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Infection of field weeds by *Colletotrichum dematium*, the causal agent of spinach anthracnose

Possible weed hosts of *Colletotrichum dematium* had not been well documented in the Texas Wintergarden production region. These were of concern with respect to being a source of inoculum that could contribute to anthracnose epidemics in spinach crops. Two locations were selected near spinach production fields, and 10 sites were selected between those two locations. The first was in Zavala Co., near the Ritchie Farm test plots, adjacent to the Nueces River and access roads. The second was off FM140 south of Uvalde, in Uvalde Co. Weeds were inoculated with a spinach derived *C. dematium* isolate via infested oats heavily (1 cup/m^2 by volume) broadcast spread onto marked weed habitat areas. Multiple plants of each species present were inoculated at each site. Inoculated weeds were then checked for symptoms (lesions, sporulation on stems, leaves, crown) and collected for verification in the lab every 2 weeks after inoculation. Lab verification included incubating plants to induce sporulation for 24-48hr, then examining specimens under the microscope. Selected samples were cultured to confirm pathogenicity (Koch's postulates). Additional samples that were not sporulating but symptomatic were cultured to check for the presence of *C. dematium* infection in the plant without evident sporulation.

Of the 25 weed species collected across the 10 sites in Zavala and Uvalde Counties, 9 species had active *C. dematium* sporulation present on the symptomatic plant tissues.

Fig 1: Images of acervuli producing classic *C. dematium* shaped conidia collected from sporulating lesion of common mallow (site 2 sample). Top left image is acervuli *in situ*, approx. 80x. Top right image is the naturally occurring *Colletotrichum* sp. observed in many mallow samples at site 1. Note the color difference. The *C. dematium* acervuli are larger and more pronounced.

Bottom left image is a smashed acervulus and spores at 100x magnification. Curved conidia (spores) and setae (whisker-like appendages) tips at 450x magnification.

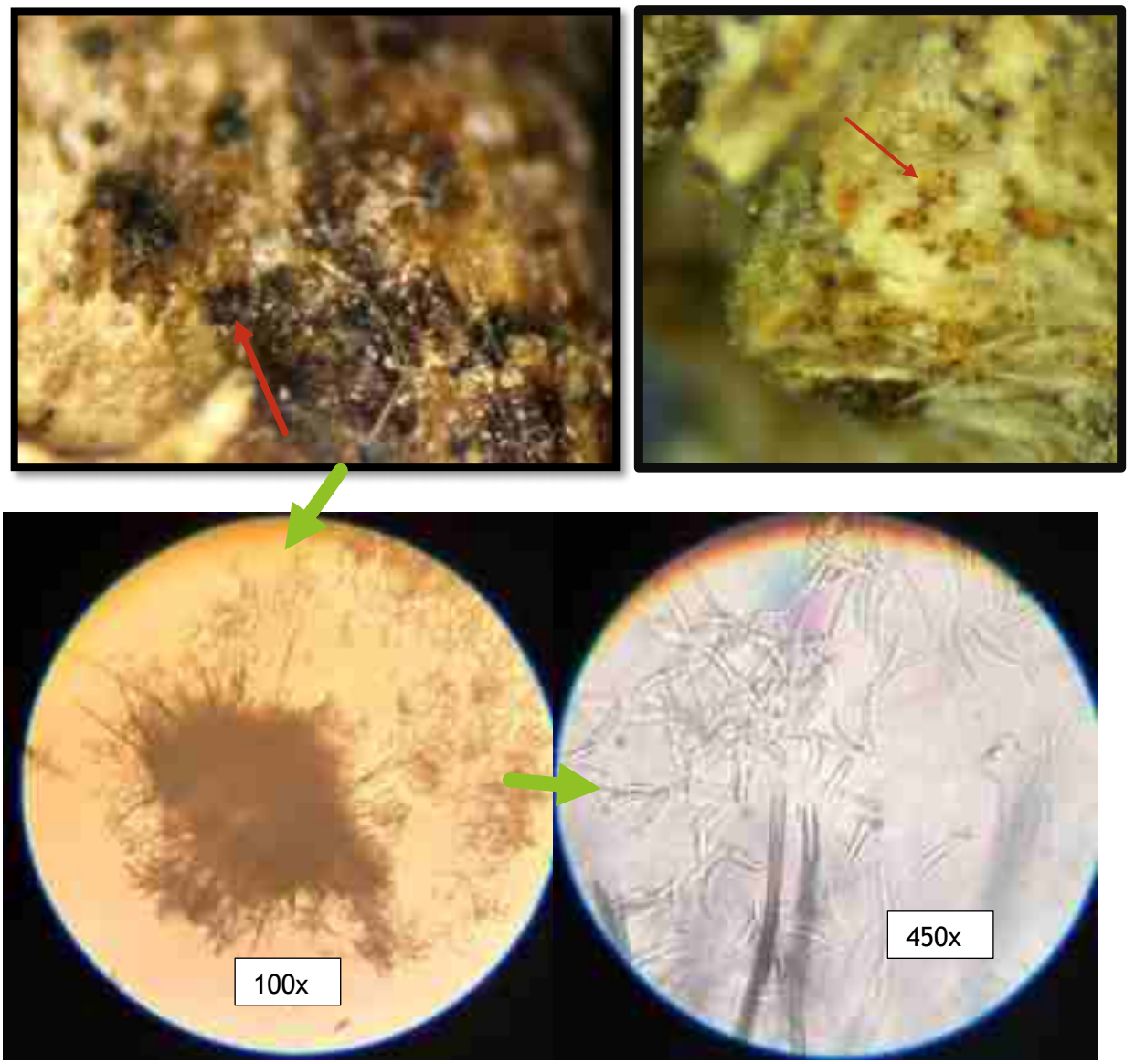

Fig 2. From ragweed, site 2.

Top left: symptomatic leaf tissue on inoculated ragweed plant, approx. 40x; Top Right: Close up of black perithecia (sexual reproductive structure) of suspected *C. dematium* embedded in the leaf tissue, approx. 80x.

Bottom Left: perithecia containing asci (oblong sac-like structures containing sexual reproductive spores) that have burst out after being smashed on a microscope slide, 100x magnification. Bottom right: oblong asci containing ascospores at 450x magnification.

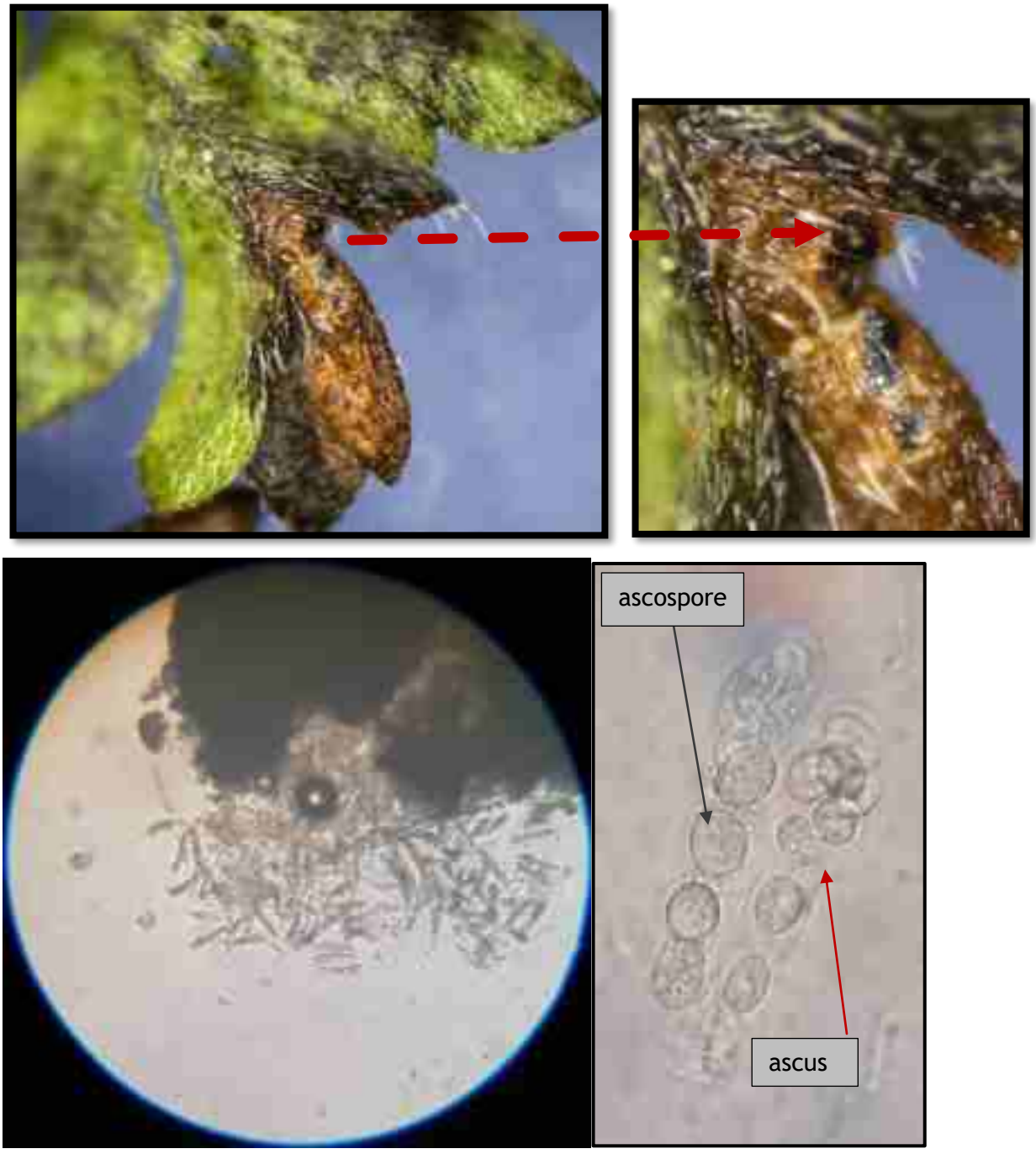

Fig 3: Ragweed from site 2 with acervuli on symptomatic stem. These had curved spores typical of *C. dematium*. ~50x magnification.

The ragweed samples were particularly interesting due to the fact that they had sexual reproductive structures suspected to be associated with *C. dematium*. Both sexual and asexual fungal reproduction were occurring simultaneously in the same plants. These sexual reproduction structures (perithecia) are more durable than the asexual acervuli in terms of longevity. More work is needed for additional insight to one way *C. dematium* could over-season in weeds.

Fig: 4: Thistle leaf with acervuli on lower leaf tissue. Crown tissue areas near the inoculation site had large lesions and rot symptoms. 80x magnification.

Fig 5: Acervuli on Yellow woodsorrel stolon. 80x magnification.

Fig 6: Acervuli on symptomatic stems from Horseherb. Approx 80 and 60x. Note in the right image you can see the “cup” shape of the acervuli and that sporulation was abundant in this host.

Fig 7: Acervuli on spiderwort stem near the crown area. Approx (Left) 30x and (Right) 60x.

Fig 8: Oat inoculum recovered from the field after 2 months with acervuli and conidial sporulation: 60x (top) side view of an acervulus can be seen and the high density of acervuli can be observed, particularly on the edge of the oat. Oats were rinsed with sterile water and cultured on 1/2 PDArad and *C. dematium* was recovered. This confirms that the inoculum distributed in the field does in fact sporulate and survive for weeks in the field in the absence of irrigation.

	Site (see Index for maps)	**Plant Inoculated**	***Colletotrichum dematium* present (y/n)**	**Observations**
1.	1acd,2bd	Common mallow	Y* *2 species of *Colletotrichum*; 1. small acervuli with oval spore† and 2. large acervuli with curved spore (*C. dematium* type) were found at site1 †Dr. Jim Correll has an isolate of the oval spore type. Pathogenicity tests on spinach using this isolate would be useful.	Site 2: both species types were observed on a single plant from site 1 (Fig 1) Plants with sporulating lesions of only the *C. dematium* spore type were found at site 2b,d
2.	2b,c	Ragweed – 2 species of ragweed; (WeakLeaf Bur Ragweed, Western Ragweed)	Y	Acervuli with curved spores present on lower symptomatic leaves Perithecia in leaves, lesions and dead leaves a base, symptomatic leaves higher up (see Fig 2,3)
3.	2acd	Thistle -Texas Thistle	Y	Acervuli on symptomatic leaf tissue; lesions at crown, lower leaves (Fig 4)
4.	2c	Yellow Woodsorrel, *Oxalis stricta*	Y	Acervuli on stolon area of plant (Fig 5)
5.	1d	Plains Poppy Mallow OR Salt Marsh Mallow-Malvaceae	N	
6.	1ef	Wild Umbellaceae (carrot family)	N	
7.	1cd	Spiderwort (possibly Ohio spiderwort, but seems more fleshy)	Y	Acervuli on lower stem, crown rot symptoms

8.	2a,b	Horseherb, straggler daisy; *Calyptocarpus vialis*	Y	Acervuli on lesions occurring on lower stems
9.	1d,2b	Goatbeard, clematis (vine)	N	
10.	1adef,2d	Nettle weed	N	
11.	1bc	Live Oak	TBD	Heavy leaf spot symptoms, but no acervuli present to ID. Heavy additional infection on some leaves. Additional site needs tested, ideally free of other foliar diseases.
12.	2d	Chickweed	N	
13.	1a,2cd	Purslane	N	
14.	2d	Mesquite sapling	N	
15.	2d	Lizard tail goura	TBD	Pending additional work- this population of plants has crown lesions but also has Phomopsis on leaves
16.	2ab	Rye Grass	N	
17.	2d	Lazy Daisy, Arkansas Lazy Daisy, Arkansas Doze-daisy *Aphanostephus skirrhobasis* OR A.*ramosissimus*	N	
18.	2b	3-lobe false mallow	Y	Acervuli & lesions on lower stem areas
19.	1f,2bc	Common Hedge Parsley	N	
20.	1abf, 2abc	Lambsquarter	Y	Symptomatic and sporulating lesions on leaves and stems
21.	2abc	Pigweed	N	
22.	2cd	Indian Blanket (wildflower)	N	
23.	2bcd	Western Horsenettle	N	
24.	2ad	Dallisgrass	N	
25.	2ab	Purple flower, 7” trailing weed-TBD	Y	Avervuli on symptomatic leaves