ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 11, pp. 14230-14236, November, 2016

International Journal of Recent Scientific Research

Research Article

INFLUENCE OF CITRUS ROOTSTOCKS ON SOIL POPULATIONS OF PHYTOPHTHORA SP. IN THE GHARB REGION IN MOROCCO

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ARTICLE INFO

Article History:

Received 16th August, 2016
Received in revised form 25th September, 2016
Accepted 23rd October, 2016
Published online 28th November, 2016

Key Words:

Seasonal measure, *Phytophthora, P. parasitica, P. citrophthora,* Root rot.

ABSTRACT

Soil samples were collected from April 2013 to June 2014 in an experimental orchard planted with 14 rootstocks, aged 17 years and associated with the variety "Valencia late". The density of *Phytophthora* spp. propagules was determined by spreading soil on a selective medium. The lowest densities were found in the vicinity of *Citrus aurantium*, *Citrus macrophylla, Poncirus trifoliata, Cleopatra mandarin*, *citrumelo 4475* and *Goutou*. By contrast, the highest densities were found in the vicinity of *Citrus volkameriana*, *sunki mandarin* and *Carrizo citrange*. In general, the values recorded ranged for 100 propagule/g of soil (in the rootzone of sour orange) to more than 1200 propagule/g of soil (in the rootzone of *Citrus vokameriana*). Furthermore, our results provided a classification of the rootstocks studied according to the season when their inoculum density peaks: Winter for *Poncirus trifoliata*, *Cleopatra mandarin* and *sunki mandarin*, and summer for *Gou-Tou*, *Citrus volkameriana*, *Citrus aurantium* and *citrumelo 4475*.

INTRODUCTION

Phytophthora sp. species including *P. parasitica* and *P. citrophthora* are reported to cause serious diseases to citrus crops in Morocco (Vanderweyenne, 1774; 1982 Benyahia, 1998 and 2007; Boudoudou *et al.,* 2015) and worldwide (Timmer and Menge, 1988; Graham, 1990). The symptoms of *Phytophthora* attack can be observed on trunk and branches, root system and fruits. To face this problem, the use of resistant rootstocks remains the most practical solution and is privileged by most researchers. Indeed, it has been established that the resistance of citrus rootstocks to biotic constraints such as Phytophthora diseases, and to abiotic constraints such as salinity and low temperatures varies among genotypes (Benyahia, 2007; Fadli *et al.,* 2014; Castle, 1987; Spiegel-Roy & Goldschmidt, 1996).

Previously, rootstocks were ranked based on their tolerance or resistance to trunk gummosis until the extent and importance of the damage in roots and feeder fibers due to root rot was established (Graham *et al.,* 1996). However, no correlation was reported earlier between resistance to gummosis and root rot. Indeed, a rootstock which is resistant to foot gummosis is not necessarily resistant to root rot (Benyahia, 2008).

In Morocco, although the density of *Phytophthora* sp. inoculum was estimated in citrus orchards after isolation in selective media (Serhini, 1986; Benyahia, 1998 and 2007; Boudoudou *et al.,* 2015), no threshold was established for the harmfulness of *Phytophthora* sp. inoculum in Moroccan orchards in contrast to other countries, including Florida, where fungicide treatments are recommended for inoculum densities of 5 to 15 propagules per cm^3 of soil (Timmer, 1991; Lutz et Menge, 1986; Sandler *et al.,* 1989). It is important to note also that fungicide treatments are usually expensive, especially when *Phytophthora* is abundant in soil (Timmer *et al.,* 1989; Lutz et Menge, 1986; Donald *et al.,* 1996).

The rootstock is among the most important environmental factors which can affect inoculum density in a citrus orchard soil as suggested by Agostini *et al.* (1991). The study of Boudoudou *et al.* (2015) in a clay soil of the Gharb region (Morocco), for example, showed a high density of *P. parasitica* inoculum in the vicinity of sour orange than that of *Citrus*

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macrophylla. This result reflects a marked effect of the rootstock on the density of the *Phytophthora* inoculum in soil.
In the current situation of Moroccan orchards, sour orange is the most used rootstock due to its satisfactory resistance to *Phytophthora* attacks. However, the resistance of this rootstock is variable depending on accessions used (Benyahia, 1983) and is affected by salinity of soil or irrigation water (Sullistyowati et Keane, 1992; Benyahia 1998; Benyahia *et al.,* 2003, Benyahia, 2007). Also, most associations using sour orange as rootstock are susceptible to tristeza, the most damaging virus disease to citrus crops.

Consequently, many rootstocks were introduced as candidates to replace sour orange and trials are underway in different Moroccan citrus producing regions to test their behavior in association with commercial varieties (Benyahia communication personnel). One of the important criteria considered when choosing a new rootstock, in addition to tristeza tolerance, is its effect on the evolution of inoculum density of *Phytophthora* in soil (Agostini *et al* 1991).

Thus, the objective of this study is to investigate the effect of 15 citrus rootstocks on the density and the evolution of *Phytophthora* sp. inoculum in a soil of the Gharb region in Morocco.

MATERIALS AND METHODS

Collection of soil samples

Soil was collected in a plot planted with Valencia late on 15 rootstocks aged 17 years (Tableau 1). Three trees were randomly selected for each rootstock and the sub-samples were collected following the cardinal directions. These were taken at one meter from the trunk and at a depth of 5 to 20 cm using an auger (Timmer *et al.,* 1988, 1993).

Table1 List of the rootstocks used in the experiment

Code	Rootstocks	Code ICVN
3	*Poncirus.trifoliata*. B6 CZ 24	*ICVN 0110139*
6	Mandarine Sunki x P.T. B2 38581	*ICVN 0110204*
5	P.T B 6 C Z 13	*ICVN 0110107*
7	Citrange Carrizo 28608	*ICVN 0110181*
11	Citrumelo 4475 B2 G3	*ICVN 110145*
16	Mandarine Sunki x P.T. 30591	*ICVN 0110211*
17	Mandarine Sunki x P.T. 30588	*ICVN 0110208*
18	Mandarine Cleopatre x P.T. 30584	*ICVN 0110155*
23	Gou-Tou SRA 506	
24	*Citrus Macrophylla*	*ICVN 0110058*
25	*Citrus Volkameriana* 28613	*ICVN 0110025*
30	Mandarine Cleopatre X C.C. 30577	*ICVN 0110223*
34	Bigaradier P6 R26 A16	
39	Mandarine Sunki x P.T. 330590	*ICVN 0110210*
41	Citrumelo 1452 B6 C	*ICVN 0110282*

Estimation of inoculum density in soil

Soil samples were screened separately using a sieve of 2 mm mesh and stored under an ambient temperature of 21-24°C (Tsao, 1983; Timmer *et al.,* 1988, 1989). To estimate the density of *Phytophthora* sp. inoculum in these soil samples, we used the dilution technique. 10 g from each sub-sample, representing a geographic orientation, were diluted in 90 ml of water agar 0.25%. After stirring for 20 min, 1 ml is spread on a Petri dish containing BARPHY 72, which is a selective growth medium for *Phytophthora* species (Benyahia *et al.,* 2004; Benyahia, 2007). Incubation of Petri dishes was made in the dark at 28°C for 48 hours (Timmer *et al*., 1988, 1989). To determinate the number of *Phytophthora* sp. propagules, the dishes were first washed with sterile distilled water to remove soil particles, then colonies were counted. These were later transferred separately into test tubes containing cornmeal agar medium (CMA). Knowing the amount of soil sown in each Petri dish, an approximate value of the number of *Phytophthora* propagules per gram of dry soil was calculated.

Identification of Phytophthora species

The identification was based on taxonomic criteria described by Waterhouse (1963) and Stamps *et al.* (1990) and morphological criteria of colonies, mycelium characteristics, the growth medium, the presence or absence of chlamydospores, the morphology of hyphae and the dimensions of sporangia. Different identification keys were used for reference, including that of Feichtenberger *et al.* (1984).

RESULTS

Phytophthora species isolated

All colonies that developed from collected samples in this study seemed to be typical of *P. parasitica* and *P. citrophthora*, with a clear dominance of *P. citrophthora* populations over those of *P. parasitica*. No other *Phytophthora* strain was observed in any of the samples analyzed.

Effect of the rootstock on the density of Phytophthora inoculum in soil

The nature of the rootstock affected the density of *Phyophthora* propagules per gram of dry soil. According to the figure 1, we note that inoculum density varies depending on the rootstock. The use of some rootstocks tends to encourage an increase in propagule density relative to others whose rhizosphere displays relatively low densities all over the year.

This result enabled us to classify the rootstocks in four groups based on the inoculum density recorded in the vicinity of their root system.

The average inoculum density was highest in the rhizosphere of *Citrus volkameriana* B2 during July. By contrast, the inoculum density recorded in the rhizosphere of sour orange (*Citrus aurantium*) was the lowest with an average of 162 propagules per gram of soil.

- ***The 1st group:*** consisting of *Citrus volkameriana* whose rhizosphere showed maximum densities of more than 1000 propagules per gram of soil (Figure 2);
- ***The 2nd group:*** including the rootstocks Sunki mandarin x PT B2 30581, Sunki mandarin x PT B2 30588, Sunki mandarin x PT B2 30591, Carrizo citrange B2 28608, Sunki mandarin x PT B2 30590 and Cleopatra mandarin x PT B2 30584 with maximum densities ranging from 500 to 1000 propagules per gram of soil (Figure 3);

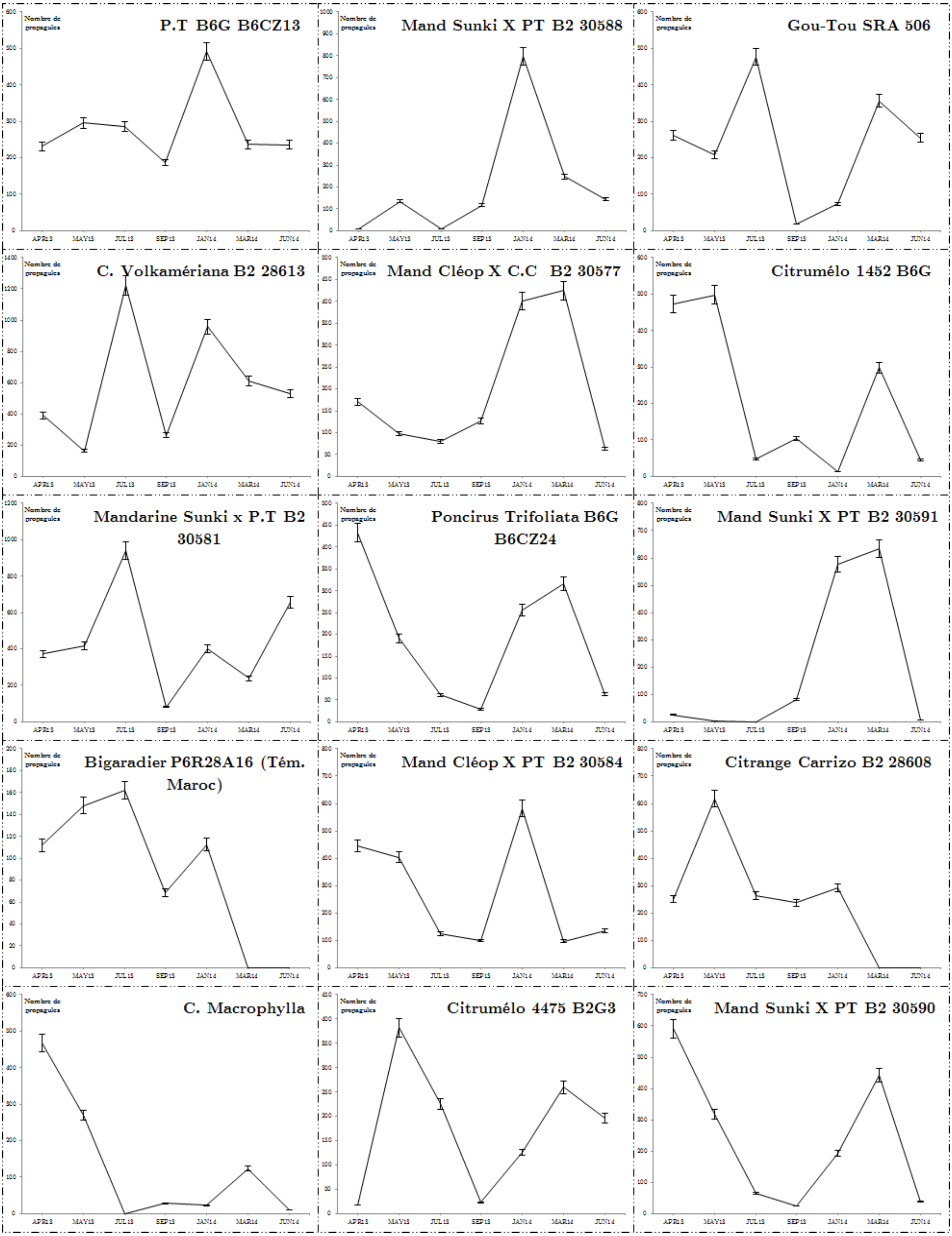

Figure 1 Seasonal variations of *Phytophthora* sp. populations at Allal Tazi experimental station between April 2013 and June 2014. For each rootstock and date, the density of propagules per gram of soil is an average of three replications. (In each point, segments represent a SD of 5% relatively to the average).

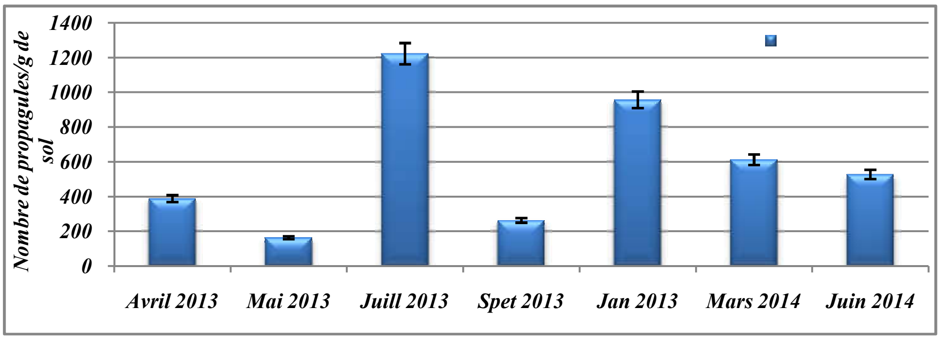

Figure 2 Seasonal variations of *Phytophthora* sp. populations in the rhizosphere of rootstocks of the 1st category between April 2013 and June 2014at Allal Tazi experimental station. (In each point, segments represent a SD of 5% relatively to the average).

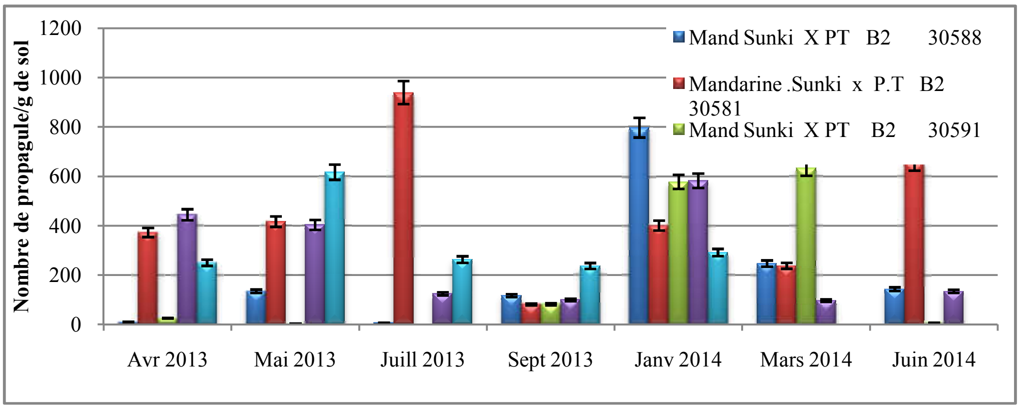

Figure 3 Seasonal variations of *Phytophthora spp.* populations in the rhizosphere of rootstocks of the 2nd category between April 2013 and June 2014 at Allal Tazi experimental station. (In each point, segments represent a SD of 5% relatively to the average).

The 3rd group: includes the rootstocks Citrumelo 1452 B2G, PT B6G B6CZ13, Gou-Tou SRA 506, *C. macrophylla*, *Poncirus trifoliata* B6G B6CZ24, Cleopatra mandarin x CC B2 30577 and Citrumelo 4475 B2G3 with maximum densities lower than 500 propagules per gram of soil (Figure 4);

- The 1st category: including the rootstocks PT B6G B6CZ13, Sunki mandarin x PT B2 30588, Cleopatra mandarin x CC B2 30577, Sunki mandarin x PT B2 30591 and Cleopatra mandarin x PT B2, which displayed maximum density values in winter.

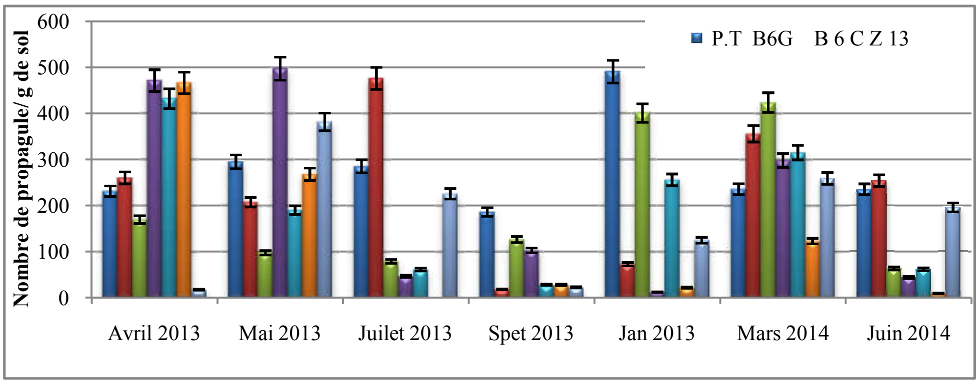

Figure 4 Seasonal variations of *Phytophthora* sp. populations in the rhizosphere of rootstocks of the 3rd category between April 2013 and June 2014 at Allal Tazi experimental station. (In each point, segments represent a SD of 5% relatively to the average).

- The 4th group: consisting of sour orange whose rhizosphere displayed significantly lower values those of all other rootstocks studied (Figure 5).

Influence of climate on Phytophthora density

In this study, we can also classify the rootstocks studied in categories based on the impact of climatic variations on maximum propagule densities recorded in their surroundings.

- The 2nd category: including the rootstocks Gou-Tou SRA 506, *C. volkameriana* B2 28613, sour orange P6R28A16 (Control Morocco), Sunki mandarin x PT B2 30581 and Citrumelo 4475 B2G3 with highest density values achieved in the summer.
- The 3rd category: including all the other rootstocks, with maximum density values reached in the spring.

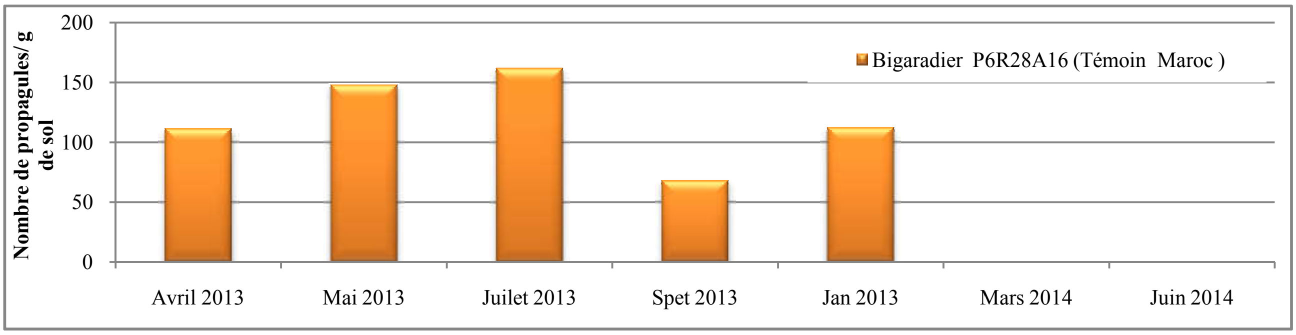

Figure 5 Seasonal variations of *Phytophthora* sp. populations in the rhizosphere of rootstocks of the 4th category between April 2013 and June 2014 at Allal Tazi experimental station. (In each point, segments represent a SD of 5% relatively to the average).

DISCUSSION

Our study revealed that the density of *Phytophthora* sp. inoculum in soil is influenced by both the nature of the rootstock and climatic conditions. Through this study, the lowest inoculum densities were found in the vicinity of orange roots, whereas the highest densities were recorded in the vicinity of *Citrus volkameriana*. In this sense, our results are consistent with the works of Afek *et al.* (1990); Benyahia (1993 and 1998). Among the other rootstocks, citrumelo 4475 B2G3 comes in the second place with an inoculum density more or less equivalent to that recorded in the vicinity of sour orange, which is in agreement with the findings of Agostini *et al.* (1991) and Bright and Graham (2004). Indeed, these authors reported a low density of *Phytophthora* sp. inoculum in the vicinity of citrumelo.

On the other hand, the highest inoculum density was recorded in the vicinity of *Citrus volkameriana* B2 roots. This result is in contrast with the findings of Bright and Graham (2004) who reported sparse *Phytophthora* populations near the roots of this rootstock. One plausible explanation is the heterogeneity of plant material since the accessions we used are different from those used by Graham. This fact was confirmed by the study of Benyahia (1993) which showed variability in the response to Phytophthora root rot among 18 sour orange accessions. However, in an earlier study, Agostini (1991) has shown Swingle citrumelo and trifoliate orange as rootstocks that were associated with sparse populations of *P. parasitica*, whereas sour orange and Cleopatra mandarin, although tolerant to gummosis, were associated with high inoculum density.

The high density of *Phytophthora* inoculum near the roots of *Citrus volkameriana* may be related to the ability of this rootstock to regenerate root fibers, which is an ideal substrate for the proliferation of *P. parasitica*. In this sense, Graham (1999) demonstrated that *C. volkameriana* is characterized by a great ability to regenerate root fibers and is concomitantly susceptible to *P. citrophthora* strains. This shows that the origin of the rootstock accession plays a key role in the determination of *Phytophthora* density in soil.

Concerning the impact of seasonal variations on the evolution of *Phytophthora* density in soil, we found in this study that the density is not constant but varies depending on the season and the rootstock used. The study of Menge *et al.* (1988), which was carried out in Californian citrus orchards, has also highlighted an effect of the season on the populations of *P. parasitica*. Indeed, high summer temperatures and low winter temperatures are known to catalyze the production of fungus inoculum (Agostini *et al.*, 1991).

Agostini *et al.* (1991) reported that high temperatures and heavy rainfall are conducive to the proliferation and sporulation of *P. parasitica*, which may explain the culminated propagule densities during April and May 2013 in our study. The same authors reported, however, sparse *Phytophthora* populations during winter seasons, which are characterized by temperature drops. Low temperatures, causing the dormancy of *P. parasitica* spores, or the absence of new roots could be direct causes of the decline in populations of the pathogen during the winter (Lutz and Menge, 1986). Similarly, Timmer *et al.*, (1987) and Mitchell (1986) were observed seasonal differences in *Phytophthora* populations in a soil that was planted with perennial plants. In contrast to the results and similarities aforementioned, Timmer *et al.* (1989) reported that differences in *Phytophthora* populations in citrus orchards are not related directly to seasonal climatic variations, but probably correlate to the extent of root rot that occurred previously.

The inconsistencies between our study and that of Timmer *et al.* (1989) may be due to differences in climatic conditions and/or cultural practices.

Furthermore, the works of Ippolito *et al.* (1992) and Dirac ***et al.*** (2003) highlighted a clear influence of climate on the pathogeneicity of different *Phytophthora* species. They reported that the severity of root infection with *P. citrophthora* was highest in winter, whereas the damage caused by *P. parasitica* was highest in autumn and moderate in winter.

The studies of Alvarez *et al.* (2009) and Dirac *et al.* (2003), using branches isolated and inoculated with two *Phytophthora* species, have also revealed seasonal variations, which supports our findings. These variations were shown even under constant temperature, which excludes temperature as a significant factor affecting growth of the pathogen.

On the other hand, the literature reports other factors which may affect the density and the evolution of *Phytophthora* inoculum. For instance, Feld *et al.* (1990) found that flood irrigation is more favorable than localized irrigation. We should note that flood irrigation was the method used in our experimental orchard and might therefore has predisposed the rootstocks to severe root attacks leading to high *Phytophthora* densities.

It appears from our study that the effect of the rootstock on the density of *Phytophthora* inoculum is evident, suggesting that the use of some genotypes can favor inoculum proliferation relative to others. Fourie (2004) demonstrated that the level of total soluble phenolic compounds increased in all citrus rootstocks once infected with *P. nicotianae.* However, the extent of this increase was higher in Troyer and Macrophylla (tolerant) than the rootstocks Rough lemon and *Citrus volkameriana* (sensitive). According to Nicholson and Hammerscmidt (1992), the rapid accumulation of phenolic compounds is a characteristic of resistance in rootstocks. In citrus, the production of phenolic compounds was widely reported (Rodov *et al.,* 1994) in peels (Dubery *et al.,* 1999), leaves (Manthey *et al.,* 2000) and roots (Feldman and Hanks, 1968). In previous investigations, mainly those by Afek and colleagues (1986; 1988; 1989; 1990; 1993; 1995), scoparone phytoalexin were involved in the resistance mechanism of citrus to crown rot cause by *Phytophthora citrophthora*. However, this study did not cover the root part. A quantification of phytoalexin production at root level following the infection is therefore necessary to accurately estimate the resistance of citrus rootstocks to root attacks caused by *Phytophthora*. Furthermore, the estimation of regeneration ability of root fibers under infection conditions would be valuable for evaluating rootstock tolerance to root rot.

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How to cite this article:

Dalal BOUDOUDOU *et al.*2016, Influence of Citrus Rootstocks on Soil Populations of Phytophthora SP. In the Gharb Region in Morocco. *Int J Recent Sci Res.* 7(11), pp. 14230-14236.