THE EFFECT OF SEED INOCULATION WITH *COLLETOTRICHUM* spp. ON EMERGENCE, SURVIVAL AND SEEDLING GROWTH OF *STYLOSANTHES HAMATA*

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ABSTRACT

Colletotrichum dematium *f. sp.* truncata *and* C. gloeosporioides, *two fungal pathogens of* Stylosanthes, *were isolated from* Stylosanthes *spp. seed harvested in Australia and Florida. When seeds of two* S. hamata *accessions were inoculated with these fungi in greenhouse tests, percent emergence and survival were significantly reduced. Root length and plant height were slightly reduced while root and shoot dry weights were significantly reduced. The implication of these results for the persistence of* Stylosanthes hamata *stands is briefly discussed.*

INTRODUCTION

Stylosanthes hamata is a legume with potential forage value in subtropical and tropical regions (Anon 1973). A *S. hamata* cultivar, "Verano" collected in Venezuela, was released in Australia in 1973 (Anon 1973). It is grown for forage and seed in northern Australia (McKeague, Miller and Anning 1978) and is being tested in Thailand (D. F. Cameron personal communication). In southern Florida, indigenous *S. hamata* accessions are presently being evaluated for forage (Brolmann 1974a, 1974b).

Colletotrichum leaf spot and stem canker, caused by *C. gloeosporioides* (Penz.) Sacc., can severely damage seed crops of "Verano" in Australia (Irwin and Cameron 1978) and moderately to severely affect indigenous *S. hamata* accessions in Florida (Lenné and Sonoda 1978c). In Florida, *C. dematium* f. sp. *truncata* (Schw.) v. Arx also affects *Stylosanthes*. *C. gloeosporioides* has been found on *S. scabra* seed in Colombia (Ellis *et al.* 1976), and pathogenic *C. dematium* f. sp. *truncata* and *C. gloeosporioides* have been isolated from seed of *S. guianensis, S. hamata, S. humilis, S. scabra* and *S. subsericea* in Florida, and from seed of *S. hamata* cv. Verano, *S. scabra* and *S. viscosa* harvested in Australia (Lenné 1977, J. M. Lenné, unpublished data).

Persistence of a stand is one of the most sought after attributes in a pasture legume (Brolmann 1977). Regeneration from seed is one of the means by which a stand persists. Factors affecting seed germination, seedling emergence and survival affect stand persistence. The study reported here was conducted to determine the effect of *Colletotrichum* spp. on seedling emergence, growth and survival.

MATERIALS AND METHODS

Isolates of Australian *C. dematium* f. sp. *truncata* and *C. gloeosporioides* were obtained from seed of *S. hamata* cv. Verano, harvested on the Atherton Tablelands, Australia in 1976. Isolates of Florida *C. dematium* f. sp. *truncata* and *C. gloeosporioides* were obtained from lesions on *S. hamata* CPI 38843 asd *S. hamata* IRFL 7303, respectively, growing in field plots at the Agricultural Research Center, Fort Pierce.

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TABLE 1

Effect of seed inoculations of Colletotrichum *spp. on emergence, survival and growth of* Stylosanthes hamata 7303†

Treatment	Emergence %	Emergence and survival %	Root length cm	Plant height cm	Root dry weight mg	Shoot dry weight mg
1) *Germinated seed; dipped in inoculum.*						
Control	67 a§	67 a	6.1 a	4.3 a	20.5 a	182.1 a
C. gloeosporioides F‡	49 b	7 d	3.0 a	2.3 b	2.1 d	16.3 e
C. gloeosporioides A	59 ab	27 c	5.9 a	5.2 a	14.6 b	95.2 c
C. dematium F	59 ab	48 b	6.1 a	5.2 a	8.7 c	76.6 d
C. dematium A	48 b	41 b	6.2 a	5.4 a	20.6 a	120.9 b
2) *Ungerminated seed; soaked in inoculum* 1 *hour.*						
Control	60 a	59 a	5.8 a	5.4 a	20.0 a	168.6 a
C. gloeosporioides F	21 b	14 b	3.9 a	3.7 a	2.2 b	20.6 c
C. gloeosporioides A	21 b	19 b	5.1 a	5.4 a	2.3 b	38.6 bc
C. dematium F	30 b	24 b	5.1 a	6.6 a	5.0 b	78.8 b
C. dematium A	20 b	17 b	3.8 a	3.8 a	3.2 b	38.5 bc
3) *Ungerminated seed; soaked in inoculum* 16 *hours.*						
Control	73 a	73 a	5.8 a	5.2 a	30.1 a	210.6 a
C. gloeosporioides F	14 b	6 b	1.0 b	0.9 b	0.5 b	8.3 c
C. goeosporioides A	41 b	14 b	3.1 a	3.2 ab	4.2 b	28.8 bc
C. dematium F	31 b	19 b	4.3 a	3.4 ab	5.5 b	46.5 bc
C. dematium A	29 b	19 b	4.8 a	4.7 a	5.0 b	62.2 b

† ARC-FP Accession Number.
‡ F=Florida, A=Australia.
§ Numbers followed by the same letters do not differ significantly at the 5% level by Duncan's Multiple Range Test.

TABLE 2

Effect of seed inoculations of Colletotrichum *spp. on emergence, survival and growth of* Stylosanthes hamata *cv. Verano* (*ungerminated seed, soaked in inoculum for one hour*).

Treatment	Emergence %	Emergence and survival %	Root length cm	Plant height cm	Root dry weight mg	Shoot dry weight mg
Control	80 a§	80 a	7.3 ab	7.6 ab	44.1 a	376.5 a
C. gloeosporioides F‡	54 b	49 b	7.9 a	8.2 a	32.8 b	272.4 b
C. gloeosporioides A	60 b	41 b	5.7 c	5.7 c	12.7 c	124.3 c
C. dematium F	87 a	86 a	6.5 bc	7.2 b	40.9 a	352.1 a
C. dematium A	49 b	46 b	6.1 c	6.8 b	18.2 c	178.7 c

‡ F=Florida; A=Australia.
§ Numbers followed by the same letters do not differ significantly at the 5% level by Duncan's Multiple Range Test.

Suspensions of 3–6 × 10^5 spores ml^{-1} distilled water were prepared from cultures on oatmeal agar (OMA).

Seeds of *S. hamata* IRFL 7303, an indigenous Florida accession, harvested at the ARC-FP in 1976 and found to be free of *Colletotrichum* spp. by plating seed samples on OMA were mechanically scarified (Lenné and Sonoda 1978a) and subjected to three treatments. In treatment 1, seeds were dipped in suspensions of *Colletotrichum* spp. or distilled water when their radicles were 3 mm or longer. In treatment 2, ungerminated seeds were soaked in inoculum or sterile distilled water in Petri plates at 20° to 25° for 1 hr. In treatment 3, ungerminated seeds were soaked as in treatment 2 for 16 hr. Seeds of "Verano" found to be free of *Colletotrichum* spp. by random sampling were subjected to treatment 2.

Treated seeds were planted in Jiffy Mix (a 1:1 peat moss: vermiculite mixture containing plant nutrients) in Styrofoam cups. Seven seeds were planted in separate 6 to 8 mm deep holes to which *Rhizobium* had been added. The seeds were loosely covered with Jiffy Mix. There were five replicates of two cups each per treatment. The cups were placed in a greenhouse at 18 to 25° C and 50–70% humidity. Seedling emergence was recorded up to 10 days, and survivors were counted 35 days after planting. Seedlings were periodically checked for disease symptoms. After 35 days, surviving seedlings were removed from cups and roots rinsed free of Jiffy Mix. Plant height and root length were measured and dry weights of roots and shoots were determined.

RESULTS AND DISCUSSION

Colletotrichum spp. inoculated on seed of *S. hamata* affected emergence, survival and seedling growth (Tables 1 and 2). Florida *C. gloeosporioides* was usually the most severe fungal isolate on *S. hamata* 7303 (Table 1) while Australian *Colletotrichum* spp. were more severe on Verano stylo than the Florida isolates (Table 2). Similar differences in pathogenicity were obtained in previous tests on seedlings of these *S. hamata* accessions when inoculated with the same fungi (Lenné and Sonoda 1978b). Dipping germinated seed in inoculum affected emergence less than soaking ungerminated seed in the same inoculum (Table 1), but all treatments significantly reduced survival of seedlings. In general, root length and plant height were not significantly reduced by *Colletotrichum* spp. (Tables 1 and 2). On the other hand, root ond shoot dry weights were considerably reduced.

Lesions on both *S. hamata* accessions were typical of *Colletotrichum* damage with pale centres and dark margins (Lenné and Sonoda 1978b, 1978c). *Colletotrichum* spp. were readily isolated from these lesions. Seedlings emerging with large (2–4 mm) cotyledonary lesions and apex lesions died within 35 days. Seedlings with small (0.5–1 mm) cotyledonary lesions generally survived. Lesions also developed on the first trifoliate leaf but rarely on subsequently formed leaves.

The results of this work suggest that persistence of some *Stylosanthes hamata* may be reduced by the presence of seed-borne *Colletotrichum* spp. Tests in the field are needed to confirm this.

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REFERENCES

ANON. (1973)—Introducing Caribbean Stylo. *Rural Research* No. 82: 7-10.
ANON. (1976)—Annual Report, Centro Internacional de Agricultura Tropical. CIAT, Cali, Colombia. pp. C5-C14.
BROLMANN, J. B. (1974a)—Growth studies in some new *Stylosanthes hamata* (L). selections. Ft. Pierce ARC Research Report. RL-1974-6. 4pp.
BROLMANN, J. B. (1974b)—The adaptability of *Stylosanthes hamata* (L.) Taub. to dune soils and Ona fine sand. Ft. Pierce ARC Research Report. RL-1974-9. 5pp.

BROLMANN, J. B. (1977)—Persistence studies in *Stylosanthes species*. *Proceedings of the Soil and Crop Science Society of Florida* **36**: 144-147.

ELLIS, M. A., FERGUSON, J. E., GROF, B. and SINCLAIR, J. B., (1976)—Transmission of *Colletotrichum gloeosporioides* and the effect of sulphuric acid scarification on internally-borne fungi in seeds of *Stylosanthes* spp. *Plant Disease Reporter*. **60**: 844-846.

IRWIN, J. A. G. and CAMERON, D. F. (1978)—Two diseases of *Stylosanthes spp.* caused by *Colletotrichum gloeosporioides* in Australia, and pathogenic specialization within one of the causal organisms. *Australian Journal of Agricultural Research* **29**: 305-317.

LENNE, J. M. (1977)—*Colletotrichum dematium* on *Stylosanthes* spp. Australian Plant Pathology Society Newsletter **6**: 53-54.

LENNE, J. M. and SONODA, R. M. (1978a)—*Rhizopus stolonifer* (Ehr. ex Fr.) Lind., a seed-borne fungus of *Stylosanthes hamata* (L.) Taub. in Florida. *Proceedings of the Soil and Crop Science Society of Florida* **37**: (In press).

LENNE, J. M. and SONODA, R. M. (1978b)—Occurrence of *Colletotrichum dematium* f. sp. *truncata* on *Stylosanthes* spp *Plant Disease Reporter* **62**: 641-644.

LENNE, J. M. and SONODA, R. M. (1979)—The occurrence of *Colletotrichum* spp. on *Stylosanthes* spp. in Florida and the pathogenicity of Florida and Australian isolates to *Stylosanthes* spp. *Tropical Grasslands* **13**: 98-105.

MCKEAGUE, P. J., MILLER, C. P. and ANNING, P. (1978)—Verano . . . A new stylo for the dry tropics. *Queensland Agricultural Journal* **104**: 31-34.

SONODA, R. M., KRETSCHMER, A. E. and BROLMANN, J. B. (1974)—Colletotrichum leaf spot and stem canker on *Stylosanthes* spp. in Florida. *Tropical Agriculture (Trinidad)* **51**: 75-79.

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