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***Alternaria cichorii* in Brazil on *Cichorium* spp. Seeds and Cultivated and Weedy Hosts**

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Received August 27, 2007; accepted October 18, 2007

Keywords: endive, leaf blight, leaf spot, seed pathology, sowthistle, spiny sowthistle, Brazil

Abstract

Alternaria cichorii was recorded for the first time in Brazil causing leaf spots on endive (*Cichorium endivia*) in 2003 based on material collected at Catalão, state of Goiás, in 2001. In 2005, *A. cichorii* was found causing severe leaf-spotting in an escarole (*C. endivia*) plantation in Viçosa, state of Minas Gerais and shortly afterwards also in Viçosa, but at a different location, in the weed hosts common sowthistle (*Sonchus oleraceus*) and spiny sowthistle (*Sonchus asper*). A description of *A. cichorii* based on specimens collected in Viçosa is provided herein. The natural occurrence of *A. cichorii* on weed hosts in Viçosa indicates that it has already become naturalized in Brazil surviving on non-cultivated composites, complicating disease management. Pathogenicity tests, complementary to those already published, were performed to better elucidate the range of potential alternative hosts in the Asteraceae of this fungus. This involved inoculation of one isolate of *A. cichorii* obtained from cichory and one obtained from common sowthistle on selected species and cultivars of 10 tribes in the Asteraceae, but concentrated in the Lactuceae. All species included in the test were shown to be susceptible to at least one of the isolates. A high incidence of leaf spot or blight resulted from inoculation of most individuals. All plants of six test species were killed after inoculation with one of the isolates of *A. cichorii*. Several of the test species have not been previously recorded as hosts for *A. cichorii*. Surprisingly, the known host-range for this fungus is restricted to only few members of the Asteraceae (*Achillea millefolium*, *C. endivia*, *C. intybus*, *Carthamus tinctorius* and *Lactuca sativa*). Our results indicate that *A. cichorii* has a wide host range within the Asteraceae and that other cultivated or non-cultivated members of this family may serve as inoculum reservoirs for this fungus in the absence of cichory, endive or escarole. An attempt to verify if contaminated seeds might be the original source for such geographically distant occurrences of *A. cichorii* in Brazil was made. Analysis

of 24 samples of cichory and endive seeds obtained from dealers in five different Brazilian states showed that 25% of these samples carried *A. cichorii* with an incidence varying from 0.6% to 13.75%. Such a result highlights the vulnerable situation in Brazil for avoiding introduction of exotic seed-carried vegetable pathogens. It is known that a very significant proportion of vegetable seeds marketed in Brazil are imported and seed inspection for pathogens is not performed regularly for such material imported for commerce. It is probable that contaminated cichory, endive and escarole seeds served as a vehicle for introduction of this fungus in Brazil and that the fungus has then become naturalized in many vegetable growing areas and is now surviving on other Asteraceae in the absence of cultivated hosts.

Introduction

Endive and escarole (*Cichorium endivia* L.: Asteraceae) are plants grown worldwide as vegetables or for their roots which are used industrially as a coffee substitute. It is well adapted in Brazil where it is often used, as well as cichory (*Cichorium intybus* L.), as a green salad alternative to lettuce when climatic conditions for the latter are adverse. It is estimated that there is a national production of *c.* 25 000 ton/year in Brazil (Camargo Filho and Mazzei, 2001).

Although considered as hardier than lettuce, there are also limiting factors for this crop in Brazil, including fungal leaf spots such as those caused by *Alternaria* spp. Endive has been mentioned as a host of *Alternaria* spp. in indexes of plant diseases in the USA (Alfieri et al., 1984; Farr et al., 1989), Brazil (Viégas, 1946; Reifschneider et al., 1983; Mendes et al., 1998) and in New Guinea (Shaw, 1984).

Alternaria cichorii Nattrass was recorded for the first time in Brazil in 2003 causing leaf spots on endive (*C. endivia*) based on material collected in Catalão, state of Goiás, in 2001 by Lima et al. (2003). These authors demonstrated the pathogenicity of this isolate

to endive and to other species of the Asteraceae, namely: three lettuce cultivars (*Lactuca sativa*), three chicory, two chicory cultivars and one of catalonha (*C. intybus*) and annual sowthistle (*Sonchus oleraceus*).

In 2005, *A. cichorii* was found causing severe leaf-spot epiphytotics in an escarole plantation at the locality of Piúna, municipality of Viçosa, state of Minas Gerais and shortly afterwards also in Viçosa, but at a different location, attacking the weed hosts common sowthistle (*S. oleraceus*) and spiny sowthistle (*Sonchus asper*). Such geographically distant occurrences of *A. cichorii* in Brazil (southeast Goiás and southeast Minas Gerais) suggested that the disease might have been disseminated in contaminated seeds. Additionally, the natural occurrence of *A. cichorii* on weed hosts in Viçosa, coupled with the previous observations by Lima et al. (2003) that their isolate of *A. cichorii* was also capable of attacking other artificially inoculated composites, was interpreted as possibly indicating that the fungus has already become naturalized in Brazil surviving on non-cultivated composites, complicating disease management. Herein, results of observations on the identity of the *Alternaria* on escarole and sowthistles in Viçosa, an investigation of the occurrence of *A. cichorii* in seeds in the Brazilian market and also an extended study of the host-range of *A. cichorii* isolates in the family Asteraceae, are presented.

Materials and Methods

Observations of escarole and sowthistle with leaf spots were made on freshly collected material obtained from two localities of the municipality of Viçosa (MG): escarole (*C. endive* cv. Escarola Lisa) from Piúna and common sowthistle (*S. oleraceus*) and spiny sowthistle (*S. asper*) from the Campus of the Universidade Federal de Viçosa. Isolations were performed either through directly transferring fungal structures from lesions onto plates containing vegetable broth agar (VBA) as described in (Pereira et al., 2003) or by surface sterilizing selected fragments of diseased tissues (2-min immersion in Chlorox) and transferring these onto plates containing VBA. Slides were mounted in lactophenol either with fungal structures obtained from diseased leaves or from pure cultures and studied with an Olympus BX 50 light microscope. Representative material was deposited in a local herbarium (Herbarium VIC).

One isolate from escarole and one from common sowthistle were tested for pathogenicity and their host-range within the Asteraceae. A series of 27 taxa in the Asteraceae was selected for that study (Table 1) including plants in the subfamilies Cichorioideae and Asteroideae and in 10 tribes, 20 genera and 22 species; five cultivars of chicory, two of lettuce were included in the list of test-plants. Besides, vegetables such as chicory, escarole, artichoke, ornamental, medicinal and weed composites (exotic and native) were included in the list of test plants. Inoculations were performed with suspensions of conidia in 0.05 % Tween 20 solution. Conidia utilized for inoculations were produced

as described by Walker (1980) with some minor changes. Ten cultures disks obtained from the margin of 7-day-old cultures grown on VBA were transferred to flasks containing 100 ml of VB (the same as VBA but without agar). The flasks were left on a shaker at 180 rpm for 7 days at room temperature. The mycelial mass was then blended with the remaining liquid medium in each flask and poured onto 20 × 28-cm aluminum trays, each containing 100 ml of solidified VBA. Trays were kept in a controlled temperature room at 26 ± 2°C under a 12-h light regime (light from two 40-W daylight fluorescent lamps and two 40-W fluorescent, near ultra-violet light lamps). After 2 days, conidia were harvested by pouring 50 ml of sterile water on the tray surface and scraping off the fungal culture with a rubber spatula. The resulting suspension was then filtered through two layers of cheese cloth and the final concentration of the suspension was evaluated and adjusted for each inoculation series.

Isolates from escarole and common sowthistle were tested separately. The inoculum of the isolate from common sowthistle was used at a concentration of 2 × 10⁶ conidia/ml. The escarole isolate was tested, but with a lower conidial concentration (2 × 10⁵ conidia/ml). Test plants were brush-inoculated with the suspensions on both sides of all leaves of the plants. After the inoculation, the plants were maintained in a dew chamber at a temperature of 25°C, for 48 h and later transferred to a greenhouse where they were observed daily for the appearance of symptoms. Plants brushed with 0.05% Tween solution served as controls. Disease incidence (number of diseased leaves/total number of leaves) and disease severity were evaluated. Disease severity was ranked with an arbitrary scale having four levels: high severity, moderate severity, low severity and absence of disease.

The experiment had three replicates, each consisting of a pot containing a plant. To confirm that *A. cichorii* would be completing its cycle on each of the test-plants, selected diseased leaves of inoculated plants were taken to a humid chamber and left there for 24 h and later observed under the dissecting microscope for the presence of new flushes of conidiophores and conidia. Slides were prepared for additional observations under the microscope whenever necessary.

The possibility of seed transmission being responsible for the widespread distribution of the disease in Brazil was investigated by the standard method of seed testing (blotter test as described in Agarwal and Sinclair, 1987). Twenty-four samples of chicory, escarole and endive available commercially were obtained from five Brazilian states. Four hundred seeds were taken randomly from each sample, divided in groups of 100 seeds and evenly distributed inside plastic boxes containing sterile filter paper soaked with sterile water and treated with 0.1% 2,4-D for inhibition of seed germination. Each box containing the seeds was taken to a shelf in a controlled temperature room at 26 ± 2°C with a 12-h light regime (light from two 40-W daylight fluorescent lamps and two 40-W fluorescent, near

Table 1
Host-range evaluation of
Alternaria cichorii isolates
from *Sonchus oleraceus*^a and
Cichorium endivia^b

Sub-family: Cichorioideae	Scientific name	P1	P2	P3	Class
Tribe Cardueae	<i>Arctium minus</i> Bernh +	Death 4/5	Death 2/5	Death 2/4	HS LS
	<i>Cynara scolymos</i> L. +	5/5	4/4	5/5	HS
Tribe Lactuceae	<i>C. endivia</i> L. + (Escarola Lisa)	Death 3/4	Death 3/4	5/5 3/4	HS MS
	<i>Cichorium intybus</i> L. + (Almeirão da Catalonha)	Death 4/6	Death 4/6	Death 4/5	HS MS
	<i>C. intybus</i> L. + (Almeirão Pão de açúcar)	5/6	5/6	5/5	MS
	<i>C. intybus</i> L. (Chicória Crespa)	4/5	4/5	4/5	MS
	<i>C. intybus</i> L. + (Almeirão)	4/5	5/5	4/5	MS
	<i>C. intybus</i> L. (Chicória Crespa)	4/4	5/5	6/6	MS
	<i>C. intybus</i> L. + (Almeirão)	Death 5/6	Death 5/6	Death 5/6	HS MS
	<i>C. intybus</i> L. + (Almeirão Verde Spadona)	5/6	5/6	6/6	MS
	<i>Crepis japonica</i> L.	4/4	4/4	4/4	MS
	<i>Lactuca sativa</i> L. + (Alface Grandes lagos)	4/6	4/5	4/6	MS
	<i>L. sativa</i> L. (Boston Branca)	1/4	2/12	0/4	LS
	<i>Sonchus asper</i> (L.) Hill +	1/4	1/11	0/4	LS
	<i>S. oleraceus</i> L. +	4/5	4/5	4/5	MS
	<i>Taraxacum officinale</i> Weber +	3/5	4/5	4/5	MS
	Tribe Mutisiae	<i>Gerbera jamesonii</i> Adlam	4/4	4/4	5/5
		3/4	4/5	4/8	LS
Tribe Vernoniae	<i>Vernonia grandiflora</i> Less.	1/6	1/5	0/6	LS
		5/8	4/7	5/8	MS
Sub-family: Asteroideae		7/7	6/6	7/7	MS
		6/7	6/6	5/7	MS
Tribe Anthemideae	<i>Chrysanthemum leucanthemum</i> L.	1/6	1/8	3/8	LS
	<i>Dendranthema grandiflora</i> Tzvelev +	1/6	1/7	1/8	LS
Tribe Calendulae	<i>Calendula officinalis</i> L. +	6/6	10/10	7/7	MS
	<i>Dimorphohteca sinuata</i> L.	–	–	–	–
Tribe Eupatoriae	<i>Mikania micrantha</i> H. B. & K. +	Death 2/4	Death 3/4	Death 4/4	HS MS
		3/6	2/6	1/5	LS
Tribe Heliantheae	<i>Bidens pilosa</i> L. +	11/14	9/11	9/13	LS
	<i>Helianthus annuus</i> L. +	7/12	7/11	10/13	LS
Tribe Plucheae	<i>Pterocaulon lanatum</i> Kuntze +	Death 4/5	Death 5/5	Death 4/4	HS HS
		9/9	9/9	9/9	MS
Tribe Senecioneae	<i>Dahlia pinnata</i> Cav. +	5/9	6/9	4/9	LS
	<i>Emilia sonchifolia</i> (L.) DC. +	4/4	4/4	4/4	MS
	<i>Senecio douglasii</i> L.	2/4	3/4	4/4	LS
		2/4	–	–	MS
		11/11	12/12	13/13	MS
		8/10	9/12	11/13	LS
		8/9	9/9	9/9	MS
		6/9	7/9	8/9	LS
		2/3	2/4	1/4	LS
		3/3	4/4	4/4	LS
		15/57	18/96	13/60	LS
		17/52	18/88	17/51	LS

HS, high severity; MS, moderate severity; LS, low severity.

^aResults for inoculations of isolate from *S. oleraceus* given on the first line following each test-plant name.

^bResults for inoculations of isolate from *C. endivia* given on the second line following each test-plant name.

P1, P2 and P3 = individual plants inoculated.

+ = sporulation of *A. cichorii* confirmed on diseased leaves left for 24 h in humid chamber.

ultra-violet light lamps). The evaluation was made 7 days later. Evaluation of the experiment consisted in counting the total number of seeds colonized by the pathogen, with the help of stereoscopic microscope. In case of doubt, slides of fungal structures were mounted in lactophenol for observation and elucidation of the fungus identity. Incidence of the pathogen was ranked

as the percentage of seeds of each group colonized by the fungus.

Results and Discussion

Leaf spots on escarole were initially small dark developing into larger necrotic lesions with concentric rings that coalesced leading to necrosis of extensive areas or

whole leaves with age (Fig. 1). On sowthistle, leaf spots were smaller (5- to 9-mm diameter) and had fainter concentric rings and were pale centrally (Fig. 2). The fungus from escarole and sowthistles had the morphology typical for *A. cichorii* as described in Ellis (1971) and was as follows: conidiophores isolate or in small groups, cylindrical, slightly geniculate, 60.0–90.0 × 6.5 µm, 3–6 septate, pale brown, smooth; conidia solitary or forming short chains (often in culture), fusiform to obclavate, 44.0–69.0 × 12.5–19.0 µm, 6–9 transverse septate, 0–3 longitudinal septate, brown, smooth, acute and long beak (up to 129 µm), attenuating towards the apex, sometimes bifurcate or trifurcate, pale brown.

MATERIAL EXAMINED: VIC 29372, campus of the Universidade Federal de Viçosa, state of Minas Gerais, Brazil, 19th May 2005 on *S. oleraceus*, B. S. Vieira; VIC 29373; campus of the Universidade Federal de Viçosa, state of Minas Gerais, Brazil, 19th

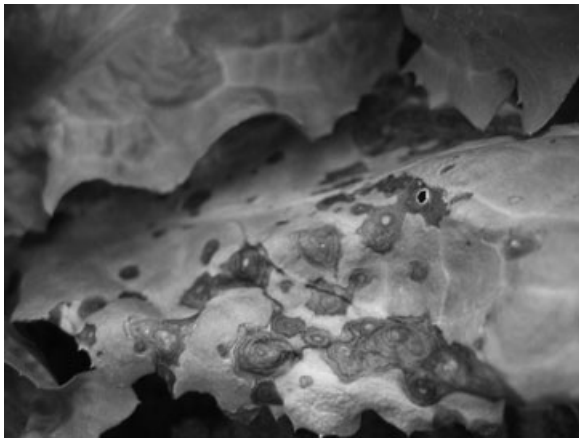


Fig. 1 Adaxial side of leaves of endive (*Cichorium endivia*) showing leaf-spot symptoms caused by *Alternaria cichorii*



Fig. 2 Adaxial side of leaves of *Sonchus oleraceus* (right) and *Sonchus asper* (left) showing leaf-spot symptoms caused by *Alternaria cichorii*

May 2005 on *S. asper*, R.W. Barreto; VIC 29374, Piúna, Viçosa, state of Minas Gerais, Brazil, 15th May 2005 on *C. endivia*, R.W. Barreto.

Sonchus asper and *S. oleraceus* are reported here for the first time as a hosts of *A. cichorii* in Brazil.

Leaf spot symptoms and sporulation of *A. cichorii* on inoculated plants included in the host-range test were visible 7 days after the inoculation. All species included in the two tests were susceptible to either the isolate from *S. oleraceus* or that from *C. endivia*, and high leaf-spot disease incidences were generally observed (Table 1). A severe blight, instead of leaf spotting resulted for some of the isolate/test-plant combinations.

Significant differences were observed for disease severity obtained by inoculating each isolate on a single test species. For instance, inoculation of the isolate from *S. oleraceus* on *Articum minus*, *Bidens pilosa* (Fig. 3), *Calendula officinalis* and *Vernonia grandiflora* (Fig. 3) led to death of all plants but the same plant species were not killed by inoculation with the isolate from *C. endivia* and *A. minus*, *B. pilosa* and *V. grandiflora* showed only moderate-to-low levels of disease when inoculated with the sowthistle isolate. Conversely, inoculations with the isolate from *C. endivia* caused death of all escarole and cichory plants, and two of the three *Cynara scolymos* plants that were inoculated whereas these test-plants only showed moderate levels of disease when inoculated with the isolate from *S. oleraceus*. Nevertheless, the majority of the inoculations resulted in equivalent levels of disease.

It is likely that the blight and unusually fast lethal reactions obtained for some of the isolate/test-plant combinations resulted from a high sensitivity of the plant to phytotoxins produced by the fungus isolates. Phytotoxins are commonly involved in disease development (Agrios, 2005) and an abnormally high level may be present in inoculum suspensions such as those used in the test. Some of these phytotoxins may have a wide action spectrum and can be non-specific. *Bidens pilosa* and *V. grandiflora* are known to be very serious weeds in Brazil (Lorenzi, 2000) and their high susceptibility to this purported phytotoxin of *A. cichorii* highlights the potential of further explorations on bioactive compound produced by this fungus to be used as a herbicide against these important weeds. A study along this line has already been performed by Stierle et al. (1993) who investigated an organic extract of an isolate of *A. cichorii*, from Russian knapweed [*Acroptilon repens* (L.) DC]. A series of zinniol-related compounds with varying degrees of phytotoxicity was isolated in that study. It is probable that the same group of compounds is present in the isolates from escarole and sowthistles from Brazil.

In the second series of inoculations, involving the isolate from *C. endivia*, an attempt was made to reduce the effect of the purported toxin present in the inoculum suspension. A lower concentration of conidia was hence utilized. Some reduction of disease levels was observed, but the host-range remained equivalent for

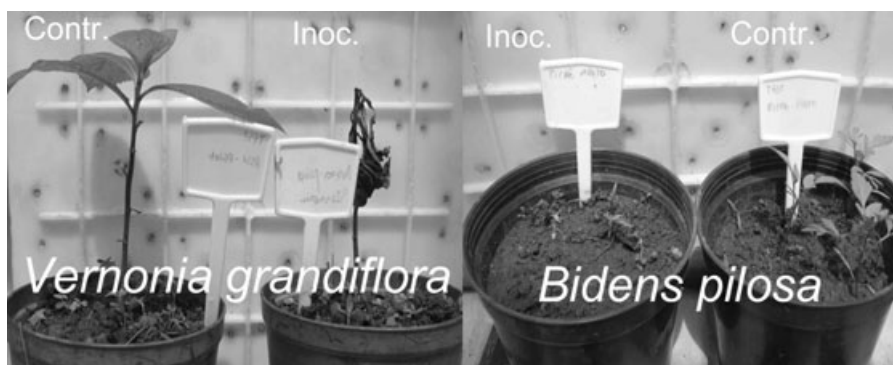


Fig. 3 *Vernonia grandiflora* (left) and *Bidens pilosa* (right) individuals completely blighted 7 days after inoculation with *Alternaria cichorii*

Table 2
Data on seed samples tested for the presence of *Alternaria cichorii*

No.	Origin (locality – state)	Fungicide treatment	Lot number	Cultivar	Fungus incidence
1	Sanga do Café – SC	Captan	49092	Chicória Crespa	0
2	Sanga do Café – SC	Captan	48092	Chicória Lisa	8.75
3	Belo Horizonte – MG		19439	Chicória Lisa	0
4	Belo Horizonte – MG	Thiram	13606A	Chicória Lisa Batavia	1.25
5	Belo Horizonte – MG	Thiram	13605	Chicória Crespa	0
6	Belo Horizonte – MG	Captan	401204	Chicória Lisa	13.75
7	Belo Horizonte – MG	Captan	49092	Chicória Crespa	0
8	São José de Ubá – RJ	Thiram	13606A	Chicória Lisa Batavia	0
9	SC	Captan	709682	Chicória Gigante Barbarella	0
10	SC	Captan	49882	Chicória Crespa	0
11	SC	Captan	803902	Chicória Escarola Biondina	0
12	Vacaria – RS	Captan	52191	Chicória Lisa	0
13	Rio de Janeiro – RJ		15876	Chicória Crespa	0
14	Rio de Janeiro – RJ	Captan	49092	Chicória Crespa	0
15	Rio de Janeiro – RJ	Thiram	13606A	Chicória Lisa Batavia	0.6
16	Rio de Janeiro – RJ	Captan	48092	Chicória Lisa	1.25
17	Rio de Janeiro – RJ		19152	Chicória Escarola Lisa	0
18	Faxinal dos Guedes - SC	Captan	805110	Chicória Escarola Lisa	0
19	Faxinal dos Guedes - SC		15781	Chicória Escarola Lisa	0
20	Viçosa – MG		18439	Chicória Escarola Lisa	0
21	Viçosa – MG	Captan	481204	Chicória Escarola Lisa	0
22	Sorriso – MT	Captan	49092	Chicória Crespa	0
23	Sorriso – MT	Captan	803902	Chicória Escarola Biondina	0
24	Viçosa – MG	Thiram	–	Chicória Escarola Lisa	2.24

the two isolates. Sporulation of *A. cichorii* was observed on diseased leaves of most test-plants, confirming that the fungus can complete its cycle in those hosts. *Alternaria cichorii* emerges from this study as having a very wide host-range in the Asteraceae. Several among the test-species have not been previously recorded as hosts for *A. cichorii*. Surprisingly, the known host-range for this fungus is restricted to only few members of the Asteraceae (*A. repens*, *C. endivia*, *C. intybus*, *Carthamus tinctorius* and *L. sativa*). Such a result indicates that *A. cichorii* is highly polyphagous within the Asteraceae and that other cultivated or non-cultivated members of this family may serve as inoculum reservoirs for this fungus in the absence of cichori, endive or escarole complicating disease management. Similarly, to what has been previously observed by Vieira and Barreto (2006) about the additional noxious aspect of both *Sonchus* species as reservoirs for the fungus causing downy mildew of lettuce – *Bremia lactucae* Regel, these are now shown to be hosts in natural conditions for the agent

of *A. cichorii* highlighting even further the need of their eradication as a part of disease management in green salad vegetable growing areas and also perhaps to other vegetables and ornamentals.

Analysis of the selection of samples of cichori and endive seeds showed that 25% (6 out of 24 samples) were carrying *A. cichorii*. Incidences varied from 0.6% to 13.75% (Table 2). The fungus was detected in samples of two different brands among the five that were included in the study and samples carrying the pathogen were obtained from commerce in three different states, among the five where samples were obtained, namely: Minas Gerais, Rio de Janeiro and Santa Catarina. Such a diversity of origins and the involvement of two large seed dealers in Brazil among the providers of *A. cichorii* carrying seeds are a worrying indication that the seeds are a primary source of inoculum for that disease in Brazil and that this relatively newly recorded pathogen is being actively introduced into new areas with such seeds. Additionally, it was observed that treatment with fungicide coating with

Thiram or Captan did not effectively control the fungus. All of the samples where *A. cichorii* was detected were treated with both Thiram and Captan. Such products are known to be ineffective at controlling fungi such as *A. cichorii* in treated seeds. This is due to the fact that fungi belonging to *Alternaria* can colonize the embryo and the endosperm of seeds becoming inaccessible to contact fungicides such as Thiram and Captan (Muniz and Porto, 1999; Reis et al., 2006).

It is probable that contaminated cichory, endive and escarole seeds served as a vehicle for introduction of this fungus in Brazil and that the fungus has then become naturalized in many vegetable growing areas and is now surviving on other native and introduced Asteraceae in the absence of cultivated hosts.

Acknowledgements

The authors wish to thank O. D. Dhingra for his suggestions on seed testing and acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Conselho Nacional do Desenvolvimento Científico e Tecnológico (CNPq) for their financial support.

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