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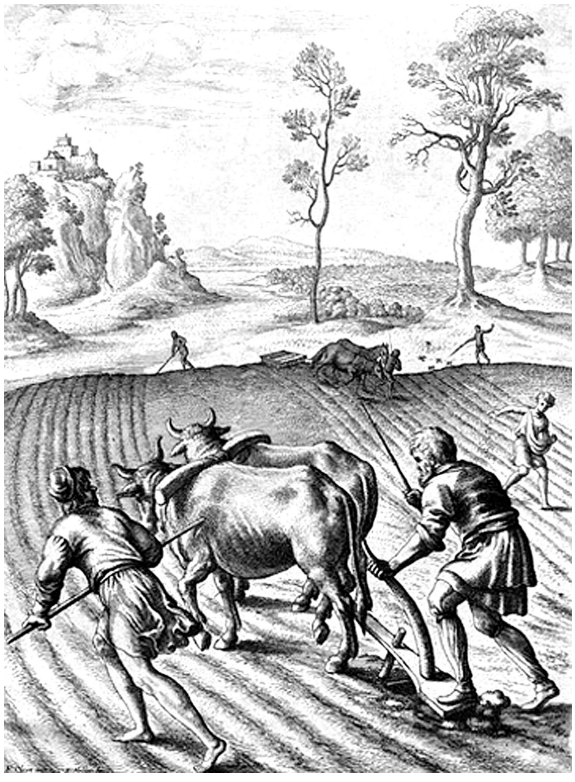

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Effect of Leaf Extracts of Multipurpose Trees on the Life Cycle of *Striga asiatica* (L.) Kuntze

A. Gomba and [1]B. Kachigunda
Department of Horticulture,
[1]Department of Agronomy, Midlands State University, P/Bag 9055, Gweru, Zimbabwe

Abstract: A laboratory study was conducted to investigate the effects of several leguminous multipurpose tree (MPT) leaf crude extracts on the different stages of the life cycle of *Striga asiatica*. The MPTs used were *Sesbania sesban*, *Luceana leucocephala*, *Acacia angustissima* and *Calliandra callothyrsus*. Stages studied were germination, haustoria initiation, attachment and penetration of *Striga asiatica* on maize seedlings. Using the agar gel assay method discs containing preconditioned *S. asiatica* seeds were immersed into 9 cm petri dishes containing two millimeters of the MPT crude leaf extracts. Autoclaved water agar at room temperature was then poured over the mixture of *S. asiatica* seeds and leaf extract. The petri dishes were swirled around to evenly distribute *S. asiatica* seeds and leaf extracts in the agar. A germinating maize seed was submerged in the solidifying agar near the edge of the petri dish, with the root tip pointing across the plate. Germination of *S. asiatica* was significantly reduced ($p< 0.05$) by all the mulch extracts. The greatest reduction in germination (49%) resulted from *S. sesban* extracts while extracts from *L. leucocephala* caused the least reduction (24%). All the leaf mulch extracts did not affect haustoria formation. Attachment was not observed in any of the dishes including the control.

Key words: *Acacia angustissima*, *Calliandra callothyrsus*, counts, emergence, *Lueccana leucocephala*, multipurpose trees, *Sesbania sesban*, *Striga asiatica*

INTRODUCTION

Striga asiatica (Witchweed) is a parasitic weed of cereal crops. In Africa, *Striga* is the greatest biological constraint to food production, a more serious problem than insects, birds or plant diseases[1]. In many parts of Zimbabwe *S. asiatica* is a growing threat to cereal production[2]. A weed survey conducted by Chivinge[2] revealed heavy *S. asiatica* infestations on maize, sorghum, pearlmillet and finger millet in all the eight provinces of Zimbabwe. The cereals attacked by *Striga* are major sources of energy in the diets of many people in Zimbabwe. Ogborn[3] stated that when maize is attacked it often produces no grain at all. Apart from yield reduction, *Striga* is also responsible for other losses of a social nature such as increasing distance of farms from settlements in an effort to locate *Striga*-free fields[4]. Arable fields are often abandoned because of prohibitive parasite population and this sometimes leads to the preferred cereal not being grown on the most infested land[5,6]. Many smallholder farmers have had to accept the current low levels of cereal production leading to nationwide food insecurity and malnutrition.

Striga spp. are characterised by prolific seed production and seed can remain viable in the soil for up to 20 years[7] making control extremely difficult. Each *Striga* plant can produce 40 000 to 90 000 microscopic seeds depending on the species and conditions for plant development[7]. Long viability in combination with high seed production results in severely infested fields. The seeds require an after-ripening period, as they cannot germinate at the end of the cropping season in which they were produced[6]. The seeds germinate in response to host and non-host stimuli[8] only after pre-conditioning which, involves keeping seed in moist condition for 1-5 weeks at a suitable temperature[9]. Once conditioned and exposed to a stimulant from a potential host, germination will occur within 24 h[10]. The seed is so small that once germination has occurred, the food reserves are quickly exhausted and without a host the seedling will die. Thus only those seeds, which are exposed to the chemical stimulant produced by the host root, germinate. The presence of a chemical stimulant signals that a suitable host is available and near enough to be reached by the *Striga* radicle. This is an advantage for *Striga* since it ensures that seeds too far away from host roots, remain un-germinated and viable hence perpetuate the seed bank.

Corresponding Author: A. Gomba, Department of Horticulture, Midlands State University, P/Bag 9055, Gweru, Zimbabwe

It is suggested that a second signal, the haustoria initiator, directs the radicle to the host root and on contact with the host root the elongation of the radicle stops and the development of the haustorium immediately begins[8]. This second signal has to be received in about 4 days of germination otherwise the *Striga* seedling dies. The haustorium is the morphological and physiological bridge between the host and the parasite and its development is the beginning of parasitic expression for *Striga*. The primary function of the haustoria is the procurement of water and mineral nutrients. Phenolics and cytokinins are the two basic substances active in haustorial promotion. Most of the attached parasites are not visible above ground, since usually only about 1-30% of the attached plants emerge. The relative success of each stage of the life cycle determines the volume of seed production.

Understanding the biology of any pest aids in devising its control. *Striga* is an obligate parasite such that interactions between the parasite and its host plants are crucial for its survival. Blocking these interactions, which occur throughout the life cycle of *Striga* offers unique opportunities for intervention in the control of this weed. At each stage of development, there is a potential opportunity for control. Control methods are aimed at reducing *Striga* multiplication by interfering at different stages in the *Striga* life cycle. Chanyowedza *et al.*[11] reported that exposing *S. asiatica* seeds to extracts from MPT mulch reduced the germination of the parasite by between 50 to 100%. Leaf extracts from *A. nilotica*, which had the highest level of soluble phenolic compounds, completely inhibited the germination of *S. asiatica* while *G. sepium* with the lowest of total phenolics reduced *S. asiatica* germination by 50 to 70%. To quantify the effects of promising control methods, it is important to measure which stages of the weed are affected. This study was carried out to investigate the effects of multipurpose trees (MPTs) leaf extracts namely, *Sesbania sesban*, *Luceana leucocephala*, *Acacia angustissima* and *Calliandra callothyrsus* on the germination, haustoria initiation and attachment and penetration of *Striga asiatica*.

MATERIALS AND METHODS

Surface sterilisation and conditioning of *Striga* seed: The Weed Research Team at Henderson Research Station supplied *Striga asiatica* seeds used. The seeds were surface sterilised by soaking them in 1% sodium hypochlorite solution for 5 min after which they were washed with distilled water until the chlorine smell disappeared. The seeds were then left to dry in a laminar flow. Eight-millimetre discs were cut from Whitman glass microfibre (What GF/C) filters using a stainless steel corkborer. The sterilised, dried *Striga* seeds were then carefully sprinkled on the discs. Two layers of the same filter paper were placed in a petri dish lid and wetted with distilled water. Discs containing *Striga* seed were placed in the lined petri dish lid and the base of the petri dish was used to cover the discs in the petri dish lid. The petri dishes were tightly wrapped with parafilm to reduce contamination, then wrapped in black polythene bags to exclude light and incubated in the dark at 25°C for 14 days.

Surface sterilisation of maize seed: Maize seeds of the variety SC 513 (20 per sample) were surface sterilised using one percent (1%) sodium hypochlorite solution for 25-30 min. The seeds were then washed with distilled water 3 times until the chlorine odour disappeared. The seeds were incubated in 90 mm petri dishes lined with moist filter paper at 25°C for 5-7 days until the radicle emerged.

Collection of leaf extracts: Five grams of ground sun dried leaves of *S. sesban*; *L. leucocephala*, *A. angustissma* and *C. callothyrsus* were suspended in 200 mL of distilled water for 24 h at room temperature. To remove the residues, the leaf suspension was passed through a 250 μm sieve and the collected extracts were filtered through ordinary Whitman filter paper after which they were filter sterilised using a 250 mL sterilising unit and stored in sterilised containers.

Assay set up: The agar gel assay was performed following the method described by Reda *et al.*[12]. Two millimetres of leaf extracts were pipetted into 9 cm petri dishes. Discs containing preconditioned *S. asiatica* seeds were immersed into the leaf extracts to remove the *Striga* seeds. Thirty millimetres of 0.7% autoclaved water agar at room temperature was then poured over the mixture of *S. asiatica* seeds and leaf extract. The petri dishes were swirled around to evenly distribute *S. asiatica* seeds and leaf extracts in the agar. A germinating maize seed was submerged in the solidifying agar near the edge of the petri dish, with the root tip pointing across the plate. The plates were covered, wrapped with vita film and then incubated in the dark at 27°C.

Data collection and statistical analysis: Assessment on induced germination and reading haustoria initiation was done after 72 h. Germination of *Striga* seed was easily visible through the bottom of the plate with a dissecting microscope. Germination was determined by examining seeds in 16 mm diameter microscope fields. The

experiment was laid out as a Completely Randomised Design with 4 replications, 4 types of MPT crude leaf extracts and a control of no leaf extracts. Data collected was subjected to analysis of variance (ANOVA) procedures for a Completely Randomised Design using MSTATC statistical package. Dunnett's test was used to compare the control to all the other treatments. Means were separated using the Least Significance Difference procedure.

RESULTS AND DISCUSSION

Germination: Mulch extracts of *C. callothyrsus*, *A. angustissma* and *S. sesban* significantly reduced ($p<0.05$) germination of *S. asiatica*. The greatest reduction (49%) resulted from *S. sesban* extracts but this was not different from the reduction caused by *C. callothyrsus* and *A. angustissma* (Table 1). Mulch extracts from *L. leucocephala* caused the least reduction (24%) in germination of *S. asiatica*. Variations among mulch types in germination inhibition of *S. asiatica* in the laboratory shows that even their effectiveness when used in the field for *Striga* control would also differ. High inhibitors would be expected to inhibit a larger percentage of *Striga* seeds to germinate. These findings agree with the results of Chanyowedza *et al.*[11]. These authors reported that exposing *S. asiatica* seeds to extracts from MPT mulch reduced the germination of the parasite by between 50 to 100%. Leaf extracts from *A. nilotica*, which had the highest level of soluble phenolic compounds, completely inhibited the germination of *S. asiatica* while *G. sepium* with the lowest of total phenolics reduced *S. asiatica* germination by 50 to 70%. However, in Zambia *S. sesban* leaves have been reported to stimulate *S. asiatica* seed germination[13]. It is highly probable that the concentration of the aqueous leaf extracts used in this experiment was inhibitory. Ariga *et al.*[13] stated that the germination percentage of *S. hermonthica* depended on the concentration of the leaf extracts. Germination percentage of *S. hermonthica* seed increased as the concentration of cowpea (*Cajanus cajan*) aqueous leaf extracts increased from 2.5 to 6.3 mg plant tissue/mL of water. Below and above the optimal concentration of the extracts relative percent germination of the parasite seeds decreased. The concentration of the extracts used in this experiment was 5 g/200 mL of water, which was equivalent to 25 mg plant tissue/mL of water. This value was well above the optimum level reported for *S. hermonthica* such that germination was reduced. Whitney[15] reported similar depressive effects at high concentration of root extract of *Vicia faba* L. in the germination of *Orobanche crenata* Forsk and *Orobanche minor* Sm. This suggests the presence of both inhibitory and stimulatory substances of *S. asiatica* from aqueous leaf extracts of MPTs[15]. Dilution of the extracts probably tips the balance in favour of the stimulant. The existence of both *S. asiatica* germination inhibitors and stimulants in leaf extracts of MPTs opens up an interesting possibility of extraction, identification and use of such plants to stop or stimulate the germination of the parasite.

Table 1: Effect of leaf mulch extracts on germination of *Striga asiatica*

Mulch type	Germination (%)	Germination reduction (%)
Sesbania sesban	40.87	48.93
Calliandra callothyrsus	44.62	44.24
Acacia angustissma	47.70	40.39
Leucaena leucocephala	60.70	24.14
Control (no extract)	80.02	
SED		8.02
LSD (0.05)		17.47
CV%		20.70

Haustoria formation, penetration and attachment: Although germination was inhibited by mulch extracts, all the mulch extracts did not affect haustoria formation. This suggests that all the parasite seeds that were induced to germinate formed haustoria normally. However, attachment and shoot formation of the parasite were not observed in all treatments including the control suggesting that these could be the stages that are affected by the leaf extracts. It is also possible that the nutrient composition in the water agar was not conducive for any further development of *S. asiatica* after haustoria formation. Attachment and shoot formation have been shown to depend on the nutrient composition of the media. In culture media containing some nitrogen sources, healthy shoots were formed. Based on these findings it is possible that lack of nutrients in the water agar hindered attachment and shoot formation of *S. asiatica*. Even if the mulches used are known to contain some level of nitrogen, the extraction time (time taken to extract leaf extract) was probably not long enough for any reasonable amounts of nitrogen to be leached out.

The MPTs studied; *S. sesban*, *C. callothyrsus* and *A. angustissima* significantly reduced germination of *S. asiatica* but haustoria formation, attachment and penetration on maize seedlings was not affected by the leaf extracts. The indication of the presence of both inhibitory and stimulatory substances of *S. asiatica* from leaf extracts of MPTs highlights the need for further studies to critically identify and quantify these contributory effects on the control of *S. asiatica* as a parasite of economic importance.

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