

# Disease Notes

## Diseases Caused by Fungi and Fungus-Like Organisms

### First Report of *Stemphylium* Leaf Spot Caused by *Stemphylium vesicarium* on Alfalfa (*Medicago sativa*) in Peru

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Alfalfa (*Medicago sativa*) is the most cultivated fodder crop in Peru with 172,000 ha cultivated (MINAM 2019), and Arequipa is the top producing region with 40% of the national production in 2015 (Santamaría et al. 2016). In January to April 2019 (average 20°C and 70% RH), most alfalfa fields in Majes-Pedregal, Arequipa, were affected by an unidentified foliar disease. One of the fields was located at the farm of the Universidad Nacional de San Agustín de Arequipa (16°19'29.6" S, 72°12'59.9" W). Symptoms appeared as elliptical light brown spots with dark brown borders. The field (~60 × 60 m) was divided into ~30 × 12-m sections, and two plants in each section were collected (20 plants total). Plants were digitized, and the leaflet diseased area was calculated with ImageJ 1.53a, from which an incidence of 100% and a severity of 38.7 ± 4.4% were estimated. Microscopical observations of the leaflet spots consistently revealed the presence of oblong multiseptated conidia (23.6 to 42.8 × 16.5 to 25.2 µm; average 33.3 × 20.9 µm; n = 40) of the genus *Stemphylium* (Simmons 1969; Woudenberg et al. 2017). We obtained 10 pure cultures by placing conidia from the spots directly onto potato dextrose agar medium with the aid of stereoscope and sterile forceps. Two isolates (UNSA-StemV01 and UNSA-StemV02) were incubated further until ascospore production at room temperature with no special light stimulus. After 45 days of growth, globose pseudothecia and ellipsoidal ascospores (25.4 to 38.7 × 11.2 to 16.6 µm; average 31.9 × 13.7 µm; n = 30) formation occurred. We extracted the DNA from these two isolates using a Wizard

Purification Kit (Promega Corp., Madison, WI) and sequenced the internal transcribed spacer 1 and 2 intervening the 5.8S rDNA subunit (GenBank accessions MT371236 to 37), and the glyceraldehyde-3-phosphate dehydrogenase (MT375513 to 14) and the calmodulin (MT375515 to 16) genes, highly resolute markers to identify *Stemphylium* species, following Woudenberg et al. (2017). We retrieved sequence data available from 43 isolates of nine *Stemphylium* species (Han et al. 2019; Woudenberg et al. 2017) and built a midpoint-rooted phylogeny with the three-loci concatenated data set. We identified our isolates as *Stemphylium vesicarium*. Koch's postulates were fulfilled by spray inoculation with conidia from isolate UNSA-StemV01 suspended in sterile water (1 × 10<sup>4</sup>/ml) to two healthy 50-day-old alfalfa plants growing on pots in the university greenhouse (average 25°C and 70% RH). Two plants sprayed with sterile water without conidia served as a control. Symptoms appeared after 21 days of inoculation, and when conidia were reisolated, they were the same as originally obtained. No symptoms developed in the control plants. This confirmed that *S. vesicarium* is the causal agent of the alfalfa disease in Majes-Pedregal, identified as *Stemphylium* leaf spot. Previous studies documented *S. vesicarium* on asparagus and onion in Peru (Castillo Valiente 2018; Vásquez Salas 2018; Vásquez Sangay 2013), but molecular characterization has only been applied to *S. lycopersici* from potatoes (Woudenberg et al. 2017). *S. vesicarium* has been documented in various crops, including alfalfa, and countries in Europe, North America, Africa, and Asia, and in Australia and New Zealand (Han et al. 2019; Woudenberg et al. 2017). This occurrence is the first report of *S. vesicarium* on alfalfa in Peru. The disease compromises the quality of this fodder crop, so actions need to be taken in Arequipa.

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