Leaf Spot of *Centrosema* Species Caused by *Cylindrocladium colhounii*

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ABSTRACT

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A previously undescribed leaf spot of *Centrosema* spp. in Colombia and Costa Rica was found to be caused by *Cylindrocladium colhounii*. Symptoms included irregular, zonate, brown leaf spots, 10–30 mm in diameter; chlorosis; and defoliation. Pastures of *Centrosema acutifolium* cv. Vichada in the eastern plains of Colombia were moderately to severely defoliated under humid conditions. The host range of the pathogen was restricted to *C. acutifolium, C. arenarium, C. brasilianum, C. macrocarpum*, and *C. pubescens*. Usable resistance was identified in accessions of *C. acutifolium* from Minas Gerais and Goias, Brazil, as well as in other *Centrosema* spp.

The genus *Centrosema*, native to the Americas, comprises about 35 species of mostly tropical, viny, perennial legumes (2). Its natural adaptation ranges from high-rainfall, humid forests to dry scrub and coastal sand dunes. Species with proven or potential value as tropical pasture legumes include *Centrosema acutifolium* Benth., *C. brasilianum* (L.) Benth., *C. macrocarpum* Benth., *C. pascuorum* Benth., *C. pubescens* Benth., and *C. virginianum* (L.) Benth. (2,8,9). These species have been the source of several productive, high-quality, tropical pasture legume cultivars in Australia (2) and South America (4).

In 1982, during periodic evaluations in germ plasm collections and pastures of *Centrosema* spp. at the ICA-CIAT Research Station, Carimagua, in the eastern plains of Colombia, a previously undetected leaf spot was observed in plots and pastures of *C. acutifolium* and in plots of *C. pubescens*. Since its detection, the leaf spot has become increasingly damaging to *C. acutifolium* cv. Vichada, causing considerable defoliation under humid conditions. This article reports the identity of the causal fungus, its isolation and growth in artificial culture, its symptomatology, its host range, its distribution on *Centrosema* spp. in Central and South America, and sources of resistance within *Centrosema* spp.

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MATERIALS AND METHODS

Survey. From 1982 to 1988, *Centrosema* spp. were evaluated periodically for diseases in over 150 trials in the Tropical Pasture Evaluation Network (RIEPT) throughout the tropical Central and South American lowlands (5,6). These trials were located in Brazil, Colombia, Costa Rica, Ecuador, Mexico, Panama, Peru, and Venezuela. Particularly intensive evaluations were made at Planaltina, Brazil; Quilichao and Carimagua, Colombia; Atenas, Guapiles, and San Isidro, Costa Rica; and Pucallpa, Peru. Where available, *Centrosema*-based pastures were also evaluated. Information was gathered on the incidence and relative severity of diseases of *Centrosema* spp.

Isolation. Leaves of *C. acutifolium* cv. Vichada with typical zonate spots were collected in Carimagua, Colombia, in 1982. Pieces of leaf tissue (2–3 mm^2) were cut from the border of these spots, surface-sterilized with 1% NaOCl for 30 sec, washed three times in sterile distilled water, plated directly on 2% water agar, and incubated at 25–27 C. Plates were inspected at 24-hr intervals, and fungal colonies growing from tissues were transferred to potato-dextrose agar (PDA), oatmeal agar, and V-8-$CaCO_3$ agar and then incubated at 25–27 C.

Pathogenicity. Three types of pathogenicity trials were carried out in the glasshouse with fungal isolates obtained from *C. acutifolium* cv. Vichada. Plants for all pathogenicity tests were prepared in the same way. Seed was scarified with concentrated sulphuric acid for 3 min and germinated on filter paper in petri plates; the resultant seedlings were transplanted in 1-kg plastic pots at the rate of two plants per pot. The potting medium was an oxisol soil ammended with a complete fertilizer (50 kg/ha P_2O_5, 40 kg/ha KCl, 30 kg/ha MgO, 10 kg/ha sulphur). For the Koch's postulates trial, 10 1-kg pots of Vichada were used; eight were inoculated with two isolates and two were used as controls. While we developed an acceptable inoculation methodology, Koch's postulates were repeated at least four times. For the pathogenicity trials to determine the host range of and the reaction of accessions of *C. acutifolium* to the pathogen, six

pots of each entry were planted; four were inoculated and two were controls. Plants were grown for 8 wk under glasshouse conditions of 24–26 C and 12 hr/day of natural daylight. In all pathogenicity trials, the three leaflets of the five youngest, fully expanded, trifoliolate leaves on each plant were inoculated. The host range of the fungus was investigated by inoculation of *Centrosema* spp. (*C. acutifolium, C. arenarium, C. brasilianum, C. macrocarpum, C. plumieri, C. pubescens, C. schiedianum, C. tetragonolobum*, and *C. virginianum*) and other important tropical pasture legumes (*Arachis pintoi, Desmodium ovalifolium, Pueraria phaseoloides, Stylosanthes capitata*, and *S. guianensis*), and the reaction of 28 accessions of the *C. acutifolium* germ plasm collection to the isolate most pathogenic to Vichada was determined. These two experiments were repeated once.

Conidia were collected from 10-day-old PDA cultures grown at 25–27 C for 10 days, filtered through cheese cloth, and adjusted to 1×10^5 conidia per ml. Using a micropipette, a 20 μl droplet of conidial suspension was placed in the center of each leaflet and secured by placing a 7-mm-diameter filter paper disc over each droplet. Droplets of distilled water were placed on leaflets of control plants. Inoculated plants were incubated in a humidity chamber at 100% relative humidity and temperatures of 27–28 C for 48 hr and then removed to the glasshouse. Leaf spot development was evaluated 5 days later according to the scale: 0 = no leaf spots; 1.0 = leaf spots < 5 mm in diameter, no chlorosis; 2.0 = leaf spots 5–10 mm in diameter, no chlorosis; 3.0 = leaf spots 10–20 mm in diameter, slight to moderate chlorosis; 4.0 = leaf spots 20–30 mm in diameter, moderate to severe chlorosis; and 5.0 = leaf death.

RESULTS AND DISCUSSION

Survey. From 1982 to 1988, Cylindrocladium leaf spot (CylLS) was detected at several sites in Colombia and Costa Rica but was not detected at RIEPT trial sites in the tropical lowlands of other countries of Central and South America. In Colombia, CylLS was common and caused moderate to severe damage to accessions of *C. acutifolium* and *C. pubescens*; it was also detected on *C. arenarium, C. brasilianum*, and *C. macrocarpum* in trials in the eastern plains of Colombia. CylLS was common and moderately to severely damaging on accessions of *C. acutifolium, C. macrocarpum*, and *C. pubescens* in San Isidro, Costa Rica, and was detected on the same species at Atenas, Costa Rica. It was not recorded at other pasture evaluation sites in these two countries.

C. acutifolium cv. Vichada and other accessions of this form of the species have a very localized distribution in the eastern plains of Colombia, near the Venezuelan border (8). Detection of CylLS on *Centrosema* spp. at Carimagua in the eastern plains of Colombia for the first time suggests that the disease may be native to this region. The occurrence of CylLS in Costa Rica is more difficult to explain. Although *C. acutifolium* is not native to Central America, *C. pubescens*, another host of *C. colhounii*, is native to the region (2). A different form of *C. acutifolium* is present in central regions of Brazil, including Mato Grosso, Goias, and Minas Gerais (9); however, to date, CylLS has not been reported on *Centrosema* spp. in Brazil. Surveys of native populations of *C. acutifolium* and other *Centrosema* spp. are necessary to answer the question of the origin of the disease. It is also possible that the native host of CylLS in Colombia and Costa Rica is not a species of *Centrosema*. CylLS has not been reported on *Centrosema* spp. in other regions of the tropics outside Central and South America.

Symptomatology. On *C. acutifolium*, symptoms first appear on leaflets as small (1–2 mm in diameter), irregular, medium-brown spots, which enlarge to 10–30 mm in diameter in zones of varying shades of brown (Figure 1). Spots may involve the whole leaflet but usually affect only part of it. Leaves become chlorotic and defoliate. On the underside of the leaflet the fungus sporulates profusely (especially under humid conditions), forming a white mesh of mycelium, conidiophores, and conidia. Stems are not affected and usually produce new leaves once humid conditions cease. Although symptoms are similar on other susceptible *Centrosema* spp., lesion zonation is usually more developed on *C. acutifolium*.

Pathogen identification. On PDA, the fungus isolated from lesions produced uniform, dark, tan-brown colonies. Conidia, produced laterally on conidiophores on the surface of colonies, were cylindrical, hyaline, three-septate, and 40–60 × 4–5.5 μm. From cultural characters and conidium/conidiophore morphology, the causal fungus was tentatively identified as a *Cylindrocladium* sp. The International Mycological Institute (IMI) confirmed its identity as *C. colhounii* Peerally.

Pathogenicity. Koch's postulates were successfully completed with two isolates of *C. colhounii* from *C. acutifolium* cv. Vichada. Zonate leaf spots, as originally observed, were reproduced under glasshouse conditions, and the same fungus was reisolated. In glasshouse inoculation studies, the host range of *C. colhounii*

Fig. 1. Zonate spotting, caused by *Cylindrocladium colhounii*, on leaves of two accessions of *Centrosema acutifolium*.

Table 1. Reaction of accessions of *Centrosema acutifolium* to *Cylindrocladium colhounii*

Origin	Number of accessions	Reaction to *C. colhounii*	
		Mean	Range
Mato Grosso, Brazil	13	3.58 a[w,x]	(4.17–3.00)
Vichada, Colombia	5	3.47 a	(3.94–2.89)
Amazonas, Venezuela	3	3.19 b	(3.39–3.06)
Goias, Brazil	6	3.12 b	(3.39–2.72)[y]
Minas Gerais, Brazil	1	2.72 c[z]	...

[w]Means followed by different letters are significantly different according to Duncan's multiple range test ($P = 0.05$).
[x]0 = no leaf lesions; 5.0 = leaf death.
[y]Mean reaction of accession CIAT 5597.
[z]Mean reaction of accession CIAT 15353.

was restricted to *C. acutifolium, C. arenarium, C. brasilianum, C. macrocarpum*, and *C. pubescens*. Accessions of *C. plumieri, C. schiedianum, C. tetragonolobum*, and *C. virginianum* and important tropical pasture legumes *Arachis pintoi, Desmodium ovalifolium, Pueraria phaseoloides, Stylosanthes capitata*, and *S. guianensis* were immune to *C. colhounii*.

C. colhounii causes pod rot of the legume cover crop *Canavalia ensiformis* (L.) DC in India (IMI, *unpublished record*) and leaf and fruit spot of custard apple (*Annona squamosa* L.) in Queensland, Australia (3). The teleomorph, *Calonectria colhounii* Peerally, causes leaf spot and defoliation of *Callistemon lanceolatus* (Sm.) DC, tea (*Camellia sinensis* (L.) Kuntze), and *Eucalyptus robusta* Sm. in Mauritius (7); it causes leaf spot of *Castanea vulgaris* and *Eucalyptus grandis* A. W. Hill ex Maiden in India and of clove (*Eugenia caryophyllus*) in Indonesia (IMI, *unpublished records*). Isolates of the pathogen from these hosts may affect *Centrosema* sp. and vice versa. *Annona* sp., native to Central and South America, may be the native hosts of *C. colhounii* in Colombia and Costa Rica, from which the pathogen has spread to *Centrosema* sp. Further inoculation studies with isolates of *C. colhounii* from a range of hosts would help to clarify relationships between specific isolates and hosts.

Among 28 accessions of the *C. acutifolium* germ plasm collection, the most susceptible to *C. colhounii* were from Mato Grosso, Brazil, and Vichada, Colombia (Table 1). Of 13 accessions from Mato Grosso and 5 from Vichada, 9 and 3, respectively, were more susceptible (mean reaction > 3.39) than the most susceptible accessions from Amazonas, Venezuela, and Goias, Brazil. The most resistant accessions were CIAT 15353 from Minas Gerais and CIAT 5597 from Goias with mean reaction to *C. colhounii* of 2.72 (Table 1). Results suggest that additional germ plasm with resistance to CylLS is more likely to be found in Amazonas, Venezuela, and Goias and Minas Gerais, Brazil, than in the other locations.

During 1986–1988, CylLS caused moderate to severe leaf spotting and defoliation, resulting in 30% dry-matter losses in grazed pastures in the eastern plains of Colombia (1). Further monitoring and evaluation are necessary to determine if CylLS affects animal production and long-term persistence and productivity of these pastures. If it is necessary to breed *C. acutifolium* for resistance to CylLS, usable resistance has been identified in several accessions of this species and also in accessions of *C. plumieri, C. schiedianum, C. tetragonolobum*, and *C. virginianum*.

LITERATURE CITED

1. CIAT. 1988. Annual Report. Tropical Pastures Program. Pages 4, 20-31. Centro Internacional de Agricultura Tropical, CIAT, Cali, Colombia.
2. Clements, R. J., Williams, R. J., Grof, B., and Hacker, J. B. 1983. *Centrosema*. Pages 69-96 in: The Role of *Centrosema, Desmodium* and *Stylosanthes* in Improving Tropical Pastures. R. L. Burt, R. P. Rotar, J. L. Walker, and M. W. Silvey, eds. Westview Press, Boulder, Colorado.
3. Hutton, D. G., and Sanewski, G. M. 1988. Cylindrocladium leaf and fruit spot of custard apple in Queensland. Australas. Plant Pathol. 18:15-16.
4. ICA. 1987. Centrosema—Vichada. Instituto Colombiano Agropecuario Boletin Tecnico 152. 13 pp.
5. Lenné, J. M., Vargas de Alvarez, A., and Torres, C. 1986. Damaging fungal diseases of promising pasture legumes in the tropical American lowlands. Pages 807-809 in: Proc. Int. Grassl. Congr. 15th.
6. Lenné, J. M., Pizarro, E. A., and Toledo, J. M. 1986. Importance of diseases as constraints to pasture legume adaptation in the tropical American lowlands. Pages 810-812 in: Proc. Int. Grassl. Congr. 15th.
7. Peerally, A. 1974. *Calonectria colhounii*. CMI Descriptions of Pathogenic Fungi and Bacteria 430. 2 pp.
8. Schultze-Kraft, R., Benavides, G., and Arias, A. 1987. Recoleccion y evaluacion preliminar de *Centrosema acutifolium* Benth. Pasturas Tropic. 9:12-20.
9. Thomas, D., and Grof, B. 1986. Some pasture species for the tropical savannas of South America. II. Species of *Centrosema, Desmodium*, and *Zornia*. Herb. Abstr. 56:511-525.