AGRIVITA VOLUME 34 No. 3 *OCTOBER – 2012* *ISSN : 0126-0537*

Striga hermonthica SEED GERMINATION THROUGH ROOT EXUDATES OF INDIGENOUS SUB-SAHARAN WEED SPECIES

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Received: July 29, 2012/ Accepted: October 12, 2012

ABSTRACT

This study was conducted to evaluate root exudates from sub-Saharan indigenous weed species to induce germination of *Striga hermonthica* (Del.) Beth., a root parasitic weed. Significant variation in Striga seed germination was observed, ranging from an absence to the induction of 74.1% Striga seeds. Direct comparison of Striga germination was obscured by differences in weed root biomass as within most of the species, a direct proportional relation between Striga seed germination and weed root dry weight was observed. Expression of Striga seed germination in % g^{-1} root dry weight *(GIC)* was found a suitable solution as stable values for *GIC* were obtained despite considerable variation in root dry weight. GIC was significant for 25 species and highest with *Commelina forskalaei* and *Sesamum alatum* (9.91; 9.78 % g^{-1} dry root, respectively). Striga seeds did not germinate following application of exudates from *Mitracarpus scaber* and *Phyllanthus pentrandus*. These results show that a substantial number of indigenous weed species may serve as alternative trap crops to control the parasites seed bank. Furthermore, the timing of weeds in the cropping system may provide a (partial) explanation for the erratic infestation levels found across fields and years that have dazed researchers for many years.

Keywords: *Striga hermonthica,* parasitic weeds, seed germination, Sub-Saharan indigenous weeds, root exudates, seed bank

INTRODUCTION

Root parasitic weeds of the genus *Striga* constitute a major biotic constraint to cereal production in sub-Saharan countries, in particular pearl millet, sorghum and maize (Yonli *et al*., 2010). *Striga hermonthica* (Del.) Benth., infests the major cereal grains and average yield losses of 25-40% could occur but complete crop failure under drought is not uncommon (Hess *et al*., 2001). A single Striga plant can produce up to 500.000 seeds which can remain viable for more than 14 years (Bebawi *et al*., 1984). This has led to the build-up of a large seed bank reserve of Striga seeds in contaminated soils.

The germination of the parasite seed is such that, fully after-ripened seeds must first undergo a period of imbibed storage in a warm environment to become sensitive to stimulants produced by host plants (Logan and Stewardt, 1995). This process of sensitization is generally termed as conditioning (Magnus *et al*., 1992). Germination of the parasite was initially believed a host specific step following from specific metabolites present in host plant root exudates. Striga hosts exude a combination of three or more stimulatory compounds (Siame *et al*., 1993) which are collectively called *"Strigolactones"* (Butler. 1995). The adaptation of obligate parasitic weeds to respond to host plant excreted germination stimulants which provide them with an evolutionary benefit that ensures the seeds only to germinate in the vicinity of active, viable host plant roots. More recent studies have shown that germination of Striga is not host specific but showed that not only do wild ancestors of sorghum and millet induce Striga seed germination (Kuiper, 1997; van Mourik, 2007), but also non-host plants, including some tree species (Ma *et al.*, 2004; Marley *et al*., 2004;

Accredited SK No.: 81/DIKTI/Kep/2011

http://dx.doi.org/10.17503/Agrivita-2012-34-3-p296-302

Yonli *et al*., 2010). Most of these non-host plants do not permit attachment of the parasite to their roots with consequence that germinated Striga seeds are not able to survive and reproduce. This process, often referred to as suicidal germination, contributes to the reduction of the Striga seed population in the soil and may provide 1) an alternative to conventional trap crop varieties and 2) a (partial) explanation to the parasites erratic infestation across fields and years.

Rainfall, Weed Growth and the Timing of Crop Planting

First rains in 2007 at ICRISAT Sahelian Centre, Niger, were highly erratic and were observed first in late April. Only 80 days later, in mid-July, a severe rain event (>20 mm day^{-1}) produced sufficient water to initiate crop planting on a large scale. Such delayed planting of the main food crop has repeatedly been associated with the occurrence of so called 'non-Striga' years; years in which the incidence of the parasite is very low and negligible compared to its incidence in regular years (Vallance, 1950; Babiker *et al.,* 1994; Hess and Williams, 1994; Bielders and Michels, 2002; Gressel *et al.,* 2004; Samake *et al.*, 2005; pers. obs.) In hindsight, it was noted that the 2007 delayed planting was associated with markedly low Striga infection levels (*Pers. Comm*. B.I.G. Haussmann). At the moment of crop planting, field weeds had been growing vigorously on residual rain (*Pers. obs.*) as fields were only cleared from weeds just before crop planting. The presence of weeds may have evoked large scale germination of Striga seeds. Such sanitation of the Striga soil seed bank at this particular time would provide a sound explanation for the high correlation between late crop planting and non-Striga years.

To what extent weed species are capable of producing Striga germination stimulants and whether large differences occur between the weed species are not known. A substantial number of non-host plants are known to induce Striga seed germination but very little is known regarding the Striga seed inducing germination potential of indigenous field weeds. The main objective of this study was to examine root exudates of common field weeds for their ability to stimulate Striga seed germination.

MATERIALS AND METHODS

Striga Seed Material

Striga seeds used in this study were harvested in 2003 from a sorghum field in Kouli, Mali and had been kept in a glass container under dark and dry conditions at 24°C since then.

Plant Material

In 2007, an experimental field at ICRISAT, Sadore, Niger was used for collection of 27 weed species. Weeds were collected on the 21st of June. To standardize the size of the plants as much as possible, plants with a height or length of approximately 10 cm were selected and exhumed from the soil. During exhumation, root damaging was avoided as much as possible. Because of the high temperatures, the exhumed plants were stored in a plastic bag and brought to the laboratory within 1.5 hour.

Root Exudates Collection

Collection of root exudates followed a modified method instead of that described by Kröschel (2001). After exhumation of weed plants, individual plants were planted in 200 ml pots filled with pure sand. Plants were kept in a laboratory for five days and covered with a transparent plastic bag to prevent transpiration loss. Average temperature in the laboratory was 24 ºC. Plastic bags were removed daily for 5 minutes for watering with regular tap water. On the 6th day, the plastic cover was removed and plants were watered every other day. On day 14, plants were removed from the pots and roots were gently washed with tap water. Roots of each plant were then immersed in a little glass pot of 100 ml, containing distilled water. On the rim of the pots, shoots were supported by a little strap of non-absorbent cotton that was wrapped around the stem under the first leaf axial. The water level in the pot was maintained at 100 ml by daily filling with dH_2O. On the third day, plants were removed from the pots and the pots were refilled with dH_2O to 100 ml and covered with aluminium foil. Pots were then stored at 5°C in the refrigerator and used in germination assay the same day. Plant roots were oven dried for 42 hours at 60°C and weighted on a balance.

Striga Seed Cleaning, Pre-conditioning and Germination Bio-Assay

160 mg of Striga seeds were surface sterilized by placing the seeds in a 50 ml flask containing 25 ml of a 1% natrium hypochlorite solution, after which the flask was gently swirled 3 to 4 times per minute. After three minutes the suspension, including seeds, was poured onto a 15 cm diameter folded filter (Schleider and Schuell GmbH, Germany). The seeds were then rinsed eight times with 10 ml dH_2O and exposed to room temperature (circa 24°C) to dry for 24 hours.

100 Striga seeds per sample were spread on a 3.0 cm diameter glass fibre filter paper including a cross (4 quarter segments) to simplify the counting of germinated seeds in a later stage. Each filter was put into a sterile, 9 cm diameter, Petri-dish lined with a water lock cut from a 9 cm diameter filter paper (Schleicher and Schuell GmbH, Germany) to prevent water vapour escaping from the Petri-dish. Both, the glass fibre filter and the water lock were wetted with 0.9 ml dH_2O after which the Petri-dishes were wrapped with parafilm. Conditioning took place in a climate room for 16 days at 30 °C in darkness.

To assess the germination inducing capacity of the collected weed root exudates, 3 ml of exudates solution was equally spread over three glass fibre filters containing 16 days pre-conditioned Striga seeds. 3 ml control treatments GR24 or dH_2O were applied. Petri-dishes were incubated in a climate room for 96 hours at 30°C in the dark. After that, filters with seeds were lifted with tweezers from the Petri-dish and put onto a 15 cm filter paper for one minute to absorb excessive moisture. Germinated seeds were counted by use of a microscope (40 × magnification). Germination was considered when a little whitish radicle had protruded the seed coat.

Experimental Set-up

The experiment was carried out as a randomized complete block design with root exudates from 27 weed species, 6 control treatments and three-time replication. Control treatments included five different concentrations of the synthetic germination stimulant GR24 (0.001, 0.01, 0.1, 1.0 and 5.0 mg L^{-1}) and one treatment with dH_2O.

Statistical Analysis

Analysis of variance was carried out on the variables maximum germination percentage *(G_{max})* and GICper weed species. Maximum germination percentage was considered when Striga seed germination in Petri-dishes stabilized. Before analysis with GenStat (12th Ed. Rothamsted), an angular transformation on both variables *(G_{max}; GIC)* was carried out to normalize the variance (Gomez and Gomez, 1984),

$$Y(x) = Arc\sin e\sqrt{\frac{B}{100}}$$

Where, *B*is the value obtained from G_{max}or *GIC.* Comparison of the data was based on the transformed scale. Means were separated using Duncan Multiple Range Test and differences between means were considered significant at $p<0.05$. $G_{max}$and*GIC* data presented in this paper were back transformed.

RESULTS AND DISCUSSION

Stimulation of StrigaSeed Germination

In the current work, it was found that a substantial number of weed species, common to sub-Saharan African fields, were able to trigger seed germination of *Striga hermonthica.* These findings confirm earlier work by Akiyama *et al.* (2005) who indicated that *Striga*stimulating exudates might be produced by a wider spectrum of plant species than the limited number of host and non-host plants identified this far.

The Striga seeds used in this study showed a good viability which was reflected in control treatments with the synthetic germination stimulant GR24. Seeds treated with GR24 alone showed a gradual declining germination response with decreasing GR24 concentrations. 71.7, 69.1, 60.4, 41.5 and 5.9 % germination followed from application of 5.0, 1.0, 0.1, 0.01 or 0.001 mg L^{-1} GR24 (Figure 1A). No Striga germination followed from application of dH_2O.

Root exudates from 25 species significantly induced Striga seed germination. The highest stimulatory effects on Striga seeds resulted from application of *Digitaria ciliaris* (74.1%) which exceeded the stimulatory effect of

the highest concentrations GR24 at 5.0 and 1.0 mg L^{-1} (Table 1).

Exudates from *Commelina forskalei, Sesamum alatum, Eleusineindica, Brachiaria distichophylla, Echinochloa crussgalli* stimulated Striga seed gerimination over 60%. Two species, *Mitricarpus scaber* and *Phyllanthus pentrandus* showed no stimulatory effects on Striga seeds.

The Role of Weed Root Biomass

To understand the role of weed root biomass in relation to inducing seed germination, Striga seed germination and weed root dry weight of individual plants were plotted against one another (Figure 1A). Within most of the species a proportional increase of Striga seed germination with weed root dry weight was observed. This observation implied that direct comparison of Striga seed stimulation between weed species was obscured by differences in their root dry weight. For this reason, Striga seed germination was expressed per gram of weed root dry weight (*GIC*; Figure 1B).The visual output illustrated that the new variable *GIC* was almost independent of weed root dry weight and therefore much better suited for a comparison of the germination inducing capacity between weed species. The only exceptions were *Echinochloa crussgalli, Sesamum alatum, Eleusine indica* and *Brachiaria distichophylla* where a gradual levelling in Striga seed germination was observed with increasing root biomass. An explanation for the observed negative correlation between root biomass and Striga seed stimulationis not known and beyond the scope of this paper but one could postulate here that root growth in general may have been at the cost of other physiological processes that produce/release Striga stimulants.

Variance analyses of GIC revealed that between species differences were highly significant (P<0.001; Table 1). Among the six species that ranked the highest in *GIC*, four belonged to the family of *Poaceae*. Kuiper *et al*. (1997) pointed to the preference of Striga seeds for root exudates produced by *Poaceae* species as they share a common genetic background with their domesticated host crops like millet, sorghum and maize.

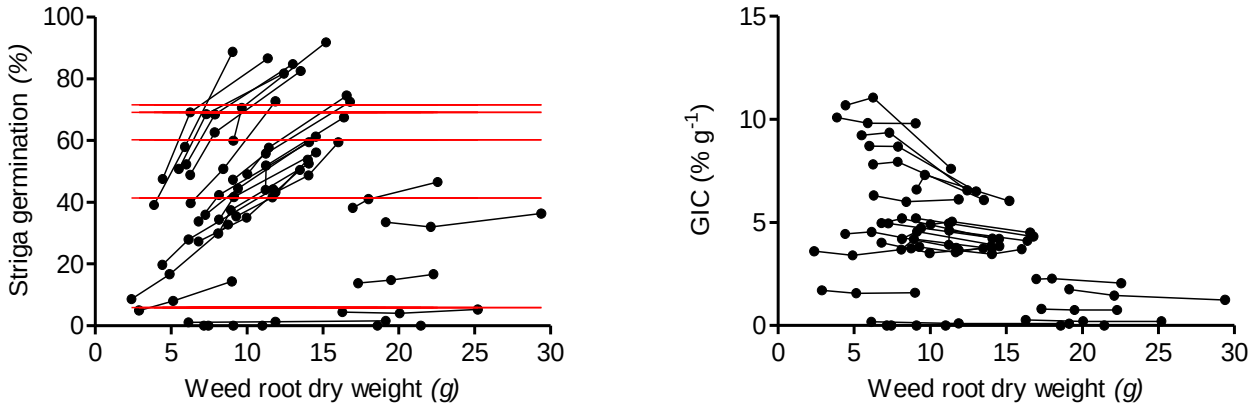

Figure 1.**(A)** Striga seed germination plotted against weed root dry weight of individual plants. Each weed species was represented by three individuals and their results were connected. Red lines indicate Striga germination following from GR24 control treatments: from bottom to top: 0.001 - 0.01 - 0.1 - 1.0 to 5.0 mg L^{-1}. **(B)** Striga seed germination expressed per gram root biomass (*GIC*) plotted against weed root dry weight for the same weed species

Table 1. Root dry weight *(R_{dw})*, induced Striga seed germination *(G)* and the Striga seed germination inducing capacity *(GIC*) of 27 weed species collected from an experimental field of ICRISAT, Sadore, Niger in 2007. Weed species were sorted in descending order of *GIC*

Botanical name	RDW (g)		G (%)		GIC (% g^{-1})	
Commelinaforskalaei	6.09	fgh	61.9	a-f	9.91	A
Sesamumalatum	7.08	e-h	67.7	abc	9.78	A
Eleusineindica	8.18	e-h	67.0	a-d	8.38	B
Brachiariadistichophylla	8.74	e-h	68.5	ab	7.96	Bc
Echinochloacruss-galli	8.97	e-h	64.6	a-e	7.28	Cd
Digitariaciliaris	11.17	c-h	74.1	a	6.64	De
Hibiscus sabdariffa	8.72	e-h	54.3	a-h	6.14	E
Pennisetumpedicellatum	12.17	b-g	58.5	a-g	4.82	F
Jacquemontiatamnifolia	12.53	b-f	60.4	a-g	4.81	Gh
Alysicarpusovalifolius	9.44	e-h	45.2	b-h	4.79	Gh
Ipomeavagans	10.18	e-h	47.2	b-h	4.63	Ghi
Indigoferastrobilifera	7.13	e-h	30.6	h-k	4.25	Ghi
Corchorustridens	12.85	b-f	54.4	a-h	4.21	Ghi
Zorniaglochidiata	10.44	d-h	44.0	c-h	4.21	Ghi
Ceratothecasesamoides	10.22	e-h	39.9	f-i	3.86	Ghi
Digitarialongiflora	9.90	e-h	37.6	g-i	3.75	Ghi
Cenchrusbiflorus	11.58	b-h	43.6	d-h	3.74	Ghi
Panicumatrosanguineum	13.95	b-e	50.5	a-h	3.60	Hi
Citrulluscolocynthis	5.02	f	19.3	i-l	3.58	I
Tephrosiagracilis	19.11	a-d	41.9	e-i	2.19	J
Fimbristylishispidula	5.40	fg	9.1	kl	1.62	Kl
Dipcadytacca	23.35	a	33.9	h-j	1.48	Kl
Merremiatridentata	19.65	abc	15.1	jkl	0.77	Lm
Merremiapinnata	20.36	ab	4.5	l	0.22	M
Cassia mimosoides	11.79	b-g	1.3	l	0.12	M
Mitracarpusscaber	11.93	b-g	0.0	l	0.00	M
Phyllanthuspentrandus	11.84	b-g	0.0	l	0.00	M
Statistical analyses						
Means	11.40		40.6		4.18	
CV%					0.69	
SE	0.91		4.6		0.57	

Remarks: Means within the same column followed by a different letter are significantly different at $p = 0.05$. R_{dw}, G and GIC means are original values

Ecological Consequences

These results pointed out that stimulation of Striga seed germination is not a host specific step. The observed germination behaviour might be characterized as opportunistic rather than host specific. This opportunistic germination strategy in the presence of weeds may enhance suicidal germination with consequence of reducing the parasites seed bank. In the presence of weeds, the parasites opportunistic germination behaviour may also provide a (partial) explanation to the erratic.

Striga infestation levels observed across years and fields (Vallance, 1951; Babiker*et al.,* 1994; Hess and Williams, 1994; Bielders and Michels, 2002; Gressel*et al.,* 2004; Samake, *et al*, 2005; pers. obs.) as common field weeds flourish on the first rains that are generally erratic and insufficient for large scale host crop planting. Postponed planting has indeed been frequently associated with low parasite severity in the field (van Ast, 2006).

Weeds and Conventional Trap Crops

Rainfall is the most important biotic factor that determines the start and ending of the growing season in the Sahel region. Here rainfall can start as early as April but can severely hamper crop production because of its unpredictability and erratic nature. The economic costs for conventional trap crop varieties to reduce the parasites seed bank, as also the current biotic constraints for farmers in the Sub-Saharan region to utilize these crops (Samake *et al*., 2006), leads to the question whether trap cropping can be achieved by alternative means. In this view, common field weeds may provide a promising alternative. The differences among weeds in their potential to induce Striga seed germination offer scope for selective weeding to maximize the trap cropping effect of the weed community.

CONCLUSION

Root exudates of a substantial number of common sub-Saharan field weeds showed stimulatory effects on Striga seed germination but strongly depended on the root biomass of the weed. Expression of Striga seed germination per gram of root dry weight *(GIC)* was found a suitable solution as stable values for *GIC* were obtained within species despite considerable variation in root dry weight. The use of local weed species by farmers may offer scope to reduce the parasites seed bank by selective weeding in the period before crop planting. In this view, but with some degree of cautiousness, the presence of common field weeds just before crop planting may provide a sound explanation for the erratic Striga infestation levels across years and fields that have dazed researchers for many years.

ACKNOWLEDGEMENTS

Many thanks go to the International Crop and Research Institute of the Semi-Arid Tropics (ICRISAT) for facilitating this research at Sadore, Niger. The author is grateful to Dr. van Ast from Wageningen University (The Netherlands) for providing GR24 and Striga seeds. Special appreciation is addressed to Dr. B.I.G. Haussmann for coordination of the research on location.

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