Journal of Applied Biosciences 37: 2453 - 2459

ISSN 1997–5902

Germination stimulation of *Striga gesnerioides* seeds from tobacco plantations by hosts and non-hosts

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Original submitted in 15th October 2010. Published online at www.biosciences.elewa.org on January 10, 2011.

ABSTRACT
Objective: Tobacco (*Nicotiana tabacum*) is an important cash crop in Zimbabwe and its production in the Mvuma area is being adversely affected by the witchweed (*Striga gesnerioides*). The germination of *S. gesnerioides*, a noxious root parasite of tobacco, cowpeas and other wild cultivars, is stimulated by exudates from the roots of both host and non-host trap plants. Thus, crop rotation with selected non-host cultivars may have potential to control *S. gesnerioides*. The objective of this study was to screen potential trap crops for *S.gesnerioides* control and to select for tolerant tobacco cultivars.
Methodology and results: Greenhouse and laboratory procedures were used to evaluate potential trap crops for their ability to stimulate *S. gesnerioides* seed germination. Germination stimulants were extracted from host and non-host root exudates grown in pots for 21 days. Results showed that germination of *S. gesnerioides* seed was also stimulated by exudates from the roots of non-host crops [sugars bean (*Phaseolus vulgaris*), ground nuts (*Arachis hypogea*) and pigeon pea (*Cajanus cajan*)]. Sugar bean stimulated significantly ($P<0.05$) more germination (78%) of *Striga* seeds than other crop species. Cowpea (*Vigna unguiculata*), a known host of *S. gesnerioides*, also stimulated seed germination even though no crop infestation was observed during the on-site surveys conducted in *Striga*- infested areas of Mvuma. This may suggest that the *S. gesnerioides* strain affecting tobacco in that area may not parasitise cowpea. Since cowpea could only stimulate *S. gesnerioides* seed germination without further parasitism (haustorial attachment), it can be an important trap crop in tobacco production. In the study to investigate the variability among twelve different *N. tabacum* genotypes for *S. gesnerioides* germination induction, all the tested cultivars had the same stimulatory effect ($p>0.05$) and germination ranged from 50-67% (LSD=20.12).
Application of findings: There is scope in the use of trap crops that stimulate *Striga* seeds in tobacco rotations as part of the integrated *Striga* control program.
Key words: Witchweed trap crops, *Striga gesnerioides*, tobacco

INTRODUCTION
The control of *Striga gesnerioides* is difficult, mainly because of the unique adaptation of the parasite to its environment, and the complexity of the host-parasite relationship (Botanga & Timko, 2005). Various methods to control *Striga* have been postulated (Kim, 1994) and even put into practice but marginal gains have been obtained principally due to high cost of the control package, unfriendly technology, and low acceptance by the end users (farmers). In the United States *S. asiatica* was successfully controlled by stimulation of the parasitic weed seeds to germinate in the absence of the host. Ethylene gas was used as *Striga* seed germination stimulant (Oswald &

Ransom, 2001). Trap cropping is one method of *Striga* control that is friendly to farmers and is a readily acceptable control strategy (Gbèhounou & Adango, 2003). Trap crops such as groundnut, cowpea, soybean, pigeon pea (*Cajanus cajan)* and cotton (*Gossypium spp*) were found to stimulate suicidal seed germination of *Striga hermonthica*. *Striga* seed will not germinate in the absence of a chemical germination stimulant. The stimulants can be host root exudates, non-host root exudates, natural leachates and synthetic germination stimulants. In previous *Striga* research, it was found that seeds of *S. gesnerioides* do not respond to synthetic stimulants (Strigol analogs), so seed characterization experiments used root exudates as germination stimulants (Lagoke *et al*., 1991).

The severity of *Striga* attachment is dependent on the amount of germination stimulants produced by different host crop varieties. The selection of host plant varieties with reduced capacity to produce germination stimulants could be a viable management measure, as there is a positive correlation between the amount of stimulant produced and the susceptibility to *Striga* infestation (Timko, 2008). In this study, it was therefore important to test different crop cultivars for their ability to stimulate *S. gesnerioides* seed germination. Selected plants could be used in rotation with the susceptible host, tobacco, in reducing *Striga* seed bank in the soil.

Legumes have an added advantage of improving soil fertility. Cowpea, pigeon pea, soya bean and groundnuts when grown in rotation with a susceptible host, have been reported to induce abortive germination of *S. hermonthica* seeds, with a consequent reduction in infestation levels (Musselman, 1991). Cotton (*Gossypium spp*), a non leguminous crop, has been reported to stimulate *Striga* seeds to germinate as well. The most efficient method of selecting trap crop cultivars is to initially screen for stimulant production in the laboratory, followed by field screening of the most promising materials. The objectives of this study were to: (i) Screen plants to identify cultivars with reduced stimulant production and thus considered tolerant to *Striga* parasitism; and (ii) identify cultivars of non host plants (particularly legumes), that act as false-hosts in stimulating germination of *S. gesnerioides* seeds, and used in rotation with tobacco in a trap cropping system.

MATERIALS AND METHODS

Collection of *S. gesnerioides* seeds from infested tobacco fields: The emergence of *S. gesnerioides* plants began at around 9 weeks after the host crop (tobacco) had been transplanted. Determining the maturity of the *Striga* plants in the field was difficult but attention was taken in avoiding fresh plants with attached petals. Plants with visible seed capsules were considered mature and every effort was made to collect only those plants with healthy intact mature capsules. Several paper bags were used to collect the mature *Striga* floral heads in order to obtain substantial quantity of seed when threshed (Fig 1).

Figure 1: *Striga* floral heads in paper bag

Figure 2: *Striga* plants surrounded with wind shields.

The bags containing *Striga* capsules were then sun-dried for 1 week by removing the plants from the paper bags and spread on a polyethylene sheet surrounded with wind shields (Fig 2). The dried floral heads were threshed by gently taping to release the seeds from the capsules and sieved through 250 micron and 100 micron sieves. Seed was then separated from chaff using a light dissecting microscope and stored in glass test tubes at room temperature for 7 months before being used in *Striga* experiments.

Viability testing: The tetrazolium red test, described by Berner & Winslow (1997), was used to detect the presence of a dehydrogenase enzyme, which indicates that the *Striga* seed is alive. When treated with tetrazolium red, viable seeds produce red to pink coloration in both the embryo and aleurone layer. Viability tests were done by dissolving 1g of 2,3, 5 triphenyl tetrazolium chloride salt in 100 ml of distilled water. The pH of the resulting solution was 6.9. The container with the solution was then covered with aluminium foil to exclude light and refrigerated. Fifty to one hundred *S.gesnerioides* seeds were put in a petri dish covered with aluminium foil and drops of tetrazolium solution added to barely cover the *Striga* seeds, and incubated at 40^{o}C for 48 hrs. The mixture was then poured into a funnel lined with a 9 cm filter paper with water rinsing the Petri dish to carry any remaining seeds into the funnel. The solution was allowed to drain and the filter paper was put in a clean Petri dish and drops of 1% NaOCl solution added to barely cover the seeds to bleach the seed coats and allow the red-stained endosperm beneath to be seen. The red-stained endosperm, an indication of viable seed, was then examined under a microscope. Very lightly stained seeds were considered non viable. Either the viable non-germinated or the non-viable non-germinated seed were counted depending on which category appears to have fewer, more easily countable seed and the percentage viability was calculated.

Conditioning and germinating seed: In addition to a chemical stimulant, *Striga* seeds also require conditioning in order to germinate. After harvest of *Striga* seed, there is a period of 4-6 months when the seeds are truly dormant and generally cannot be conditioned to germinate. After this period, it takes 7-21 days of exposure to moisture to precondition the seeds so that they will respond to a germination stimulant. In conditioning, 1% sodium hypochlorite was prepared and 30 ml decanted into a large petri dish. One drop of Tween 80 was added to break surface tension. *Striga* seeds were added and stirred for 2 minutes. The floating seeds and debris were discarded. The mixture was then poured into a funnel lined with filter paper and clean, ideally sterile, water was used to wash the seed. The surface disinfested *Striga* seeds were then placed in 30 ml of sterile water in a sterile Petri dish and the seeds stirred to force them to sink. The Petri dish was placed in a dark place for 14 days, and water changed after every 2 days. After this the seeds were spread on moist filter paper in a new Petri dish, with a small paintbrush being used to spread the seed evenly over the surface of the filter paper. The conditioned seeds were then used in screening to identify potential trap crops and resistant cultivars.

Production of germination stimulant: The most efficient method of selecting trap crop cultivars is to initially screen for stimulant production in the laboratory, followed by field screening of the most promising materials. Green house and laboratory assays were conducted to screen potential trap crops that could induce abortive germination of *S. gesnerioides* seeds. Cowpea, cotton, soybean, groundnuts, pigeon pea, sugar beans and tobacco were grown in separate 'double pot' systems and this required two tapered pots of the same size. The bottom pot was perforated and fitted into a second un-perforated pot. The upper pot was then filled with vermiculite and the desired seeds were planted. Water was allowed to percolate through the vermiculite and the holes in the upper pot and collected in the bottom pot. Seedlings were grown for 3 weeks and water frequently discarded. Refilling with 100 ml of water was done and the subsequent leachate collected in the bottom pot. The collected drops of the host root exudates were then used to stimulate the already conditioned *Striga* seeds. *Striga* seed germination was observed after 48 hrs and water was used as a control in each of these tests. Variability between treatments was accounted for by expressing germination induced by host root exudates as a percentage relative to that induced by water, as outlined in the IITA *Striga* Research Manual (Berner & Williams, 1998).

Selecting hosts producing low levels of stimulant: It was of interest to screen selected cultivars, breeding lines, wild types and wild *Nicotiana* species for *Striga* germination induction. There is scope in selection for host cultivars producing low levels of stimulant. Selecting varieties with different parental lines was done to increase genetic variability. The following tobacco varieties were used, with the parental lines in brackets: KE1, KRK 1 (KE1 x STNCB), KRK 22 (RW x KM10), KRK 26 (K326 x RW), KRK 28 (XM x XM),

K30R (AW3R x SpG28), T64 (XS x XZ), T66 (ONC x MG), K35 (AW x WZ), T 29, Land race 11 and Land race 12. Water was used as the negative control in the study. The varieties were grown, and leachates obtained, in a double pot system as outlined in the trap cropping experiment above.

Statistical analyses: The data was subjected to Analysis of Variance (ANOVA) using Genstat version 9.2 and statistically significant treatment effects were separated using least significant differences (LSDs) at $p<0.05$.

RESULTS

***Striga* seed germination induction from tobacco cultivars:** A 64% viability rate was found for the *S. gesnerioides* seeds tested. All the tobacco cultivars stimulated *Striga* seed germination (Fig. 3), but no significant differences were noted in their degree of stimulation ($p>0.05$). The negative control of the trial was water and as expected, no *Striga* germination induction was observed. The two landraces (LR11 and LR12) were more susceptible to *Striga* in field trials compared to the commercial cultivars (T66, KRK26, KRK28), but in terms of stimulant production, no significant differences were established.

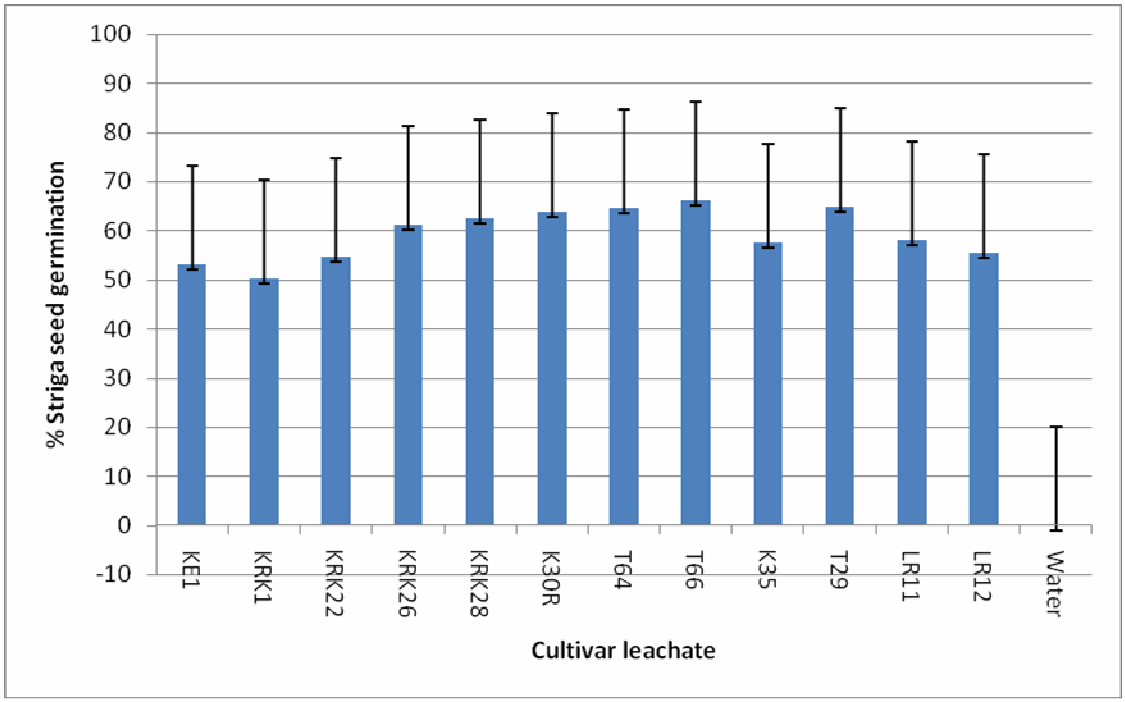

Figure 3: Germination induction of *Striga* by tobacco genotypes, Error bars show the LSD value, $p<0.001$

Effect of trap crop leachate on *Striga* seed germination: *Phaseolus* leachate stimulated *Striga* germination the most (75%) whilst the lowest (15%) stimulation between the legume leachates was from *Arachis. Glycine* and *Gossypium* species failed to stimulate germination of *S. gesnerioides* seeds (Fig 5). *Vigna*, a known host of *S. gesnerioides* also stimulated germination of *Striga* seeds (59%) but in field surveys conducted in the infested areas *S. gesnerioides* could not be found in cowpea fields.

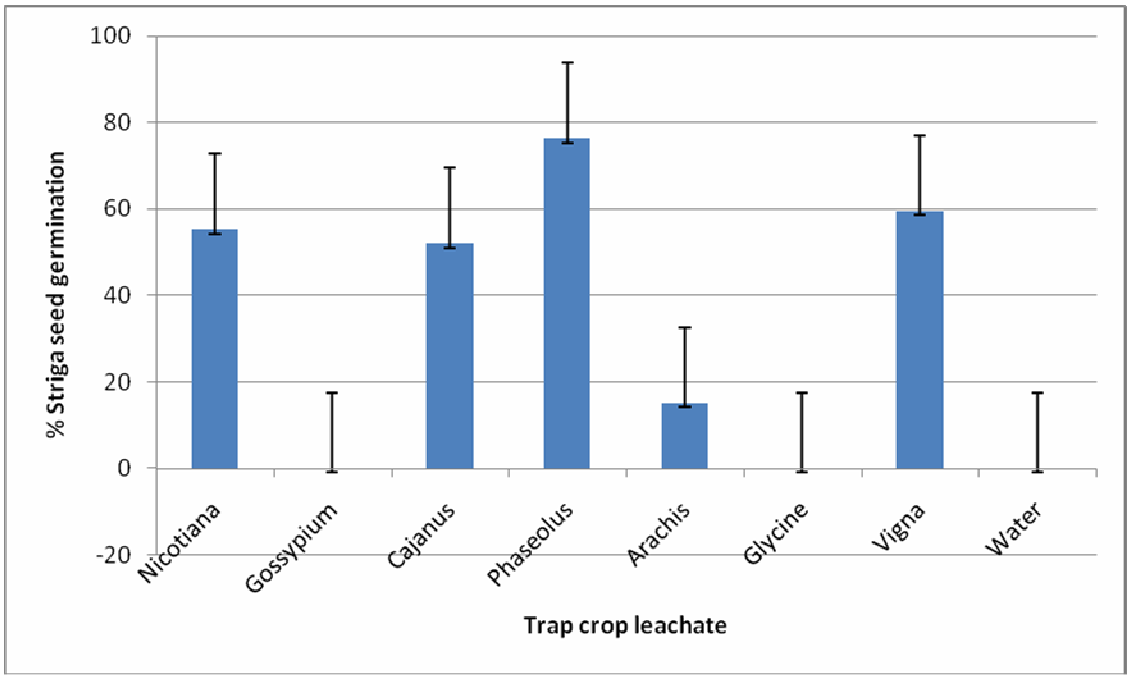

Figure 4: Germination induction of *S. gesnerioides* by leachate of various crop species
The error bars show the LSD value, $p<0.001$.

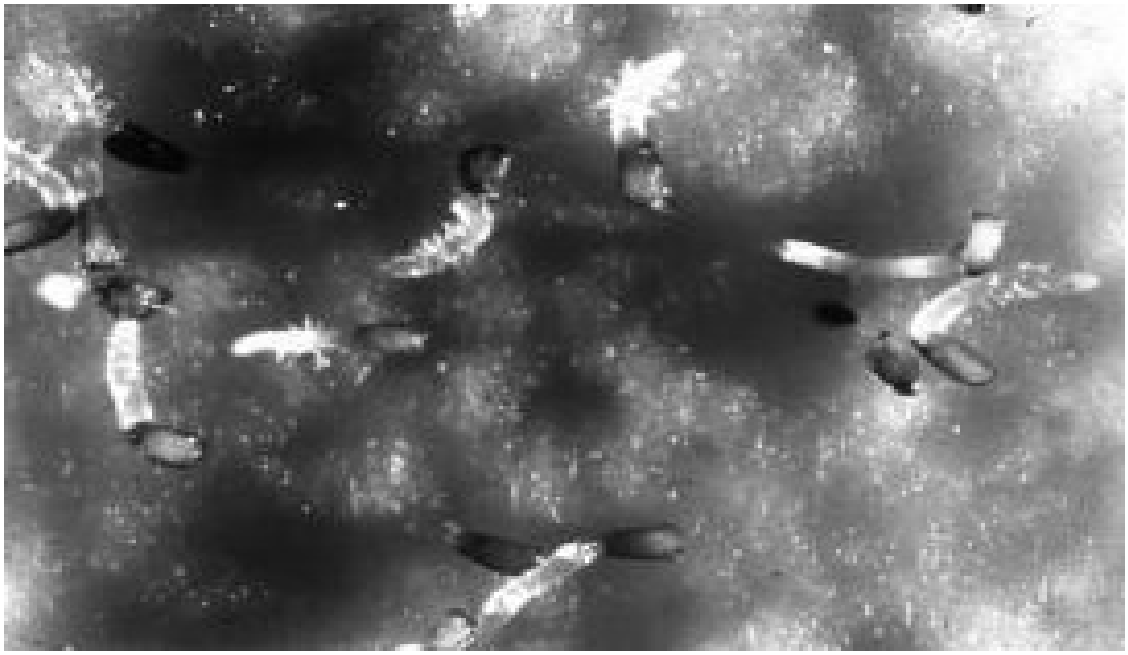

Figure 5: Growing radicles from germinated *S. gesnerioides* seeds exposed to stimulant from *Nicotiana*

Growing radicles of germinated *S. gesnerioides* seeds are shown in Fig 5. The percentage germination was calculated by counting the emerged radicles against the total number of *Striga* seeds exposed to the germination stimulant. After germination, a series of chemical signals directs the radicle to the host root where it attaches and penetrates. However, since there was no host around for haustorium attachment the seedling perished within 3–5 days of radicle development.

DISCUSSION

The first indication of the possible use of trap crops was the synthesis of a potent germination stimulant, strigol (Berner & Winslow, 1997), a compound extracted from the roots of a non-host crop, cotton. Crops which stimulate more *Striga* seeds to germinate possibly produce more germination stimulant (Gbèhounou & Adango, 2003). In the present study, *Phaseolus*, *Vigna* and *Cajanus* produced more *Striga* germination stimulants and since haustorial initiation requires an additional unique chemical signal from the specific host, these crops could be used as trap crops in an integrated *Striga* control program. Cotton seeds, which failed to stimulate germination of *S. gesnerioides* seeds may not be conclusively ruled out as a stimulant producer. They might actually produce a germination stimulant that is simply not recognized by the receptors of seeds of the particular strain of *Striga* (Botanga *et al*., 2001).

In previous research, some varieties of cowpea, soybean, and groundnut have also been shown to control *S. hermonthica* through a combination of mechanisms ranging from induction of suicidal germination of *Striga* seeds, N fixation, and smothering effect (Reiss & Bailey, 1998). Sunflower (*Helianthus annuus*) and sweet potato (*Ipomea batatas*) also have been reported to support the growth and flowering of *S. gesnerioides* in Florida in pot tests, but there were no reports of such attacks under field conditions (Botanga & Timko, 2005). This could be the case with cowpea stimulating germination of *S gesnerioides* but not supporting establishment under field conditions. The *Striga* biotype parasitizing tobacco in Mvuma area could be different from the one that parasitize cowpea. Germination, haustorial induction, attachment to and penetration of the host root vascular system are all critical events in the *Striga* life cycle. Of interest are factors and processes that are required for the establishment of compatible host-parasite interactions, and how resistant host and non-host plants avoid being parasitized. It also has been reported that exudates from cowpea stimulated the germination of a strain of *S. gesnerioides* that parasitizes Morning glory (*Jacquemontia tamnifolia)* but failed to parasitize cowpea. It is known that *S. gesnerioides* is among the most variable of the witchweeds and that there is both host specificity and differential resistance responses within host species. There have been few attempts to analyze genetic variability of *Striga* species and the relationship between genetic variability of the parasite and its host range and virulence is not known (Singh, 2000).

Seven distinct races of *S. gesnerioides* that parasitize cowpea have been identified across West and Central Africa including isolates of the parasite that are specific for different wild legume species as well as non-leguminous dicots such as tobacco (Musselman, 1991). Several races of *S. gesnerioides* have been identified based on differential resistance reactions of cowpea cultivars (Lagoke *et al*., 1991). Since *Striga* seedlings must attach to a host root within 3 to 5 days after germination or they die (Wigcher, 1999), a sustainable *Striga* control option for the resource-poor farmers in Mvuma is the use of trap crops, particularly legumes that stimulate germination of the parasite's seeds but are non-hosts, in rotation with tobacco.

All the tobacco cultivars stimulated relatively high percentage of *S. gesnerioides* seed germination, despite visual differences observed in field experiments conducted in the infested areas of Mvuma. This indicates that tolerance probably involves many factors and metabolic reactions involving both the host crop and the parasite. In the field trials the tobacco cultivar (T66) was more tolerant to *Striga* while the two landraces (LR11, LR12) were more susceptible. Breeding for *Striga* resistance has relied on selection of host plants that allow germination and emergence of few parasitic plants and show little or no loss of productivity. In cowpea studies done previously, no information was available on the variability among cultivars in their ability to stimulate seed germination of different *S. gesnerioides* isolates (Berner & Winslow, 1997). There are several mechanisms of resistance to *S. gesnerioides* and these can be expressed as different types of post-attachment hypersensitive responses (Berner *et al*., 1995). All the tested cultivars may have stimulated *Striga* seeds to germinate but the absence of haustorial induction compounds and the complex interchange of signals in root exudates could be the reason why some cultivars were tolerant in the field compared to others. Studies by Berner and Williams (1998) showed that a cowpea cultivar, generally regarded as susceptible to *S. gesnerioides*, may be capable of stimulating germination of only a fraction of viable seeds from populations of *S. gesnerioides* to which it is exposed. Appearance of *Striga* on host plants in the field is the eventual expression of a series of interactive events between the parasite and its host from the earliest stages of *Striga* development.

ACKNOWLEDGEMENTS

Special thanks go to the Tobacco Research Board and National University of Science and Technology for supporting this research work.

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