Group D groups isolates with a latency between 60-70 hours and more particularly condor and desire. Group E or register a latency period of 70-80 hours in Kondor and Bartina against no strain in Spunta and desire.





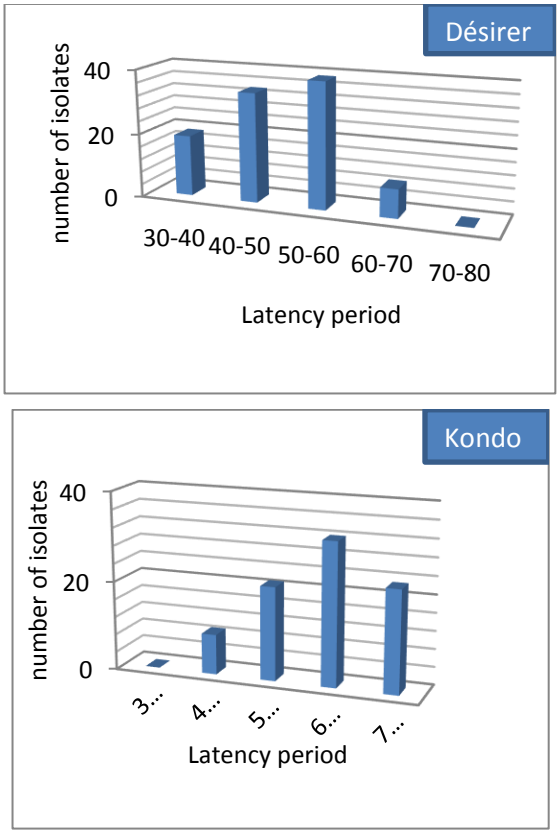


Fig. 4. Distribution of isolates according to their latency periods recorded on the foliage of the potato varieties Spunta, Desirée Bartina, Kondor.

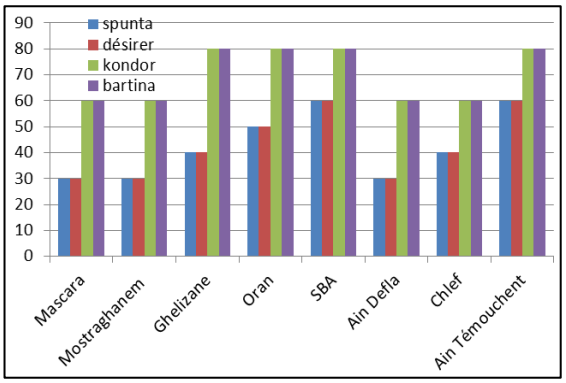


Fig. 5. Average latency recorded for isolates, foliage of potato varieties (Spunta, Désirée, Kondor and Bartina) according to their origin.

Fig. 3 shows mean latency times for isolates from potato by source. The shortest mean values are observed in potato isolates collected from Mascara, Mostaghanem Chelef and Ain Defla while for isolates from Oran. Ain Témouchent and Sidi BelAbbes the first signs of infections appear after a longer latency 70-80 h. Intermediate values of latency periods are found in isolates collected from Ain Témouchent. Generally, infections caused by the majority of isolates appear more quickly on the leaves of Spunta varieties and desire, while on the leaflets of the variety Kondor and Bartina the fungus takes longer to manifest itself. Nevertheless, some similarities are observed between isolates from different localities such as latencies recorded on the foliage of the Bartina variety after inoculation by isolates collected from the Mascara region. The latency characteristic, which is frequently used in pathotypes aggression studies, has once again revealed the variability in *P. infestans* populations in these areas. This variability is observed even for isolates collected from the same plot.

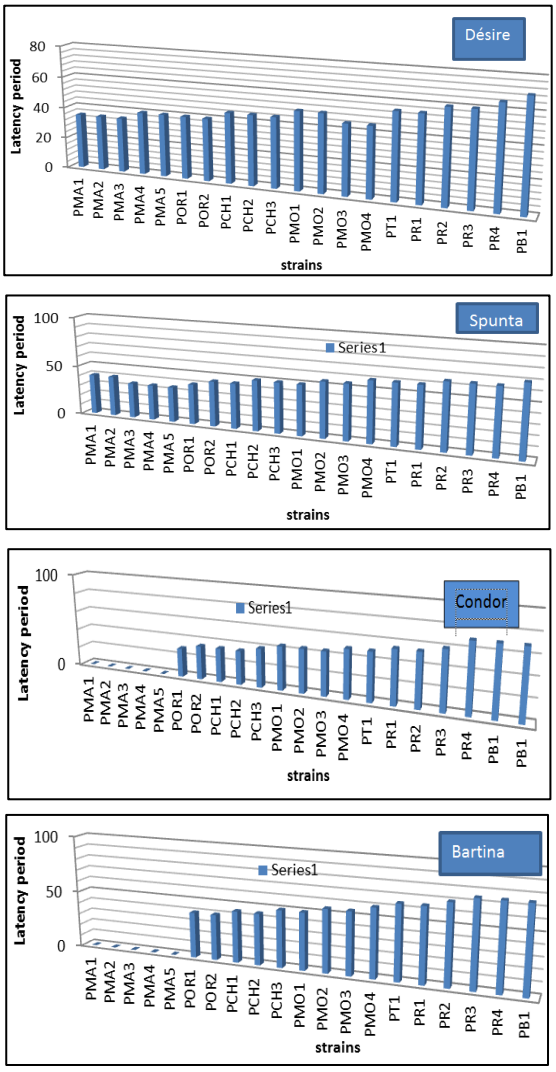


Fig. 6. Distribution of 20 isolates according to their latency periods recorded on the foliage of potato varieties Spunta, Desirée, bartina, Kondor.

Fig. 4 shows the lag times corresponding to 20 isolates from the potato according to their origin. The shortest average values are observed in isolates PMA1.PMA2.PMA3.PMA4 PMA5 POR1. POR2 PCH1. PCH 2. PCH3 collected from Mascara, Oran, Chlef regions. In strains (PR1.PR2.PR3PR4 AND PB1). The first signs of infections appear after a longer latency. Between 70-80h Generally, the infections caused by the majority of isolates appear more quickly on the leaves of the varieties Spunta and Désirer, while on the leaflets of the variety Kondor and Bartina.

The fungus takes longer to appear

Size of the lesion

The size of the lesions developed on the leaflets - inoculated by the different isolates tested after 7 days of incubation at 20°C and a photoperiod of 1600h -. These values show that the dimensions of the lesions vary according to the isolates and varieties tested. Isolates developed significant lesions. This again shows the performance of the isolates on the potato varieties tested. The smallest lesions formed on the leaflets of the Kondor variety, whereas the lesions developed on the foliage of the bartina variety are intermediate and relatively comparable, whereas the lesions on the Spunta and Désirée varieties are.

Fig. 5 and 6 show the lesions developed and the distribution of the isolates according to the dimensions of the lesions developed on each of the varieties tested. It is a heterogeneous distribution illustrating the variability of the behavior of the isolates with respect to relatively different varieties of sensitivity. By determining the average size of the lesions developed on all the varieties used, the isolates can be distributed in 4 groups:

Group A, the average lesion size of which is between 200 and 400 mm 2.

- The group B meets the average size of the lesions varies between 400 and 600 mm2.
- The group c is formed of the average size of the lesions is between 600 and 800 mm2.
- Group E contains dimensions that vary between 1000 and 1200 mm2- The group F is formed of average dimensions vary between 1200 and 1400 mm2 the group G represents the dimensions between 1400 and 1600 mm2

Fig. 6 summarizes the results of the mean dimensions of the lesions developed on the foliage of the 4 varieties by the isolates according to their origin. Fig. 6 summarizes the results of the average dimensions of lesions developed on the foliage of the 4 varieties by the isolates according to their origin

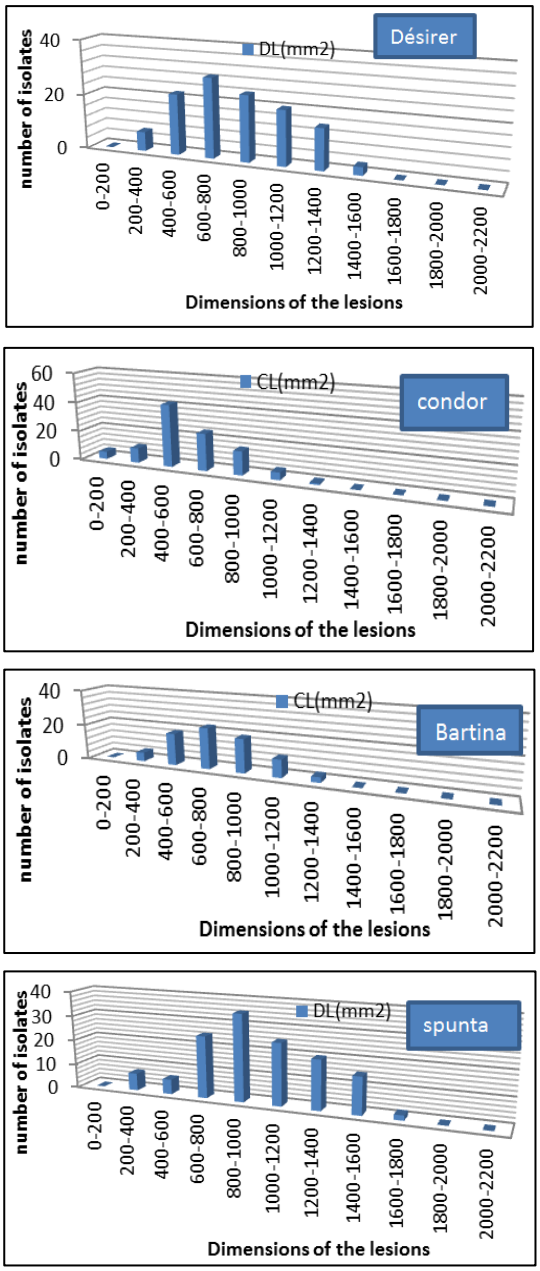


Fig. 7. Distribution of *P. infestans* isolates according to the size of the lesions developed on the foliage of the potato varieties Spunta, Desirée, Bartina and Kondor.

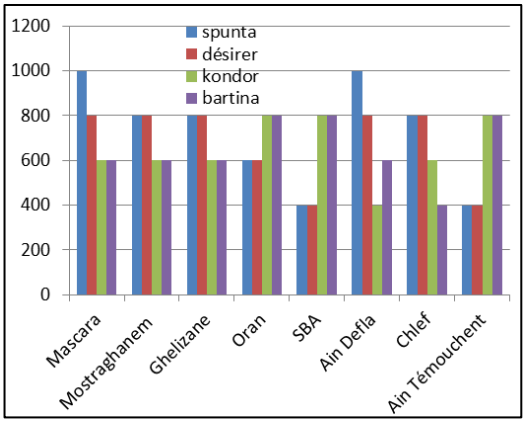


Fig. 8. Dimension de lésions moyennes enregistrées pour les isolats, sur le feuillage des variétés de pomme de terre (Spunta, Désirée, Kondor et Bartina) selon leurs origines.

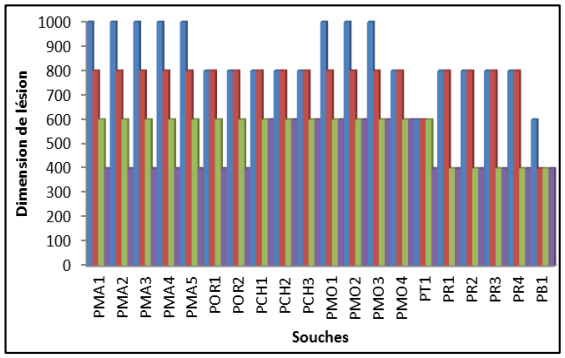


Fig. 9. Distribution of 20 isolates of *P. infestans* according to the size of the lesions developed on the foliage of potato varieties Spunta, Desirée, Bartina and Kondor.

As with the previous aggression parameter (latency), the largest lesion sizes were recorded in isolates collected from Mascara, Mostaghanem Chlef and Ain Defla on all varieties tested. The values recorded for the isolates collected from Ain Témouchant localities in Sidi Bel Abbes are relatively low. The study of the variability of the dimensions of lesions developed by the isolates by contribution to the time of the atence did not give any differences. This can be explained by the existence for each phenotype of a specific degree of aggressiveness whatever the infected variety.

Sporulation intensity

The sporocyst production by the isolates on the foliage of the inoculated varieties was evaluated after 8 days of incubation. The results obtained for all 40 isolates. This intensity of sporulation is variable according to the isolates. In fact, for the collected isolates of the potato, the in vivo sporulation capacity varies between 3×10^4 and 25×10^4 sporocysts ml -1

Fig. 8 shows the grouping of 40 isolates according to their in vivo sporulation abilities on each of the potato varieties. As for the other characterization parameters previously studied, the distribution of the isolates according to their in vivo sporulation is very heterogeneous for each of the varieties.

Considering the general averages found for the 4 varieties, the isolates are classified Group A includes strains with an average sporulation intensity of between 2 and 5×10^4 sporocysts / ml. Group B contains a sporulation ability of between 5 and 10×10^4 sporocysts / ml. Group C contains isolates whose sporulation intensity varies between 10 and 15 $\times$ 10^4 sporocysts / ml. - Group D collects the strains whose in vivo sporulation intensity varies between 15 and 20×10^4 sporocysts / ml;

Whatever the variety tested, isolates collected from the Mascara, Mostaghanem, Ain Defla and Chlef regions have the greatest ability to produce sporocysts compared to isolates from other localities (Fig. 9).

The sporulation capacities determined for the isolates collected from the areas of Oran, Sidi Bel Abbes and Ain Témouchent are relatively comparable but are relatively lower. In the light of these results recorded following the evaluation of the latency, the size of the lesion and the sporulation intensity of the isolates, the sensitivity of the varieties tested is high.

Nevertheless, a growing order of sensitivity has been highlighted which is as follows: the variety of potato Spunta and desire are the most susceptible to P. infestans, kondor has an intermediate sensitivity while Bartina has the most sensitive level of sensitivity The strains from Mascara, Mostaghanem, Chlef and Ain Defla are more aggressive and have a virulent pathogenicity.

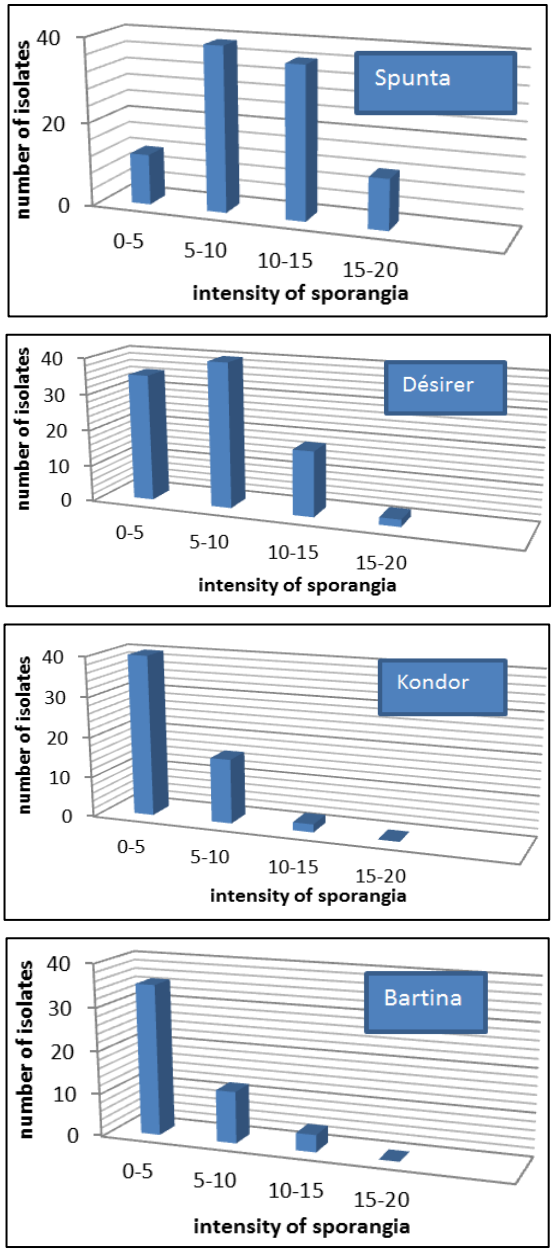


Fig. 10. Distribution of isolates according to their sporulation intensities on the foliage of potato varieties Spunta, Desirée, Bartina and Kondor.

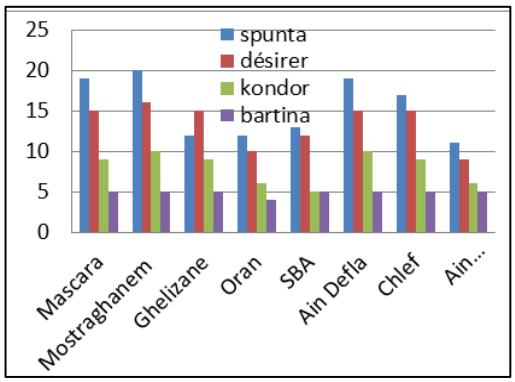


Fig. 11. In vivo sporulation of foliar isolates of potato varieties according to their localities of origin.

Discussion

During this crop year, the distribution of incidence, severity and frequency of the disease in the localities surveyed was heterogeneous. Nevertheless, the spatial importance of culture could also be a factor influencing the importance of the disease. Indeed, in the Mascara, Mostaghnem, Ain Defla and Chlef regions, the cultivated areas are larger than those of the other localities.

In addition, the rate of infected tubers was found to be very low or even absent in the various plots surveyed at the end of the vegetative cycle. In fact, there were no significant crop losses due to this disease during this farming season (2015-2016) compared to the 2014-2015 season. Nevertheless, tubers harvested from different infected plots would be potential sources of inoculum during the following agricultural seasons and could contribute to the post-marketing dispersion of the disease (Montarry 2007). The absence of necrosis on tubers harvested from infected plots will be due to a late onset of downy mildew in these plots APRIL 2015-APRIL 2016. Despite the strong foliage attack by late blight observed in some potato plots, high soil temperature could prevent Tuber tuber infection. 2015 Characterization of isolates collected using certain parameters revealed the existence of a great variability of expression of the populations studied. This variability is observed either within the same parcel of potato or between parcels located in the same locality or different localities this result is consistent with the work of (Nicolat 2016).

The results obtained in this study showed essentially that the isolates collected from Mascara, Mostaghnem, Ain Defla and Chlef regions perform better than isolates collected from other localities. This geographical variability is reported by several haters (hammi, 2003, Montarry 2007 and Sanju *et al.*, 2013). The evaluation of the aggressiveness of the different isolates under controlled conditions revealed the existence of large variations between these isolates on the potato varieties. This study showed a spectrum of aggression ranging from a low level (long

latency, small lesion and low sporulation) that characterizes mainly isolates of the potato, to a high level in other isolates. are reported by (Volk 2001, Gloria 2008, Andrivon *et al.,* 2011) These variations in aggressiveness are noticed in the isolates collected from the same plot/locality and different plots/ localities. Isolates collected from the Mascara, Mostaghnem, Chlef and Ain defla regions showed greater aggression (short latency, large lesion, and significant sporulation) than other isolates from other parts of the west. In addition, this isolate aggressiveness study of loose leaves has shown that potato varieties have high levels of susceptibility to the Zwankhhuien pathogen, 1998. Nevertheless, the four potato cultivars can be further subdivided into 3 groups. The Spunta variety is the most sensitive, Kondor and Désirée are moderately susceptible, while the Bartina variety has shown the lowest level of sensitivity the same results have been cited by (Ferjaoui *et al.*, 2010). This great sensitivity noted in the tested varieties might suggest the presence in the Algerian field of a new population of the very aggressive pathogen that has replaced the old population characterized by its relatively low aggressiveness.

The number of isolates studied in this study is certainly not representative of the totality of the existing population in Algeria (in western Algeria). Nevertheless, this investigation is evidence of the very heterogeneous nature of the population structure of P. infestans present. This heterogeneity is observed in a population of pathogen belonging to either the same plot or plots located in different areas (Andrivon and Lebreton 1997). With the coexistence of the two sex types A1 and A2 in the Algerian fields, the new population of the pathogen will acquire the potential for sexual reproduction. This evolution is at the origin of the variability highlighted in this study (Volk 2001). This heterogeneity of the population will be manifested by the appearance of several genotypes very aggressive and genetically more adapted (Fry *et al.*, 1992). Nevertheless, the real contribution of sexual reproduction in this evolution in Algeria remains to be confirmed.

Conclusion

During this crop year, the distribution of incidence, severity and frequency of the disease in the surveyed sites was heterogeneous. Nevertheless, the spatial importance of culture could also be a factor influencing the importance of the disease. Indeed, in the Mascara, Mostaghnem, Ain Defla and Chlef regions, the cultivated areas are larger than those of the other localities. On 20 infected and prospected plots, we managed to isolate the pathogen from plants from only 14 plots. Successive transplants allowed us to purify *P. infestans* and obtain 40 isolates.

The latency characteristic, which is frequently used in pathotypes aggression studies, has once again revealed the variability in *P. infestans* populations in these areas. This variability is observed even for isolates collected from the same plot The shortest average values are observed in isolates PMA1. PMA2. PMA3. PMA4. PMO1. PMO2. PMO3. PMO4 collected from the Mascara, Mostaghanem and Ain defla regions whereas for the POR1. POR2. PCH1. PCH2. PCH3 isolates from Oran and Chlef. The first signs of infections appear after a longer latency. Intermediate values of latency periods are found in isolates collected from Ain Témouchent and Ghlizane (PR1. PR2. PR3. PR4. AND PB1). Generally, infections caused by the majority of isolates appear more quickly on the leaves of the varieties Spunta and Désirer, while on the leaflets of the variety Kondor and Bartina the fungus takes longer to manifest itself. As with the previous aggression parameter (latency), the largest lesion sizes were recorded in isolates collected from Mascara, Mostaghanem Chlef and Ain Defla on all varieties tested. The values recorded for the isolates collected from the Ain Témouchant localities of Sidi Bel Abbes are relatively small Whatever the variety tested, the isolates collected from the Mascara, Mostaghanem, Ain Defla and Chlef regions have the greatest ability to produce sporocysts in comparison with the isolates of the isolates. other localities. The sporulation capacities determined for the isolates collected from the areas of Oran, Sidi Bel Abbes and Ain Témouchent are relatively comparable but are In the light of these results recorded following

the evaluation of the latency, the size of the lesion and the sporulation intensity of the isolates, the sensitivity of the varieties tested is high. Nevertheless, a growing order of sensitivity has been highlighted which is as follows: the variety of potato Spunta and desire are the most susceptible to *P. infestans*, kondor has an intermediate sensitivity while Bartina has the most sensitive level of sensitivity low.

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