REVISTA DE INVESTIGACIONES AGROPECUARIAS
RIA

ISSN edición impresa 0325-8718
ISSN en línea 1669-2314
Vol. 46 N.º 2, Agosto 2020
Buenos Aires, Argentina

Alimentos del futuro:
crece la demanda de proteínas alternativas a la carne

CONSTRUYEN UNA BIBLIOTECA DE NANOANTICUERPOS DE LLAMAS CONTRA LA COVID-19

ENTREVISTA A CARLOS PARERA: "HAY MUY POCAS INSTITUCIONES EN EL MUNDO COMO EL INTA"

CAMAS BIOLÓGICAS: UNA HERRAMIENTA VERSÁTIL Y PRODUCTIVA PARA EL USO ADECUADO DE FITOSANITARIOS

ENTREVISTA A YÉSICA CHAZARRETA: "EL DESAFÍO ES GENERAR HERRAMIENTAS QUE LLEGUEN AL PRODUCTOR"

INTA Ediciones

RIA
REVISTA DE INVESTIGACIONES AGROPECUARIAS

ISSN 0325-8718
ISSN 1669-2314

Institución Editora:
Instituto Nacional de Tecnología Agropecuaria (INTA)

Cuatrimestral

Vol. 46 N.º 2
Agosto 2020, Buenos Aires, Argentina

Propiedad Intelectual
Propiedad Intelectual N.º 5231354

SEDE EDITORIAL

Chile 460 2.º piso
Ciudad Autónoma de Buenos Aires, Argentina
CP (C1098AAJ)
+54 (011) 4339-0600
http://ria.inta.gob.ar
revista.ria@inta.gob.ar

La Revista de Investigaciones Agropecuarias (RIA) es una publicación de información científica editada por el INTA. Los artículos presentados son admitidos por un Comité Editorial compuesto por prestigiosas figuras del ámbito académico y científico agropecuario. Abarca diferentes temas preparados por profesionales del INTA y de otras instituciones, y está destinada a la comunidad científica nacional e internacional.

Lo expresado por autores, corresponsales o columnistas no necesariamente reflejan el pensamiento del Comité Editorial, de la revista o de su institución editora.

Indexada en:
Scopus
Latindex (Folio 3346)
CABI Publishing
DOAJ (Directory of Open Access Journals)
Redalyc.org
Núcleo Básico
Thomson Reuters
Dialnet
Scielo
CiteFactor

MIEMBROS DEL CONSEJO DIRECTIVO DEL INTA

Presidenta:
Ing. Agr. Susana Mirassou

Vicepresidente:
Ing. Agr. Tomás Schlichter

Consejeros Nacionales:
Dr. Manuel Pedreira. MinAgro
Ing. Agr. Liliana Monterroso. Facultad de Cs. Agronómicas
Méd. Vet Humberto Luis José Occhi. Facultades de Ciencias Veterinarias
Ing. Agr. Alejandro Conci. AACREA
Ing. Agr. Mario Nicolás Carlino. CONINAGRO
Ing. Agr. Sergio Melgarejo. CRA
Dr. Pablo Paillole. FAA
Sr. Santos Zuberbhuler. SRA

Director Nacional:
Ing. Agr. Carlos Parera

Coordinador Nacional Técnico:
Ing. Agr. José Luis Sponton

Directores Nacionales Asistentes:
DN Asistente de Investigación, Desarrollo y Planificación
Ing. Agr. Martín Irurueta

DN Asistente de Transferencia y Extensión
Ing. Agr. María Rosa Scala

DG de Sistemas de Información, Comunicación y Procesos
Ing. Fernando Ricitelli

DG de Capital Humano
Ing. Prod. Agr. Eduardo Alberto Quargnolo

DG de Administración
CPN Lic. Rosendo Tarsetti

CN de Vinculación Tecnológica y Relaciones Institucionales
Ing. Zootecnista Juan Sebastián Llorens

COMITÉ EDITORIAL

Presidente:

Dr. Carlos Di Bella. Universidad de Buenos Aires (UBA) y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Argentina.

Vocales:
Ing. Agr. Jorge Mariotti. Universidad Nacional de Salta (UNSa). Argentina.

Dr. Tomás Miguel Schlichter. Instituto Nacional de Tecnología Agropecuaria (INTA). Argentina.

Dra. Ana María Romero. Universidad de Buenos Aires (UBA). Argentina.

Ing. Agr. M. Sc. María Eugenia Beget. Instituto Nacional de Tecnología Agropecuaria (INTA). Argentina.

Dr. Miguel Ángel Taboada. Instituto Nacional de Tecnología Agropecuaria (INTA) y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Argentina.

Dr. Martín Durante. Instituto Nacional de Tecnología Agropecuaria (INTA). Argentina.

Dr. Frédéric Goulet. Instituto Nacional de Tecnología Agropecuaria (INTA) y Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD). Francia.

Dr. Ariel Pereda. Instituto Nacional de Tecnología Agropecuaria (INTA) y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Argentina.

Dr. Laureano S. Frizzo. Universidad Nacional del Litoral (UNL).

Dr. Claudio Machado. Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA). Argentina.

Dr. Juan Wahren. Instituto de Investigaciones Gino Germani (IIGG). Facultad de Ciencias Sociales. Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Argentina.

Dra. Karina Trono. Instituto Nacional de Tecnología Agropecuaria (INTA) y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Argentina.

Dra. Gabriela Posse. Instituto Nacionas de Tecnología Agropecuaria (INTA). Argentina.

STAFF

Dirección:
Dra. Norma Pensel
Lic. Máximo Bontempo

Coordinación editorial:
Per. Giselle Miquet
Mg. Mario Migliorati

Asistente editorial:
Lic. Gustavo Ciuffo
Lic. Cecilie Esperbent

Producción Editorial:
Asesores técnicos y de contenido
Ing. Agr. Cristian Zuchini
Dr. Martín Irurueta
Dra. Mariana Insani

Diseño y Edición:
Comunicación Visual, Gerencia de Comunicación e Imagen Institucional

Fotografía:
Audiovisual, Gerencia de Comunicación e Imagen Institucional

COMITÉ ASESOR

Ing. Agr. M. Sc. Marina Di Filippo. Instituto Nacional de Tecnología Agropecuaria (INTA). Argentina.

Ing. Agr. M. Sc. Emiliano Quiroga. Instituto Nacional de Tecnología Agropecuaria (INTA). Argentina.

Dr. Darío Fernández. Instituto Nacional de Tecnología Agropecuaria (INTA). Argentina

Ing. Agr. M. Sc. Alejandro Rago. Instituto Nacional de Tecnología Agropecuaria (INTA). Argentina.

M. Sc. María Isabel Tort. Instituto Nacional de Tecnología Agropecuaria (INTA). Argentina.

Ing. Agr. M. Sc. Damián Vega. Universidad de Buenos Aires (UBA). Argentina.

Ing. Agr. Sergio E. Feingold. Instituto Nacional de Tecnología Agropecuaria (INTA). Argentina.

INTA
Ministerio de Agricultura, Ganadería y Pesca
Argentina

Esta publicación es propiedad del Instituto Nacional de Tecnología Agropecuaria. Rivadavia 1439. CABA. Buenos Aires, Argentina.

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

MARANGI, M.J.[1,4]; TEMPERINI, C.V.[1,4]; FERNÁNDEZ, D.[3]; PARDO, A.G.[2,4]; POSE, G.N.[1,4,a]

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 arborícola 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 almendro 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 arborícola pv. pruni en frutos de almendro en otras partes del mundo. Se determinó la presencia de Xanthomonas arborícola 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 almendro en Argentina.

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

[1]Universidad Nacional de Río Negro, Río Negro, Argentina. Mitre 331, (8336) Villa Regina, provincia de Río Negro, Argentina. Correo electrónico: mjmarangi@gmail.com
[2]Universidad Nacional de Quilmes, Buenos Aires, Argentina. Roque Sáenz Peña 352, (B1876) Bernal, provincia de Buenos Aires, Argentina.
[3]Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria (EEA) Alto Valle, Agencia de Extensión Rural (AER) Valle Medio. Villa Galense 575, (8361) Luis Beltrán, Río Negro.
[4]Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Argentina.
[a]Institución actual: Universidad Nacional de Quilmes, Laboratorio de Micología y Cultivo de Hongos Comestibles - INTECH (CONICET).

Received September 10th 2019// Accepted May 27th 2020 // Published online November 04th 2020

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 agronomic behavior and its good profitability and growth possibilities (Iannamico, 2015).

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

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 (GCGAGATCAATGCGACCTCGTC) and XarbQR (GGTGACCACATCGAACCGCGCA) 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

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 production (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.

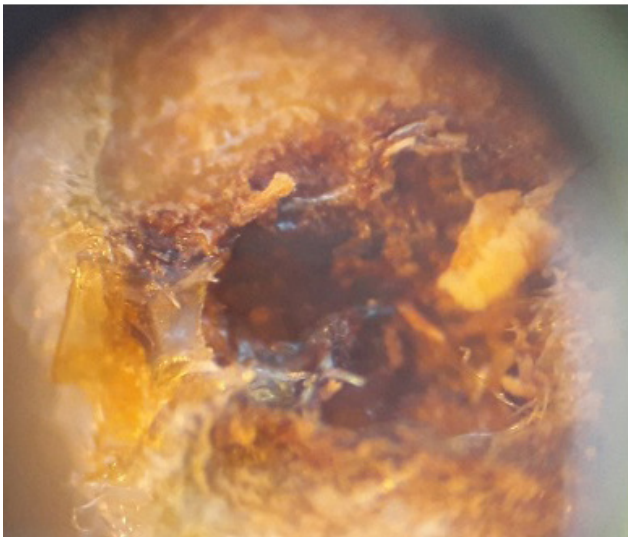

Figure 2. Gum production observed under a stereoscopic microscope (40X).

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.

REFERENCES

ADASKAVEG, J.; HOLTZ, B.; DUNCAN, R.; DOLL, D. 2015. Bacterial spot of almond in California: Update on the disease and management. California. (Available at: http://thealmonddoctor.com/2015/01/01/bacterial-spot-of-almond-management/ verified: July 13th 2019).

CICCARONE, A. 1959. Notes on the pathology of the almond tree, with special reference to Sicily. Abstract in Review of Applied Mycology (38), 757-758.

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION. 2006. Diagnostics. *Xanthomonas arboricola* pv. *pruni*; Bulletin (36), 129-133.

IANNAMICO, L. 2015. Documento técnico del INTA: Cultivo del almendro. En: *Instituto Nacional de Tecnología Agropecuaria* (Eds.). INTA, Centro Regional Patagonia Norte. Estación experimental Agropecuaria Alto Valle-Allen, Río Negro, Argentina. 4-5 pp.

Samples	Morphological characterization		Molecular test	Patogenicity 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 - Mucoid - Well defined edges	(+)	(+)	(+)	(+)
ALM 3	Yellow - Mucoid - Well defined edges	(+)	(+)	(+)	(+)
ALM 4	Yellow - Mucoid - Well defined edges	(+)	(+)	(+)	(+)
ALM 5	Yellow - Mucoid - Well defined edges	(+)	(+)	(+)	(+)

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

ISHIYAMA, S. 1923. Studies of blackspot disease of plum. Japanese Journal of Botany (1), 21.

LAMICHHANE, J.R. 2014. *Xanthomonas arboricola* disease of stone fruit, almond, and walnut tres: Progress toward understanding and management. Plant Disease (98), 1600-1610.

PALACIO-BIELSA, A.; ROSELLÓ, M.; CAMBRA, M.A.; LÓPEZ, M.M. 2010. First report on almond in Europe of bacterial spot disease of stone fruits caused by *Xanthomonas arboricola* pv. *pruni*. Plant Disease (94), 786.

POTHIER, J.F.; PAGANI, M.C.; PELLUDAT, C.; RITCHIE, D.F.; DUFFY, B. 2011. A duplex - PCR method for species - and pathovar - level identification and detection of the quarantine plant pathogen *Xanthomonas arboricola* pv. *pruni*. Journal of microbiological methods (86), 16-24.

SMITH, E.F. 1903. Observations on a hither to unreported bacterial disease, the cause of which enters the plant through ordinary stomata. Science (17), 456-457.

TEMPERINI, C.V.; PARDO, A.G.; POSE, G.N. 2017. First report of apical necrosis in walnut cultivars from Northern Argentinean Patagonia. Journal of Plant Pathology and Microbiology (8), 414-419.

YOUNG, J.M. 1977. *Xanthomonas pruni* in almond in New Zealand. New Zealand Journal of Agricultural Research (20), 105-107.

YUNG-ANN, L.; AI-NING, S.; TZU-FEN, L.; YUNG-SHAN, L. 2009. Combination of chromogenic differential médium and estA-specific PCR for isolation and detection of Phytopathogenic *Xanthomonas* spp. Applied and Environmental Microbiology (75) 6831-6838.