

Recent studies of fungal pathogens of onion in Puerto Rico¹

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ABSTRACT

First descriptions of fungal pathogens of onions in Puerto Rico were reported by J. A. B. Nolla, J. Matz, R. A. Toro and C.M. Tucker during the 1920s. At that time onions were grown in the northern region of the island and fungal pathogens such as *Alternaria allii*, *Aspergillus niger*, *Colletotrichum dematium*, *C. gloeosporioides*, *Rhizoctonia* sp. and *Stemphylium botryosum* were reported causing important fungal diseases under field conditions. From 1999 to 2000 a broad survey of fungi associated with onion was conducted in the southern region of the island. Pathogenicity tests were conducted with 25 fungal isolates obtained from soil, mature onion bulbs and foliage from fields at Guánica, Juana Díaz, and Santa Isabel, Puerto Rico. The pathogenicity tests were performed on onion tissues of cultivars Excalibur and Nikita under laboratory and greenhouse conditions. Tests showed that *A. niger*, *Phoma sorghina*, *Phoma* sp., *Penicillium purpurogenum* and *Sclerotium rolfsii* were pathogenic to mature bulbs; *Alternaria alternata*, *A. tenuissima*, *Fusarium acuminatum*, *F. equiseti*, *F. oxysporum*, *Stemphylium* sp. and *S. herbarum* to foliage; *Phoma* sp. and *Phoma sorghina*, pathogenic to young roots and bulbs. This is the first report of *Phoma sorghina*, *Penicillium purpurogenum* and *Sclerotium rolfsii* causing diseases in onions in Puerto Rico and the Caribbean.

Key words: fungal pathogens, onions, *Allium cepa*, pathogenicity tests, Puerto Rico

RESUMEN

Estudios recientes de hongos patógenos de cebolla en Puerto Rico

Las primeras descripciones de hongos patógenos de cebolla en Puerto Rico se publicaron por J.A.B. Nolla, J. Matz, R. A. Toro y C.M. Tucker durante los años 1920. En aquella época las cebollas se producían en la región norte de la isla y se informaron hongos patógenos tales como *Alternaria allii*, *Aspergillus niger*, *Colletotrichum dematium*, *C. gloeosporioides*, *Rhizoctonia* sp. y *Stemphylium botryosum* causando importantes enfermedades fungosas en condiciones de campo. Durante 1999 al 2000, se realizó un catastro de hongos asociados a la cebolla en la región sur de la isla. Se realizaron pruebas de patogenicidad con 25 aislados de hongos obtenidos de suelo, bulbos maduros y follaje de cebolla de fincas localizadas en Guánica,

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Juana Díaz, y Santa Isabel, Puerto Rico. Las pruebas de patogenicidad se realizaron en tejidos de cebolla de los cultivares Excalibur y Nikita en condiciones de laboratorio e invernadero. Las pruebas demostraron que *A. niger*, *Phoma sorghina*, *Phoma* sp., *Penicillium purpurogenum* y *Sclerotium rolfsii* fueron patógenos a los bulbos maduros; *Alternaria alternata*, *A. tenuissima*, *Fusarium acuminatum*, *F. equiseti*, *F. oxysporum*, *Stemphylium* sp. y *S. herbarum* al follaje; *Phoma* sp. y *Phoma sorghina*, a raíces y bulbos jóvenes. Este es el primer informe de *Phoma sorghina*, *Penicillium purpurogenum* y *Sclerotium rolfsii* causando enfermedades en cebolla en Puerto Rico y el Caribe.

Palabras clave: hongos patógenos, cebollas, *Allium cepa*, pruebas de patogenicidad, Puerto Rico

INTRODUCTION

Onion production in the Caribbean increased dramatically from 45,843 metric tons in 1995 to 200,215 t in 2006. Puerto Rico was the fourth largest Caribbean producer, after Haiti, Dominican Republic and Cuba during 2005 (FAO, 2006). According to the Puerto Rico's Department of Agriculture, the island produced 3,632 mt during fiscal year 2004-2005. This crop's revenues contributed around \$1.6 million to the island's annual gross agricultural income (Anonymous, 2006). Onion production has a great potential for increasing because of local demand and markets for exportation. However, onion is a perishable commodity that suffers yield losses due to diseases and pests, and to post-harvest handling and storage. Yield and storage losses in onion as a result of plant diseases can reach up to 50% or more per year, depending upon the location, the environment and the causal agents, most of which are microscopic fungi (Schwartz and Mohan, 1995).

Limited research has been conducted in Puerto Rico addressing fungi pathogenic to onion ever since the first reports in the 1920s (Matz, 1921; Toro, 1923; Nolla, 1926; Tucker, 1927). The sporadic and limited nature of *Allium cepa* L. production in Puerto Rico for most of the 20th century accounts for the scant research record. Various fungal diseases have been observed in onion fields in Puerto Rico: wilting and bulb black rot caused by *Stemphylium botryosum* Wallr.; purple blotch caused by *Alternaria porri* (Ell.) Cif.; black mold caused by *Aspergillus niger* van Tieghem; anthracnose caused by *C. gloeosporioides* Penz. & Sacc. and *C. chardonianum* Nolla; and root rot caused by *Fusarium solani* (Mart) Sacc. (Toro, 1923; Nolla, 1926, 1927; Cook, 1939). In addition, plant diagnostic clinic reports have described *Alternaria* sp. and *Stemphylium* sp. as causes of leaf spots (R. Rodríguez, 1993 and 1994, Department of Crop Protection, UPR-Mayaguez, personal communication) and basal rot caused by *Fusarium* sp. (R. Woodward, 1999, Micro Macro Analytical Laboratories, Georgia, personal communication). Thus, increasing onion production under tropical conditions has re-

newed the importance of studying fungal pathogens limiting yield in the Caribbean Basin.

Recently, a survey showed that a diverse group of fungi were associated with plant tissue and with the air and soil of onion fields located in the southern region of Puerto Rico (Vélez et al., 2004). Fungi such as *Alternaria* spp., *Aspergillus niger*, *Bipolaris* sp., *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Fusarium* spp., *Myrothecium* sp., *Phoma* spp., *Penicillium* sp., *Rhizopus* sp., *Sclerotium rolfsii* and *Stemphylium* spp., were frequently isolated (Vélez et al., 2004). However, the pathogenic potential of these fungi must be examined. This study reports the pathogenicity of this diverse group of fungi commonly associated with onions in the southern region of Puerto Rico.

MATERIALS AND METHODS

Fungal isolates: Twenty-five fungal isolates obtained in the above mentioned survey (soil, mature onion bulbs and foliage) conducted in the southern region of Puerto Rico were selected for pathogenicity tests (Table 1). The survey was conducted during 1999-2000 in experimental and commercial fields located at Guánica, Juana Díaz, and Santa Isabel, Puerto Rico (Vélez et al., 2004). The following fungi were evaluated: *Alternaria* spp., *A. alternata*, *A. tenuissima*, *Aspergillus niger*, *Bipolaris* sp., *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Curvularia* sp., *Fusarium acuminatum*, *F. equiseti*, *F. oxysporum*, *Penicillium* sp., *P. purpurogenum*, *Phoma* spp., *Phoma sorghina*, *Stemphylium* sp., *S. herbarum* and *Sclerotium rolfsii*. Taxonomic keys were used to identify fungal isolates (Barnett and Hunter, 1998; Domsch and Gams, 1980; Mordue, 1971; Sutton, 1980; Watanabe, 1994). Identification of the following isolates was corroborated by CABI Bioscience Identification Services⁴ (Bakeham Lane, Egham, Surrey, UK): *Alternaria alternata*, *A. tenuissima*, *Fusarium acuminatum*, *F. equiseti*, *F. oxysporum*, *Penicillium purpurogenum*, *Phoma sorghina* and *Stemphylium herbarum*.

Pathogenicity Tests

Onion bulbs: Pathogenicity tests were conducted on mature onion bulbs cv. Nikita under laboratory conditions. Mature onion bulbs were provided by a commercial private farm in Santa Isabel, Puerto Rico. First leaves were removed from bulbs and these were superficially ster-

⁴Fungal identification service was mentioned to provide specific information and mention of same does not constitute a warranty by the University of Puerto Rico, nor is this mention a statement of preference over other identification services.

TABLE 1.—Number and origin of fungal isolates tested during the pathogenicity tests conducted in different onion tissues.

Fungal isolates	No. of isolates	Isolated from ²	Tested on ²	Inoculation method		
				Conidial/ml		Mycelial disks (cm)
				1 st test	2 nd test	
<i>Alternaria tenuissima</i> (isolate no. 1)	one	F	F ³ , MB ⁴	1.1 × 10 ⁵	1.0 × 10 ⁵	0.4
<i>A. alternata</i> (isolates no. 2 and 5)	two	F	F, MB	1.0 × 10 ⁵	1.2 × 10 ⁵	0.4
<i>Alternaria</i> sp. (isolate no. 3 and 4)	two	F	F	2.0 × 10 ⁵	1.5 × 10 ⁵	—
<i>Aspergillus niger</i> (isolates no. 1 and 2)	two	Soil	MB	—	—	0.4
<i>Bipolaris</i> sp.	one	F	F	1.1 × 10 ⁵	—	—
<i>Cladosporium</i> sp.	one	F	F	3.3 × 10 ⁶	—	—
<i>Colletotrichum gloeosporioides</i>	one	Soil	F, YB ⁵	4.0 × 10 ⁶	—	0.4
<i>Curvularia</i> sp.	one	F	F	1.0 × 10 ⁵	—	—
<i>Fusarium acuminatum</i> (isolates no. 1 and 4)	two	F	F, MB, R ⁶	2.0 × 10 ⁶	6.9 × 10 ⁵	0.4
<i>F. oxysporum</i> (isolate no. 2)	one	Soil	F, R	1.1 × 10 ⁵	8.7 × 10 ⁵	—
<i>F. equiseti</i> (isolate no. 3)	one	F	F, MB, R	5.2 × 10 ⁵	5.6 × 10 ⁵	0.4
<i>Penicillium</i> (isolate no. 1)	one	MB	MB	—	—	0.4
<i>Penicillium purpurogenum</i> (isolate no. 2)	one	Soil	MB	—	—	0.4
<i>Phoma</i> spp. (isolates no. 1, 3, 4 and 5)	four	Soil	MB, YB, R	—	—	0.4
<i>Phoma sorghina</i> (isolate no. 2)	one	Soil	MB, YB, R	—	—	0.4
<i>Sclerotium rolfsii</i>	one	MB	MB, R	—	—	0.4
<i>Stemphylium</i> sp. (isolate no. 1)	one	F	F	1.0 × 10 ⁴	2.0 × 10 ⁴	—
<i>S. herbarum</i>	one	F	F	1.0 × 10 ⁴	1.0 × 10 ⁴	—

¹Mycelial disks or conidial suspension (conidia per milliliter) were used during pathogenicity tests. Fungal colonies were grown on acidified potato dextrose agar (APDA) and incubated at 28° C. Controls were inoculated with APDA disks or sprayed with sterile dionized double distilled water.

²Foliage = F; Mature bulbs = MB; Young bulbs = YB; and Roots = R.

³Pathogenicity tests were conducted on foliage of 42-day-old plants (cvs. Nikita and Excalibur). A conidial suspension from sixteen-day-old fungal colonies grown on APDA was sprayed on leaf tissues for each fungal isolate evaluated. Sterile dionized double distilled water was used as control.

⁴Pathogenicity tests were conducted on mature onion bulbs cv. Nikita under laboratory conditions. Mycelial disks from 14-day-old fungal colonies grown on APDA were used in the pathogenicity tests.

⁵Pathogenicity tests were conducted on 55-day-old onion plants (cv. Nikita). Leaves were removed leaving the bulbs and 6-cm-long roots. Eighteen-day-old fungal colonies were used as source of inoculum.

⁶Twenty milliliters of mycelia and spore suspensions from each fungal isolate were applied directly into the soil close to the roots of 42-day-old onion plants (cvs. Nikita and Excalibur).

ilized with 1% sodium hypochlorite for 5 min. Two onion bulbs were placed in a plastic box (34 × 20 × 10 cm) as moist chambers, and were inoculated with each of the sixteen fungal isolates. Three punctures (0.6-cm depth) were made on bulb tissues with a needle tip before inoculation with a 0.4-cm mycelial disk of the fungal isolate. Control group was inoculated with acidified potato dextrose agar (APDA) mycelial disks. The same procedure was followed with non-wounded bulbs.

Fourteen-day-old fungal colonies grown on APDA and incubated at 28° C were used in the pathogenicity tests. Sixteen fungal isolates were evaluated in this assay. These were two *Alternaria alternata* isolates and one *A. tenuissima* isolate from foliage; two *Aspergillus niger* isolates from soil; two *Fusarium acuminatum* isolates and one *Fusarium equiseti* isolate from foliage; one *Penicillium* sp. isolate from mature bulbs and one *P. purpurogenum* isolate from soil; four *Phoma* spp. and one *Phoma sorghina* isolates from soil; and one *Sclerotium rolfsii* isolate from bulbs (Table 1). Bulbs were placed in moist chambers under laboratory conditions in a random complete block design with four repetitions each. Temperature ranged from 20 to 26.6° C. Daily observations were made for a month. After a month, symptoms were described and photographs were taken. Koch's postulates were completed after reisolation of the inoculated fungi on acidified PDA.

Onion plant foliage: Onion seeds from cvs. Nikita and Excalibur were planted in plastic trays (25 × 50 cm with 128 holes of 1.8 × 1.8 × 5 cm) containing sterilized Sunshine Mix # 1® (Sun Grow Co., Canada)⁵. Trays were kept in a greenhouse and plants were fertilized with 17-5-17 (N-P-K) + 3-2 (Ca-Mg). Plants were irrigated with sprinklers when needed. After 35 days, onion plants were transplanted to 10-cm pots. Pathogenicity tests were conducted on foliage of 42-day-old plants by using a suspension of 10⁴ to 10⁵ conidia per milliliter from sixteen-day-old fungal colonies grown on acidified PDA. Three wounds were made, one each at the tip, center and base of mature leaves, before inoculation. Unwounded leaves were also inoculated. Fourteen fungal isolates were evaluated in this assay. These were *Alternaria* spp. (two isolates), *A. alternata* (two isolates), *A. tenuissima* (one isolate), *Bipolaris* sp. (one isolate), *Colletotrichum gloeosporioides* (one isolate), *Cladosporium* sp. (one isolate), *Curvularia* sp. (one isolate), *Fusarium acuminatum* (isolate no. 1), *F. oxysporum* (one isolate), *F. equiseti* (one isolate), *Stemphylium* sp. (one isolate) and *S. herbarum* (one isolate)

⁵Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

(Table 1). All fungal species tested in this assay were isolated from foliage with the exception of *C. gloeosporioides* and *F. oxysporum*, which were isolated from soil. Conidial suspension was sprayed on leaf tissues for each of the fourteen fungal isolates evaluated. Double sterile distilled water was used as control.

Each plant was covered with a plastic bag (32.5 × 15.0 cm) and kept for four days under laboratory conditions. A complete randomized experimental design was used with three repetitions. Temperature ranged from 23 to 28° C and relative humidity (RH) from 59 to 63%. Inoculated foliage was examined daily and careful observations were made on symptom development. Koch's postulates were completed after reisolation of inoculated fungi on APDA. A second pathogenicity test was conducted as previously described to corroborate findings using onion plants cv. Nikita only. Nine fungal isolates were evaluated during the second assay (Table 1). Plants were kept for three to six days under laboratory conditions at 24 to 28° C and 60 to 65% RH. Then plants were transferred to a greenhouse at 26 to 35° C with 62 to 88% RH.

Young onion roots and bulbs in vitro: Foliage of 55-day-old onion plants cv. Nikita was removed, leaving the bulbs with 6-cm-long roots. Foliage tissue was superficially disinfected with sodium hypochlorite 0.05% for 3 min. Two bulbs and roots were placed inside a glass petri plate (150 × 15 mm) acting as a moisture chamber. One of the two bulbs was wounded at the center by using a sterile needle whereas roots were not wounded. Bulbs, root tips and basal ends were inoculated with 0.4-cm mycelial disks of the fungal isolates evaluated. Eighteen-day-old fungal colonies were used as source of inoculum. Four fungal isolates were evaluated in this assay: *Phoma* spp. (isolates no. 1 and 3), *P. sorghina* and one isolate of *Colletotrichum gloeosporioides* from soil. Controls were inoculated with acidified PDA disks.

A complete randomized block experimental design with three replications was used. Temperature ranged from 23 to 28° C with 58 to 66% RH. Observations of symptom development on bulbs and roots were made five to nine days after inoculation. Photographs were taken of symptom development, and Koch's postulates were completed after reisolation and identification of inoculated fungal isolates.

Inoculum applied directly into soil close to the roots: Fungal isolates were grown in APDA for 14 days at 28° C. Twenty milliliters of mycelia and spore suspensions from each fungal isolate were applied directly into the soil close to the roots of 42-day-old onion plants (cvs. Nikita and Excalibur). Eight fungal isolates were evaluated in this assay: *Fusarium acuminatum* (two isolates), *F. equiseti* (one isolate) from foliage; *F. oxysporum* (one isolate), *Phoma* spp. (isolates no. 1 and 3), *Phoma sorghina* (one isolate) isolated from soil and *Sclerotium rolfsii*

(one isolate) from bulbs. Control plants were treated with sterile distilled water. Plants were kept in a greenhouse in a completely randomized block design at 21 to 40° C with 56 to 103% RH. Four replicates for each treatment were included. After three weeks, onion bulbs and stem basal area were wounded with a sterile needle and re-inoculated with mycelial disks (0.6 cm) from each fungal isolate tested. Controls were inoculated with acidified PDA disks. Plants were kept at 20 to 38° C with 58 to 103% RH.

Data of relative humidity and temperature under laboratory and greenhouse conditions were determined with a hygrothermograph (Oakton Model 37250-00, Cole Parmer Instrument Co., Chicago, Illinois).

RESULTS AND DISCUSSION

Onion bulbs: Thirty days after inoculation, five out of 16 tested isolates were pathogenic to mature onion bulbs: *Aspergillus niger* (two isolates), *Penicillium purpurogenum*, *Phoma* sp. (isolate no. 1) and *Sclerotium rolfsii* produced symptoms on onion bulbs cv. Nikita. Non inoculated (control) bulbs did not show symptoms.

Two isolates of *Aspergillus niger*, obtained from soil, produced profuse black mycelial growth and black sporulation around inoculation points in onion bulbs. Tissues underneath showed yellowish coloration and water soaked lesions. Black mold in onion has been previously described by Sumner (Schwartz and Mohan, 1995). *Aspergillus niger* has been reported in onion bulbs and leaves in Puerto Rico (Stevenson, 1975; Minter et al., 2001). In addition, we found *A. niger* frequently associated with onion seeds of cultivars Nikita, Mercedes and Excalibur, thus implicating the seed as the primary source of inoculum (Vélez et al., 2004).

Phoma sp. (isolate no. 1) caused lesions limited to bulb surface, and mycelial growth was observed around inoculation points (Figure 1A). Lesions were small on the external layers of wounded onion bulbs; this finding agrees with studies conducted by Kreutzer (1941) with *Phoma terrestris*. At the beginning of the experiment onion bulbs had a tender tissue layer which was susceptible to pathogen attack, and eventually the external layer dried out.

Penicillium purpurogenum isolated from soil produced a reddish pigmentation on agar media and reddish lesions on bulb surface that turned dark around the inoculation point. First bulb layers showed soft watery lesions with reddish to purple coloration but as disease developed, soft rotten bulbs were observed. Profuse greenish mycelial growth with heavy sporulation occurred 30 days after inoculation (Figure 1B). This is the first report of *P. purpurogenum* affecting onion bulbs in Puerto Rico

and the Caribbean. Blue mold symptoms caused by several *Penicillium* spp. have been described by Schwartz and Mohan (1995). *Penicillium aurantiogriseum* (syn. *P. cyclopium*), *P. citrinum*, *P. digitatum*, *P. expansum*, *P. hirsutum* (syn. *P. corymbiferum*), *P. funiculosum* and *P. oxalicum* have been related to the development of such symptoms.

Sclerotium rolfsii completely macerated onion bulbs, producing abundant white mycelia with brownish sclerotia. It is well known that this pathogen secretes a wide range of pectolytic enzymes, celluloses and oxalic acid responsible for tissue maceration (Schwartz and Mohan, 1995). Even though *S. rolfsii* has not previously been reported, in this study *S. rolfsii* caused soft rot of onion bulb.

The following evaluated fungi were non pathogenic to mature onion bulbs: *Phoma* spp. (isolates no. 3, 4 and 5) and *P. sorghina* isolated from soil; *Fusarium acuminatum* (isolates no. 1 and 4), *Fusarium equiseti* (isolate no. 3), *Alternaria alternata* (isolates no. 2 and 5) and *A. tenuissima* (isolate no. 1) isolated from foliage; and *Penicillium* sp. (isolate no. 1) isolated from mature bulbs.

Onion foliage: Nine out of 14 fungal isolates from foliage and soil caused symptoms on leaves during the first pathogenicity test: *Alternaria* sp. (isolate no. 4), *A. tenuissima*, *A. alternata* (isolates no. 2 and 5), *Fusarium acuminatum* (isolate no. 1), *F. oxysporum*, *F. equiseti*, *Stemphylium* sp. and *S. herbarum*.

All *Alternaria* spp. evaluated (except isolate no. 3), *Stemphylium* sp. and *S. herbarum* caused ellipsoidal sunken lesions that eventually developed into brownish to purple lesions that extended through the tip of the leaf. This tip blight was commonly observed seven days after inoculation. Leaves showed mycelial growth and sporulation on tissues. Twelve days after inoculation, *A. tenuissima* ellipsoidal lesions measured 0.6×0.3 cm. Lesions were light brown with a dark halo at the margins and with profuse mycelial growth and sporulation at the center. Similar symptoms were observed with *A. alternata*, in which brownish to purple lesions were developed (Figure 2).

Even though typical *A. allii* foliar lesions were observed decades ago in onions in the northern region of Puerto Rico as reported by Nolla (1927), this species was not isolated during the present study. In contrast, *A. tenuissima* and *A. alternata* were frequently isolated from onion foliage in the southern region of Puerto Rico. In Colorado, *A. porri*, *A. alternata* (synonymous with *A. tenuis*) and *A. tenuissima* have been reported by Skiles (1953) as causing purple and brownish foliar lesions in onion.

Stemphylium spp. caused lesions of 0.3×0.2 cm seven days after inoculation. After 12 days, lesions extended to the leaf tip producing necrosis and tip blight, with profuse mycelial growth and sporulation.

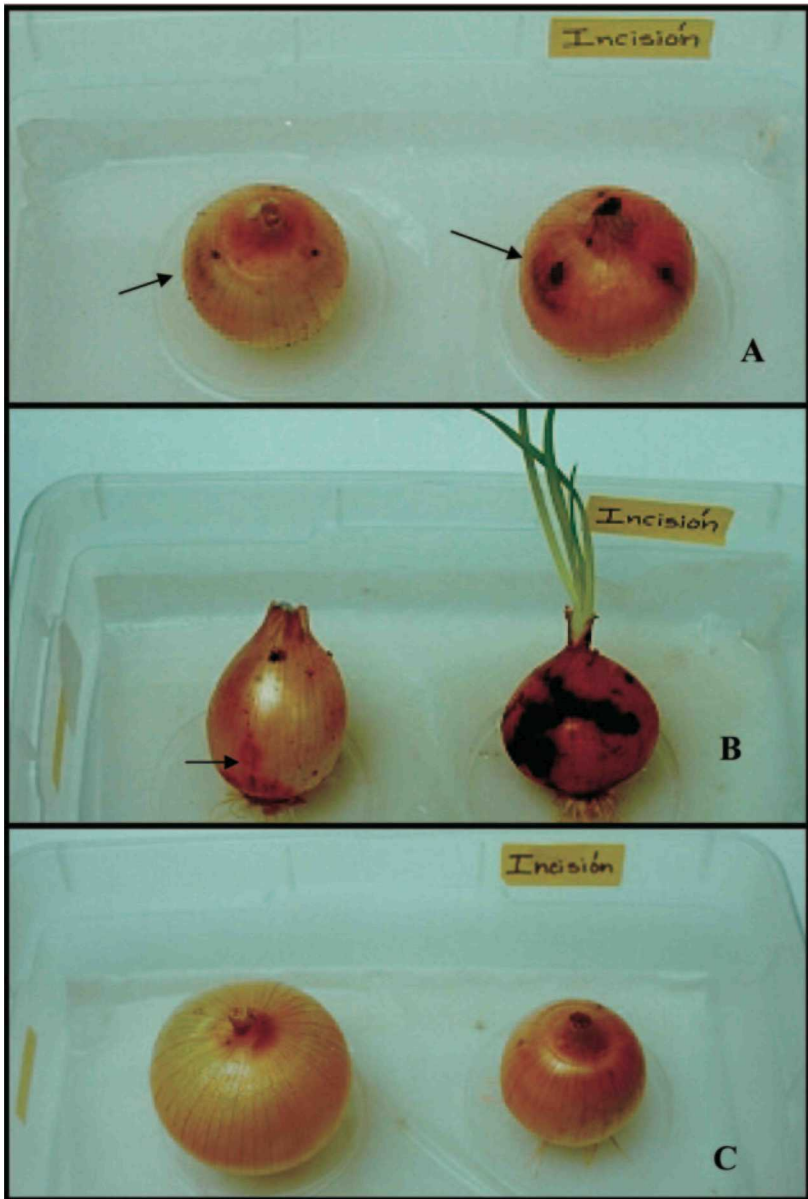


FIGURE 1. Symptoms observed in unwounded (left) and wounded (right) mature onion bulbs, 30 days after inoculation with A) *Phoma* sp. (isolate no. 1) which caused necrotic lesions; B) *Penicillium purpurogenum* caused soft watery lesions with reddish coloration (arrow); and C) Controls.

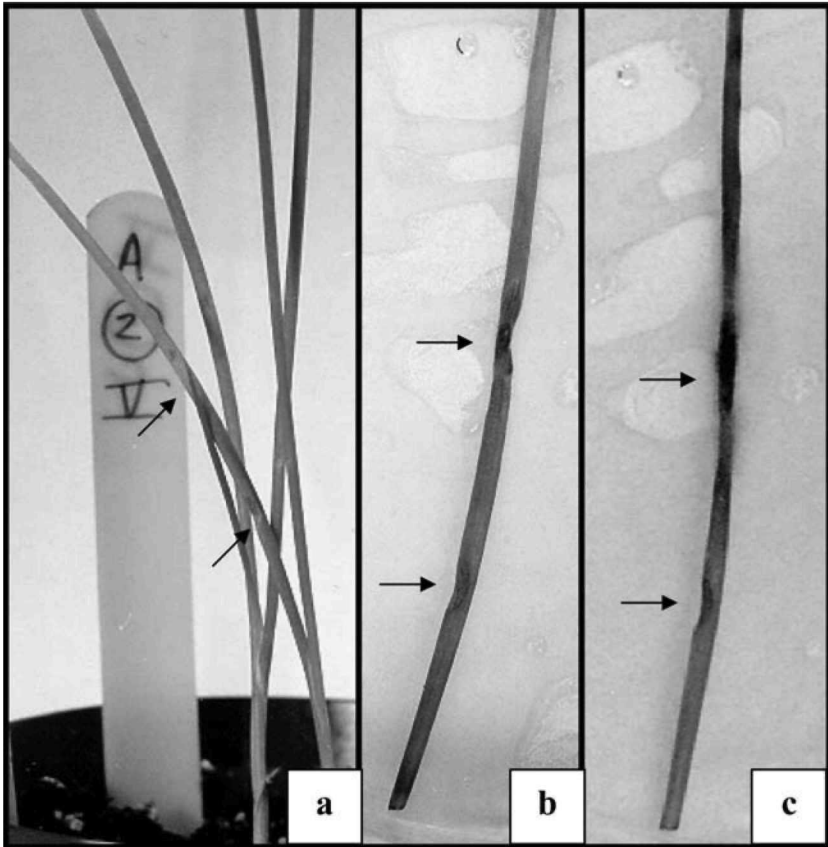


FIGURE 2. Symptoms observed in 42-day-old onion plants cultivar 'Nikita' under greenhouse conditions caused by *Alternaria alternata*. Observed development of symptoms three, six and nine days (a to c) after inoculation: (a) sunken leaf spot, (b) development of brown ellipsoidal lesions, and (c) necrosis and leaf blight.

Fungal isolates of *Stemphylium* sp. and *S. herbarum* produced blights very similar to those caused by *Alternaria* spp. in the field. In the past, *S. botryosum* has been reported causing foliar lesions of onion in Puerto Rico (Toro, 1923). However, *S. herbarum*, a foliar pathogen of alfalfa, has not been reported in onion (Chaisrisook et al., 1995). Further studies on the characterization of *Alternaria* spp. and *Stemphylium* spp. occurring in onion foliage in Puerto Rico are necessary to clarify these findings.

Tip blight was observed in onion foliage of cv. Nikita inoculated with *F. acuminatum*, *F. equiseti* and *F. oxysporum* (Figure 3). Initially leaves showed chlorosis, and eventually necrosis. The most virulent, *F. acumi-*

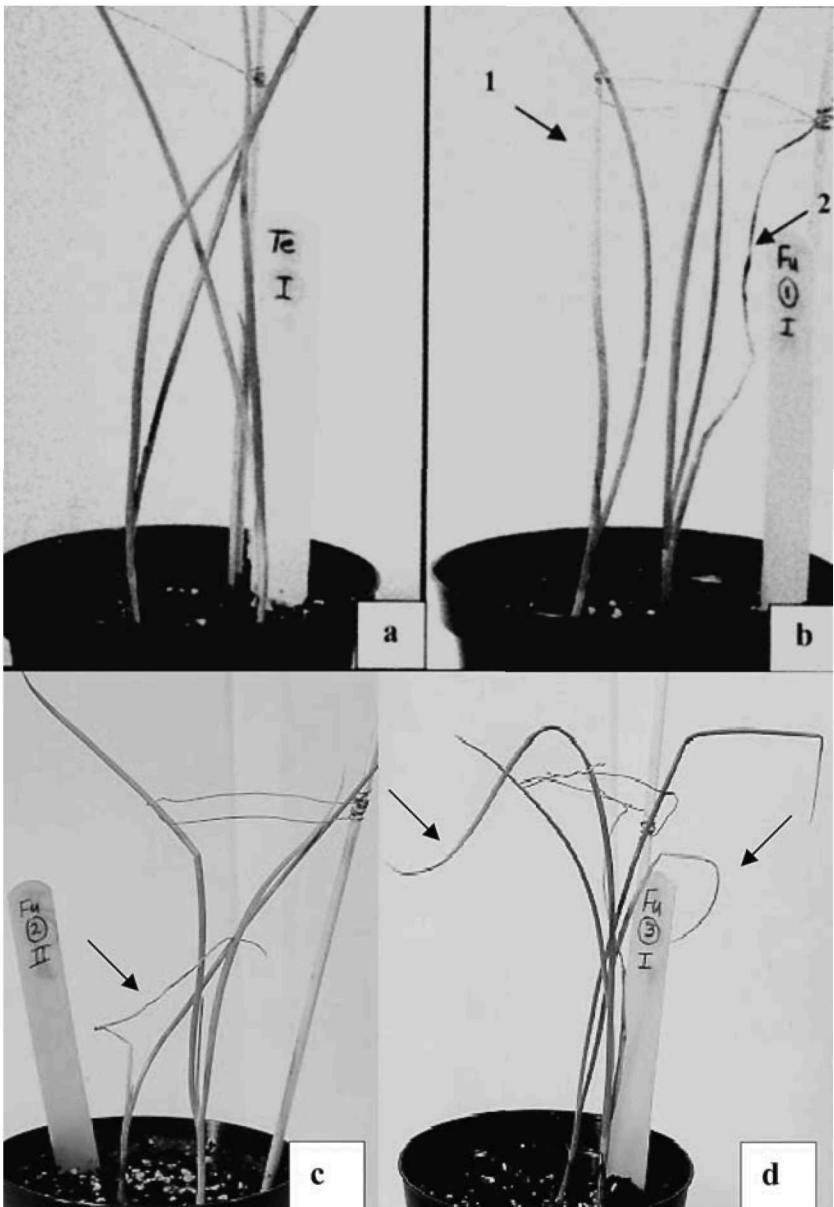


FIGURE 3. Tip blight (→) observed on onion foliage of cv. Nikita six days after inoculation with *Fusarium* spp. a) Non-inoculated plants (controls); and b) inoculated with *Fusarium acuminatum* (1) chlorosis and (2) necrosis; c) *F. oxysporum*; and d) *F. equiseti*.

natum, produced severe symptoms three days after inoculation. The other two species of *Fusarium* evaluated, *F. equiseti* and *F. oxysporum*, caused severe symptoms six days after inoculation. *Fusarium equiseti* produced lesions on older leaves with profuse mycelial growth. This species was the only one affecting cv. Excalibur and has not been reported causing disease symptoms in onion. In Canada and Sudan, *F. oxysporum* f. sp. *cepae* has been reported affecting onion roots and stems (Yassin et al., 1982; Thornton and Mohan, 1996). *Fusarium acuminatum* caused crown bud rot of alfalfa but has not been associated with onion tissues (Hawn, 1959).

All fungal isolates that caused symptoms on foliage during the two assays were reisolated on acidified PDA medium with the exception of *F. acuminatum*. Many pathogens are pure colonizers of culture media thus making isolation difficult. *Fusarium acuminatum* pathogenicity on plants of cv. Nikita requires further investigation. Neither *Curvularia* sp., *Bipolaris* sp., *Cladosporium* sp., *Colletotrichum gloeosporioides* nor *Alternaria* sp. (isolate no. 3) caused lesions on onion foliage.

Onion cv. Nikita was more susceptible to the fungal isolates evaluated than cv. Excalibur, which was susceptible only to *F. equiseti* and *Stemphylium* spp. Wounded tissues were more susceptible to fungi than non-wounded tissues. Plants of cultivar Nikita showed symptoms on foliage without wounds when inoculated with *F. oxysporum* and *A. alternata*.

Onion cultivar, plant age and mechanical damage are important factors that must be considered during pathogenicity studies. In the field, herbivores such as insects are crucial in the development of diseases and as vectors of important pathogens (McKenzie et al., 1993; Dillard et al., 1998; Gitaitis et al., 2003).

Young roots and bulbs in vitro: Direct inoculation in vitro of young bulbs and roots was an effective method for evaluating the pathogenicity of a diverse group of fungi. Symptoms were observed in young onion bulbs and roots of cultivar Nikita inoculated with *Phoma* sp. (isolate no. 3) and *P. sorghina*, five and nine days after inoculation. Control treatments and those inoculated with *C. gloeosporioides* did not show any symptoms.

Phoma sp. (isolate no. 1) caused lesions in mature bulbs, but did not cause lesions in young bulbs. *Phoma sorghina* caused a gray lesion in young bulbs that extended 1.5×1.2 cm from the inoculation point and into the first layers of the bulb. A pink coloration was also observed at inoculation sites in the roots (Figure 4A, B). This coloration was also observed with *Phoma* sp. (isolate no. 3). *Phoma sorghina* and *Phoma* sp. (isolate no. 3) were reisolated from roots and young bulbs in APDA and

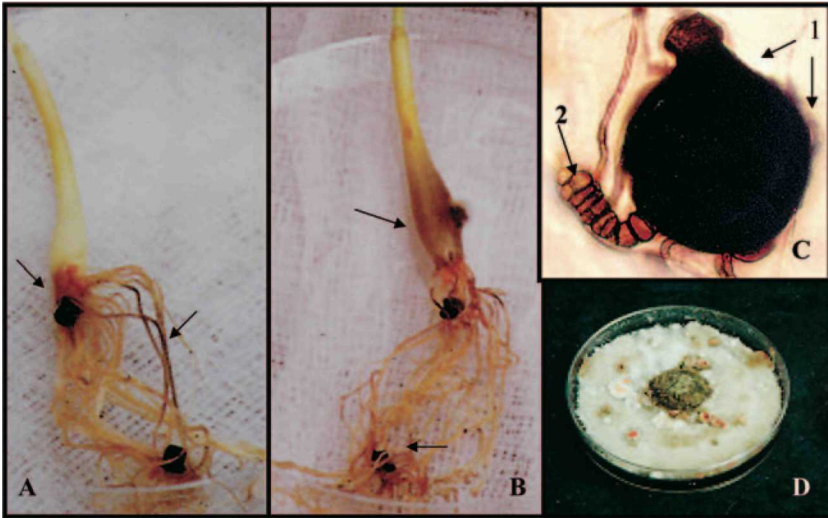


FIGURE 4. Symptoms observed on young bulbs and roots of onion cv. Nikita, nine days after inoculation with *Phoma sorghina*: (A) Observed pink pigmentation; (B) necrosis of bulbs and roots; (C) *Phoma sorghina*: pycnidia (1) and dyctiochlamydospores (2) at 40X; (D) growth in acidified potato dextrose agar. Note aerial mycelium with characteristic salmon pink areas.

water agar, where pycnidia, conidia and dyctiochlamydospores were produced (Figure 4C). In culture, *P. sorghina* produced aerial mycelium with characteristic salmon pink areas (Figure 4D). Pink to purple coloration observed in plant tissues caused by *Phoma* sp. (isolate no. 3) and *Phoma sorghina* was similar to that reported for *P. terrestris* by Hansen (1929). *Phoma terrestris* was included in the annotated list published by Minter et al. (2001) occurring in onion bulbs and roots in Puerto Rico but was not found in this study. This is the first report of *P. sorghina* affecting onion bulbs, even though this species is reported causing foliar lesions and affecting seeds of weeds, rice and sorghum (Frederiksen, 1986; Prabhu and Bedendo, 1988; Venkatasubbaiah et al., 1992).

Inoculum applied directly into soil close to the roots: Roots and bulbs treated with fungal inoculum applied directly into the soil did not show symptoms 21 days after inoculation under greenhouse conditions. Even though this technique has proven to be successful in Nigeria when inoculating *Glomerella cingulata* (teleomorph of *C. gloeosporioides*) in onions (Ebenebe, 1980), it was not effective under our experimental conditions. Apparently, fungal isolates tested did not adapt to changes in RH (56 to 103%) and temperatures (21 to 40° C) prevailing in the greenhouse during the experiments.

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