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First Report of Rose Bent Neck Caused by *Sclerotinia sclerotiorum* on Commercial Cut Roses (*Rosa hybrida* L.)

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Abstract: Cut roses are highly valuable ornamentals and their profitability depends on the flower postharvest performance. Bent neck symptoms in roses make them unmarketable and are typically related to physiological disorders, bacteria accumulation in the vase solution, and *Botrytis cinerea* infection. Unusual bent neck symptoms were observed in 4.7% of 'Orange Crush' roses from two commercial shipments, resulting in complete flower collapse. This research was aimed to determine the causal agent of the bent neck symptoms. Following incubation in a humid chamber, symptomatic roses evolved in water-soaked lesions with the presence of white mycelium and sclerotia development. Fungal isolations and molecular characterization were performed and *Sclerotinia sclerotiorum* was identified as the causal agent of rose bent neck. Therefore, when bent neck symptoms are observed, *S. sclerotiorum* incidence should be considered to avoid possible outbreaks.

Keywords: floriculture; pathogenicity; vase life; disease management

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1. Introduction

Roses are perennial, woody shrubs that have been domesticated for over 500 years. They are a highly valuable ornamental crop with tremendous economic, social, and cultural impact [1]. The wholesale value of cut roses in the U.S. exceeded USD 19 million for 29 million stems sold in 2019 [2]. Vase life duration is one of the main parameters used by customers to measure cut rose quality. Vase life reduction or termination for cut roses is often related to water stress symptoms, oxidative stress damage [3], *Botrytis cinerea* infection, rapid senescence associated with ethylene responses [4], and bent neck symptoms connected with water stress and different microorganisms [5]. Bent neck refers to the collapse of the stem tissue at the base of the receptacle. Several biotic and abiotic factors can contribute to the development of bent neck, including insufficient lignification when flowers are harvested before maturity [6], malfunctioning stomata on flowers grown in high relative humidity conditions [7], differential stomatal sensitivity to water signals [8], bacteria accumulation occluding the vascular system [9], and *B. cinerea* infections at the base of the pedicel [4].

White mold, soft rot, blossom blight, and over 60 disease symptoms have been related to *Sclerotinia sclerotiorum* infection in several crops [10]. *S. sclerotiourum* is a cosmopolitan fungus with a broad host range that comprises over 600 plant species worldwide including fruit, vegetable, tree, oil, fiber, and ornamental crops [11], and can infect virtually all plant organs [12]. *S. sclerotiorum* can persist and survive in the soil for a prolonged time in the form of sclerotia [13]. The sclerotia can produce mycelia (myceliogenic germination, asexual) or ascospores (carpogenic germination, self-fertilized sexual reproduction) [14] and both can serve as inoculum sources for disease development [15]. In 2020, *S. sclerotiourum* was described as a newly emergent pathogen for garden roses (*Rosa chinensis*) in Bangladesh [16]; the reported symptoms were described as flower and blossom blight. In the fall of 2020, bent neck symptoms were observed during the postharvest of 'Orange Crush' roses from a farm located in Colombia. Incubation under high-humidity conditions revealed petal

discoloration and the presence of white mycelia and sclerotia in the surface and at the interior of the flowers without sporulation. The objective of this research was to determine the causal agent of the bent neck symptoms observed in roses.

2. Materials and Methods

Initial assessment: Two commercial shipments of 80 'Orange Crush' roses were received at Clemson University from a commercial farm located in Colombia, South America (4°59′16.9″ N, 73°59′36″ W; 2650 MASL). The roses were produced in hydroponic systems using rice husks as substrate and were cultivated under plastic greenhouse conditions without a fully controlled environment. The roses received standard postharvest procedures that included quick stem dip after harvest in sodium hypochlorite, classification by quality and size, leaf and thorn removal from the lower third of the stem, bouquet formation and wrapping in clear polypropylene, rehydration in a commercial solution (FloraLife®, Kent, OH, USA) for 2 h, and packing in cardboard boxes. The flowers received forced air cooling until internal temperature of the box was 2 °C. Then, the flowers were shipped via refrigerated airfare to Miami airport and then ground transported to their final destination. The roses arrived six days after their harvest. Upon arrival, an average of 4.7% of the roses showed bent neck symptoms. Symptomatic and asymptomatic roses were placed in a humid chamber at 100% relative humidity, after removing 5 cm from the base of the stem to avoid potential air embolisms. Inside the humid chamber, 3 cm of the base of the stems were kept in water to avoid dehydration. The conditions in the humid chambers were 22 °C and a 12 h photoperiod. Symptom development was evaluated for 7 days.

Fungal isolation: Five petal and five receptacle tissues were collected from symptomatic and asymptomatic roses, respectively. Collected tissue was surface-sterilized by immersion for 1 min in sodium hypochlorite solution (0.525%) followed by 1 min immersion in sterile deionized water and air-dried for 5 min. Tissue pieces of approximately 5 mm^2 were cut with a sterile scalpel, placed in Petri dishes containing potato dextrose agar (PDA) medium (Difco Laboratories, Sparks, MD, USA), and sealed with parafilm. Morphological characteristics of the fungal colonies were assessed after 7 days of growth at 22 °C and 12 h darkness/12 h light intervals.

Molecular identification: Isolates for DNA extraction were cultured in PDA plates with cellophane paper (Research Products International Corp., Mount Prospect, IL, USA) covering about 50% of the media surface. Then, genomic DNA was extracted [17] and PCR amplification was performed using the ribosomal internal transcribed regions ITS1 and ITS4 [18] and the elongation factor 1-alpha region EF1-728F and EF1-986R [19], and then purified and Sanger sequenced. Sequences were assembled and edited using Geneious Prime (Dotmatics, version 2023.0.4). Consensus and reference sequences were saved as FASTA files. A set of ten publicly available *S. sclerotiorum* sequences from different locations (accession numbers: JQ618848, MN105884, JX648201, JF277567, MT378215, MT378216, LC318706, LC318707, KP340898, JN013184), together with two *S. minor* (accession numbers: AB516662, AY195574), two *S. trifolium* (accession numbers: EU0824364, AY187068), two *S. nivalis* (JN415129, MW692801), one *S. glacialis* (accession number: Z99669), one *S. tetraspora* (accession number: Z99671), and one *Botrytis cinerea* (accession number: KF859918, used as outgroup) sequences were downloaded from GenBank and were used to confirm identity and evaluate phylogenetic relationships with other *Sclerotinia* isolates. Sequences were MUSCLE aligned [20] and a phylogeny tree was built using the Geneious Tree Builder function (Geneious Prime, Dotmatics, version 2023.0.4). A maximum likelihood tree was created using the RAxML 8.2.11 function. Branching confidence was evaluated using 1000 bootstrap replicates.

Pathogenicity assays: Two assays were performed to evaluate pathogenicity. During the first assay, commercially harvested 'Orange Crush' cut roses were inoculated by placing one mycelial plug of 8 mm diameter (n = 5 per inoculated tissue) or one piece of sclerotia (~2 mm) from pure culture (n = 5 per inoculated tissue) between petals in the flower head or on the receptacle after puncturing it with a scalpel and covering it with parafilm. Controls

were inoculated with a PDA plug of 8 mm diameter or wounded without inserting the sclerotia. Inoculated flowers were placed in a humid chamber with the base of the stems submerged in water, and evaluation of symptom development was performed 5, 7, and 9 days after inoculation (DAI); the experiment was repeated twice. During the second assay, one-year-old garden rose plants from cultivars 'At Last®' and 'Oso Easy Double Red®' were inoculated with sclerotia pieces in the flower head (n = 5 plants) and receptacle (n = 5 plants) as described for the first experiment. Garden roses were used as a proof-of-concept to determine that the symptoms will develop in whole plants, when possible water stress conditions are removed from the equation as a possible cause for bent neck symptoms. After inoculation, the flowers were covered with sealable quart plastic bags with a moistened paper tissue to provide humidity. Control plants included flowers covered with sealed bags and moistened paper tissue with and without wounded receptacles (n = 5 plants wounded, n = 5 non-wounded receptacles). Evaluation of symptom development was performed at 5, 7, and 9 DAI; the experiment was repeated twice. Symptomatic tissue from both experiments was collected, surface-sterilized, placed in PDA as described above, and morphological observations were performed after 7 days.

3. Results

Initial assessment: After five days of incubation in a humid chamber, the symptomatic roses exhibited discolored, water-soaked lesions with the presence of small lumps (2–8 mm diameter) of white mycelia. After 7 days of incubation, the receptacles and interior of the flowers presented bleached, watery, rotten tissue (Figure 1a) with more abundant mycelial lumps and presence of black and hard sclerotia at the interior of the flower head (Figure 1b), ranging from 5 to 13 sclerotia per flower.

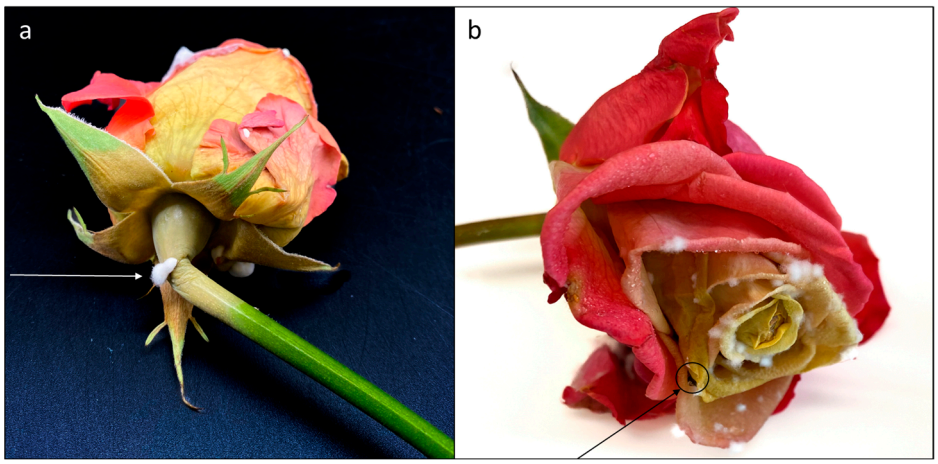

Figure 1. Symptom development for roses exhibiting initial bent neck: (**a**) bleached sepals and receptacle; the arrow indicates the place where the bent neck occurred with the mycelial lumps over the decayed tissue; (**b**) symptom development in the interior of the flower, the arrow points to sclerotia formation.

Fungal isolation: The colonies from symptomatic tissue displayed a thick, white, cottony, loose mycelium, with conspicuous black sclerotia formation (1.5 to 4.7 mm in diameter) that appeared predominantly at the periphery of the dishes. Sclerotia were semi-rounded to irregularly shaped. The number of sclerotia present after 10 days varied between 9 to 35. No teleomorph was observed, and no colonies emerged from non-symptomatic tissue. Based on the morphological observations, we tentatively identified the causal agent as *Sclerotinia sclerotiorum* [21].

Molecular identification: The sequence from the ITS1/ITS4 region (accession no. OM810147) showed 100% identity (510/510 bp) with *S. sclerotiorum* (MN105884.1) while the sequence from the EF1-728F/EF1-986R region (accession no. OM802503) was 99% identical (329/330 bp) to *S. sclerotiorum* (AF040088.1). The phylogenetic analysis of the bent neck

isolate from roses and the other *Sclerotinia* isolates from GeneBank showed the closest relationship to other *S. sclerotiorum* isolates, with the isolate from Florida, USA (MT378215.1) being the most closely related (Figure 2). Based on molecular and morphological characteristics, we identified the causal agent of the rose bent neck as *S. sclerotiorum.*

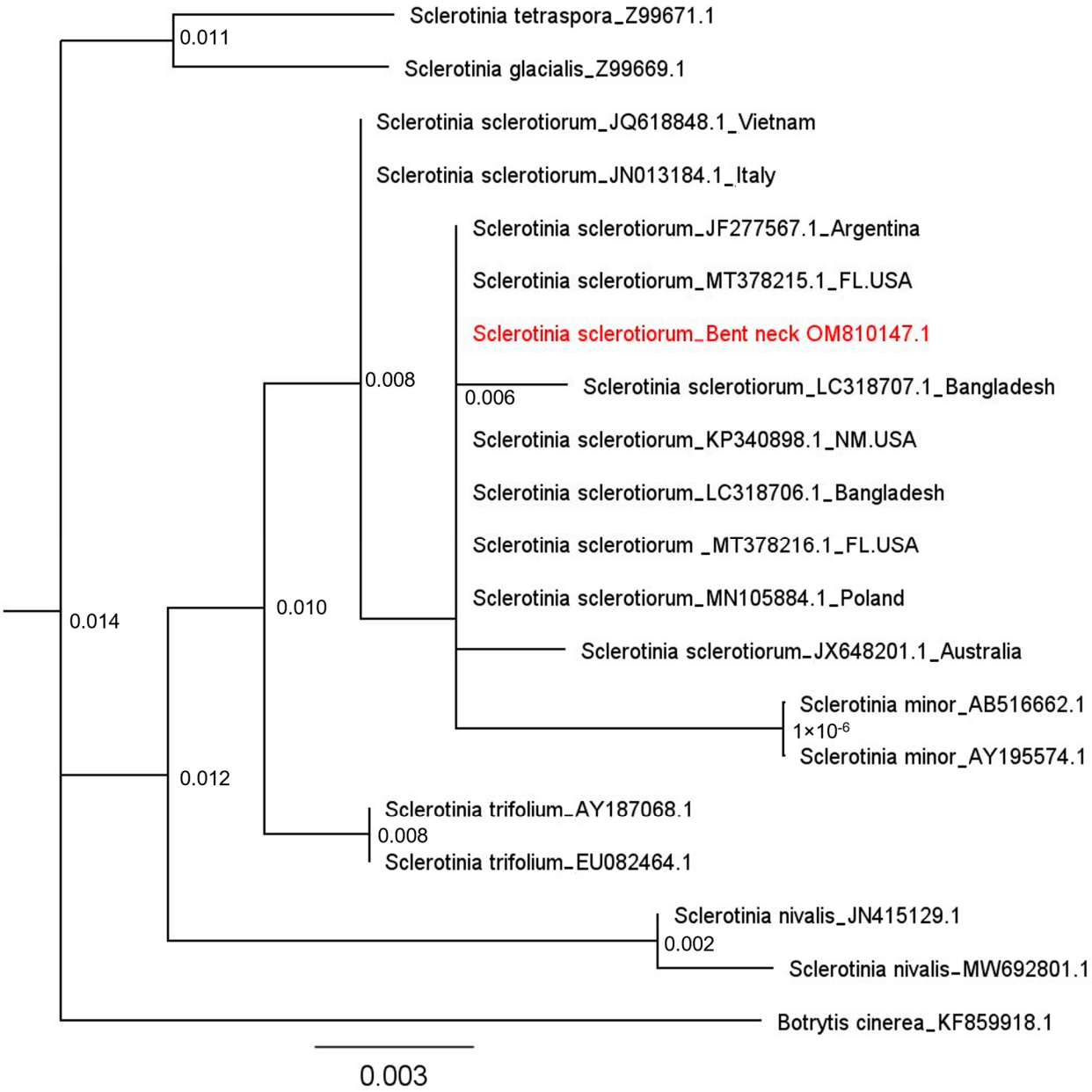

Figure 2. The maximum likelihood tree shows phylogenetic relationships between the bent neck isolate OM810147, for which sequence is highlighted in red, and other *Sclerotinia* sequences obtained from GeneBank. The place of origin for the *S. sclerotiorum* isolates is shown after the accession number. Bootstrapping of 1000 replicates was considered for assessment of branching pattern. Analysis was performed in Geneious Prime (Dotmatics, version 2023.0.4).

Pathogenicity assays: Inoculated petals initially displayed petal discoloration, followed by water-soaked lesions that progressively developed into lumps (2–12 mm diameter) of white mycelium. At the interior of the flower, sclerotia formation developed and ranged from 8 to 19 sclerotia per flower head. When the infection started at the receptacle, necrotic water-soaked lesions appeared 5 DAI at the receptacle and enlarged towards the sepals. As the infection progressed, the petals became bleached and water soaked, with

the appearance of white mycelia over the affected areas. At 9 DAI, the flowers presented sclerotia formation at the receptacle and interior of the flower head. At the end of the evaluation, 60% of the inoculated roses showed completely collapsed flower heads, i.e., bent neck. No difference in symptom development or severity was observed between the flowers inoculated with agar plugs and those inoculated with sclerotia pieces. Symptom development was similar to that the observed during the first experiment in both petals and receptacle for both cultivars. 'At Last' roses also exhibited flower collapse and flower petal shattering, which exposed necrotized stamens. The fallen petals were necrotized and water-soaked. No symptoms were observed in the control flowers. The resulting colonies were morphologically identical to the original isolates.

4. Discussion

In recent years, climatic changes, including increasing rain in tropical regions and variable winter conditions in subtropical regions, have led to an increased incidence of *S. sclerotiorum* outbreaks [22]. *S. sclerotiorum* can cause severe economic losses by pre- and post-harvest damage to many crops [23]. To our knowledge, this is the first report of *S. sclerotiorum* causing bent neck symptoms in roses, the first report of *S. sclerotiorum* in cut rose production, and the first report in roses from Colombia. The main reason behind bent neck symptoms in cut roses is adverse water conditions. However, other causes for bent neck are associated with bacteria plugging the vascular system, *B. cinerea* infection, and physiological disorders related to tissue maturity. During this research, we found that *S. sclerotiorum* infection can also cause bent neck symptoms in roses, which has not been reported before. Ignoring the presence of this pathogen in the crop could lead to the underestimation of possible outbreaks and possible unsuccessful management practices.

In the majority of crops, *S. sclerotiorum* management relies on the application of fungicide; however, the efficacy of those applications can be impeded by the development of fungicide resistance, which is reported for different chemical classes [24,25]. This is especially important considering the high number of fungicide applications that occur in cut rose production, which has led to the development of fungicide resistance in other fungi such as *B. cinerea* [26]. Future research evaluating the most promising practices, including cultural, biological, and chemical strategies to avoid and manage potential disease outbreaks, is necessary to create pertinent management programs. Currently, the disease is rare and during this evaluation was limited to a small percentage (4.7%) of the commercial roses; however, monitoring its incidence and severity over time will help prevent its potential threatening spread.

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