PROCEEDINGS OF THE 5TH INTERNATIONAL SYMPOSIUM OF PARASITIC WEEDS

Nairobi, Kenya.
24-30 June 1991

J. K. Ransom, L. J. Musselman,
A. D. Worsham, and C. Parker
editors

Sponsored by:

The International Maize and Wheat Improvement Center (CIMMYT), the Kenya Agricultural Research Institute (KARI), the International Parasitic Seed Plant Research Group (IPSPRG) the Pan African Striga Control Network (PASCON), and Old Dominion University, Norfolk, Virginia.

25 years of CIMMYT

1966 - 1991

CIMMYT is an internationally funded, nonprofit scientific research and training organization. Headquartered in Mexico, the Center is engaged in a worldwide research program for maize, wheat, and triticale, with emphasis on improving the productivity of agricultural resources in developing countries. It is one of 13 nonprofit international agricultural research and training centers supported by the Consultative Group on International Agricultural Research (CGIAR), which is sponsored by the Food and Agriculture Organization (FAO) of the United Nations, the International Bank for Reconstruction and Development (World Bank), and the United Nations Development Programme (UNDP). The CGIAR consists of a combination of 40 donor countries, international and regional organizations, and private foundations. CIMMYT receives core support through the CGIAR from a number of sources, including the international aid agencies of Australia, Austria, Brazil, Canada, China, Denmark, Germany, Finland, France, India, Iran, Ireland, Italy, Japan, Mexico, the Netherlands, Norway, the Philippines, Spain, Switzerland, the United Kingdom, and the USA, and from the European Economic Commission, Ford Foundation, Inter-American Development Bank, OPEC Fund for International Development, UNDP, and World Bank. CIMMYT also receives non-CGIAR extra-core support from Belgium, the International Development Research Center, the Rockefeller Foundation, and many of the core donors listed above.

Correct Citation:

Ransom, J.K., L.J. Musselman, A.D. Worsham, and C. Parker, eds. 1991. Proceedings of the 5th International Symposium of Parasitic Weeds. Nairobi: CIMMYT.

How to cite a paper in the volume (example):

Friesen, G.H. and G.R. Korwar. 1991. Effect of phenolic acids and mixed cropping on *Striga asiatica* infestations in sorghum. pp. 10-13 in Ransom J.K., L.J. Musselman, A.D. Worsham, and C. Parker (eds). Proceedings of the 5th International Symposium of Parasitic Weeds. Nairobi: CIMMYT.

ISBN 968-6127-58-5

RESEARCH INTO GERMINATION OF *STRIGA* SEED BY SORGHUM ROOT EXUDATES *

D. E. Hess [1], G. Ejeta [2] and L. G. Butler [3]

This research was undertaken to develop a laboratory assay to permit screening of sorghum lines for low production of sorgoleone, the first germination stimulant of *Striga* seed to be isolated from host root exudate. Our findings led us to assay for other substances in sorghum root exudate which control germination of *Striga* seed. A convenient petri dish assay, described elsewhere, permits host selection for low stimulation of germination. Work to isolate, assay and characterize these compounds is underway at Purdue University.

INTRODUCTION

Witchweeds (*Striga* spp. Lour.) are angiospermous obligate root parasites of many important cereals and legumes. As a result of successful adaptation to the parasitic habit witchweeds produce abundant, tiny, long-lived seed that generally do not germinate unless aged, conditioned and stimulated by an exogenous germination stimulant (Worsham and Egley, 1990). Stimulants are exuded by the roots of host and non-host plants (Doggett, 1988). Germination of *Striga* seed is stimulated by other compounds (Visser, 1989) which may occur widely in nature (Dale and Egley, 1971).

Two witchweed species, *S. hermonthica* (Del.) Benth. and *S. asiatica* (L.) Kuntze, cause serious yield losses to sorghum (*Sorghum bicolor* (L.) Moench) and pearl millet (*Pennisetum glaucum* (L.) R. Br.) in the semi-arid tropics (Doggett, 1982; Gilliver, et al., 1985). Breeding resistant genotypes is the most promising approach to reducing these losses but requires effective screening techniques. The complex interactions between host, parasite and environment which influence establishment on the host root and subsequent growth have slowed the development of reliable methods for large-scale screening of host genotypes in the field. Vasudeva Rao (1987) has discussed the laboratory and field methodologies currently used.

The best-characterized of the several mechanisms of resistance to *Striga* which have been proposed (Doggett, 1988) is unusually low production by host plant roots of compounds stimulating germination of *Striga* seed (Vasudeva Rao, 1987). "Sorgoleone" is the first *Striga* seed germination stimulant to be isolated and identified from a natural host (Netzly and Butler, 1986; Chang et al., 1986; Netzly et al., 1988). We have since investigated the production of sorgoleone by a variety of sorghum cultivars and report here the apparent lack of relationship between sorgoleone production under optimum conditions in the laboratory and susceptibility/resistance to *Striga* in the field.

Sorgoleone, a series of four alkyl-substituted benzoquinones active in their reduced hydroquinone form, are chemically unstable and virtually insoluble in water. Thus they do not account for the more-stable and water-soluble germination stimulants previously reported to be exuded by sorghum roots, as summarized by Egley (1990).

An assay utilizing preconditioned *Striga* seeds embedded in water agar was developed by Riopel and Baird (1987) to investigate germination, radicle elongation and haustorial initiation in *Striga*. We have adopted this technique in order to more effectively screen for the capacity of water-soluble root exudates to stimulate germination of *Striga* seeds, rather than assay for a particular stimulant such as sorgoleone (Hess et al., 1991). In order to study the production of water-soluble root exudates and obtain samples for analysis we have modified the double pot technique first described by Parker et al. (1977) for identification of low stimulant producing

---

Approved as a conference paper (CP619) by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), B.P. 12404, Niamey, Niger.

1. *ICRISAT Sahelian Center, B.P. 12404, Niamey, Niger*
2. *Agronomy Department, Purdue University, West Lafayette, IN 47907, USA*
3. *Biochemistry Department, Purdue University, West Lafayette, IN 47907, USA*

sorghum lines. Both techniques mimic soil conditions more closely than the sorgoleone assay because the host roots are in a moist substrate rather than in air.

SORGHUM ROOT EXUDATES

Sorgoleone. Sorgoleone is exuded on root hair tips of sorghum seedlings grown in the dark in Petri dishes (Netzly et al., 1988). We observed that excessive moisture on the filter paper substrate strongly inhibits sorgoleone production by the roots of three- to seven-day-old sorghum seedlings. Sorgoleone production was therefore carefully evaluated under identical levels of moisture for all samples tested. The amount of sorgoleone produced by differing sorghum genotypes was the same within a factor of two (Table 1). Sorgoleone production under these conditions does not correlate with host reaction to *Striga* in the field. As a result we have not further refined or simplified the method of screening for sorgoleone production.

Water-soluble exudates. When grown in water agar containing preconditioned *Striga* seeds, sorghum genotypes germinating a large proportion of the *Striga* seeds also germinated seeds much farther away from the host root than those causing the germination of only a few *Striga* seeds. Between- and within-line variability of germination distance was studied using one resistant and two susceptible sorghum cultivars (Table 2). Variability observed was primarily due to differences between lines and not to variation within lines. To test the reproducibility of the assay over days, we tested 43 F_6 progenies from the cross SRN39 X P954063. Two samples from each progeny and of both parents were tested at intervals of one week for four weeks. The average germination distance for weeks 1-4, respectively, was 0.86, 1.16, 1.37, 0.99 cm (S.E. difference = 0.08), indicating that significant differences existed among weeks. Progeny differences for stimulation of *Striga* seed germination were also found (Table 3), allowing their separation into high or low classes.

Measuring the interval between the host root and the most distant germinated *Striga* seed is more rapidly accomplished than determining percent germination of *Striga* seed. Since germinated and ungerminated *Striga* seeds are not counted, results are not influenced by percent viability of *Striga* seed samples. The short time period of 72 hours required for the assay permits the screening of large numbers of seeds. These features constitute a major improvement over previous techniques (Vasudeva Rao, 1987). This assay, unlike the one recently described by Ramaiah et al. (1990), makes possible the screening of individual seedlings, a prerequisite for evaluation of early generation breeding progenies.

The agar gel assay reveals large differences in the capacity of these sorghums to stimulate *Striga* seed germination in contrast to their rather uniform production of sorgoleone (Table 1). These results indicate that the assay is measuring not the production of sorgoleone, but *Striga* seed germination due to other as yet unidentified stimulants whose production is favored by the aqueous medium.

Despite reports to the contrary (Fate et al., 1990; Lynn and Chang, 1990), we have been unable to demonstrate the production of sorgoleone in agar gels. This is presumably attributable to inhibition by the aqueous medium of sorgoleone production.

It is important to understand that the agar gel assay does not specifically assay germination stimulant production but is a measure of the many interactions of host root and conditioned *Striga* seed which result in germination of the seed. These interactions may involve production by the host root of compounds that stimulate or inhibit *Striga* seed germination, stability and mobility of the chemical signals, and specificity of receptor sites on the seed, any or all of which may be altered by the environment. For example, the incubation temperature of the assay strongly influences the results. Greater levels of germination were observed at 30 °C than at 27 °C for the sorghums studied (Table 4). Susceptible and resistant cultivars were more easily differentiated at 27 °C than at 30 °C (Table 4).

The large differences in capacity of the different genotypes to stimulate *Striga* seed germination (Table 1) correlate well with resistance and susceptibility to *Striga*. Sorghums reported to be susceptible to *Striga* all stimulated germination at distances greater than 1 cm from the host root. Most resistant cultivars stimulated germination no more than 1 cm from the root at 27 °C. High stimulation of *Striga* seed germination by resistant cultivars N13 and P967083 supports previous reports that their resistance is due to other mechanisms (Maiti et al., 1984; Cherif-Ari et al., 1990).

The utility of the assay was investigated by testing 62 F_6 progenies from intercrosses among SRN39 (resistant), Framida (resistant) and P954063 (susceptible), selected under *Striga*-free conditions on the basis of yield and grain quality. The results are shown in Table 5. Low stimulant producing progeny resulted from crosses between two low stimulant lines, whereas progenies from low X high stimulant genotypes included varying numbers of low and high stimulant producers.

A subsequent field test of these same progenies provided preliminary evidence that low *Striga* seed germination in the laboratory assay corresponds very closely to field resistance. The Petri dish assay for *Striga* seed germination is an effective screening technique allowing selection of materials to be tested in naturally-infested fields for agronomic adaptation and reaction to *Striga* attack.

The agar gel assay has significant advantages over previous assays for characteristics contributing to *Striga* resistance (Vasudeva Rao, 1987). Minimal equipment is required for the rapid screening of large numbers of

seedlings. The technique is carried out on individual seeds, which, in locations where quarantine of *Striga* seed is not a concern, can be removed from the agar plates following the assay and transplanted into soil. We obtained a survival rate of 100% for seedlings recovered from *Striga*-free agar plates after 72 hr. Although our data is not yet complete on this point, the screening technique should work as well on other crops which are hosts for *Striga*.

ACKNOWLEDGEMENTS

This work was partially supported by USAID Grant No. DAN 1254-G-00-0021-00 through INTSORMIL, the International Sorghum and Millet CRSP and by Grant No. GA AS 8905 from the Rockefeller Foundation. We are grateful to Dr. J. Riopel and Bruce Deipe for invaluable assistance.

REFERENCES

Chang, M., D. H. Netzly, L. G.Butler, and D. G. Lynn. 1986. Chemical regulation of distance: Characterization of the first natural host germination stimulant for *Striga asiatica*. Journal. of the American Chemical Society 108: 7858-7860.

Cherif-Ari, O., T. L. Housley, and G.Ejeta. 1990. Sorghum root length density and the potential for avoiding *Striga* parasitism. Plant and Soil 121: 67-72.

Dale, J. E. and G. H. Egley. 1971. Stimulation of witchweed germination by run-off water and plant tissues. Weed Science 19: 678-681.

Doggett, H. 1982. Factors reducing sorghum yields - *Striga* and birds. pp 313-320 in ICRISAT. Sorghum in the Eighties, Vol. I. ICRISAT Patancheru P.O.: International Crops Research Institute for the Semi-Arid Tropics.

Doggett, H. 1988. Sorghum (2nd Ed.). New York: John Wiley & Sons.

Egley, G. H. 1990. Isolation and identification of witchweed seed germination stimulants from natural sources. pp 37-45 in Sand, P.F., Eplee, R.E., and Westbrooks, R.G. Witchweed Research and Control in the United States. Champaign: Weed Science Society of America.

Fate, G., M. Chang, and D. G. Lynn. 1990. Control of germination in *Striga asiatica*: Chemistry of spatial definition. Plant Physiology 93: 201-207.

Gilliver, B., M. J. Vasudeva Rao and P. Venkateswarlu. 1985. A design and methods of analysis to monitor crop growth conditions illustrated with sorghum screening trials for resistance to *Striga*. Experimental Agriculture 21:233-240.

Hess, D. E., G. Ejeta and L. G. Butler. 1991. A simple assay for selecting *Striga*-resistant sorghum genotypes. (Submitted to Phytopathology).

Lynn, D. G., and M. Chang. 1990. Phenolic signals in cohabitation: Implications for plant development. Annual Review of Plant Physiology and Plant Molecular Biology 41: 497-526.

Maiti, R. K., K. V. Ramaiah, S. S. Bisen and V. L. Chidley. 1984. A comparative study of the haustorial development of *Striga asiatica* (L.) Kuntze on sorghum cultivars. Annals of Botany 54: 447-457.

Netzly, D. H. and L. G. Butler. 1986. Roots of *Sorghum bicolor* exude hydrophobic droplets that contain biologically active materials. Crop Science 26: 775-778.

Netzly, D. H., J. L. Riopel G. Ejeta and L. G. Butler. 1988. Germination stimulants of witchweed (*Striga asiatica*) from hydrophobic root exudate of sorghum (*Sorghum bicolor*). Weed Science 36: 441-446.

Parker, C., A. M. Hitchcock and K. V. Ramaiah. 1977. The germination of *Striga* species by crop root exudates: techniques for selecting resistant crop cultivars. pp 67-74 in Proceedings of the Sixth Asian-Pacific Weed Science Society Conference. Jakarta: Asian-Pacific Weed Science Society.

Ramaiah, K. V., V. L.Chidley, and L. R.House. 1990. Inheritance of *Striga* seed-germination stimulant in sorghum. Euphytica 45: 35-38.

Riopel, J. L. and Wm. V. Baird. 1987. Morphogenesis of the early development of primary haustoria in *Striga asiatica*. pp 107-125 in Musselman, L.J. Parasitic Weeds in Agriculture I. *Striga*. Boca Raton: CRC Press.

Worsham, A. D., and G. H. Egley. 1990. Physiology of witchweed seed dormancy and germination. pp 37-45 in Sand, P.F., Eplee, R.E., and Westbrooks, R.G. Witchweed Research and Control in the United States. Champaign: Weed Science Society of America.

Vasudeva Rao, M. J. 1987. Techniques for screening sorghums for resistance to *Striga*. pp 281-304 in Musselman, L.J. Parasitic Weeds in Agriculture I. *Striga*. Boca Raton: CRC Press.

Visser, J. H. 1989. Germination requirements of some root-parasitic flowering plants. Naturwissenschafter 76:253-261.

Table 1: Stimulation of *Striga* seed germination by sorghum.

Cultivar	*Striga* reaction [1]	Agar Gel Assay distance (mm)[2]	Sorgoleone A_{284}/g dry root
P954063	S	22	15.7
Babadia Fara	S	29.5	16.4
SRN39	R	0	26.4
Framida	R	4.3	21.0
N13	R	27	17.8
P967083	R	27	17.7
S.E.		3.0	
CV (%)		17.3	

1: S = susceptible, R = resistant.

2: Maximum distance from the host root at which *Striga* seed germinated.

Table 2: Within-line variation of three sorghum lines for the agar gel assay.

	Germination Distance, cm [1]			
Line	minimum	maximum	mean [2]	S.E.
SRN39	0	1.90	0.71	0.134
P954063	1.15	2.30	1.80	0.096
Shanqui Red	1.50	3.30	2.40	0.136

1: Maximum distance from the host root at which *Striga* seed germinated.

2: Average of 15, 15 and 13 replications, respectively, for SRN39 P954063 and Shanqui Red.

Table 3: Stimulation of *Striga* seed germination by 43 sorghum progenies from the cross SRN39 X P954063.

	Germination Distance [1]
Progeny minimum	0.09 cm
Resistant parent mean	0.24 cm
No. progeny < 1 cm [2]	19
Susceptible parent mean	1.78 cm
Progeny maximum	2.10 cm
Progeny mean	1.10 cm
Standard deviation	0.65

1. Maximum distance from the host root at which *Striga* seed germinated.
2. Germination distance for high and low classes r 1 cm and < 1 cm, respectively.

Table 4: Effect of genotype and incubation temperature on germination of *Striga asiatica* seed embedded in water agar.

Temperature	Line	Germination distance [1] minimum	maximum	mean [2]	S.E.
27 °C	SRN39	0	0.9	0.14	0.070
	Framida	0	0.6	0.04	0.040
	P954063	1.15	2.30	1.80	0.096
30 °C	SRN39	0	1.70	0.93	0.118
	Framida	0.60	2.00	1.35	0.125
	P954063	1.45	3.30	2.35	0.133

1: Maximum distance from the host root at which *Striga* seed germinated.
2: Average of 15 Petri dishes.

Table 8: Stimulation of germination of *Striga asiatica* seed embedded in water agar by water soluble root exudates from F_8 progenies of intercrosses among resistant (SRN39 and Framida) and susceptible (P954063) sorghum lines.

Cross	Germination distance, cm [1]						
	0.25	0.75	1.25	1.75	2.25	2.75	3.25
SRN39 X P954063	120 [2]	36	67	65	38	14	40
Framida X P954063	23	4	3	1	1	0	0
Framida X SRN39	20	0	0	2	0	0	0

1: Maximum distance from host root at which *Striga* seed germinated.

2: Number of plants.