Hongos Asociados a la Parte Aérea del Arándano en Los Reyes, Michoacán, México

Fungi Associated to Blueberry Foliage in Los Reyes, Michoacan, Mexico

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Resumen. El objetivo del estudio consistió en identificar morfológicamente los hongos asociados a sintomatologías presentes en hojas, tallos y frutos del arándano y determinar su incidencia estacional en la principal zona productora de esta frutilla en Michoacán, México. Entre marzo 2009 y febrero 2010 se tomaron al azar, en el 5 % de plantas de tres huertos comerciales, se colectaron muestras de hojas, tallos y frutos con síntomas atribuidos a hongos. A partir de tejido dañado se hicieron aislamientos en PDA y se obtuvieron cepas puras de hongos. Para la identificación de los hongos a nivel de género se emplearon claves especializadas. Se identificaron 12 hongos asociados a 12 sintomatologías, entre éstas: pudrición del fruto (*Alternaria, Colletotrichum*), manchas foliares (*Alternaria, Colletotrichum, Neofusicoccum, Pestalotiopsis, Phyllosticta, Stemphyllium*), roya (*Pucciniastrum*), atizonamiento y cáncer del tallo (*Alternaria, Bipolaris, Colletotrichum, Curvularia, Neofusicoccum, Phomopsis, Pestalotiopsis, Phoma*) y tizón de yemas (*Chaetomium, Phoma*). La roya presentó la mayor incidencia (84.39 %), principalmente durante primavera. Los hongos aislados con mayor frecuencia fueron *Colletotrichum* y *Alternaria*.

Palabras clave adicionales: *Vaccinium* sp., diagnóstico, incidencia.

El arándano (*Vaccinium* spp.) en la última década ha adquirido una gran importancia por su alto valor nutritivo y demanda a nivel mundial. En México esta frutilla es de reciente introducción, teniéndose registradas 200 ha en Michoacán. Diversos hongos han sido reportados induciendo daños en arándano en todos los órganos de la planta en diferentes zonas productoras del mundo, entre los que destacan: *Alternaria* sp., *Curvularia* sp. *Microsphaera vaccinii, Phomopsis vaccinii, Stemphyllium* sp. (Cline y Schilder, 2006), *Bipolaris cynodontis* (Sisterna *et al*., 2009), *Botrytis cinerea* (Bristow y Milholland, 1995), *Colletotrichum gloeosporioides* y *C. acutatum* (Milholland, 1995a ;Wharton y Schilder, 2008), *Dothichiza caroliniana*

Abstract. The aim of the study was to identify morphologically the fungi associated to symptomatology present in leaves, stems and blueberry fruits and determine its seasonal incidence in the main producing area of this berry in Michoacán, Mexico. Between March 2009 and February 2010, some leaves, stems and fruits with symptoms attributed to fungi were randomly collected from 5% of the plants of three commercial orchards. Isolates in PDA were made from damaged tissue and the pure fungal strains were obtained. Specialized keys were used for fungi identification at genus level. Twelve fungi were identified associated to 12 symptomatologies, among them: fruit rot (*Alternaria, Colletotrichum*), leaf spot (*Alternaria, Colletotrichum*, *Neofusicoccum*, *Pestalotiopsis, Phyllosticta, Stemphyllium*), rust (*Pucciniastrum*), blighting and stem canker (*Alternaria, Bipolaris, Colletotrichum, Curvularia, Neofusicoccum, Phomopsis, Pestalotiopsis, Phoma*) and bud blight (*Chaetomium, Phoma*). The rust showed the highest incidence (84.39%), mainly during spring. The most frequently isolated fungi were *Colletotrichum* and *Alternaria*.

Additional Keywords: *Vaccinium* sp., diagnosis, incidence.

In the last decade, blueberries (*Vaccinium* spp.) have become very important because of their high nutritional value and worldwide demand. In Mexico this fruit has been recently introduced with reports of 200 ha in Michoacan. Several fungi have been reported to induce damage in blueberry and all plant organs in different producing areas of the world, among them: *Alternaria* sp., *Curvularia* sp. *Microsphaera vaccinii*, *Phomopsis vaccinii, Stemphyllium* sp. (Cline and Schilder, 2006), *Bipolaris cynodontis* (Sisterna *et al*., 2009), *Botrytis cinerea* (Bristow and Milholland, 1995), *Colletotrichum gloeosporioides* and *C. acutatum* (Milholland, 995a; Wharton and Schilder, 2008), *Dothichiza caroliniana* (Milholland, 1995b), *Phyllosticta vaccinii* (Szmagara, 2009), *Gloeocercospora inconspicua* (Milholland, 1995c), *Exobasidium vaccinii* (Nickerson, 1995), *Pucciniastrum vaccinii* (Dal Bello and Perello 1998), *Phoma* sp. (Szmagara 2009), *Cercospora* sp. (Milholland, 1995e), *Godronia cassandrae* (Ramsdell, 1995), *Gloeosporium minus* (Milholland, 1974), *Septoria*

(Milholland, 1995b), *Phyllosticta vaccinii* (Szmagara, 2009), *Gloeocercospora inconspicua* (Milholland, 1995c), *Exobasidium vaccinii* (Nickerson, 1995), *Pucciniastrum vaccinii,*(Dal Bello y Perellò 1998) *Phoma* sp. (Szmagara 2009), *Cercospora* sp. (Milholland, 1995e), *Godronia cassandrae* (Ramsdell, 1995), *Gloeosporium minus* (Milholland, 1974), *Septoria albopunctata* (Milholland, 1995d), *Botryosphaeria* sp., *Pestalotiopsis* sp., (Espinoza *et al*., 2009). En Michoacán, México, es importante conocer las enfermedades en huertos comerciales de arándano, para establecer estrategias de control y manejo que contribuyan al mejoramiento de su productividad. El objetivo de esta investigación fue identificar morfológicamente, a nivel de género, los hongos asociados a hojas, tallos y frutos del arándano y determinar la incidencia estacional de las mismas en Los Reyes Michoacán. Esto solo como una medida preventiva y exploratoria para iniciar con el estudio formal de las enfermedades más importantes del arándano.

Las colectas de material enfermo se hicieron de 2009 a 2010 en las estaciones de primavera, otoño e invierno, suspendiéndose en el verano por ser la época de poda. Se determinó la incidencia estacional de cada uno de los síntomas identificados en tres sitios de colecta (huertos: El Barreno, La Alameda y La Loma) con un tamaño de muestra del 5 % de las plantas de la población (7 ha). En laboratorio, las muestras colectadas se procesaron mediante las técnicas comunes (Agrios, 2005) de aislamiento de hongos en PDA (Bioxon®) e incubadas a temperatura ambiente. La identificación de los hongos obtenidos se llevó a cabo con base en características de forma, color y apariencia de las colonias miceliales, y forma de estructuras reproductivas; ello mediante el uso de literatura especializada (Barnett y Hunter, 1998; Crous *et al*., 2006; Hanlin, 2001; Maharachchikumbura *et al*., 2011; Wikee *et al*., 2011).

De las muestras colectadas se identificaron a 12 hongos asociados a la parte aérea del arándano. En los frutos se observaron dos tipos de síntomas: "pudrición seca" (*Alternaria* sp.) y "pudrición suave" (*Colletotrichum* sp.). En hojas se observaron cinco tipos de sindromes: "mancha plateada" (*Alternaria* sp., *Colletotrichum* sp., *Neofusicoccum* sp. y *Stemphyllium* sp.), "mancha cobriza" (*Colletotrichum* sp., *Phyllosticta* sp.), "tizón foliar" (*Colletotrichum* sp., *Pestalotiopsis* sp.), "mancha marrón", (*Colletotrichum* sp.) y roya (*Pucciniastrum* sp.). En los tallos se detectaron tres enfermedades: entre ellas "tizón de tallo", (*Colletotrichum* sp., *Pestalotiopsis* sp.)., "costra del tallo" (*Colletotrichum* sp., *Curvularia* sp.) y "cancro" (*Alternaria* sp., *Colletotrichum* sp., *Neofusicoccum* sp., *Phoma* sp., *Phomopsis* sp.). Las yemas presentaron dos tipos de síntomas: "tizón de yemas" (*Bipolaris* sp., *Chaetomium* sp., *Phoma* sp.) y "cancro de yemas" (*Colletotrichum* sp.). La incidencia estacional de los síntomas se presenta en el Cuadro 1.

De los 12 géneros de hongos identificados en este estudio, 11 han sido reportados en arándano (Bristow y Milholland, 1995; Cline y Schilder, 2006; Dal Bello y Perelló 1998; Espinoza *et al*., 2009; Hanlin, 2001; Milholland, 1995 a, b, c, d, e; Nickerson, 1995; Ramsdell, 1995b; Schilder, 2006; Schilder, 2008; Sisterna *et al*., 2009; *albopunctata* (Milholland, 1995d), *Botryosphaeria* sp., *Pestalotiopsis* sp., (Espinoza *et al*., 2009). It is important to know the blueberry diseases in commercial orchards in Michoacan, Mexico in order to establish control and management strategies that contribute to improve the productivity. The aim of this research was to identify morphologically, at genus level, the fungi associated to blueberry leaves, stems and fruits and to determine their seasonal incidence at Los Reyes, Michoacan. This is just a preventative and exploratory research to start with the formal study of the most important blueberry diseases.

The diseased material collections were made from 2009 to 2010 during spring, autumn and winter seasons, and they were suspended during summer because of the pruning time. Seasonal incidence was determined for each of the symptoms identified in three collection sites (Orchards: El Barreno, La Alameda and La Loma) with a 5% sample size of the total plants in the population (7 ha). The collected samples were processed in the lab using common techniques (Agrios, 2005) for isolation of fungi in PDA (Bioxon®) and incubated at room temperature. Fungi identification was done based on characteristics such as shape, color and appearance of the mycelial colonies and form of the reproductive structures; always with help of specialized literature (Barnett and Hunter, 1998; Crous *et al.* 2006; Hanlin, 2001; Maharachchikumbura *et al.* 2011; Wikee *et al.,* 2011).

From collected samples, 12 fungi were identified associated to the aerial part of the blueberry. In fruits, two types of symptoms were observed: "dry rot" (*Alternaria* sp.) and "soft rot" (*Colletotrichum* sp.). In leaves, five symptomatologies were observed: "silver stain" (*Alternaria* sp., *Colletotrichum* sp., *Neofusicoccum* sp., and *Stemphyllium* sp.), "copper stain" (*Colletotrichum* sp., *Phyllosticta* sp.), "leaf blight" (*Colletotrichum* sp.,

Cuadro 1. Incidencia estacional de los síntomas en órganos aéreos de plantas de arándano durante el período 2009 - 2010 en Los Reyes, Michoacán.

Table 1. Seasonal incidence of symptoms in aerial organs of blueberry plants during the 2009 - 2010 in Los Reyes, Michoacan.

Enfermedad	Incidencia (%)[z]		
	Primavera	Otoño	Invierno
Cáncer de yemas	0.86	1.44	1.63
Cancro del tallo	36.72	41.26	37.06
Costra del tallo	32.97	13.00	6.76
Mancha cobriza	11.58	4.86	3.96
Mancha marrón	9.80	7.60	10.62
Mancha plateada	90.00	54.00	55.48
Pudrición seca de fruto	1.03	3.40	2.08
Pudrición suave de fruto	0.00	1.94	1.95
Roya	100.00	88.62	64.57
Tizón de yemas	1.78	4.75	3.96
Tizón del tallo	24.72	18.40	36.13
Tizón foliar	0.00	25.74	8.62

[z] Porcentaje de las sintomatologías observadas en el 5 % (2450 plantas) de la población muestreada.

Szmagara, 2009; Wharton); solamente el género *Chaetomium* no ha sido aislado de este cultivo en trabajos similares.

De los síntomas descritos, la roya de la hoja registró la mayor incidencia estacional. Se ha señalado (Bristow y Strech, 1995) que esta enfermedad puede provocar defoliaciones intensas que reducen la producción del cultivo en el año siguiente. En este estudio, el daño de la roya al arándano sólo se observó como una defoliación parcial de los arbustos. Por otro lado, el cancro del tallo se presentó con una incidencia de hasta 41 %, con la frecuente presencia de *Neofusicoccum*. En otros estudios, el cancro del tallo ha sido atribuido a *Phomopsis* (Wilcox, 1939; Weingartner y Klos, 1974). De igual forma, *Pestalotiopsis* (Espinoza *et al*., 2009) y *Phoma* (Szmagara, 2009) se han reportado causando tizón del tallo del arándano, por lo que su presencia se debe considerar a fin de determinar su importancia en el desarrollo de esta enfermedad. Otros hongos aislados del follaje del arándano fueron *Bipolaris, Curvularia, Phyllosticta* y *Stemphyllium. Phyllosticta* se señala atacando otras especies de *Vaccinium* (Szmagara, 2009) en las que provoca pudrición del fruto (Oudemans *et al*., 1998). En este trabajo, *Phyllosticta* fue aislado de la "mancha cobriza". *Bipolaris*, *Curvularia y Stemphyllium* ya han sido reportados causando síntomas similares a los observados en este estudio (Cline y Schilder, 2006); sin embargo, la incidencia de las enfermedades a las que estos patógenos estuvieron asociados fue baja. El cancro de yemas lo describió Milholland (1974), quien lo atribuyó a *Gloeosporium minus*; en esta investigación, del cáncer de yemas se aisló a *Colletotrichum*. A *Chaetomium* se le ha señalado causando pudriciones de madera en cultivos ornamentales (Alexopoulos *et al*., 1996), aunque es reconocida su condición de saprófito (Crous *et al*., 2009). Las cepas de todos los hongos aislados se conservan en viales con agua destilada estéril, bajo condiciones de refrigeración (4 °C) en el Laboratorio de Fitopatología II de la Facultad de Agrobiología "Presidente Juárez" de la UMSNH.

CONCLUSIONES

En las hojas se presentaron cinco síndromes, donde destacó la roya por su incidencia. En el tallo se encontraron cuatro sintomatologías, con la mayor incidencia para el cancro del tallo. En general, todas las sintomatologías observadas se presentaron en las tres estaciones de muestreo, con excepción de la pudrición suave de fruto y el tizón foliar, los cuales no se presentaron durante la primavera. De las 12 sintomatologías identificadas en los órganos del arándano analizados, *Colletotrichum* se encontró asociado a nueve y *Alternaria* a cuatro de ellas.

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Pestalotiopsis sp.), "brown stain" (*Colletotrichum* sp.) and rust (*Pucciniastrum* sp.). Regarding the stems, three diseases were detected, among them the "stem blight" (*Colletotrichum* sp., *Pestalotiopsis* sp.) "stem scab" (*Colletotrichum* sp., *Curvularia* sp.) and "canker" (*Alternaria* sp., *Colletotrichum* sp., *Neofusicoccum* sp., *Phoma* sp., *Phomopsis* sp.). The buds showed two types of symptoms: "bud blight" (*Bipolaris* sp., *Chaetomium* sp., *Phoma* sp.) and "bud canker" (*Colletotrichum* sp.). The seasonal incidence of symptoms is presented in Table 1.

From the 12 fungi genus identified in this study, 11 have already been reported in blueberries (Bristow and Milholland, 1995; Cline and Schilder, 2006; Dal Bello and Perelló 1998; Espinoza *et al*., 2009; Hanlin, 2001; Milholland, 1995 a, b, c, d, e; Nickerson, 1995; Ramsdell, 1995b; Schilder, 2006; Schilder, 2008; Sisterna *et al.,* 2009; Szmagara, 2009; Wharton), and only *Chaetomium* genus has not been isolated and reported on previous studies.

From the symptoms described, the leaf rust had the highest seasonal incidence. It has been previously reported (Bristow and Stretch, 1995) that this disease can cause severe defoliation that reduces the crop production in the following year. In this study, the damage caused by rust to blueberries was only observed as partial shrubs defoliation. On the other hand, stem canker was observed with an incidence of up to 41 %, with frequent *Neofusicoccum* occurrence. In other reports, stem canker has been attributed to *Phomopsis* (Wilcox, 1939; Weingartner and Klos, 1974). Similarly, *Pestalotiopsis* (Espinoza *et al.,* 2009) and *Phoma* (Szmagara, 2009) have been reported causing blueberry stem blight; therefore, their presence should be considered in order to determine their importance in the development of this disease. Other fungi isolated from blueberry leaves were *Bipolaris, Curvularia, Phyllosticta* and *Stemphyllium*. *Phyllosticta* has been observed attacking other *Vaccinium* species (Szmagara, 2009) that causes fruit rot (Oudemans *et al.,* 1998). In this work, *Phyllosticta* was isolated from the "copper stain". *Bipolaris, Curvularia* and *Stemphyllium* have already been reported causing similar symptoms to those reported in this study (Cline and Schilder, 2006); however, the incidence of diseases that were associated with these pathogens was low. Buds canker was described by Milholland (1974) and he attributed it to *Gloeosporium minus*; in this research, *Colletotrichum* was isolated from buds canker. *Chaetomium* has been considered the cause of wood rot in ornamental crops (Alexopoulos *et al.,* 1996), although its saprophyte capacity has also been recognized (Crous *et al.,* 2009). Strains of all isolated fungi were preserved in vials with sterile distilled water under refrigeration (4 °C) in the laboratory of Plant Pathology II at "Presidente Juárez" Agrobiology Faculty (UMSNH).

CONCLUSIONS

Five symptomatologies were observed in the blueberry leaves, and rust had the highest incidence. Four symptomatologies were found on the stems and stem canker had the highest incidence. In general, all observed symptomatologies were present in the three sampling seasons, except fruit soft rot and leaf blight, which did not

mayor importancia económica".

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appear during spring. From the 12 identified symptomatologies in the blueberry organs analyzed, *Colletotrichum* was associated to nine of them and *Alternaria* only to four of them.

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