Identification of Fusarium oxysporum causing sweet potato rot (*Ipomoea batatas* L & Lamb) in Uruguay

Identificación de Fusarium oxysporum causando podredumbre en boniato (*Ipomoea batatas* L & Lamb) en Uruguay

Identificação de Fusarium oxysporum causando podridão en batata doce (*Ipomoea batatas* L & Lamb) en Uruguay

Scattolini, A.[1]; Hernández Rodriguez, L.[1]; González Idiarte, H.[2]

[1]*Universidad de la República, Facultad de Agronomía, Unidad de Fitopatología, Montevideo, Uruguay.*

[2]*Universidad de la República, Facultad de Agronomía, Unidad de Mejoramiento Genético de Plantas, Montevideo, Uruguay.*

Editor

Joanna Lado
Instituto Nacional de Investigación Agropecuaria (INIA), Montevideo, Uruguay.

Correspondence

Agueda Scattolini,
agueda@fagro.edu.uy

Received *03 Sep 2019*
Accepted *27 Jan 2020*
Published *15 Jun 2020*

Citation
Scattolini A, Hernández Rodriguez L, González Idiarte H. Identification of Fusarium oxysporum causing sweet potato rot (Ipomoea batatas L & Lamb) in Uruguay. Agrociencia Uruguay [Internet]. 2020 [cited yyyy mm dd];24(1):124. Available from: http://agrocienciauruguay.uy/ojs/index.php/agrociencia/article/view/124

doi:
https://doi.org/10.31285/AGRO.24.124

Abstract

Sweet potato (*Ipomoea batatas* L & Lamb) is an important crop for Uruguay, both for the number of producers participating in its production and the per capita consumption of the population. It is harvested between January and April, and it is commercialized after a conservation period that lasts until November. During this period some losses happen due to bad conservation, most of them for microbiological causes. One of the main problems is a dry, superficial, rounded, and slightly sunken rot that as elapses it can increase its size and eventually mummify the root. To identify the causative agent of these injuries, a directed sampling was conducted, and the samples were sent to the Diagnostic Clinic of the Phytopathology Unit of the Agronomy Faculty. Fungi with *Fusarium oxysporum* characteristic was frequently isolated from roots showing initial rot symptoms and its identity verified by morphological and molecular techniques. Pathogenicity tests were performed and, once the symptom was recorded, the causal relationship of *F. oxysporum* with respect to the observed symptoms was verified accomplishing Koch's Postulates. This result contributes to the knowledge of the disease and to adapt management practices to reduce losses and the commercial quality of sweet potato in local conditions.

Keywords: sweet potato, fusariosis, post-harvest, conservation

Resumen

El boniato (*Ipomoea batatas* L & Lamb) es un cultivo importante para Uruguay tanto por la cantidad de productores que participan en su producción como por el consumo per cápita de la población. Se cosecha entre enero y abril y se comercializa escalonadamente tras un período de almacenamiento que se extiende hasta noviembre. Durante ese período se producen pérdidas por mala conservación, muchas de ellas por razones microbianas. Entre los problemas detectados, el más frecuente es una podredumbre seca, superficial, circular, oscura, levemente hundida, de tamaño pequeño, que aumenta de tamaño y puede llegar a momificar la raíz. Para identificar el agente causal de estas lesiones se realizaron muestreos dirigidos y se enviaron a la Clínica de Diagnóstico de la Unidad de Fitopatología de Facultad de Agronomía. A partir de síntomas incipientes de esta podredumbre se aisló reiteradamente un hongo con características similares a *Fusarium oxysporum*. En este trabajo, mediante la utilización de técnicas morfológicas y moleculares y prueba de patogenicidad, se verificó la relación de causalidad de *F. oxysporum* con respecto a los síntomas observados, completándose de esa forma los Postulados de Koch. Este resultado contribuye al conocimiento de la enfermedad y a adecuar las prácticas de manejo para reducir las pérdidas y la calidad comercial del boniato en nuestras condiciones de producción.

Palabras clave: camote, batata, fusariosis, poscosecha, conservación

Resumo

A batata-doce (*Ipomoea batatas* L & Lamb) é um cultivo importante para o Uruguai, tanto pela quantidade de agricultores que participam da sua produção, como por seu consumo per capita. É colhida de janeiro a abril e se comercializa de forma escalonada, tendo um período de armazenamento que se estende até novembro. Durante esse período são verificadas perdas por má conservação, muitas vezes provocadas por microrganismos. Entre os problemas detectados, o mais frequente é uma podridão seca, que apresenta lesão superficial, de forma circular, escura, levemente deprimida, de tamanho pequeno, a qual pode aumentar, chegando a mumificar a raiz. Para identificar o agente causal dessas lesões foram realizadas amostragens dirigidas, as

quais foram enviadas a Clínica de Diagnóstico da Unidade de Fitopatologia da Faculdade de Agronomia. A partir de sintomas incipientes dessa podridão foi isolado de forma repetida um fungo com as características similares ao *Fusarium oxysporum*. Neste trabalho, mediante a utilização de técnicas morfológicas, moleculares e prova de patogenicidade foi verificada a relação causal associada a *F. oxysporum* a partir dos sintomas observados, completando-se dessa forma os Postulados de Koch. Este resultado contribui ao reconhecimento da doença e na adequação de práticas de manejo para reduzir as perdas e a qualidade comercial da batata-doce em nossas condições de produção.

Palavras clave: batata doce, fusariosis, pós-colheita, conservação

1. Introduction

Among the horticultural items commercialized in 2017 in the Wholesale Market of Montevideo, Uruguay, the sweet potato (*Ipomoea batatas* L & Lamb) takes the fifth place if the physical volume is taken into account, and the sixth place when considering the monetary value. Uruguayans are estimated to consume 5 to 7 kg annually[(1)]. It is produced by 1,150 family businesses, of which 93.4% cultivate between 0.5 and 3 hectares of sweet potato[(2)]. 86% of these small farmers are located in the southern region[(3)], mainly in the department of Canelones.

National production supplies the demand of the Uruguayan population in the period that runs from January to November. From September until the following harvest, the national supply decreases and it is necessary to import, although in reduced volumes. From 2015 to 2018, annual imports ranged between 0 and 13% of the volume that entered the Wholesale Model Market (2018, conversation with P. Pacheco and Romero, unreferenced).

The harvest takes place between January and April and must be followed by immediate “curing” before storage, so that the healing periderm can be formed. This process is carried out in the field under conditions of temperature and relative humidity that depend on the climate and soil variability. Conditions are not always adequate to achieve correct healing of the wounds produced during harvest because they are frequently outside the optimal healing temperature range determined between 29 and 32 ºC, and around 85% RH.[(4)(5)], producing a slower and incomplete suberization that favors *F. oxysporum* penetration in the reserve root[(5)(6)].

Many producers in the department of Canelones adopt the strategy of staggered commercialization, that allows them to obtain periodic monetary income for several months. For this purpose, part of the production is conserved in storage structures that differ between producers and even in the same farm, all without artificial regulation of temperature or relative humidity. This determines an important variability in both variables in the storage period, which are frequently outside the optimal range of 13-16 ºC and 85% relative humidity, adequate to maintain the physiological and sanitary quality of the stored sweet potato[(4)(5)(6)(7)].

Different pathogens that infect the reserve root penetrate mainly during harvest and develop during conservation, producing different types of rot that are an important cause of losses that occur in storage, which are greater the longer this is.

Maeso and Vilaró [(8)] identified *Pellicularia filamentosa, Sclerotinia sclerotiorum, Sclerotium rolfsii, Plenodomus destruens, Monilochaetes infuscans, Albugo ipomoeae-panduratae, Rhizopus stolonifer*, and *Fusarium oxysporum* causing sweet potato diseases. Monteiro[(9)] identified the presence of *Plenodomus destruens, Phomopsis batatis, Ceratocystis fimbriata, Monilochaetes infuscans, Pythium* spp, *Fusarium* spp, and *Rhizopus nigricans* in stored sweet potatoes.

During sweet potato storage, the frequent appearance of dry, circular, dark, slightly sunken cankers of variable size was observed in recent years. They are not normally visible at first sight when harvested, but become visible and increase in

quantity and size during storage, and may coalesce.

This type of lesions is similar to that described by Clark and Moyer[4] for the superficial rot produced by *F. oxysporum,* described as circular, dark brown to pale, firm, dry, superficial, and external to the vascular ring. When extended storage, the tissues in and around the lesions dry out and wrinkle, and the roots can mummify. Root rot caused by *F. solani* is also described, producing circular lesions and commonly showing dark and pale brown concentric rings, surpassing the vascular ring through the center and being able to take the entire root. The tissue turns orange to pale brown on the margin of the lesion. Older lesions become dry and lens-shaped cavities appear in the pulp, with white hyphae sometimes visible inside. In some cases, surface rot is a preliminary stage of root rot. The two species can be differentiated by the morphology of the colonies, asexual spores and molecular methods[4][9][10][11].

Several authors have identified different Fusarium species that infect the sweet potato reserve root[4][9]. In a survey carried out in North Carolina (USA), six species of Fusarium were identified, corresponding to *F. solani* 43% and *F. oxysporum* 39% of the total isolations[7].

Regarding *F. oxysporum*, the soil is the inoculum source from which it infects the reserve root. It survives as chlamydospores when environmental conditions are adverse, or in the absence of host plants, allowing the fungus to be present in the soil for a long time. However, little is known about the population dynamics in the soil[4][9][12].

Unlike *F. solani*, that causes cankers on the stem of the transplanted cuttings decreasing the yield of sweet potatoes, this pathogen does not spread from the infected root used as “seed” to the shoots (cuttings) it produces[4].

F. oxysporum requires wounds to infect the reserve root and in storage, it does not spread from infected roots to other healthy roots unless new wounds occur[4]. When the infected sweet potatoes are stored under optimal temperature and relative humidity conditions (13-16 ºC and 85% RH), the disease progresses slowly[4][12], given that the cardinal optimal temperatures of fungus development are between 25 ºC and 30 ºC[10]. Genetic resistance to *F. oxysporum* has been studied by different researchers, evaluating the behavior of commercial cultivars and selections. Research shows differences in the degree of susceptibility, but the scientific evidence is not consistent enough with respect to materials that genetically possess qualitative resistance[5][6][12][13].

The objectives of this study were: to identify the species of the isolated pathogen from the predominant lesions observed in sweet potatoes stored under different conditions of Canelones production; to verify the causal relationship between the pathogen and the symptoms; to contribute to the study of its epidemiology, and to design management strategies that minimize losses at harvest and during storage.

2. Material and methods

From 2012 to 2016, a directed sampling was carried out at four storage sites of producers Cerrillos, San Antonio, Sauce, and Progreso regions of Canelones department. A total of twenty sweet potato samples with symptoms of dry circular, dark, slightly sunken cankers were taken from the cultivars Cuarí, Baqueano, Beauregard, Cuará, and Cambará. Each sample, which consisted of 10 sweet potatoes, was identified with the variety and date and was sent to the Diagnostic Clinic of the Plant Protection Department of the Agronomy Faculty, University of the Republic, Uruguay.

The symptoms were observed macro and microscopically, externally and internally, and photographed. To identify the fungi by morphology, the signs were studied directly on the sample in the cases that were detected, but in all of them, the structures were studied from isolates[10][11].

To start the isolation process, a sweet potato with incipient and characteristic symptoms was chosen from the sample. After disinfection, cuts from the transitional tissue between the affected and healthy areas were removed. Those sections were sterilized, immersing them for one minute in 70% alcohol, rinsed twice with sterile distilled water, dried in a laminar flow chamber, and seeded in

Potato Dextrose Agar culture medium (PDA, Oxoid. CMO139). Subsequently, they were incubated at 24 ºC and in the dark for approximately five days. The developed colonies were replicated to obtain pure cultures and to begin with the morphological identification that was carried out with colonies between 5 and 7 days old[10][11]. The fungi that appeared most frequently were kept on sterile filter paper. They were subsequently recovered to perform molecular characterization and pathogenicity tests.

The DNA was extracted from two colonies with seven days of growth in PDA medium for molecular identification, using the protocol described by Delgado-Cerrone[14]. For each isolate part of the elongation factor one alpha gene (EF1α) was amplified and sequenced with the primers EF1-728F / EF1-986R[15]. Amplifications were performed using a MultiGene™ Mini thermocycler (Labnet International Inc., USA) in a final volume of 20 µL. Amplified products were purified and sequenced at Macrogen Inc. (Seoul, Korea). The sequences obtained were aligned with Clustal W included in the MEGA 7.0.26[16] program. The phylogenetic reconstruction was performed for the EF1α region data set with the Maximum Likelihood (ML) method, including sequences from similar species and close to those obtained, extracted from the GenBank database. The missing spaces or data generated in the alignment were considered complete deletions. The estimation of the value of the nodes was obtained through 1000 Bootstrap repetitions.

Sweet potatoes without visible symptoms of disease were used to carry out the pathogenicity test. Each one was superficially disinfected with 70% alcohol, just like the instruments used. The ends were discarded and the rest was cut into three sections. Half were inoculated with the most frequently obtained fungus and the rest remained uninoculated as control.

To inoculate the sweet potato sections, 2 cm diameter discs of the culture medium were placed on the cut area with 6-day-old fungal colony mycelium. The plant material thus inoculated was kept in a humid chamber at 23 ºC and a 12-hour photoperiod. Symptoms were documented after about a month and a half. The fungus was re-isolated and the same identity of the inoculated fungus could be verified with its morphological identification, thus fulfilling Koch's postulates.

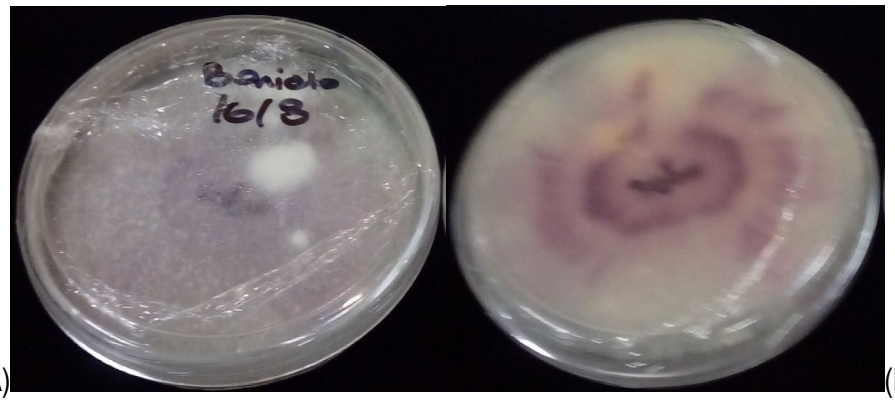
3. Results and discussion

All received samples presented the symptoms of superficial circular cankers that minimally affected the underlying tissue, from pale brown to blackish and dry colors, with an average diameter of 2 to 4 mm at the beginning, that could grow and coalesce, generating more extensive dark areas (Figure 1). In some of these injuries, a detachment of the skin between the affected and the healthy tissue was observed.

Figure 1. Superficial and slightly sunken circular lesions.

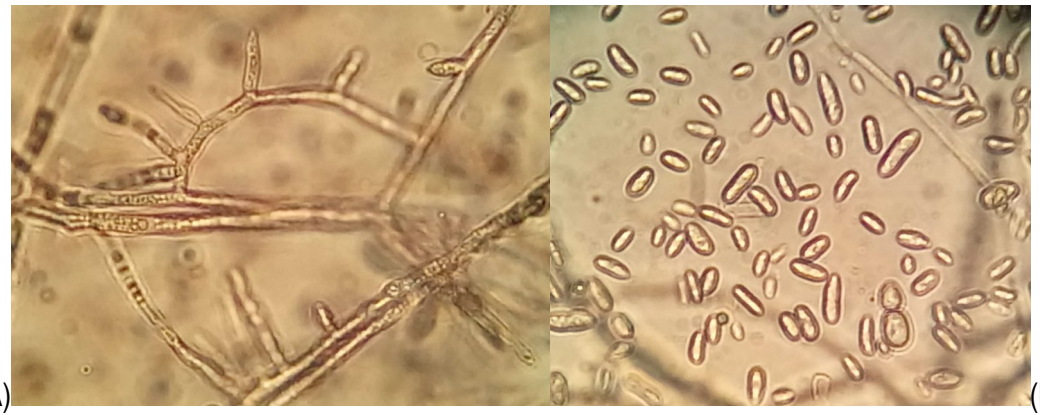
38 fungal isolates were obtained from the seeding of the transitional tissue sections in the culture medium. 85% of these were white colonies with purplish-pink tones characteristic of many *Fusarium* species (Figure 2). The rest of the isolates corresponded to the genera *Alternaria, Verticillium,* and *Penicillium*.

Figure 2. Obverse (A) and reverse (B) view of *Fusarium* colony.

(A)  (B)

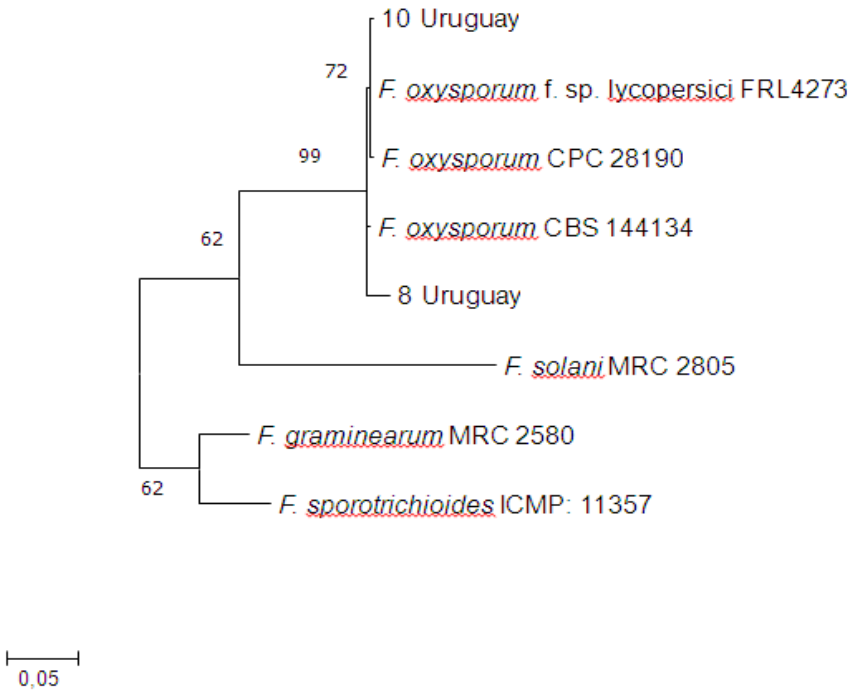
In the morphological identification of the colonies, the presence of predominantly oval microconidia of 6.4 by 3 um in the aerial mycelium was found in false heads on short monofialides, and scarce medium-sized macroconidia of 27 by 3.8 um and straight (Figure 3). The mentioned characteristics corresponded to *Fusarium oxysporum*[11][12], confirmed by molecular identification as explained below.

Figure 3. Short monofialides (A), and conidia and mesoconidia (B)

(A)  (B)

An amplification of approximately 560 bp was obtained from the elongation factor one alpha region of two sweet potato isolates. Sequence analysis by ML resulted in a tree with three clades (Figure 4). Isolates 8 and 10 were included in the clade that groups the species *F. oxysporm,* with Bootstrap support of 99%.

Figure 4. Tree generated from the phylogenetic analysis of EF1α sequences by Maximum likelihood of two sweet potato isolates obtained in this study, and sequences of reference isolates obtained from GenBank. The values in the nodes correspond to Bootstrap support (%) for 1000 repetitions.



In the pathogenicity test, dry rot was obtained that tended to grow towards the center of the sectioned sweet potato and in re-isolation *F. oxysporum* was identified again. Therefore, the pathogenicity of *F. oxysporum* and its causal relationship with the cankers described in the studied samples were confirmed. This rot matches with Clark and Moyer[4] description of the injuries caused by *F. oxysporum*.

The five studied cultivars presented lesions caused by *F. oxysporum*, which show that none of them possessed qualitative resistance. This agrees with the investigations of Nielsen[5], Scott and others[12], and Nielsen and Johnson[6], who found no qualitative resistance among the materials they evaluated, although they did identify different susceptibility degrees. One of the practices to consider in integrated crop management is choosing cultivars with a lower susceptibility level to *F. oxysporum*. For this, work on genetic improvement should include this evaluation, both of the materials under selection and of the cultivars currently planted.

F. oxysporum persists in the soil for a long time, mainly as chlamydospores, and penetrates mainly through the wounds caused by harvesting[4]. Producers harvest the sweet potato under variable conditions of temperature and relative humidity, and the “curing” is carried out in the field, frequently outside the optimum temperature range of 29 ºC and 32 ºC and around 85% RH, which allow rapid and complete healing of the periderm[4][5][6][17]. This condition substantially favors infection.

Technically, artificial “curing” would be the best option, particularly if the sweet potato is meant to be stored for a long time, but currently, it is not economically feasible to invest in structures specially built for that purpose. Therefore, careful management of the harvest to minimize injuries and the identification of the optimal harvest date so that curing is carried out with the temperature and relative humidity closest to optimal are subjects to study.

In storage, temperatures between 13 and 16 ºC and relative humidity of 85% slow down the development in the already infected sweet potatoes. The

pathogen does not spread from infected roots to healthy ones unless new wounds occur[4][5][6][7].

Therefore, it is necessary to study the type of structures that allow the temperature and relative humidity to be kept as close as possible to the optimal values mentioned, affordable by the family producers of Canelones.

It is suggested to continue deepening the knowledge of the epidemiological factors that affect the infection and virulence of the pathogen during cultivation, harvest, "curing" and storage. These elements will contribute to establishing the most appropriate management and control practices to avoid or reduce post-harvest losses and deterioration in the commercial quality of the sweet potato.

4. Conclusions

The techniques used for the morphological and molecular characterization, and the reproduction of the symptoms in the pathogenicity test allowed to identify *F. oxysporum* and to confirm that it is the causal organism of superficial circular cankers that minimally affect the underlying tissue. Pale brown to blackish and dry, with an average diameter of 2 to 4 mm, which may coalesce, generating more extensive dark areas, and skin detachment may occur between the affected and the healthy tissue.

The five sweet potato cultivars studied presented the characteristic lesions produced by *F. oxysporum*, which indicate that they are all susceptible.

The identification of the causal agent of the described symptoms is a contribution to future epidemiological studies, it contributes to a better understanding of post-harvest rot and to define a more precise combination of appropriate management practices for the integrated control of this disease in Canelones production conditions.

Authors contribution

A. Scattolini carried out the diagnostic analyzes and the application of Koch's postulates; L. Hernández Rodríguez carried out the DNA extraction and the sequence study of the phytopathogenic fungi with the highest appearance frequency, and H. González Idiarte monitored the crops and the harvest storage.

References

1. Comisión Administradora del Mercado Modelo, Dirección General de la Granja. Boniato: situación y perspectivas [Internet]. [place unknown]: Observatorio Granjero; 2015, [cited 2020 Mar 25]. 6p. Available from: https://bit.ly/3cEgLes.

2. Ministerio de Ganadería, Agricultura y Pesca, DIEA (UY). Censo General Agropecuario 2011: resultados definitivos [Internet]. Montevideo: MGAP; 2012 [cited 2020 Feb 19]. 146p. Available from: https://bit.ly/361mTL8.

3. Ministerio de Ganadería, Agricultura y Pesca, DIEA (UY). Anuario Estadístico Agropecuario [Internet]. 21nd ed. Montevideo: MGAP; 2018 [cited 2020 Feb 19]. 210p. Available from: https://bit.ly/3aH1fgO.

4. Clark CA, Moyer JW. *Fusarium* Root Rot and Stem Canker and Surface Rot. In: Clark CA, Ferri DM, Smith TP, Holmes GJ, editors. Compendium of sweet potato diseases, pests, and disorders. 2nd ed. St Paul (MN): American Phytopathological Society; 2013. p. 37-40.

5. Nielsen LW. Harvest practices that increase Sweetpotato Surface Rot in Storage. Phytopathology. 1965;55:640-4.

6. Nielsen LW, Johnson JT. Postharvest temperature effects on wound healing and surface rot in sweetpotato. Phytopathology. 1974;64:967-70.

7. Scruggs AC, Quesada Ocampo LM. Etiology and epidemiological conditions promoting Fusarium Root in Sweetpotato. Phytopathology. 2016;106:909-19.

8. Maeso D, Vilaró F. Enfermedades del cultivo de boniato en el Uruguay: descripción y control. In: Boniato: cultivares, semillas, enfermedades. Las Brujas: CIAAB; 1984. p. 21-35. (Miscelánea, Nº 59).

9. Monteiro C. Identificación de enfermedades en boniato en el Sur. In: INIA. Producción y uso del

boniato (*Ipomoea batatas)*. Montevideo: INIA; 1991. p. 60-1.

10. Arbelaéz G. Algunos aspectos de los hongos del género *Fusarium* y de la especie *Fusarium oxysporum*. Agron colomb. 2000;17:11-22.

11. Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O. Introduction to food-borne fungi. Baarn: Centraalbureau voor Schimmelcultures; 1995. 322p.

12. Booth C. The genus Fusarium. London: Commmonwealth Micological Institute, 1971. 237p.

13. Scott LE, Kantzes J, Boukamp JC. Clonal differences in the incidence of surface rot (*Fusarium* spp*)* on sweetpotato. Plant Dis Rep. 1972;56(9):783-4.

14. Clark CA, Randle WM, Pace CS. Reactions of sweet potato selections to Fusarium root and stem canker caused by *Fusarium solani*. Plant Dis. 1986;70:869-71.

15. Delgado-Cerrone L. Caracterización de especies de Botryosphaeriaceae asociadas al cultivo de manzano en Uruguay [master's thesis]. Montevideo (UY): PEDECIBA Biología; 2013. 78p.

16. Carbone I, Kohn LM. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia. 1999;91:553-6.

17. Kumar S, Stecher G, Tamura K. Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets: MEGA7. Mol Biol Evol. 2016;33:1870-4.