

## Occurrence and Pathogenicity of *Alternaria brassicicola* in Brazil

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### ABSTRACT

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A leaf spot disease of cabbage (*Brassica oleracea* var. *capitata*) caused by *Alternaria brassicicola* was found in central Brazil in April 1982. The seedborne pathogen was pathogenic to several crucifers and was not eliminated from cabbage seeds by captan treatment.

During our studies of postharvest diseases of fruits and vegetables in April 1982 (2,10,11), we frequently encountered a foliar disease of cabbage (*Brassica oleracea* var. *capitata* L.) sold at wholesale markets in Brasilia that resulted in approximately 5% market losses. Unidentified bacteria were among the organisms associated with leaf lesions, but the most frequent and consistent organism isolated was *Alternaria brassicicola* (Schw.) Wiltsh. Although the pathogen has been reported on various crucifers, including crambe (*Crambe abyssinica* Hochst. ex Fries) (8,9), from different parts of the world (4,5,7,12), as far as we can ascertain this is

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the first report on the occurrence of *A. brassicicola* in Brazil.

The objectives of our study were to record the disease and evaluate the host range of *A. brassicicola*. We also investigated the origin of the disease.

### MATERIALS AND METHODS

**Isolation.** Samples (1 × 2 mm) from diseased cabbage leaves were washed under running tap water, surface-sterilized for 3 min in 1% sodium hypochlorite (NaOCl), then rinsed in sterile water and plated on potato-dextrose agar amended with 240 µg/ml of chloramphenicol (PDA + C). Isolation plates were incubated on a laboratory bench at room temperature (22 ± 2 C) for 5–7 days and isolated organisms were subcultured on PDA + C. Ten single-conidium isolates of *A. brassicicola* were selected randomly and compared. No variation was observed, so one single-conidium isolate was used in further studies.

**Pathogenicity and host range tests.** Conidia of *A. brassicicola* were obtained by flooding 1-wk-old cultures on PDA + C in petri plates with 1–2 ml of sterile water and rubbing the fungal colony with a U-shaped sterile glass rod to loosen

conidia. Detached cabbage leaves of an unknown cultivar were surface-sterilized in 1% NaOCl for 20 min, rinsed twice in sterile water, and inoculated by evenly spraying 1-ml water suspensions of conidia (1.6 × 10<sup>6</sup> conidia/ml) with an atomizer. Inoculated leaves were immediately placed in polyethylene bags, held at room temperature (22 ± 2 C) for 48 hr, then removed from the bags and placed on moist filter papers in covered plastic boxes (22 × 9 × 32 cm) serving as moist chambers. Leaves similarly prepared but sprayed with 1 ml of sterile water were used as controls. The leaves from all treatments were examined and the symptoms recorded every day for 7 days. There were four replicates with two leaves per replicate per treatment.

Host range was evaluated by inoculating detached leaves of cauliflower (*B. oleracea* var. *botrytis* L.), mustard (*B. juncea* (L.) Coss), Chinese cabbage (*B. pekinensis* (Lour.) Rupr.), and turnip (*B. rapa* L.) and heads of cauliflower as previously described for leaves of cabbage.

**Seed assay.** Seeds of three commercial cabbage cultivars (Redondo da Hollanda, Chato de Quintal, and Louco de Verão), mustard, cauliflower, and Chinese cabbage were assayed for presence of *A. brassicicola*. The seeds were obtained from a supermarket and had been commercially treated with 0.2% captan 75W. The seeds were washed in sterile water to remove the fungicide, then 400 from each cultivar were plated on PDA media, 10 per plate. Seeds were examined

for *A. brassicicola* after 5 days of incubation on a laboratory bench at room temperature (22 ± 2 C).

## RESULTS

**Symptoms.** The first symptoms on cabbage leaves appeared as small (0.5–1 mm in diameter) but conspicuous circular black spots (Fig. 1A) as early as 36 hr after inoculation. The lesions were numerous and scattered irregularly over the leaf surface. As the disease progressed, the spots became larger (up to 2 cm in diameter) and oval (Fig. 1B), and tended to coalesce to form large black areas. Severely affected areas of the leaf became desiccated. On veins, the lesions started as black flecks or streaks and enlarged

into elliptic spots (Fig. 1A,B).

**Pathogenicity and host range tests.** *A. brassicicola* was pathogenic on all crucifer species tested. The plants did not vary in susceptibility to the pathogen, and with the exception of Chinese cabbage, leaf symptoms were similar to those described for cabbage. On Chinese cabbage, the black spots remained small (1–2 mm in diameter) and did not coalesce (Fig. 1C) after 7 days of incubation. On heads of cauliflower, *A. brassicicola* caused black spots that coalesced to produce large black areas (Fig. 1D). *A. brassicicola* was reisolated from all infected leaves. None of the control leaves developed symptoms.

**Seed assay.** *A. brassicicola* was present in seeds of all cabbage cultivars and of

cauliflower but not of mustard and Chinese cabbage (Table 1). Percentage of seeds with the pathogen varied from 3.5% for the cabbage cultivar Redondo da Hollanda to 37.5% for cauliflower (Table 1). The other organisms found in seeds of the crucifers tested were species of *Alternaria* (including *A. alternata* (Fr.) Keissl.), *Aspergillus* (including *A. niger* van. Tiegh.), *Aureobasidium*, *Cladosporium*, *Curvularia*, *Fusarium*, *Gilbertella*, *Phoma*, *Rhizopus*, and *Stemphylium* and unidentified bacteria. Of these, *Aspergillus niger* and the unidentified bacteria predominated (Table 1).

## DISCUSSION

Although this is the first report of *A. brassicicola* in Brazil, the pathogen probably is not new in that country. The disease probably has been confused with the much less severe disease caused by *A. brassicae* (Berk.) Sacc. or simply referred to as *Alternaria* disease without identification of the causal agent (*A. Takatsu*, University of Brasilia, *personal communication*). In the present study, identification of *A. brassicicola* was based on the description given by Ellis (7).

Our seed assay showed that the cabbage cultivars and cauliflower were not free from *A. brassicicola*. Captan at the rate used did not eliminate *A. brassicicola* from seeds of crucifers, and infected seeds were probably responsible for the introduction of *A. brassicicola* in central Brazil. Because of its pathogenicity to such a wide range of crucifer species, *A. brassicicola* should be regarded as a potential problem in Brazil. Most crucifer seeds used in Brazil are imported and then packed in the state of Minas Gerais before distribution. Precautions must be taken, therefore, to import noncontaminated seeds. Furthermore, before shipment the seeds must be treated with a fungicide effective against *A. brassicicola*.

No pathogenicity tests were done to determine how many of the other isolated genera of fungi and bacteria would be pathogenic to cabbage. Some of the genera isolated, however, are known pathogens (1,3,6,13) and might be implicated in seed and crop failure in the field.

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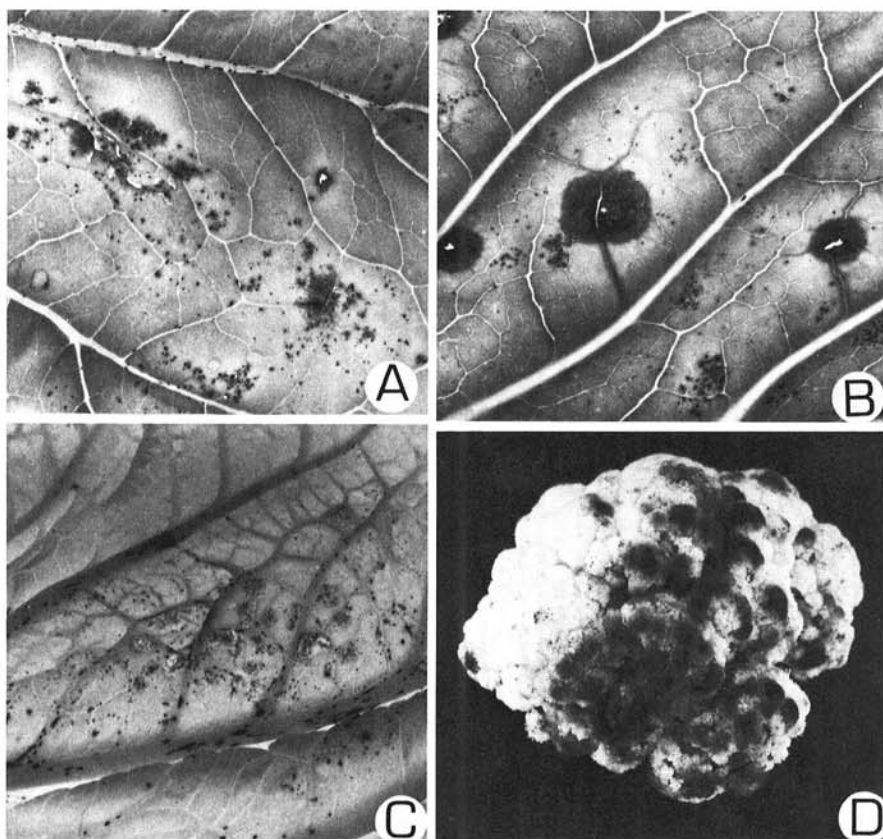


Fig. 1. Symptoms incited by *Alternaria brassicicola* on (A and B) cabbage leaves, (C) Chinese cabbage leaf, and (D) cauliflower head.

Table 1. *Alternaria brassicicola* and other organisms in seeds of crucifers in Brazil

Crucifer	Percentage <sup>a</sup> of seeds with:			
	<i>Alternaria brassicicola</i>	<i>Aspergillus niger</i>	Others <sup>b</sup>	Unidentified bacteria
Cabbage cultivar				
Chato de Quintal	10.5	8.5	9.0	0.3
Redondo da Hollanda	3.5	0.3	4.8	0
Louco de Verão	6.5	4.5	1.3	22.5
Mustard	0	0	0.3	0
Cauliflower	37.5	0	21.0	0
Chinese cabbage	0	2.5	12.6	47.0

<sup>a</sup>Based on 400 seeds per cultivar.

<sup>b</sup>Species of *Alternaria* (including *A. alternata*), *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Curvularia*, *Fusarium*, *Gilbertella*, *Phoma*, *Rhizopus*, and *Stemphylium*, combined.

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