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First report of Bacterial Spot with gum production in almond fruits of Argentina

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ABSTRACT

Bacterial Spot of Almond is a pathology caused by *Xanthomonas arboricola* pv. *pruni* responsible for significant yield losses. During 2017, young symptomatic almond fruits were observed in orchards of Northern Patagonia, Argentina. Symptoms consisted of small watery circular lesions, from which an amber gummy substance emerged. The objective of this work was to determine the etiology of this pathology considering that the symptoms were coincident with those previously described for the bacterial spot with gum production caused by *Xanthomonas arboricola* pv. *pruni* on almond fruits in other parts of the world. The presence of *Xanthomonas arboricola* in damaged fruits was determined confirming their identity by molecular analysis. Symptomatology and gum production were demonstrated by performing pathogenicity tests. As a result, Bacterial Spot with gum production is reported for the first time on almond fruits in Argentina.

Keywords: Bacterial Spot, amber gum, almond, *Xanthomonas arboricola* pv. *pruni*.

RESUMEN

La mancha bacteriana de la almendra es una patología causada por *Xanthomonas arboricola* pv. *pruni* y es responsable de importantes pérdidas en el rendimiento productivo. Durante 2017, en plantaciones del norte de la Patagonia Argentina, se observaron frutos jóvenes de almendra con pequeñas lesiones circulares, acuosas, de las cuales emergía una sustancia ámbar gomosa. El objetivo de este trabajo fue determinar la causa de esta patología, considerando que los síntomas eran coincidentes con los previamente descriptos para la mancha bacteriana con producción de goma, causada por *Xanthomonas arboricola* pv. *pruni* en frutos de almendra en otras partes del mundo. Se determinó la presencia de *Xanthomonas arboricola* en los frutos lesionados confirmándose la identidad del patógeno por análisis molecular. A través de pruebas de patogenicidad se demostró la sintomatología y la producción de goma en frutos sanos inoculados con la bacteria. A partir del estudio realizado se reporta por primera vez la mancha bacteriana con producción de goma en frutos de almendra en Argentina.

Palabras clave: mancha bacteriana, goma ámbar, almendra, *Xanthomonas arboricola* pv. *pruni*.

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INTRODUCTION

Bacterial Spot of Almond is a pathology caused by *Xanthomonas arboricola* pv. *pruni*. This disease occurs on leaves, branches and fruits of almost all *Prunus* species. On almond trees, it can be observed on leaves and buds, but the most evident symptoms are observed on fruits (Adaskaveg *et al.*, 2015). Regarding symptoms on immature fruits, lesions begin in the shell as small watery brown spots that slowly increase in diameter (2 to 4 mm, generally smaller than 5 mm). These lesions produce a light to dark amber gummy substance, whose color is important because it helps to distinguish bacterial spot from other types of damage (Adaskaveg *et al.*, 2015).

Bacterial Spot caused by *Xanthomonas arboricola* pv. *pruni* was described for the first time in the United States on Japanese plum (Smith, 1903) and, lately, it is being reported in almost every continent where stone fruits are grown (EPPO, 2006). It has been determined on almond trees in Japan (Ishiyama, 1923), Sicily (Italy) (Ciccarone, 1959), New Zealand (Young, 1977), Spain (Palacio-Bielsa *et al.*, 2010), Australia and California (United States) (Adaskaveg *et al.*, 2015). Previous studies indicate that this pathogen led to reduction in the quality and marketability of fruit, reduction in orchard productivity and increases in production costs, resulting in significant economic losses (Lamichhane, 2014).

The leading almond producer in the world is the United States, followed by Spain. Also, Australia emerges as a strong growing producer. In Argentina, in recent years, the sector has been recovered and modern plantations with late bloom varieties were incorporated (Iannamico, 2015). Currently, there are about 4,200 cultivated hectares, mainly distributed in the provinces of Mendoza, San Juan, La Rioja, Salta, Río Negro and Neuquén. In Río Negro (Northern Patagonia), growing areas are mainly located in the High Valley productive region, while smaller orchards are located

in the Lower Valley and Middle Valley productive regions (Iannamico, 2015). Although the almond trees integrate a group of fruit trees that are not traditionally grown in Northern Patagonia, it has enormous potential due to its agroeconomic behavior and its good profitability and growth possibilities (Iannamico, 2015).

During October 2017 in cultivars of Luis Beltrán, a productive region of the Middle Valley of Río Negro, young almond fruits with external lesions were observed. Symptoms consisted of small watery circular lesions, from which an amber gummy substance emerged (fig. 1). The objective of this work was to determine the etiology of this pathology, considering that the symptoms were coincident with those previously described for diseases caused by *Xanthomonas arboricola* pv. *pruni* and the fact that *Xanthomonas arboricola* pv. *juglandis* had previously been reported in the region as a causal agent of Walnut Blight and Brown Apical Necrosis (Temperini *et al.*, 2017).

MATERIALS AND METHODS

A total of 5 symptomatic immature almond fruits were chosen for analysis. The fruits were washed with tap water, superficially disinfected by soaking them in a solution of 1% Sodium Hypochlorite (NaClO) for 3 minutes, and rinsed twice with sterile distilled water. Then, they were placed individually in closed plastic jars, containing a piece of sterilized cotton soaked in water to create a wet chamber to allow microbial development. They were incubated at 27 °C for 14 days. After the incubation period, internal pieces of injured tissue were aseptically removed and placed in Eppendorf tubes containing sterile physiological solution. After homogenization, 0.1 ml of the suspension was inoculated in Petri dishes containing Luria Bertani (LB) medium. They were incubated at 27 °C for 4 days. Colonies that showed morphological characteristics equal or similar to those described for the genus *Xanthomonas* on LB medium were cultured on a differential medium for this genus, Xan-D (Yung-Ann *et al.*, 2009). They were incubated at 27 °C for 7 days. The colonies that were positive on the Xan-D medium were confirmed by molecular techniques.

DNA extractions were performed using the "DNeasy blood and tissue mini kit" with a pre-treatment protocol for gram-negative bacteria as for the manufacturer's instructions (Qiagen, Intl.). The extracted genomic DNA was quantified with a fluorimeter Qubit 2.0 (Life Technologies, Intl.). PCR amplifications were performed using *Xanthomonas* species-specific primers XarbQF (GCGAGATCAATGC-GACCTCGTC) and XarbQR (GGTGACCACATCGAACCG-CGCA) for *qumA* gene, a quinate dehydrogenase (Pothier *et al.*, 2011), according to Temperini *et al.* (2017). After molecular identification, *Xanthomonas* isolates were stored at -80 °C in broth Yeast Extract Peptone Dextrose.

Pathogenicity tests were conducted on 8 healthy immature almonds fruits, 40 days after the setting, and the results were verified according to Koch's postulates. The disinfected fruits were injured at a depth of 0.1 mm in the



Figure 1. External symptoms of the disease on an immature almond fruit.

equatorial area with a sterile needle and inoculated in the same way with bacterial culture grown for 4 days in Nutrient Agar. The fruits were placed individually in closed plastic jars in a humid chamber, as previously described, and incubated at 25 °C for 15 days. Controls were inoculated with sterile distilled water. After the incubation period, the injuries produced were evaluated and the production of gum was observed under a stereoscopic microscope (40X).

RESULTS AND DISCUSSION

The presence of *Xanthomonas arboricola* was determined in 4 of the 5 affected fruits under study. Regarding pathogenicity tests, all the fruits inoculated showed the characteristic symptomatology, necrosis and gum produc-

tion (fig. 2). The pathogenicity was demonstrated according to Koch's postulates. These results are summarized in table 1. As a result, we can confirm *Xanthomonas arboricola* pv. *pruni* as the causal agent of Bacterial Spot with gum production in almond fruits of the Middle Valley of Río Negro. This is the first report of the disease in Argentina.

Among the pathogens that attack *Prunus* species, *Xanthomonas arboricola* has become very important in recent decades. Outbreaks caused by this bacterium have increased in recent years, and the speed at which they occur suggests the possibility of future epidemics, which has made it a worldwide concern (Lamichhane, 2014). In the region, *Xanthomonas arboricola* was previously reported in walnut trees and is the causal agent of bacteriosis and Brown Apical Necrosis. These pathologies are responsible for significant losses in the production sector, and are very severe in seasons of predisposing climatic conditions (Temperini *et al.*, 2017).

This report alerts to the presence of the pathogen in the Northern Patagonia and the need to address preventive measures to avoid the emergence and spread of the disease.

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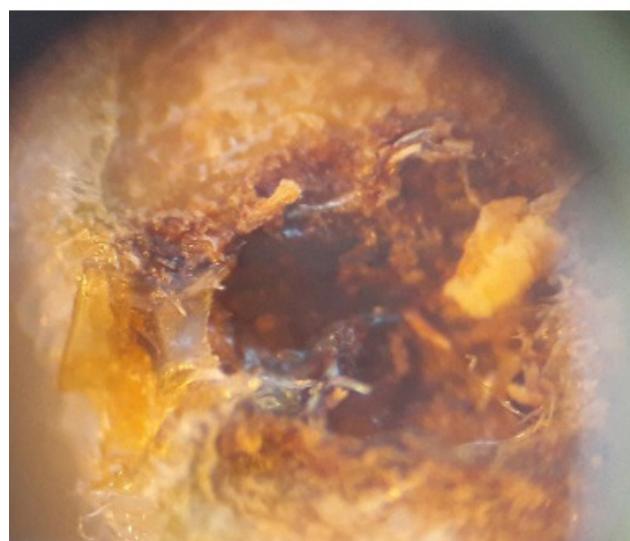


Figure 2. Gum production observed under a stereoscopic microscope (40X).

Samples	Morphological characterization		Molecular test	Pathogenicity test	
	Characteristics of the colonies on LB medium	Growth on Xan-D medium		Necrosis at the inoculation point	Gum production
ALM 1	ND	-	-	-	-
ALM 2	Yellow - Mucoïd - Well defined edges	(+)	(+)	(+)	(+)
ALM 3	Yellow - Mucoïd - Well defined edges	(+)	(+)	(+)	(+)
ALM 4	Yellow - Mucoïd - Well defined edges	(+)	(+)	(+)	(+)
ALM 5	Yellow - Mucoïd - Well defined edges	(+)	(+)	(+)	(+)

Table 1. Characterization and identification of *Xanthomonas arboricola* in affected fruits. Controls were negative for necrosis and gum. ND: Not detected.

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