INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 11, No. 5, p. 229-236, 2017
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Aggressiveness of *Phytophthora infestans* populations in western Algeria

Ameziane El Houcine*, Lamara Sid Ahmed, Ayad Amar, Benyoucef Soumia, Dergal Nadir Boudjlal, Abi-Ayad Sidi-Mohammed El-Amine, Baba Hamed Med Bey

Laboratory of Aquaculture and Bioremediation (AQUABIOR), Department of Biotechnology, Faculty of Natural and Life Sciences, University of Oran 1- Ahmed Ben Bella, El M'Naouer, Oran, Algeria

Article published on November 30, 2017

Key words: *Phytophthora infestans*, Epidemiology, Sexual reproduction, Metalaxyl resistance, Aggressiveness

Abstract

The present work has shown that attacks of potato blight caused by *P. infestans* in western Algeria can be severe on foliage levels when infection is early and climatic conditions are favorable. Consequently, the impacts, severities and frequencies recorded in this region were remarkably high during the 2013-2016 crop years. The characterization of 56 potato isolates collected from different localities in western Algeria revealed a high variability in the *P. infestans* population. This variability resides essentially in the coexistence of the two sexual types A1 and A2 in the same plot or in different plots and by the emergence of metalaxyl-resistant isolates. For example, isolates collected from potatoes, those from the wilaya of Mascara and Ghelizane, those resistant to metalaxyl, and those of the A2 type have more biological and epidemiological characteristics than those of other isolates in terms of mycelial growth In vitro, the aggressiveness of the strains showed that the infection can be carried out 66h to 16°. The assessment of the level of sensitivity of 4 varieties of potatoes showed that the majority of these varieties possess sensitive foliages and tubers with moderate susceptibility to new local strains of *P infestans*.

***Corresponding Author:** Ameziane El Houcine ✉ houssine31000@yahoo.fr

Introduction

Phytophthora infestans mushroom has two types of mating compatibility designated as A1 and A2, in the absence of any of them, the fungus cannot multiply asexually Nicolat, 2016. Due to the low genetic diversity within the populations thus multiplied, the control strategies put in place by the man have a more or less lasting effect. For this reason, control of the fungus is therefore more effective in this case. Potato yield losses due to late blight vary from 20 to 50% in developed countries and can lead to total loss of crop in severe attacks of susceptible varieties (Andrivon *et al.* 1997, 1998, Zwankhuien, 1998). Given the progression and impact of the disease worldwide, demonstrated mainly by the rapid evolution of causative agent populations in recent years (Fry *et al.*, 1992, Hammi, 2003), it is important to study the aggressive characteristics and virulence of the pathogen populations in Algeria (western Algeria), as well as the susceptibility assessment of foliage and tubers of four commercial potato varieties to -vis new pathogenic

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 2013-2016 crop year After isolation and cultivation, isolates were characterized by their coupling types (A1 or A2), metalaxyl resistance, virulence profile and Their aggressiveness. It is tested and carried out on isolates178.

Isolation of Phytophthora infestans

According to the method BaKonyi *et al*, 2002

Leaves, stems or infected tomatoes 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 Fig 1. It is subcultured several times until purification of the isolate Fig 2.

Fig. 1. Isolation of *Phytophthora infestans* from infected leaves of potato.

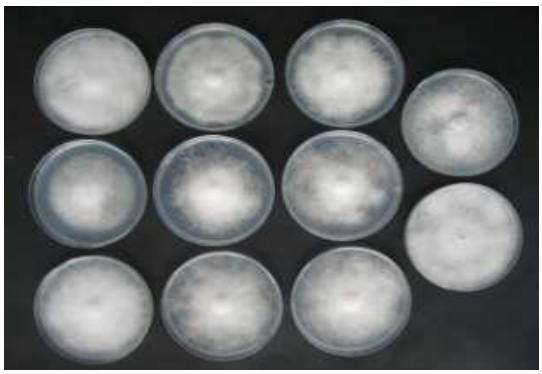

Fig. 2. The different isolates of *Phytophthora infestans.*

Mating type

Determination of mating type is effected by confrontation in Petri dish of clarified V8 agar medium of the stub to be tested with A1 and A2 reference stubs.

Observation of the presence or the absence of oospores (sexual crossing the certifying body) on the mycelia convergence zone determines the mating type (Mukalazi, 2002).

Resistance to metalaxyl

Resistance to metalaxyl, active substance of Ridomil, systemic fungicide of choice in the plant protection armory is a criterion to characterize populations and it is also information on the setting of chemical strategies is effective in respect of the fungus (Bacon, 2002). With its ability to be translocated through the whole plant (systems engineering) for optimum protection against infection, metalaxyl molecule is a strong interest for the fight against mildew; however, due to its high specific mode of action (Unisite), its propensity to select, within mildew populations, individuals resistant to its action is well known. Also, information about the ability of people to resist the action of metalaxylis useful if the active substance is used wisely without making accidents during culture (Gloria, 2008).

The measurement of the behavior of the collected isolates is performed on leaf discs of the desired variety spunta and set afloat on solutions of 5 metalaxyl (Ridomil WP25) their concentrations are known and range from 0 to 100ppm. On each leaf disc is deposited a suspension drop containing the isolate to be tested (*Muchiri et al.,* 2009).

The observation of lesions developed by the isolate carried out after 7 days of incubation (18°C, 16h photoperiod): EC50 (concentration of 50% metalaxyl ensuring protection) is determined and the isolates are classified as "sensitive" if EC50 < 0,01ppm, "intermediate" if 0,01ppm < EC50 < 10ppm,"Resistant" if 50 % protection is not assured in solutions than in a concentration of 10ppm metalaxyl (Hammi, 2003).

Aggressiveness

The aggressiveness of the pathogen results in its ability to colonize more or less healthy tissue of the host compliant quickly. Aggression can be divided into four components: FI infection rate (% of successful infections), the PL10 latency period (time between infection and sporulation 10%), growth rate of the lesions on the CLmax sheet tested and intensity of sporulation PS (amount of sporangia produced by the lesion).

The test is carried out in the laboratory on entire sheets of the desired variety and spunta levied on intermediate floors high greenhouse plants; a micro-droplet containing suspended sporangia fixed concentration (1x104 sporangia / ml) of the test isolate is deposited on each of the leaflets forming the sheet; the thus treated sheet is placed in incubation (18°C, photoperiod of 16h) and observed at 3, 4, 5, 6 and 7 days (Bertrand, 2015). FI corresponds to the percentage of the inoculation sites that developed lesions after 7 days of incubation. PL10 corresponds to the number of hours after inoculation necessary for 10% of sporulate contamination (Gloria, 2008) is the slope at the inflection point of the fitted curve (growth rate). PS is the natural logarithm of the number of sporangia per cm2 measured A global index of aggressiveness can be calculated using the formula: IA = 1/PL10*FI*Clmax*PSStudy of the behavior of varieties in field.

The main types of behavior

Initially, the model of development of the epidemic mildew and treatment decisions has been calibrated on the behavior of the susceptible variety "Bintje". To adapt the rate of treatment, usually supported for sensitive varieties, and eventually allow further processing on the economy more resistant or tolerant varieties, studies of the varieties behavior are useful (Bertrand, 2015).

The extent of the infection in the foliage is generally carried out in untreated experimental devices. Notations are made by observing the time of onset of the first symptoms on the foliage and then at regular intervals by measuring the destruction of the foliage in a predetermined scoring scale (Rekad, 2009).

Results and discussion

Isolation of Phytophthora infestans

Forty infected plots were surveyed; we managed the isolation of the pathogen from plants from 19 plots only. Only the technique of direct planting of cysts developed on lesions has enabled us to isolate quickly the fungus. Other techniques used isolations (direct and indirect) would not prove reliable for pathogen cultivation on artificial substrate.

This can be explained by the use of pea-based culture medium, which is non-selective and low competitiveness of P. infestans against saprophytes. Successive subcultures have allowed us to purify *P. infestans* and get 56 isolates Fig 2. The isolation of *P. infestans* from the foliage of infected potatoes is more successful than the one made from the stems. This can be mainly explained by the presence of a fructification of the most important pathogen on leaves and the stems, which makes transplanting easier cysts.

Mating type

The results in the following Table (1) shows the presence of the A2 type compatible with the traditional type A1 should not be overlooked in our area because it can be the source of greater aggressiveness of people Fig 3.

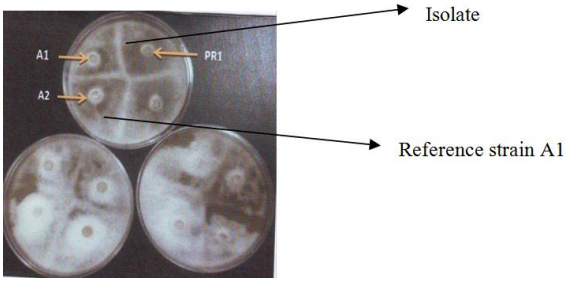

Fig. 3. comparison test between the reference strains (A1 and A2) and isolates on medium PP at 22°C for 7 days.

Table 1. Occurrence of A2 blight strains in populations in west Algeria.

year	Number of isolates	Frequency of the A2
2013	18	10
2014	19	11
2015	10	7
2016	8	5

Resistance to metalaxyl

Table 2 presents the results obtained during the period 2013-2016. One can notice that the number of susceptible isolates to metalaxyl remains relatively constant from one year. This observation clearly indicates the danger posed by a thoughtless use of this product. That is why it is strongly recommended to book as Ridomil specific uses (in vegetative growth and only when weather conditions do not allow the use of conventional contact products or application time required) and in any case not to repeat its application more again Fig 4.

Table 2. Classes of sensitivity to the métalaxyl of the isolates taken From the west of Algeria during the period 2013-2016.

Year	Resisting	Intermediary	Sensitivity	Nombre isolats tested
2013	9	3	6	18
2014	9	3	7	19
2015	7	-	3	10
2016	6	-	2	8

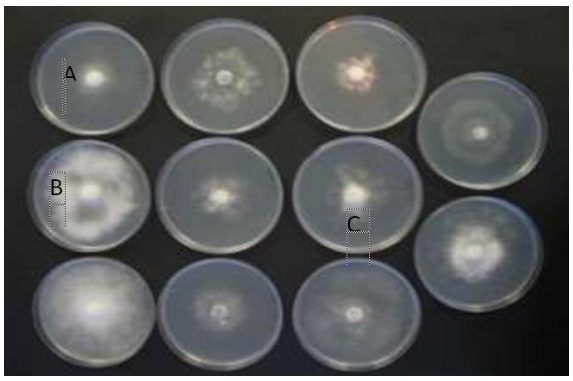

Fig. 4. Sensitivity and resistance strains of *Phytophthora infestans* metalaxyl A: Resistant strain B: Sensitive strain C: Intermediate strain.

Aggressiveness

The following graph shows the distribution of aggression indices obtained, following the examination of 56 isolates in 2013. 2016, no significant difference was observed in the behavior of people for that character. An obvious interest should be based on the study of the evolution of aggression populations for large time intervals

The study of the index of aggressiveness allowed , however, to show the existence of isolates able to perform a complete cycle "infection-fruiting" in less than 66h at 18°C while the simulation model used by cycle the warning takes into account service at this temperature, an average value for the latent period of 84h Fig 5 and Fig 6. The following Fig. shows that 28% of isolates tested have latency lower than this value this is also the reason why the incubation values have been slightly modified in the model to take into account these observations Fig 7.

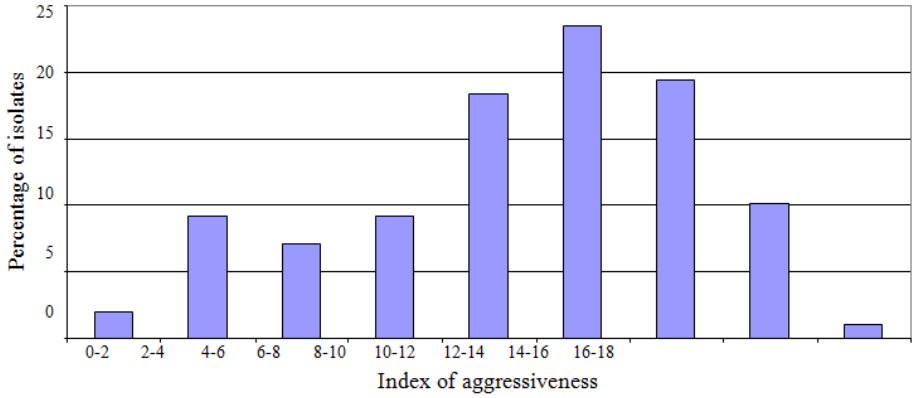

Fig. 5. Distribution of the index of aggressiveness for the isolates 2013-2016.

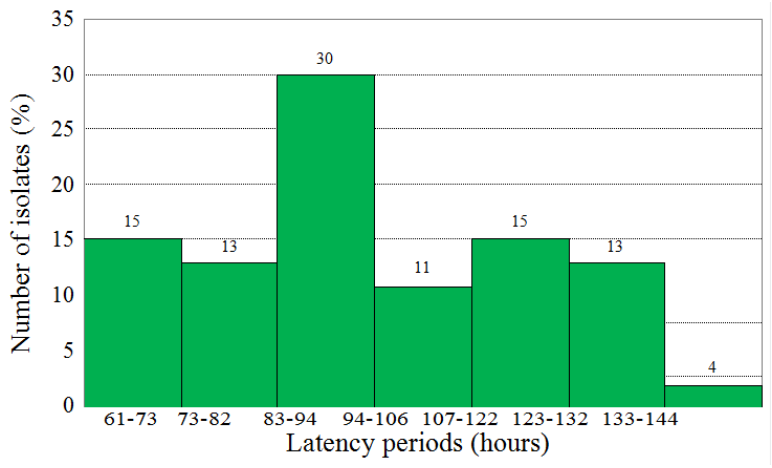

Fig. 6. Distribution of frequencies for the latency period of isolates observed at 18°C.

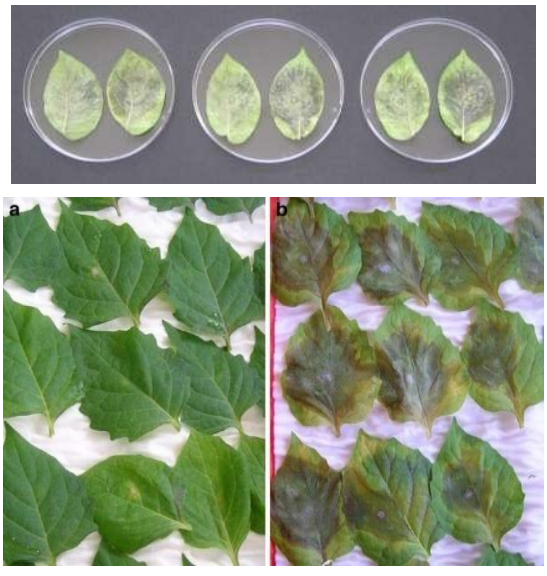

Fig. 7. Aggressive strains of *Phytophthora infestans* after 66h after 16h of incubation.

Behavior of varieties in the field

The following Fig. shows the evolution of the destruction of the foliage of three varieties with typical behavior at this level. First, the sensitive spunta and desirer variety is quickly attacked and once this mildew, the speed of development in the foliage (destruction kinetics) is very fast: it is the typical behavior of varieties having no factors mildew resistance. The second variety condor, the first symptoms of this attack almost simultaneously, but then the destruction kinetics is much slower, this is the typical behavior of varieties with some tolerance or resistance to the field guided by the Action of a complex of minor genes determining a horizontal type of resistance. The first symptoms observed in the third variety Bartina and condor are later, but once installed, mildew are progressing very rapidly so that by the end of the race, the destruction is almost equivalent to the sensitive variety spunta: this is the typical behavior of varieties with one or more major

resistance genes determining a vertical type of resistance Fig. 8. These are effective until the selection pressure that these varieties fly into mildew population result.

In the selection of physiological race able to circumvent the resistance. Once selected race, then there are no more obstacles to its action, the plant rapidly destroyed.

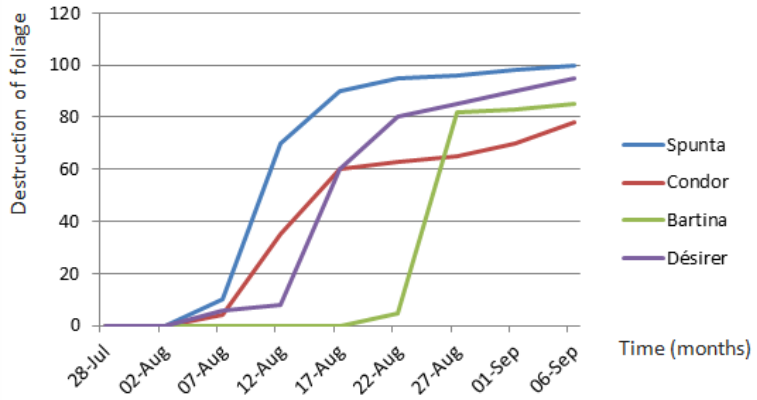

Fig. 8. Kinetics of leaf destruction to 4 different varieties developing a late blight resistance mechanism.

These different behaviors can induce specific control strategies. If the first leaves little freedom for the protection (the fight must begin as soon as the amount of inoculum in the environment is able to achieve a general infection of crops, either from the 3rd generation of mildew detected by the model after the observation of symptoms on waste pile), the following two possible to consider some reduction in the intensity of the struggle.

By spacing the treatments or reducing the quantities of active substances used for treating (Agria type tolerant varieties) or delaying the implementation of the first treatments, thereby providing savings of one to three interventions early in the season (varieties having major resistance genes).

Discussion

During the period of this study (2013-2016) the disease was manifested during four agricultural campaigns in western Algeria. However, this occurred only late in three campaigns. The follow-up of the evolution of the infection made it possible to determine its distribution in time and in space. Indeed, the climatic conditions that characterized this period (prolonged wetting hours, average daily temperatures) favored the appearance of the infection Mildew, due to Oomycete hétérothalique *Phytophthora infestans*, is a major biotic constraint, the most common in the potato crop, causing serious damage to the production and performance of the latter when conditions are favorable. During our surveys carried out for the period 2013-2016 in the different regions where are cultivated potato: AinTémouchent (Terga, ChenttoufSidi ben Adda), Mascara (SidiKada, Ghriss), Mostaganem (Sayad, Boughirat, Stidia), Chlef, Relizane, Oran (Boutliliss, Drill Mssila, Hassibounif, Ain Karma) was noticed the appearance of late blight symptoms in percentages ranging between 25% in the locality of Boutliliss, Hassibounif (Oran) and 8-12% in other areas of different provinces.

In our work we have isolated, purified, characterized phenotypically (macroscopic and microscopic) strains of *Phytophthora infestans* from potatoes plants (leaves) with the characterization of isolates collected from different localities confirms the existence of the two mating types A1 and A2 in western Algeria, reported since at least 1995. This study confirmed the emergence of resistant strains of *P. infestans* (in vitro and in vivo) metalaxyl Anonyme, 2013. A rate of 10% of the isolates collected was found to be resistant to metalaxyl (*in-vivo*). Large variability characterization parameters are within the same field or different field namely sexual type,

resistance to metalaxyl (*in-vitro* and *in-vivo*), mycelial growth of pea and pathogenicity on 3 apple varieties land. This heterogeneity varies from one locality to another. Thus the distribution of the two mating types A1 and A2 is often homogeneous at an even plot but heterogeneous throughout the study area. This study showed that there is a good correlation between resistance to metalaxyl and A2 mating types. For the different parameters studied the behavior of isolates varies according to their origin, since collected the mascara locality isolates are more efficient than those collected from other localities. Great variability is observed in isolates according to their phenotypes. In fact, metalaxyl resistant isolates (A1 or A2) have biological and epidemiological characteristics higher than those sensitive to metalaxyl. Regarding the comparative study of isolates on the basis of their sexual types, A2 isolates are relatively more efficient than sexual type isolates A1 Montarry, 2007.

Conclusion

Despite the limited number of isolates (56) collected in western Algeria, the isolation of A2 type strains is a proof of the presence of a new *P. infestans* population in Algeria that replaced the former population of the only sexual type A1. The sexual compatibility tests revealed the coexistence of the two sexual types A1 and A2 in *P. infestans* populations in the studied areas. Indeed, given that *P. infestans* is a heterothallic species, the isolation of autofertile strains (2 isolates) was a first indication of the presence of sexually compatible strains in the region The in vitro and in vivo susceptibility to metalaxyl in the isolates revealed the presence of four phenotypes in the P. infestans population of the study area, namely sensitive, moderately sensitive, moderately resistant and resistant isolates.

The isolates collected from the potato have variable behavior with respect to this fungicide. Moreover, the appearance of metalaxyl-resistant strains in this zone could also be the result of an adaptation mechanism followed by Tran's migration Region since metalaxyl is used as a treatment against downy mildew in several potato production areas.

The evaluation of the aggressiveness of the different isolates under controlled conditions revealed large variations between these isolates on the potato varieties. This study showed a spectrum of aggressiveness ranging from a low level (long latency, small lesion and weak sporulation). These variations of aggressiveness are observed in the isolates collected from the same plot/locality and from plots/localities. The isolates collected from the wilya of Mascara and Mostaganem showed greater aggressiveness (short latency, large lesion and sporulation) than the other isolates. In addition, regardless of the sexual type, metalaxyl-resistant isolates are more aggressive than metalaxyl-sensitive isolates regardless of the potato cultivar tested. In addition, this study of the aggressiveness of isolates on the detached leaves showed that potato varieties exhibit high levels of susceptibility to the pathogen. Nevertheless, the four potato cultivars can be subdivided into 3 groups. The Spunta variety is the most sensitive; Nicola and Désirée are moderately sensitive, while the Kondor variety showed the lowest level of sensitivity. Tomato variety Daniela has a level of sensitivity comparable to that of the Spunta potato variety. This high sensitivity observed in the varieties tested suggests the presence in the western Algerian region of a new population of the very aggressive pathogen which replaced the old population characterized by its relatively weak aggressiveness

Reference

Andrivon D, Lebreton L. 1997. Mildiou de la pomme de terre, ou en sommes-nous Aprés 150 ans Phytoma **494,** 24-27.

Andrivon D. 1995. Biology, ecology and epidemiology of late blight potato pathogen *Phytophtora infestans* in soil, Phytopathology **85,** 1053-1056.

Andrivon D. 1996. The origin of *Phytophtora infestans* populations present in Europe in the 1980s: a critical review of historical and scientific evidence. Plant Pathology **45,** 1027-1035.

Bacon R. 2002. Gestion de la résistance aux fongicides. Agriculture et agroalimentaire Canada.

BaKonyi J, Laday M, Dula T, and Ersek T. 2002. Characterization of isolates of *Phytophthora infestans* from Hungary. European Journal of Plant Pathology **108,** 139-146.

Beninal L. 2011. Diversité génétique de *Phytophthora infestans* agent du mildiou de la pomme de terre. Thèse de Magister en Science Agronomoque INRA.

Bertrand C. 2015. Etude des symptômes dans le cadre des interactions entre *Solanum tuberosum* L et *Phytophthora infestans*. Thèse de doctorat en Sciences Biologique.

Fry WE, Goodwin SD, Matuzak JM, Spielman LJ, Milgroon MG. 1992. Population genetics and intercontinental migration of *Phytophtora infestans*. Annual Review of Phytopathology **30,** 107-129.

Gavino PD, Smart CD, Sandrock R w, Miller JS, Hamm PB, Lee TY, Davis RM Fry WE. 2000. Implication of sexuel reproduction for *Phytophtora infestans* in the United States : Generation of an aggressive lineage. Phytopathology **84,** 731-735.

Gloria A, PhD. 2008. Methods for identification of *Phytophtora* species.Unites states department of Agriculture. Lead Scientist .Molecular diagnostics laboratory.

Goodwin SB. 1998. DNA polymorphism in *Phytophtora infestans*: The cornell experience. In *Phytophtora* Lucas, J. A., Shattok, R. C., Shaw, D. S. and Cooke, L. R., Eds. Cambridge, Cambridge University Press pp, 256-271.

Hammi AE. 2003. Caractérisation de population de *Phytophtora infestans* (Mont) Barry dans la région de Sais. Thèse de doctorat en Phytopathologie, Fès 272-274.

Montarry J. 2007. Réponse adaptative des populations de *Phytophtora infestans*, agent du mildiou de la pomme de terre, au déploiement en culture de son hôte *Solanum tuberosum*. Thèse doctorat en biologie et agronomie de l'école national supérieure agronomie (ENSAR) de Rennes, France 177p.

Muchiri FN, Narla RD, Olanya RO,Nyankanja, and Ariga ES. 2009. Efficacy of fungicide mixtures for the management of *Phytophthora infestans* on potato.

Mukalazi J. 2002. Metalaxyl résistance, mating type end pathogenicity of *Phytophtora infestans* in Uganda departement of Crop science, Makerence University.

Nicolat M. 2016. Caractéristique et évolution des traits d'histoire de vie de Phytophtora infestans et conséquence gestion des Mildiou de la pomme de terre Thèse de doctorat en sciences.

Rekad EZ. 2009. Caractérisation des isolats de *Phytophtora infestans* (Mont) de Barry,agent causal du mildiou de la pomme de terre et de la tomate dans la région nord-ouest d'Algérie. Mémoire de magister en science agronomie de la faculté des sciences de l'ingénieur de Mostaganem.

Volk TJ. 2001. *Phytophtora infestans* cause of late blight of potato and the Irish potato famine.

Zwankhhuien MJ. 1998. Potato late blight epidemics and population structure of *Phytophtora infestans*. PhD Thesis, Wageningen Agricultural University, Netherlands 147 pp.