Identification of *Alternaria* spp. as Pathogenic on the Native Species *Terminalia australis* and *Salvia guaranitica*

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ABSTRACT

This work is the result of activities included in the cooperative project between Asociación Ribera Norte (ARN) and the Department of Plant Pathology, School of Agronomy, University of Buenos Aires. ARN is a non-governmental organization created in 1993 mainly for the management of the *Reserva Ecológica Municipal Ribera Norte*, a preservation area located in San Isidro (North of Buenos Aires), within the *Provincia Paranaense* region. The aims were to identify diseases in native plant species growing there. Light brown leaf spots with thin brown margins and narrow yellow halos were observed on *Terminalia australis* (mean disease severity: 25%). The pathogen was isolated, inoculated on healthy plants, reisolated from infected leaves and identified as *Alternaria tenuissima*. Similarly, another leaf-spot disease was observed on *Salvia guaranitica*, characterized by reddish brown large spots developed from the apexes and margins towards the centre of the leaves, reaching the veins in some cases (mean severity: 20%). Pathogen isolation, inoculation and reisolation led to the identification of *A. alternata* as the causal agent of the disease. Unusual heavy rain is analyzed as disease conductive.

Keywords: *Alternaria tenuissima*; *Terminalia australis*; *A. alternata*; *Salvia guaranitica*; Diagnosis

1. Introduction

*Terminalia australis* Cambess., belonging to Combretaceae, is known as “*palo amarillo*” or “*tanimbú*”. It is a tree or shrub with grayish red cortex [1] and deciduous elliptical leaves, native to the Northeast of Argentina [2], and restricted in range to the more northerly provinces [3]. It is traditionally used to obtain wood [4] that is yellow, compact, elastic, with remarkable durability [5] because of the astringent properties of boiled leaves [6]. This species is also used as an antiseptic agent, as it was shown to possess antifungal properties against species of *Aspergillus* and *Candida* [7,8].

*Salvia guaranitica* A. St.-Hil. ex Benth. is a rhizomatous species belonging to the Lamiaceae [2], usually referred to as “*salvia azul*” [9]. It is a semi-woody perennial shrub that exhibits a bushy, somewhat open habit with upright, branching, square, dark green stems. The leaves are dark green, ovate, wrinkled, pointed and lightly-toothed. This species blooms from mid-summer to fall, producing long terminal spikes with bilabiate, tubular, deep blue flowers with purple-blue calyxes. It is an ornamental and medicinal plant originally from South Brazil, Paraguay, Uruguay and Argentina [10], traditionally used in Latin America as sedative [11]. Sedative and hypnotic properties of the ethanolic extracts [12] have been associated with the presence of high concentrations of the flavonoid cirsimil and caffeic acid ethyl ester [13]. Aerial parts also contain essential oils [9,14]. Extracts from this species are innocuous to honey bees [15].

*Salvia guaranitica* has ornamental value [16] that has led to the evaluation of procedures for its propagation and domestication [17]. *T. australis* is also considered to have ornamental characteristics [18]. *T. australis* has been recorded in the provinces of Buenos Aires, Corrientes, Entre Rios y Misiones; and *S. guaranitica* in Buenos Aires, Corrientes, Entre Rios, Misiones, Salta and Tucumán [19]. Although there is scarce information on conservation status of the flora in Argentina, both species have been found to be included in a list of endangered trees and shrubs in the province of Entre Rios (Northeast of Argentina) [20]. These species were originated in the ecological region called *Provincia Paranaense* [21].
which is a riverside forest located at the margins of Paraná River, later included in the Provincia Pampeana region [22]. That environment has become threatened because of the shift of natural areas to intensive agricultural use, urbanization and dam construction. As a consequence, habitat fragmentation may determine extreme vulnerability of surviving species [23].

Asociación Ribera Norte (ARN) is a non-governmental organization created in 1993 mainly for the management of the Reserva Ecológica Municipal Ribera Norte, a preservation area located in San Isidro (North of Buenos Aires), within the Provincia Paranaense region. The conservation of riverside environments is in the scope of the association. Since 1997, native plants are grown in an orchard created there, and used to restore natural environments and to provide specimens to be planted in urban areas. More than 190 riverside native species, including endemic and widely distributed ones, are agroecologically cultivated [24] nowadays. Plants become diseased when the ability of the cells or a plant part to carry out one or more essential functions is interfered by a pathogenic organism or an adverse environmental factor [25]. Pathogens are associated with virtually all plant species, from a diverse array of habitats. Although we know the most about diseases of economically important plants, insufficient research has been focused on the host-pathogen interactions in natural or unmanaged systems outside of the crop field or forestry plantation [26]. So as to learn more about diseases of native plants in the area, a cooperative project was signed between ARN and the Department of Plant Pathology, School of Agronomy, University of Buenos Aires, in 2010. The aims of this work were to identify diseases in plants growing there.

2. Materials and Methods

2.1. Disease Observation, Description of Symptoms and Isolation of Microorganisms

Monthly disease surveys began in January 2010. Samples of diseased leaves were randomly taken from the symptomatic plants, and disease severity was estimated. To determine the causal agents of the described symptoms, small pieces of diseased tissues were surface disinfected by immersion in 2% (v/v) of Cl as NaOCl during 1 minute, plated on PDA (pH 7), incubated at 22˚C and examined for pathogen development [27,28]. Fungal colonies that emerged from incubated diseased leaf portions were isolated and identified to genus.

2.2. Pathogenicity Tests and Identification of Pathogens

Suspensions of $1 \times 10^6$ conidia ml$^{-1}$ were prepared for each isolate. Groups of 10 healthy plants of $T. australis$ and $S. guaranitica$ and other 10 healthy plants with needle-punctured leaves were separately sprayed with each inoculum suspension. Undamaged and needle-punctured control plants were sprayed with sterilized distilled water. All the plants were individually covered with polyethylene bags and kept in a climatic chamber under fluorescent light (12-hr photoperiod). The bags were removed after 72 hr. The plants were observed regularly so as to detect and describe the evolution of symptoms. The inoculated fungi were re-isolated from the leaves that developed symptoms, and the pathogens were identified to species so as to fulfill Koch’s postulates. For fungal identifications, the isolates were cultivated on potato carrot agar (PCA) at 22˚C during 7 days, so as to observe their sporulation patterns under a dissecting microscope [29] and on malt extract agar (MA) for the observation and measurement of conidia [30,31].

3. Results

3.1. Disease Observation, Description of Symptoms and Isolation of Microorganisms

Leaf spots were observed on different species during surveys carried out at the end of March 2010. Their characteristics are described below.

*Terminalia australis*: Pale green to chlorotic large areas appeared on the margins of the leaves, and often developed towards the main vein forming wedge shaped lesions. The spots were finally light brown with thin brown margins and narrow yellow halos. Leaf roll or distortion was observed in the spotted areas (Figure 1). The lesions covered up to 50% of the leaves. Mean severity was estimated at 25%.

*Salvia guaranitica*: Reddish brown large spots developed from the apexes and margins towards the centre of the leaves, reaching the veins in some cases. Smaller, internerval, ovoid to irregular spots of the same color.
appeared scattered on young leaves. They measured 2.83 \times 5.30 \text{ mm}, mostly parallel to secondary veins (Figure 2). Incidence was 50\%, with a mean severity of 20\%.

The isolates obtained from the two diseased species produced chains of ovoid or obclavate, beaked, brown conidia, that were multicelled, with transverse and longitudinal septa, originated from free conidiophores, and were identified as belonging to the genus *Alternaria* Nees [32,33].

### 3.2. Pathogenicity Tests and Identification of Pathogens

Final symptoms were in both cases coincident with the ones observed during the surveys. Symptoms on undamaged leaves appeared 14 days after those on punctured leaves. Controls remained symptomless. The inoculated isolates were recovered from diseased leaves, thus confirming their pathogenicity.

*Terminalia australis*: Marginal lesions and also tiny spots appeared on the leaves 20 days from inoculation (Figure 3). Most of the foliage showed necrosis and loss of turgency within two weeks. Severely affected leaves dropped-off in most cases. The isolate had olive-buff hyphae, showed a pattern of sporulation typical of Group 1 [29] and produced single conidial chains of short to moderate length during the first week of growth on PCA (Figure 4). Air mycelium was loose, pale gray in the center of the plates on MA. Conidia measured 12.5 - 37.5 (24.0 \mu m) \times 7.5 - 12.5 (10.0 \mu m), with 0 - 3 septa (mainly longitudinal) and a beak of 0 - 12.5 (4 \mu m). These observations are coincident with the descriptions for *Alternaria tenuissima* (Kunze) Wiltshire [30]. A specimen of the fungal culture was deposited in the fungal collection of the Department of Plant Pathology, School of Agronomy, University of Buenos Aires.

*Salvia guaranitica*: Spots of 1-mm diameter developed on the leaves 14 days after inoculation (Figure 5). Colonies were olive brown to black. The isolate had a pattern of sporulation that belonged to Group 4 [29], with solitary branching chains that turned into low bushy groups.

Figure 2. Large marginal spots on leaves of *S. guaranitica*.

Figure 3. Initial symptoms on *T. australis*.

Figure 4. Sporulation pattern of *Alternaria tenuissima* isolated from *T. australis*.

Figure 5. Initial symptoms on *S. guaranitica*.
of branched chains of spores (Figure 6). Conidia measured 17.0 - 40.0 (25.0 µm) × 5.0 - 17.0 (11.0 µm), with 0 - 4 septa (mainly longitudinal) and a beak of 0 - 7.5 (2.0 µm). The morphological characteristics of the conidia and conidiophores fit the description of *A. alternata* (Fr.) Keissl. [31]. A specimen of the fungal culture was deposited in the fungal collection of the Department of Plant Pathology, School of Agronomy, University of Buenos Aires.

4. Discussion

The pathogenic species identified in this work show cosmopolitan distribution. A total of 336 fungus-host combinations, and 568 records of *A. tenuissima* and its synonyms can be found in the literature. Similarly, there are 914 fungus-host combinations, and 1496 records for *A. alternata* around the world [34]. No citations have been found on *T. australis* and *S. guaranitica*; only *A. alternata* on *S. nemorosa* in Poland [35, cited by 34]. As a result of this work, *A. tenuissima* and *A. alternata* are reported on *T. australis* and *S. guaranitica*, respectively, as causal agents of leaf spots. To our knowledge, it is the first report of the pathogens on these species, and might be the first pathogens identified on them.

The period of incubation differed between the two diseases; 20 and 14 days for the pathosystems *T. australis-A. tenuissima* and *S. guaranitica-A. alternata*, respectively. Wounds accelerated symptom appearance by two weeks for both pathologies. Local wounds are known to increase infection by all *Alternaria* pathogens [36]. However, foliage injury cannot be considered a prerequisite for fungal penetration for these diseases. In fact, both pathogens succeeded to infect unwounded leaves, although with extended incubation period when compared with the infection on punctured foliage.

It is generally accepted that vigorously growing plants are more resistant to infection by *Alternaria* pathogens than poorly developing ones, and that soil moisture is a major factor affecting the conditions of growth [36]. Both diseases identified in this work were observed after unusual rainy conditions that occurred in February 2010, when accumulated rain reached 419.6 mm. Consequently, soil moisture should be considered as conductive for disease development in these cases. In addition, rain may have facilitated spore germination and penetration by supplying free moisture on leaves or by increasing relative humidity [37]. *Alternaria* spores are dispersed by wind currents and are eventually deposited on the surface of susceptible tissues [38]. The germination of spores under high humidity conditions may take only few hours [39].

Our results constitute the first step in the management of plant diseases, that is the identification of their etiology and the estimation of damages. Infectious disease is a major causal factor in the demography of human, plant and animal populations [40]. An intensive work has to be done in order to identify the causal agents of new diseases on the studied hosts or other ones, and to understand the conducive factors, so as to evaluate tools to preserve native species.

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