

Chapter 06

Traditional and Molecular Studies of the Plant Pathogen *Phytophthora Capsici*: A Review

Arturo Castro-Rocha¹, Juan Pedro Flores-Márgez¹, Marisela Aguirre-Ramírez², Sylvia Patricia Fernández-Pavía², Gerardo Rodríguez-Alvarado² and Pedro Osuna-Ávila¹

¹Departamento de Ciencias Básicas, Instituto de Ciencias Biomédicas, Universidad Autónoma de Ciudad Juárez, Anillo Envoltante del PRONAF y Estocolmo s/n, México

²Laboratorio de Patología Vegetal, Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, México

***Corresponding Author:** Pedro Osuna-Ávila, ¹Departamento de Ciencias Básicas, Instituto de Ciencias Biomédicas, Universidad Autónoma de Ciudad Juárez, Anillo Envoltante del PRONAF y Estocolmo s/n, Ciudad Juárez, Chihuahua, C. P. 32310, México, Email: posuna@uacj.mx

First Published **February 12, 2018**

This Book Chapter is an excerpt from an article published by Pedro Osuna-Ávila, et al. at *Journal of Plant Pathology & Microbiology* in November 2014. (Rocha-Castro A, Flores-Margez JP, Aguirre-Ramirez M, Fernandez- Pavia S, Rodriguez-Alvarado, et al. (2014) Traditional and Molecular Studies of the Plant Pathogen *Phytophthora capsici*: A Review. *J Plant Pathol Microb* 5:250. doi:10.4172/2157-7471.1000250)

Copyright: © 2018 Pedro Osuna-Ávila, et al.

This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source.

Abstract

Phytophthora capsici is a pathogen that limits the production of diverse crops of economic interest, mainly of the Solanaceae and Cucurbitaceae families. Many studies of this pathogen have been carried out due to its impact on agricultural production systems. This review covers studies on topics such as isolation methods, fungicide resistance, pathogenicity and virulence, physiological races, genetic diversity of its populations, and studies of the genetic expression during the plant-pathogen interaction. The objective of this review is to abridge the information generated on these topics in order to guide future research on this pathogen.

Keywords

Effector; Fungicide; Hemibiotroph; Oomycete; Pathogenicity; Virulence

Introduction

Phytophthora capsici is responsible for large worldwide economic losses and is considered one of the limiting factors in the production of many crops [1]. More than 50 vegetable species have been identified as hosts of this plant pathogen [1,2]. Recently, two leguminous species, *Phaseolus vulgaris* [3], and *Phaseolus lunatus* [4], have been identified as hosts of this oomycete. Losses of up to 100% of the crop can occur in fields due to the incidence of this pathogen [5].

In Mexico, like in the rest of the world, chili wilt caused by *P. capsici* is a serious problem. The presence of this soil borne pathogen has been reported in the states of Aguascalientes, Chihuahua, Oaxaca, Puebla, Queretaro, State of Mexico, and Zacatecas [6-12]. The virulence of the isolates of *P. capsici* found in Mexican fields does not follow a defined distribution pattern and isolates of different levels of virulence can co-exist within the same space [13].

Due to its agronomic importance, numerous studies have been carried out about this plant pathogen which address its isolation [14], morphological characteristics [15], virulence [16], physiological races [17], fungicide resistance [18], the genetic diversity of its populations [19], and its genetic expression during the plant-pathogen interaction [20]. The objective of this review is to abridge the information that has been generated on these topics in order to guide future research on this pathogen.

Isolation and Morphologic Characterization

It is possible to isolate *P. capsici* from plant tissues or soil samples by using selective media such as PARP (pimaricin, ampicillin, rifampicin, and pentachloronitrobenzene) [21], NARPH (Nistatin, ampicillin, rifampicin, pentachloronitrobenzene, and hymexazol) [22], BARP (benomyl, ampicillin, rifampicin, and pentachloronitrobenzene) [23], or with supplemented carrot medium (penicillin, methyl benzimidazol-2-carbamate, pentachloronitrobenzene, and rifampicin) [24].

P. capsici has also been reported in irrigation water. As a model to isolate *P. capsici* from water both pear and cucumber fruits have been used as baits. The fruits are placed in the irrigation water for intervals of 3 to 7 days. Those portions of the fruits that manifest wounds are transferred to a culture media supplemented with ampicillin and rifampicin [14]. In a similar procedure, pear and eggplant fruits can be used as baits and the wounded parts of the fruits are cultured on water agar media with rifampicin and ampicillin. However, placing the samples of the wounded fruits on the stems of susceptible pepper plants

can increase isolation yields. This method can increase isolation percentage between 53%-77% depending on the baiting fruit used [21]. Once the isolation has been carried out successfully, the isolates can be maintained on media such as corn meal agar, potato dextrose agar, V8 agar, oat agar, rye B agar, or carrot agar [24-28].

Isolating directly from susceptible hosts is the ideal situation as this helps to confirm that the symptoms observed are caused by the pathogen. However, monitoring soil samples and irrigation water can help evaluate the epidemiology of the disease which can thus allow a better management of the pathogen.

Morphological studies of *P. capsici* isolates recovered from natural populations have shown a high degree of variability respecting the size of the sporangia, pedicels, and oospores [29]. The presence of chlamydospores has been reported on some isolates [5]. Other variable characteristics among isolates are the form of the colony, the form of the sporangia, the abundance of sporangia produced, compatibility type, and optimal growing temperature [15,24,25,30-34]. The colony form can be determined as cottony, rosaceous, petaloid, or stellate [25]. The compatibility types (A1 and A2) of each isolate can be known by co-culturing each isolate with other isolates of known compatibility type and observing the presence of oospores [35]. Homothallic isolates have been occasionally reported. The presence of both compatibility types on the same field is required so that *P. capsici* can complete its sexual reproductive cycle [1].

Phenotypic variance is one of the first indicators of variability within a population. Of special interest to the study of *P. capsici* is to determine if both compatibility types are present within a population. The presence of both types would indicate the possibility of sexual reproduction and the appearance of new lineages within the population. Sexually reproductive populations implicate different management given that sexual reproduction makes it possible to fix certain traits in the population that may be advantageous to the pathogen, such as fungicide resistance.

Fungicide Resistance

Oomycetes possess genome plasticity that allows them to develop fungicide resistance [36]. Such is the case of metalaxyl, a systemic fungicide that interferes with the incorporation of uridine during RNA synthesis [37]. The resistance of *P. capsici* to metalaxyl and mefenoxam has been studied in laboratory and field conditions [38-39]. Most studies of *P. capsici* indicate the level of susceptibility of the isolates used to metalaxyl [15-16,21,22,34]. It has been shown that *in vitro* tests for susceptibility at 100 µg/ml can reliably predict resistance in the field [40].

Fungicide resistance in *P. capsici* has been formally studied for at least two decades (Table 1). When *P. capsici* populations show resistance to mefenoxam and metalaxyl, it is possible to use novel fungicides such as zoxamide, fluopicolide, ametoctradin, mandipropamid, and cymoxanil in alternation to prevent the appearance of resistant isolates [5]. The European and Mediterranean Plant Protection Organization recommends the application of the fungicides etridiazole, propamocarb, clorotalonil, and copper oxychloride [41]. The effects of copper on the development and infection capacity of different *Phytophthora* species has been extensively studied and shown to be an effective fungicide [42-44].

Mandipromamid and dimetopmorph are mandelamide type fungicides that inhibit the synthesis of lipids and membranes as well as the synthesis of cellulose and cell walls [45-47]. Cyazofamid is a cyanoimidazole type fungicide that inhibits cellular respiration at the complex III of the electron transport chain [48]. Continuous *in vitro* exposition to dimetomorph has resulted in resistant mutants [49]. Likewise, fluopicolide, flumorph, and pyrimorph resistant mutants have been reported [50-52]. Isolates resistant to pyrimorph present the mutation Q1077K in their cellulose synthase (CesA3) gene [52]. These studies suggest the possibility of generating resistant isolates to these novel fungicides if continuous exposure occurs in the field.

Management of *P. capsici* requires and appropriate use of fungicides. Determining which fungicides are effective against isolates of a give population is important to establish a correct use of such fungicides. However, constant application of one fungicide can lead to the development of resistance within the population. Therefore, special consideration must be taken on which fungicides to apply and at what intervals prevent this from happening.

Pathogenicity and Virulence

The pathogenicity and virulence of *P. capsici* has been reported for Solanaceous, Cucurbitaceous, and Leguminous species of economic interest such as chili pepper, tomato, cucumber, squash, pumpkin, snap bean, and for Fraser fir and weeds associated with crop fields [2-3,7,15-17,22,24,27,34,53-65]. Inoculation can be done with zoospores [21,64,66] or mycelia [2,16,55]. The capacity of infection is affected by the amount of inoculum, the higher the concentration of the inoculum the higher the aggressiveness of the infection [58,67,68]. When an isolate of *P. capsici* is obtained from a new host it is necessary to confirm its pathogenicity towards the host and corroborate Koch's postulates. The pathogenicity of *P. capsici* isolates obtained from the roots of *Geranium carlinianum*, *Solanum americanum*, and *Portulaca oleracea* were evaluated on chili plants, *S. nigrum*, *S. carolinense*, and *S. capsicoides*. Only *S. nigrum* presented mortality when infected by the pathogen [22]. Isolates of *P. capsici* have also been recovered from snap bean fields (*Phaseolus vulgaris*) and their pathogenicity has been confirmed on cultivars of *P. vulgaris* and *P. lunatus*, as well as soy plants (*Glycine max*) [3]. The susceptibility of Fraser fir to *P. capsici* has also been documented [2]. *Arabidopsis thaliana* has been successfully used as an experimental host [69].

Ristaino carried out one of the key studies on *P. capsici* in 1990 [29]. She evaluated the virulence of isolates obtained from seven pepper fields and seven cucurbits fields in North Carolina. She found that some isolates obtained from chili and cucurbits were highly virulent against chili, but that other isolates from cucurbit were less virulent.

Thus, this study made it clear that not all isolates have the same virulence level and that the host from which the isolates are obtained may play a role in their virulence towards other hosts.

Temperature, humidity, and the age of the fruit can affect the infection capacity of *P. capsici* over cucumber [55,57] and pepper fruits [70]. The disease is most severe at 25 °C, 4 days after the inoculation. In general, higher temperature and humidity increase the infection capacity of the pathogen. However, ripened fruits are less susceptible. Granke and Hausbeck [71] evaluated the effect of temperature, inoculum concentration, and zoospore age (measured as the number of days after being released from the sporangia) of *P. capsici* on the infection of cucumber fruits. They were susceptible to infection by zoospore suspensions that had a temperature between 9 and 32 °C. The highest infection capacity was exhibited when applying zoospores with concentrations equal to or higher than 5×10^3 zoospores/ml and temperatures higher than 12 °C. Zoospores of up to a maximum of 5 days of age were able to infect the fruits.

The virulence of a worldwide collection of *P. capsici* isolates was tested on zucchini, tomato, and chili peppers, by inoculating the fruits with V8 agar discs infested with mycelia. The diameter of the wound, the diameter of the pathogen's growth on the fruit, and the sporulation density on the fruit were determined. It was concluded that the host of origin from which the isolates were obtained had an effect over the capacity of infection of the isolates on the fruits of other hosts [72].

Virulence and pathogenicity of *P. capsici* has been studied worldwide on a wide range of crops in an effort to determine the interaction between isolates of this pathogen and its wide range of hosts. Virulence and pathogenicity tests are also important to local growers and breeders. These tests allow them to make decisions on which crops are better suited for their local environment and to develop breeding programs to generate resistant or tolerant crops. However, breeding

resistant crops to *P. capsici* is difficult due to the presence of physiological races within isolates of this pathogen.

P. capsici Physiological Races

The specificity of plant-pathogen interactions is determined by the host and pathogen genotype. This specificity is easier to study on plants that have been subjected to genetic improvement, these plants are known as cultivars. A specie can present cultivars that are susceptible to a pathogen and cultivars that are resistant to the same pathogen [74]. This phenomenon where isolates of a pathogen have different infection capacities towards diverse cultivars of the same host is known as physiological races and was described by Stakman in 1913 [74].

In order to determine the physiological races of a pathogen it is necessary to determine its interaction with a series of differential hosts [63]. The minimum recommended host age to determine susceptibility to *P. capsici* is when plants have 4 true leaves [66]. *P. capsici* can cause multiple syndromes as it can infect roots, foliage, stems, and fruits [17]. Investigations indicate that different genetic mechanisms are responsible for the resistances to root rot, crown rot, foliar blight, and fruit rot [75]. This allows for the possibility to find physiological races of *P. capsici* for each of the syndromes mentioned in different host species. The studies that have been carried out about *P. capsici* physiological races are listed in Table 2. There are two types of studies: those in which commercial cultivars have been used as differential hosts, and those where the “New Mexico Recombinant Inbred Lines” (NM-RIL) chili pepper lines have been used. It is also noteworthy that in all of the studies only one Mexican isolate has been used [60]. The only study in which this physiological race phenomena has been tested in field conditions is the one reported by Hwang and Kim [76], the rest of the studies have been carried out in greenhouse conditions. Only one study has evaluated the effect of inoculum concentration on

physiological race typing [53]. It was determined that inoculations with 10,000 or 100,000 zoospores yielded the same physiological races of Brazilian *P. capsici* isolates when using the NM-RIL as differential hosts. Most studies focus on the median value of the disease index to rate a specific cultivar as resistant or susceptible to a specific isolate, although mortality rate and area under the disease progress curve (AUDPC) for each of the interactions are sometimes also reported.

Until now, efforts to produce cultivars universally resistant to all isolates of *P. capsici* have not been successful. This in large part due to the presence of multiple physiological races of this pathogen across the world. Therefore, breeders are encouraged to develop cultivars based on tests on isolates that represent physiological races present in the growing fields of their interest. Breeding universal resistance into a host may be something unattainable by breeding programs, but breeding hosts resistant to a determined number of physiological races can be a more surmountable challenge.

Genetic Diversity

Genetic diversity studies allow us to analyze the population structure of the pathogen and to confirm the possible genetic exchange among isolates. The confirmation of this phenomenon in each geographical area where the pathogen thrives is key in order to implement a successful management program.

Within the first studies to evaluate the genetic diversity of *P. capsici* restriction fragment length polymorphism (RFLP) assays were carried out for genomic and mitochondrial DNA [78]. However, it was not possible to correlate the generated patterns with a geographic region or host of origin. Numerous studies have reported a wide range of molecular tools to determine the genetic diversity of *P. capsici*. Among these are included molecular markers such as random amplified polymorphic DNA (RAPD) [65], amplified fragment length polymorphism (AFLP) [79], and micro satellites or single sequence

repeats (SSR) [80]. One of the advantages of using AFLP is the large number of markers (50-70) that can be resolved per reaction [81]. A characterization of 107 oospores obtained from a cross of two isolates with distinct AFLP genotyping indicated that AFLP marker segregation was Mendelian [38].

Another genetic marker that can be used to genotype *P. capsici* isolates are single nucleotide polymorphisms (SNP) [79], this can be achieved with high resolution DNA melting analysis (HR-DMA) [19]. The use of this technique allows the construction of multi locus single nucleotide polymorphism genotype profiles [81]. These profiles, besides assessing the genetic diversity of a population of *P. capsici*, generate information about the population dynamics and possible genetic material exchange. *P. capsici* population studies have demonstrated that SNP profiles match genotyping with 40 AFLP markers [82]. It is estimated that the *P. capsici* genome has a SNP substitution rate of one polymorphic site for every 40 bases [83].

An alternative to study the genetic structure of *P. capsici* populations is to compare the sequence of distinct polymorphic nuclear and mitochondrial loci such as Cox1, Cox2, Nad1, Nad5, β -tubulin, EF-1 α , Enolase, HSP90, TigA, Ura3, and ITS [22-23]. Protein patterns obtained by isoelectric focus have also been used to identify *Phytophthora* species [84], as taxonomic criteria [33], or to evaluate *P. capsici* diversity [15]. These profiles are performed from total mycelia protein, from specific enzyme systems like the acid phosphatases, or from isoenzyme systems of esterases, malate dehydrogenases, and superoxide dismutases [85-86].

Population genetics studies of *P. capsici* can help us understand the dynamics underlying the epidemiology of this pathogen. Higher diversity in a population indicates a higher probability of sexual reproduction within the population. Low diversity could indicate clonal lineages propagation. Genotyping individuals can help track their origin and explain their dispersal through a given geographical area. The sequencing of the *P. capsici* genome [83] has given way to the

development of SNP markers that allow fine scale studies on the genetic diversity of this organism. The use of these tools can lead to better management of the disease by allowing us to understand how the pathogen is being propagated and what forces are driving its population structures.

Evaluation of the Genetic Expression during the Plant-Pathogen Interaction

Most functional genomics studies of *Phytophthora* species have been carried out by genetic transformation, heterologous expression systems, gene silencing, or mutagenesis directed gene disruption [87-90]. However, these type of studies focus on only a handful of genes. It is presumed that most of the processes involved in the infection and colonization of hosts are regulated by multiple genes [91-92].

One of the ways to identify the genes involved in a particular development stage of an organism is to study the transcriptional changes that take place during said stage. The identification of these genes has been facilitated by differential identification methods such as genomic DNA or cDNA libraries hybridizations and subtractive hybridizations [93]. The *P. capsici* genome has been recently sequenced [83]. It is now possible to consult a database that includes all of the open reading frames (ORFs) that have been predicted in the genome of this pathogen (<http://genome.jgi-psf.org/Phyca11/Phyca11.download.ftp.html>).

Eighty-four effector protein encoding genes of the “crinkler” (CRN) family, related with the infection process, have been identified. The expression of these genes was evaluated during the plant-pathogen interaction of *P. capsici* and tomato at 0, 8, 16, 24, 48, and 72 hours after the inoculation with the help of a microarray composed of the sequences reported for the *P. capsici* genome. The expression levels analysis revealed that some the genes are related to the early stages of the infection while others are related to the late stage. The

fusion of some these effector proteins with green fluorescent proteins (GFP) allowed their localization within the plant cell, where they were found to aggregate around the nucleus and sub nuclear structures [20]. This is consistent with what was previously suggested about effector proteins, which are believed to be the ones in charge of deactivating the defense mechanisms of the plant cell [94-95]. The changes in the genetic expression during the different phases of the infection process have been related to the hemibiotrophic lifestyle of *P. capsici*. The modulation of the genetic expression of this oomycete during its interaction with tomato plants has allowed the identification of four classes of RxLR effectors that are expressed during different physiological process and that are related with changes in the infection process [96]. Three cDNA libraries of *P. capsici* obtained from three different physiological states (mycelia, zoospores, and germinated cysts) have been sequenced. The number of genes expressed during each state corresponded to 13,901 for mycelia, 14,633 for zoospores, and 14,695 for cysts. The large difference in expressed genes during each state is attributed specific genes required for each of the development states, including 98 genes that codify for effector proteins [97]. Another group of genes related with the infection process that have been recently defined are the “Necrosis Inducing Phytophthora Proteins” (NPP), which participate in the necrosis of the plant tissues [98]. Eighteen genes that encode proteins of the NPP family have been identified active in the mycelia of *P. capsici* [99].

The gene *phcnlp1* codifies for a Nep1 like protein of 476 amino acids with a predicted mass of 51.75 kDa. It was isolated from the *P. capsici* isolate Phyc12. The expression of this gene was evaluated during the interaction of the isolate with pepper leaves where it was observed that the product of this gene induced visible wounds. The same effect was produced in tabaco (*Nicotiana tabacum*) leaves. These results suggest that *phcnlp1* is directly related to the pathogenesis of *P. capsici* [100].

The gene *pcpme6* codifies for a 348 amino acid protein of the

pectin methylesterase family with a predicted mass of 38.18 kDa. This gene was isolated from a cDNA library of the *P. capsici* isolate SD33. It was demonstrated that the expression of this gene increases during the infection of pepper leaves, degrading the cell walls and producing necrotic lesions [101]. Similarly, the gen Pcpel1 was obtained from a genomic DNA library of the *P. capsici* SD33 isolate. This gene encodes a 410 amino acid pectate lyase of a predicted mass of 43.8 kDa and has a high level of expression during the interaction of the pathogen with pepper leaves [102]. These studies are consistent with what was reported by Jia et al [103] which demonstrated a correlation between the virulence of isolates of *P. capsici* and the activity of the enzymes polygalacturonase, pectate lyase, and pectin methylesterase of the isolates cultured *in vitro* in the presence of pepper fruits extracts. The expression of pectin methylesterase genes present in *P. capsici* differs according to the host upon which the infection is taking place [104].

While the use of advanced genomics tools such as microarrays and next generation sequencing have allowed the identification of multiple genes that are suspected to be involved in the infection process of *P. capsici*, much work is needed to validate the functions of these genes. In order to truly understand which of this genes play a significant role in determining the susceptibility of a host it is necessary to determine what are the products of this genes, what their function is, how they internalize into the host, and what their target is within the host. Much work is needed in this area before the information being generated can be successfully used for the development of resistant hosts.

Conclusion

The studies about *P. capsici* can be categorized in two main groups: those related to aspects of the integrated management of this pathogen (virulence, pathogenicity, and fungicide resistance), and

those that allow us to understand the genetic underpinnings of this organism (genetic diversity and genetic expression).

Studies on pathogenicity and virulence allow breeders and growers to select crops that are better suited to their needs. The information generated by these studies can allow us to discern the isolates into specific physiological races which can be studied in greater detail to improve breeding programs in generating resistant cultivars. Studies focused on the identification of physiological races may have an impact on the management of the disease caused by *P. capsici*, as a large number of physiological races coupled with the presence of both compatibility types in the same field could indicate a zone where the management of the disease is more complicated. The presence of both compatibility types could also facilitate the transmission of fungicide resistance genes, which should be considered when administering fungicides to a field.

Genetic diversity studies help us to understand the population dynamics of this pathogen and its dispersal patterns. This study could also help us to deduce the forces driving the structure of its populations. Studies that evaluate the genetic expression during the plant-pathogen interaction have helped to identify those genes that are directly related to the infection process. These studies have highlighted the importance of effector proteins, which are attributed the capacity to take control over the plant cell during the infection process, and cell wall degrading enzymes. However, much work is needed to validate the numerous infection related genes that have been identified in *P. capsici* in order use that knowledge to develop resistant crops.

References

1. Hausbeck MK, Lamour KH. *Phytophthora capsici* on vegetable crops: research progress and management challenges. Plant Disease. 2004; 88: 1992-1303.
2. Quesada-Ocampo LM, Fulbright DW, Hausbeck MK. Susceptibility of Fraser fir to *Phytophthora capsici*. Plant Disease. 2009; 93: 135-141.

3. Gevens AJ, Donahoo RS, Lamour KH, Hausbeck MK. Characterization of *Phytophthora capsici* causing foliar and pod blight of snap bean in Michigan. *Plant Disease*. 2008; 92:201-209.
4. Davidson CR, Carroll RB, Evans TA, Mulrooney RP. First report of *Phytophthora capsici* infecting lima bean (*Phaseolus lunatus*) in the Mid-Atlantic region. *Plant Disease*. 2002; 86: 1049.
5. Granke LL, Quesada-Ocampo LM, Lamour KH, Hausbeck MK. Advances in research on *Phytophthora capsici* on vegetable crops in the United States. *Plant Disease*. 2012; 95: 1588-1600.
6. Guigón-López C, González-González P. Estudio regional de las enfermedades del chile (*Capsicum annum*, L.) y su comportamiento temporal en el sur de Chihuahua, México. *Revista Mexicana de Fitopatología*. 2001; 19:49-56.
7. Morán-Bañuelos SH, Aguilar-Rincón VH, Corona-Torres T, Zavaleta-Mejía E. Resistance to *Phytophthora capsici* Leo. of chilli pepper landraces of southern Puebla, México. *Revista Fitotecnia Mexicana*. 2010; 33: 21-26.
8. Pérez-Moreno L, Durán-Ortiz L, Ramírez-Malagón R, Sánchez-Palé R, Olalde-Portugal V. Compatibilidad fisiológica y sensibilidad a fungicidas de aislamientos de *Phytophthora capsici* Leo. *Revista Mexicana de Fitopatología*. 2003; 21: 19-25.
9. Silva-Rojas HV, Fernández-Pavía SP, Góngora-Canul C, Macías-López BC y Ávila-Quezada GD. Distribución espacio temporal de la Marchitez del chile (*Capsicum annum* L.) en Chihuahua e identificación del agente causal *Phytophthora capsici* Leo. *Revista Mexicana de Fitopatología*. 2009;

27:134-147.

10. Vásquez-López A, Tlapal-Bolaños B, Yáñez-Morales M, Pérez-Pacheco R, Quintos-Escalante M. Etiología de la marchitez del “chile de agua” (*Capsicum annuum* L.) en Oaxaca, México. Revista Fitotecnia Mexicana. 2009; 32: 127-134.
11. Velásquez-Valle R, Medina-Aguilar M, Luna-Ruiz J. Sintomatología y géneros de patógenos asociados con las pudriciones de la raíz del chile (*Capsicum annuum* L.) en el norte-centro de México. Revista Mexicana de Fitopatología. 2001; 19:175-181.
12. Zapata-Vázquez A, Sánchez-Sánchez M, del Río-Robledo A, Silos-Espino H, Perales-Segovia C, et al. *Phytophthora capsici* epidemic dispersion on commercial pepper fields in Aguascalientes, Mexico. The Scientific World Journal. 2012; 341764.
13. Rodríguez-Moreno VM, Luna-Ruiz J, Valle-García P, Tiscareño-López M, Ruiz-Corral JA. Caracterización patogénica y sexual de *Phytophthora capsici* Leonian y análisis de su distribución especial en el centro-norte de México mediante un sistema de información geográfica. Revista Mexicana de Fitopatología. 2004; 22: 72-81.
14. Gevens AJ, Donahoo RS, Lamour KH, Hausbeck MK. Characterization of *Phytophthora capsici* from Michigan surface irrigation water. Phytopathology. 2007; 97: 421-428.
15. Fernández-Pavía SP, Biles CL, Waugh ME, Onsurez WK, Rodríguez AG, et al. Characterization of Southern New Mexico *Phytophthora capsici* Leon isolates from pepper (*Capsicum annuum* L.). Revista Mexicana de Fitopatología. 2004; 22: 82-89.
16. Enzenbacher TB, Hausbeck MK. An evaluation of cucur-

- bits for susceptibility to cucurbitaceous and solanaceous *Phytophthora capsici* isolates. *Plant Disease*. 2012; 96: 1404-1414.
17. Oelke L, Bosland P, Steiner R. Differentiation of race specific resistance to *Phytophthora* root rot and foliar blight in *Cap-sicum annuum*. *Journal of the American Society for Horticultural Science*. 2003; 128: 213-218.
 18. Foster JM, Hausbeck MK. Managing *Phytophthora* crown and root rot in bell pepper using fungicides and host resistance. *Plant Disease*. 2010; 94: 697-702.
 19. Gobena D, McGrath MT, Lamour K. Survival and spread of *Phytophthora capsici* on Long Island, New York. *Mycological Progress*. 2012; 11: 761-768.
 20. Stam R, Jupe J, Howden AJM, Morris JA, Boevink PC, et al. Identification and characterization CRN effectors in *Phytophthora capsici* shows modularity and functional diversity. *PLoS ONE*. 2013; 8: e59517.
 21. Wang Z, Langston DB, Csinos AS, Gitaitis RD, Walcott RR, Ji P. Development of an improved isolation approach and simple sequence repeat markers to characterize *Phytophthora capsici* populations in irrigation ponds in southern Georgia. *Applied and Environmental Microbiology*. 2009; 75: 5467-5473.
 22. French-Monar RD, Jones JB, Roberts PD. Characterization of *Phytophthora capsici* associated with roots of weeds on Florida vegetable farms. *Plant Disease*. 2006; 90: 345-350.
 23. Quesada-Ocampo LM, Granke LL, Hausbeck MK. Temporal genetic structure of *Phytophthora capsici* populations from a creek used for irrigation in Michigan. *Plant Disease*.

2011; 95: 1358-1369.

24. Li Z, Long W, Zheng J, Lei J. Isolation and Identification of *Phytophthora capsici* in Guangdong Province and measurement of their pathogenicity and physiological race differentiation. *Frontiers of Agriculture in China*. 2007; 1: 377-381.
25. Islam SZ, Babadoost M, Lambert KN, Ndeme A, Fouly HM. Characterization of *Phytophthora capsici* isolates from processing pumpkin in Illinois. *Plant Disease*. 2004; 89: 191-197.
26. Kim DS, Chun SJ, Jeon JJ, Lee SW, Joe GH. Synthesis and fungicidal activity of ethaboxam against oomycetes. *Pest Management Science*. 2004; 60: 1007-1012.
27. Kim ES, Hwang BK. Virulence to Korean pepper cultivars of isolates of *Phytophthora capsici* from different geographic areas. *Plant Disease*. 1992; 76: 486-489.
28. Lucas JA, Greer G, Oudemans PV, Coffey MD. Fungicide sensitivity in somatic hybrids of *Phytophthora capsici* obtained by protoplast fusion. *Physiological and Molecular Plant Pathology*. 1990; 36: 175-187.
29. Ristaino JB. Intraspecific variation among isolates of *Phytophthora capsici* from pepper and cucurbit fields in North Carolina. *Phytopathology*. 1990; 80: 1253-1259.
30. Aragaki M, Uchida J. Morphological distinctions between *Phytophthora capsici* and *P. tropicalis* sp. nov. *Mycologia*. 2001; 93: 137-145.
31. Bowers JH, Martin FN, Tooley PW, Luz EDMN. Genetic and morphological diversity of temperate and tropical isolates of *Phytophthora capsici*. *Phytopathology*. 2007; 97: 492-503.
32. Granke LL, Quesada-Ocampo LM, Hausbeck MK. Variation in phenotypic characteristics of *Phytophthora capsici*

- isolates from a worldwide collection. *Plant Disease*. 2011; 95: 1080-1088.
33. Mchau GRA, Coffey MD. Evidence for the existence of two distinct subpopulations in *Phytophthora capsici* and a re-description of the species. *Mycological Research*. 1995; 99: 89-102.
 34. Tamietti G, Valentino D. Physiological characterization of a population of *Phytophthora capsici* Leon from northern Italy. *Journal of Plant Pathology*. 2001; 83: 199-205.
 35. Truong N, Liew E, Burgess L. Characterization of *Phytophthora capsici* isolates from black pepper in Vietnam. *Fungal Biology*. 2010; 114: 160-170.
 36. Tyler B. Genetics and genomics of the oomycete-host interface. *Trends in Genetics*. 2001; 17: 611-614.
 37. Davidse LC, Hofman AE, Velthuis GCM. Specific interference of metalaxyl with endogenous RNA polymerase activity in isolated nuclei from *Phytophthora megasperma* f. sp. *medicaginis*. *Experimental mycology*. 1983; 7: 344-361.
 38. Lamour KH, Hausbeck MK. The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers. *Phytopathology*. 2001; 91: 553-557.
 39. Parra G, Ristaino JB. Resistance to mefenoxam and metalaxyl among field isolates of *Phytophthora capsici* causing *Phytophthora* blight of pepper. *Plant Disease*. 2001; 85: 1069-1075.
 40. Lamour KH, Hausbeck MK. Susceptibility of mefenoxam-treated cucurbits to isolates of *Phytophthora capsici* sensitive and insensitive to mefenoxam. *Plant Disease*. 2003; 87: 920-922.

41. EPPO. Solanaceous crops under protected cultivation. EPPO Bulletin. 2004; 34: 65-77.
42. Halsall DM. Effects of certain cations on the formation and infectivity of *Phytophthora* zoospores. 2. Effects of copper, boron, manganese, molybdenum, and zinc ions. Canadian Journal of Microbiology. 1977; 23: 1002-1010.
43. Slade SJ, Pegg GF. The effect of silver and other metal ions on the in vitro growth of root-rotting *Phytophthora* and other fungal species. Annals of Applied Biology. 1993; 122: 233-251.
44. Toppe B, Thinggaard K. Influence of copper ion concentration and electrical conductivity of nutrient solution on *Phytophthora cinnamomi* in ivy grown in ebb-and-flow systems. Journal of Phytopathology. 2000; 148: 579-585.
45. Blum M, Boehler M, Randall E, Young V, Csukai M, et al. Mandipropamid targets the cellulose synthase-like PiCesA3 to inhibit cell wall biosynthesis in the oomycete plant pathogen *Phytophthora infestans*. Molecular Plant Pathology. 2010; 11: 227-243.
46. Kuck KH, Gisi U. FRAC mode of action classification and resistance risk of fungicides. In: Krämer W y Schirmer U, editor. Modern crop protection compounds. Weinheim: Wiley-VCH Verlag GmbH and company KGaA. 2008; 415-432.
47. Kuhn PJ, Pitt D, Lee SA, Wakley G, Sheppard AN. Effects of dimetomorph on the morphology and ultrastructure of *Phytophthora*. Mycological Research. 1991; 95: 333-340.
48. Mitani S, Araki S, Yamaguchi T, Takii Y, Ohshima T, et al. Antifungal activity of the novel fungicide cyazofamid against *Phytophthora infestans* and other plant pathogenic fungi in vitro. Pesticide Biochemistry and Physiology. 2001;

70: 92-99.

49. Sun H, Wang H, Stammler G, Ma J, Zhou Mingguo. Baseline sensitivity of populations of *Phytophthora capsici* from China to three carboxylic acid amide (CAA) fungicides and sequence analysis of cholinephosphotransferases from a CAA-sensitive isolate and CAA-resistant laboratory mutants. *Journal of Phytopathology*. 2010; 158: 244-252.
50. Lu XH, Hausbeck MK, Liu XL, Hao JJ. Wild type sensitivity and mutation analysis for resistance risk of fluopicolide in *Phytophthora capsici*. *Plant Disease*. 2011; 95: 1535-1541.
51. Meng QX, Cui XL, Bi Y, Wang Q, Hao JJ, et al. Biological and genetic characterization of *Phytophthora capsici* mutants resistant to flumorph. *Plant Pathology*. 2011; 60: 957-966.
52. Pang Z, Shao J, Chen L, Lu X, Hu J, et al. Resistance to the novel fungicide pyrimorph in *Phytophthora capsici*: Risk assessment and detection of point mutations in Cesa3 that confer resistance. *PLoS One*. 2013; e56513.
53. da Costa Ribeiro SC, Bosland PW. Physiological race characterization of *Phytophthora capsici* isolates from several host plant species in Brazil using New Mexico Recombinant Inbred Lines of *Capsicum annuum* at two inoculum levels. *Journal of the American Society for Horticultural Science*. 2012; 137: 421-426.
54. Foster JM, Hausbeck MK. Resistance of pepper to *Phytophthora* crown, root, and fruit rot is affected by isolate virulence. *Plant Disease*. 2010; 94: 24-30.
55. Gevens AJ, Ando K, Lamour KH, Grumet R, Hausbeck MK. A detached cucumber fruit method to screen for resistance to *Phytophthora capsici* and effect of fruit age on susceptibility.

- ity to infection. *Plant Disease*. 2006; 90: 1276-1282.
56. Glossier B, Ogundiwin E, Sidhu G, Sischo D, Prince J. A differential series of pepper (*Capsicum annuum*) lines delineates fourteen physiological races of *Phytophthora capsici*. *Euphytica*. 2008; 162: 23-30.
 57. Granke LL, Hausbeck MK. Effects of temperature, humidity, and wounding on development of *Phytophthora* rot of cucumber fruit. *Plant Disease*. 2010; 94: 1417-1424.
 58. Lee BK, Kim BS, Chang SW, Hwang BK. Aggressiveness to pumpkin cultivars of isolates of *Phytophthora capsici* from pumpkin and pepper. *Plant Disease*. 2001; 85: 497-500.
 59. Monroy-Barbosa A, Bosland PW. Identification of novel physiological races of *Phytophthora capsici* causing foliar blight using the New Mexico recombinant inbred pepper lines set as a host differential. *Journal of the American Society for Horticultural Science*. 2011; 136: 205-210.
 60. Polach FJ, Webster RK. Identification of strains and inheritance of pathogenicity in *Phytophthora capsici*. *Phytopathology*. 1971; 62: 20-26.
 61. Quesada-Ocampo LM, Hausbeck MK. Resistance in tomato and wild relatives to Crown root rot caused by *Phytophthora capsici*. *Phytopathology*. 2010; 100: 619-627.
 62. Silvar C, Merino F, Díaz J. Diversity of *Phytophthora capsici* in northwest Spain: Analysis of virulence, metalaxyl response, and molecular characterization. *Plant Disease*. 2006; 90: 1135-1142.
 63. Sy O, Steiner R, Bosland P. Recombinant inbred line differential identifies race-specific resistance to *Phytophthora* root rot in *Capsicum annuum*. *Phytopathology*. 2008; 98: 867-870.

64. Tian D, Babadoost M. Host range of *Phytophthora capsici* from pumpkin and Pathogenicity of isolates. Plant Disease. 2004; 88: 485-489.
65. Yin J, Jackson KL, Candole BL, Csinos AS, Langston DB, et al. Aggressiveness and diversity of *Phytophthora capsici* on vegetable crops in Georgia. Annals of Applied Biology. 2012; 160: 191-200.
66. Bosland P, Lindsey D. A seedling screen for *Phytophthora* Root Rot of pepper, *Capsicum annuum*. Plant Disease. 1991; 75: 1048-1050.
67. Lee J, Lee WP, Kang B-C, Yoon JB. Inheritance of resistance to *Phytophthora* root rot in chili pepper depending on inoculum density and parental genotypes. Korean Journal of Breeding Science. 2012; 44: 503-509.
68. Koç E, Sülün Üstün A, İşlek C, Kaşko Arıcı Y. Defense responses in leaves of resistant and susceptible pepper (*Capsicum annum* L.) cultivars infected with different inoculum concentrations of *Phytophthora capsici* Leon. Scientia Horticulturae. 2011; 128: 434-442.
69. Wang Y, Bouwmesster K, Van de Mortel JE, Shan W, Govers F. A novel Arabidopsis-oomycete pathosystem: differential interactions with *Phytophthora capsici* reveal a role for camalexin, indole glucosinolates and salicylic acid in defense. Plant, Cell and Environment. 2013; 36: 1192-1203.
70. Biles CL, Wall MM, Waugh M, Palmer H. Relationship of *Phytophthora* fruit rot in fruit maturation and cuticle thickness of New Mexican-type peppers. Phytopathology. 1993; 83: 607-611.
71. Granke LL, Hausbeck MK. Effects of temperature, concentration, age, and algacides on *Phytophthora capsici* zoospore infectivity. Plant Disease. 2010; 94: 54-60.

72. Granke LL, Quesada-Ocampo LM, Hausbeck MK. Differences in virulence of *Phytophthora capsici* isolates from a worldwide collection on host fruits. *European Journal of Plant Pathology*. 2012; 132: 281-296.
73. Madriz K. Mecanismos de defensa en las interacciones planta-patógeno. *Manejo Integrado de Plagas (Costa Rica)*. 2002; 3: 22-32.
74. Stakman EC. A study in cereal rusts: physiological races. Doctoral thesis. University of Minnesota. 1913.
75. Sy O, Bosland PW. Inheritance of *Phytophthora* stem blight resistance as compared to *Phytophthora* root rot and foliar blight in *Capsicum annuum* L. *Journal of the American Society for Horticultural Science*. 2005; 30: 75-78.
76. Hwang BK, Kim CH. *Phytophthora* blight of pepper and its control in Korea. *Plant Disease*. 1995; 79: 221-227.
77. Forster H, Oudemans P, Coffey MD. Mitochondrial and nuclear DNA diversity within six species of *Phytophthora*. *Experimental Mycology*. 1990; 14: 18-31.
78. Hulvey J, Gobena D, Finley L, Lamour K. Co-occurrence and genotypic distribution of *Phytophthora* species recovered from watersheds and plant nurseries of eastern Tennessee. *Mycologia*. 2010; 102:1127- 1133.
79. del Castillo-Múnica J, Cárdenas M, Pinzón A, Castañeda A, et al. Developing a taxonomic identification system of *Phytophthora* species based on microsatellites. *Revista Iberoamericana de Micología*. 2013; 30: 88-95.
80. Blears MJ, De Grandis SA, Lee H, Trevors JT. Amplified fragment length polymorphism (AFLP): A review of the procedure and its applications. *Journal of Industrial Microbiology and Biotechnology*. 1998; 21: 99-114.

81. Gobena D, Roig J, Galmarini CR, Hulvey J, Lamour K. Genetic diversity of *Phytophthora capsici* isolates from pepper and pumpkin in Argentina. *Mycologia*. 2012; 104: 102-107.
82. Hurtado-Gonzales OP, Aragon-Caballero L, Apaza-Tapia W, Donahoo R, et al. Survival and spread of *Phytophthora capsici* in coastal Peru. *Phytopathology*. 2008; 98: 688-694.
83. Lamour KH, Mudge J, Gobena D, Hurtado-Gonzales OP, Schmutz J, Kuo A, et al. Genome sequencing and mapping reveal loss of heterozygosity as a mechanism for rapid adaptation in the vegetable pathogen *Phytophthora capsici*. *Molecular Plant-Microbe Interactions*. 2012; 25: 1350-1360.
84. Oudemans P, Coffey MD. Isozyme comparison within and among worldwide sources of three morphologically distinct species of *Phytophthora*. *Mycological Research*. 1991; 95: 19-30.
85. Erselius L, Vallavieille C. Variation in protein profiles of *Phytophthora*: Comparison of six species. *Transactions of the British Mycological Society*. 1984; 3: 463-472.
86. Nwaga D, Normand M LE, Citharel J. Identification and differentiation of *Phytophthora* by electrophoresis of mycelial proteins and isoenzymes. *EPPO Bulletin*. 1990; 20: 35-45.
87. Kamoun S, van West P, Vleeshouwers VG, de Groot KE, Govers F. Resistance of *Nicotiana benthamiana* to *Phytophthora infestans* is mediated by the recognition of the elicitor protein INF1. *The Plant Cell*. 1998; 10: 1413-1426.
88. van West P, Kamoun S, van't Klooster JW, Govers F. Inter-nuclear gene silencing in *Phytophthora infestans*. *Molecular Cell*. 3: 339-348.
89. Vleeshouwers VG, Driesprong JD, Kamphuis LG, Torto-Alalibo T, Van't Slot KA, et al. Agroinfection-based high-

- throughput screening reveals specific recognition of INF elicitors in *Solanum*. *Molecular Plant Pathology*. 2006; 7: 499–510.
90. Whisson SC, Avora AO, van West P, Jones JT. A method for double-stranded RNA-mediated transient gene silencing in *Phytophthora infestans*. *Molecular Plant Pathology*. 2005; 6: 153–163.
 91. Prakob W, Judelson HS. Gene expression during oosporengesis in heterothallic and homothallic *Phytophthora*. *Fungal Genetics and Biology*. 2007; 44: 726–739.
 92. Tani S, Yatzkan E, Judelson HS. Multiple pathways regulate the induction of genes during zoosporogenesis in *Phytophthora infestans*. *Molecular Plant-Microbe Interactions*. 2004; 17: 330–337.
 93. Shan W, Marshall JS, Hardham AR. Gene expression in germinated cysts of *Phytophthora nicotianae*. *Molecular plant pathology*. 2004; 5: 317–330.
 94. Kale SD, Tyler BM. Entry of oomycete fungal effectors into plant and animal host cells. *Cellular Microbiology*. 2011; 13: 1839–1848.
 95. Schornack S, van Damme M, Bozkurt TO, Cano LM, Smoker M, et al. Ancient class of translocated oomycete effectors targets the host nucleus. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107: 17421–17426.
 96. Jupe J, Stam R, Howden AJM, Morris JA, Zhang R, et al. *Phytophthora capsici*-tomato interaction features dramatic shifts in gene expression associated with hemi-biotrophic lifestyle. *Genome Biology*. 2013; 14: R63.

97. Chen XR, Xing YP, Li YP, Tong YH, Xu JY. RNA-Seq reveals infection related gene expression changes in *Phytophthora capsici*. PLoS One. 2013; 8: e74588.
98. Lamour KH, Stam R, Jupe J, Huitema E. The oomycete broad-host-range pathogen *Phytophthora capsici*. Molecular Plant Pathology. 2012; 13: 329-337.
99. Feng BZ, Li PQ, Fu L, Sun BB, Zhang XG. Identification of 18 genes encoding necrosis-inducing proteins from the plant pathogen *Phytophthora capsici* (Pythiaceae: Oomycetes). Genetics and Molecular Research. 2011; 10: 910-922.
100. Feng BZ, Li PQ. Molecular characterization and functional analysis of the Nep1-like protein-encoding gene from *Phytophthora capsici*. Genetics and Molecular Research. 2013; 12: 1468-1478.
101. Feng B, Li P, Wang H, Zhang X. Functional analysis of Pcpme6 from oomycete plant pathogen *Phytophthora capsici*. Microbial Pathogenesis. 2010; 49: 23-31.
102. Fu L, Wang HZ, Feng BZ, Zhang XG. Cloning, expression, purification and initial analysis of a novel pectate lyase Pcpell from *Phytophthora capsici*. Journal of Phytopathology. 2013; 161: 230-238.
103. Jia YJ, Feng BZ, Sun WX, Zhang XG. Polygalacturonase, pectate lyase and pectin methylesterase activity in pathogenic strains of *Phytophthora capsici* incubated under different conditions. Journal of Phytopathology. 2009; 157: 585-591.
104. Li P, Feng B, Wang H, Tooley PW, Zhang X. Isolation of nine *Phytophthora capsici* pectin methylesterase genes which are differentially expressed in various plant species. Journal of Basic Microbiology. 2011; 51: 61-70.

105. Qi R, Wang T, Zhao W, Li P, Ding J, et al. Activity of ten fungicides against *Phytophthora capsici* resistant to metalaxyl. *Journal of Phytopathology*. 2012; 160: 717-722.
106. Daniell IR, Falk CL. Economic comparison of *Phytophthora* root rot control methods. *Crop Protection*. 1994; 13: 331-336.
107. Biles CL, Lindsey DL, Liddell CM. Control of *Phytophthora* root rot of chile peppers by irrigations practices and fungicides. *Crop Protection*. 1992; 11: 225-228.
108. Jackson KL, Yin J, Ji P. Sensitivity of *Phytophthora capsici* on vegetable crops in Georgia to mandipropamid, dimethomorph, and cyazofamid. *Plant Disease*. 2012; 96: 1337-1342.
109. Agosteo GE, Raudino F, Cacciola SO. Resistance of *Phytophthora capsici* to metalaxyl in plastic-house capsicum crops in southern Italy. *EPPO Bulletin*. 2000; 30: 257-261.