Potential of Imazaquin Seed Treatment for Control of *Striga gesnerioides* and *Alectra vogelii* in Cowpea (*Vigna unguiculata*)

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ABSTRACT

Berner, D. K., Awad, A. E., and Aigbokhan, E. I. 1994. Potential of imazaquin seed treatment for control of *Striga gesnerioides* and *Alectra vogelii* in cowpea (*Vigna unguiculata*). Plant Dis. 78:18-23.

The herbicide imazaquin was tested for efficacy in *Striga gesnerioides* and *Alectra vogelii* control when applied as a cowpea (*Vigna unguiculata*) seed treatment. Four sets of experiments were conducted in the laboratory and screenhouse. Two cowpea cultivars, three geographical isolates of *S. gesnerioides* from two hosts, and two geographical isolates of *A. vogelii* from two hosts were used. Preliminary experiments indicated cowpea seed treatments of 5-min duration in aqueous solutions of the ammonium salt of imazaquin, ranging from 1.8 to 7.2 mg a.i./ml, fit our test criteria of 50% germination and observable radicle growth inhibition. Treated cowpea seeds were dried and planted in soil-filled pots infested with 3,000 germinable *S. gesnerioides* or *A. vogelii* seeds. All experiments showed imazaquin seed treatments to significantly reduce numbers of attached (emerged and unemerged) parasites. Imazaquin seed treatments resulted in increased total cowpea dry weight in *S. gesnerioides*-infested pots in all experiments. Increases were significant at $P < 0.05$ in two experiments. Observations in vitro, combined with screenhouse data, showed apparently normal parasite germination and attachment, indicating postattachment demise of both parasites. Increasing imazaquin rates led to delays in cowpea flowering; and increased soak times, at 3.6 mg a.i./ml and higher concentrations, led to reductions in cowpea seedling emergence. By prolonging seed soak times at an imazaquin concentration of 1.8 mg/ml, good parasite control was obtained. The lower rates at longer soak durations would provide both economical ($2.31 to $3.85 per hectare) control and the flexibility in treatment necessary for implementation on African farms. Field trials on farmer fields are under way to tailor specific seed treatment recommendations.

Cowpea, *Vigna unguiculata* (L.) Walp., is one of the most important and widely grown legume crops in the savanna and Sahel regions of Africa (20). It is used for both food and forage and is traditionally intercropped with cereals (2,5). The relatively high protein content and quality of cowpea makes it an important supplement to the diet of African peoples who consume cereals, roots, and tubers high in carbohydrates and low in protein content (4). From an agronomic standpoint, rotations and crop mixes with cowpea are beneficial in maintaining soil fertility and tilth, especially under the harsh environmental conditions found in Africa. Cowpea is also effective as a rotational crop for the control of some of the parasitic flowering plants plaguing cereals in Africa (5,15, 23), and this makes cowpea additionally attractive in a cereal rotation.

A major obstacle to cowpea production is parasitism by *Striga gesnerioides* (Willd.) Vatke and *Alectra vogelii* Benth., parasitic flowering plants of the Scrophulariaceae family (11). Yield reductions due to *S. gesnerioides* on susceptible cultivars have been reported to range from 41 to 83% (1,5). Complete crop failure due to *A. vogelii* has been observed (17). The two parasites are often found within the same cowpea field in Nigeria, and single plant infections by both parasites are not uncommon (1,5, 17). *S. gesnerioides* has recently been reported in the southern United States (22).

General control measures for *Striga* spp. and *A. vogelii* include trap crops, hand pulling, cultivation, resistant cultivars, ethylene, and herbicides (6,10,12,14, 16,18). Control of the cereal-parasitizing species of *Striga* by trap crops (nonhosts that stimulate parasite seed germination) shows promise, but no trap crops have been identified for *S. gesnerioides* or *A. vogelii.*

Hand pulling and cultivation are labor intensive and increasingly expensive for the African farmer. Resistant cultivars of plant types acceptable to farmers are not widely available, and recent research indicates the possibility of races of *S. gesnerioides* (13) making control through host plant resistance more difficult. Recently, pre- and postemergence applications of the imidazolinone herbicide, imazaquin, were shown to be effective in controlling the parasitic plants *Orobanche crenata* Forsk. in broadbean (7) and *Cuscuta* spp. in soybean (9). Berner et al (3) developed methodology using imazaquin that was effective in controlling soilborne fungi, which, in many aspects, are similar to the flowering root parasites. However, herbicides are not widely used in Africa because of the direct expense of the herbicide and the higher indirect expense of the application equipment. Seed treatment would be a cost-effective method of application of a herbicide for control of root parasites, as it would eliminate the need for application equipment and reduce the volume of active ingredient required for control. Because imazaquin is metabolized by tolerant crops (19) a suitably dilute seed treatment might prove noninjurious to the host while providing protection against the parasites.

The objectives of this study were to devise a simple seed treatment methodology, determine imazaquin concentrations for cowpea seed treatment that would give maximum parasite control with minimum host damage, and determine optimum treatment exposure durations.

MATERIALS AND METHODS

Establishment of seed treatment methodology and effects on parasitism in vitro. To determine seed treatment exposure durations and imazaquin concentrations to be used in subsequent experiments, cowpea (cv. 83s 728-5) seeds were soaked for 5, 50, and 100 min in aqueous solutions of imazaquin (from the commercial herbicide formulation Scepter, American Cyanamid Company, Wayne, NJ) at concentrations of 0.018, 1.8, 18, and 180 mg a.i./ml. Experiments were conducted in the laboratory at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Germination percentages of each treatment were tested on moist filter paper in five petri dishes per treatment combination with 20 seeds per petri dish. To ensure that the herbicide was being absorbed at sublethal levels, an appropriate set of experimental conditions was defined as the treatment that would allow at least 50% germination while exhibiting a visible inhibitory effect on radicle growth. The experiment was repeated using 5-, 50-, and 100-min soak times in aqueous imazaquin solutions of 1.8, 3.6, 7.2, 14.4, and 28.8 mg a.i./ml. All petri dishes were incubated in darkness for 72 hr at 28 C. Each treatment was replicated five times in petri dishes with 20 seeds per dish, and germination percentages were determined. The highest concentrations and the shortest soak times that allowed 50% seed germination were selected for subsequent experimentation.

Accepted for publication 25 August 1993.

To determine whether imazaquin allowed attachment of the parasites or inhibited parasite seed germination, cowpea seeds were treated for 5 min in 3.6 mg a.i./ml solution (an intermediate rate used throughout these studies), dried overnight, and germinated and grown under in vitro conditions described by Lane, et al (8). In this procedure, parasite seeds are conditioned for 7 days, after which they are placed on the roots of test plants grown in plastic petri dishes. A cut is made in the edge of the petri dish, and the stem and leaves of the host plant extrude from the dish. The petri dish is covered with aluminum foil to exclude light, and the host roots and parasite seeds are kept moist by covering with filter paper periodically moistened with nutrient solution. Using this procedure, *S. gesnerioides* and *A. vogelii* seeds were placed on growing roots from treated cowpea seeds and were examined under a dissecting microscope at 3-day intervals to check for germination and attachment. These observations were completed on 20 petri dishes.

The relative amount of seed imbibition of a 3.6 mg/ml imazaquin solution was measured to assay relative costs of treatment. This was done by adding 100 g of dry cowpea seed to 200 ml of 3.6 mg a.i./ml solution; soaking for 5, 10, and 15 min; decanting the solution and seeds through a sieve; and measuring the remaining solution.

Effects of seed treatment in pot culture. All subsequent experiments were conducted from December 1991 to May 1992 in an insect-excluding screenhouse with a rain-shield roof that allowed control of watering (72 ml/pot/day). Seeds of *S. gesnerioides* on *Tephrosia* spp. in Maiduguri, Nigeria, and seeds of *A. vogelii* from groundnut (*Arachis hypogaea* L.) in Bama, Nigeria, were collected during 1989. Parasite seed germination was tested in October 1991 by the methods described by Worsham (21). After testing germination, soil (ca. 3 kg/pot) in individual 15-cm-diameter pots was infested at a rate sufficient to guarantee a minimum of 3,000 germinable parasite seed per pot. Ten pots were infested with *S. gesnerioides* and 10 with *A. vogelii*. The soil was watered and the seeds were allowed to condition for 7 days prior to planting. The day before planting, cowpea (cv. 83s 728-5; 75–80 day maturity, susceptible to both parasites) seeds were treated by soaking for 5 min in a 3.6 mg a.i./ml aqueous solution of imazaquin. Control seeds were soaked for 5 min in distilled water. After treating, the seeds were placed on fine mesh sieves, air-dried overnight, and planted in the infested pots (10 seeds per pot). Treatments were 0 and 3.6 mg a.i./ml of imazaquin seed treatment, with five pots per parasite per treatment and final stands varying between one and nine plants per pot. The time of initial parasite emergence was recorded, as were the total numbers of emerged parasites per pot and the time of cowpea flowering. At 73 days after planting (DAP), the soil and plants were removed from the pots, and the soil was gently washed off. Below-ground attached parasites were counted on the remaining roots after washing. The parasite and host plant materials (above and below ground) were oven-dried for 48 hr, and dry weights were determined. Because of difficulty in physically separating individual host root systems in each pot, statistical analysis was done on pots using data averaged over the number of plants per pot. The effects of surviving plant density per pot on each of the variables were analyzed as covariants in a completely randomized design.

Effects of imazaquin rates. Seeds of *S. gesnerioides* were collected in 1990 from the parasite on cowpea in Sokoto and Potiskum, Nigeria. Seeds of *A. vogelii* were collected in 1990 from the parasite on cowpea in Kano, Nigeria, and in 1989 from the parasite on groundnut in Bama, Nigeria. The effects of cowpea seed treatment on each parasite isolate were tested separately. Parasite seed germination was determined in January 1992, and pot soil was infested at a rate of 3,000 germinable seed per pot. The soil was watered after infestation, and the seeds were allowed to condition for 7 days prior to planting cowpea. One day before planting, cowpea (cv. IT84s 2246-4; 60–65 day maturity, susceptible to both parasites) seeds were soaked for 5 min in aqueous imazaquin solutions of 0, 1.8, 3.6, and 7.2 mg a.i./ml. After soaking, seeds were placed on fine mesh sieves and air-dried overnight. Five seeds were planted per pot. Each treatment–parasite isolate combination was replicated three times, and three pots for each isolate were left as an uninfested control. The final plant stand varied from two to four plants per pot. The time of initial parasite emergence was recorded, as were total numbers of emerged parasites per pot; cowpea flowering dates were also recorded. At 63 DAP, the soil and plants were removed from the pots, and the soil was gently washed off. Unemerged attached parasites were counted. The parasite and host plant materials were oven-dried for 48 hr, and dry weights were determined. Statistical analysis was done on pots using data averaged over the number of plants per pot.

Effects of seed treatment exposure duration. To test the effects of longer soak times with various imazaquin concentrations on seedling emergence, host development, and parasitism, cowpea seeds (cv. IT84s 2246-4) treated with aqueous imazaquin solutions of 0, 1.8, 3.6, and 5.4 mg a.i./ml for 5, 10, and 15 min were planted in soil in 15-cm-diameter pots. Seven days prior to planting, the soil in each pot was infested with 3,000 germinable *S. gesnerioides* seeds collected from plants parasitizing cowpea in Sokoto, Nigeria. There were five replications of each treatment containing 10 cowpea seeds per pot. At 10 DAP, the seedling emergence in each pot was recorded, and the plant stand was thinned to two seedlings per pot. At 55 DAP, the plants were harvested, dried as in the other experiments, and the numbers of emerged and unemerged attached *S. gesnerioides* plants recorded.

RESULTS

Establishment of seed treatment methodology and effects on parasitism in vitro. In preliminary evaluations, seed soaks of 5-min duration in imazaquin solutions ranging in concentration from 1.8 to 7.2 mg a.i./ml allowed at least 50% seed germination while producing some

Table 1. Means of cowpea–parasite parameters[a] from *Vigna unguiculata* cv. 83s 728-5 plants from seed treated[b] with imazaquin solutions of either 0 or 3.6 mg a.i./ml and planted in pots infested with 3,000 germinable seeds of either *Striga gesnerioides* or *Alectra vogelii*

Parasite	Aboveground host dry wt. (g)		Host root dry wt. (g)		Emerged parasites per plant (no.)		Attached parasites per plant (no.)[c]		Parasite dry wt. (g)	
	0	3.6	0	3.6	0	3.6	0	3.6	0	3.6
S. gesnerioides	1.4	2.8	1.8	2.2	3.7	0*[d]	4.8	0.1*	1.7	0*
A. vogelii	1.0	1.5	1.4	2.0	2.8	0*	5.1	0.6*	0.5	0*
Least squares mean	1.2	2.1	1.6	2.1	3.2	0*	5.0	0.3*	1.1	0*

[a] Any emerged parasites were recorded weekly after planting. Other parameters were measured at harvest, 73 days after planting. Plant materials were weighed after oven drying for 48 hr.

[b] Seeds were treated by soaking for 5 min in the respective solutions and air-drying overnight on the day prior to planting.

[c] Attached parasites are both emerged and below-ground parasites, the latter determined at cowpea harvest.

[d] * = Paired means are significantly different at $P < 0.05$.

visible inhibition on radicle growth. Concentrations outside of this range either had no visible effect on radicle growth or inhibited more than 50% seed germination. A 5-min soak duration was not significantly ($P < 0.05$, used through remainder of text) different from longer soaks based on germination and radicle growth, and was used for subsequent studies in experiments 1 and 2.

Microscopic examination of parasite seeds placed on growing cowpea roots in vitro showed that seeds of both parasites germinated normally and attached to the host roots.

Average seed imbibition with 5-, 10-, and 15-min soaks of a 3.6 mg a.i./ml imazaquin solution were 0.317, 0.450, and 0.493 ml/g seed. A significant fit of the data was obtained by the following: ml imbibed solution = 0.244 + 0.018 * soak time, $R^2 = 0.87$, as a proportion of the total sum of squares.

Effects of seed treatment in pot culture. Regression and covariance analyses of surviving plant density per pot showed no density effects on any of the variables. The emergence of *S. gesnerioides* and of *A. vogelii* was first observed at 39 and 49 DAP, respectively, on cowpea plants from untreated seeds. There were no emerged *S. gesnerioides* or *A. vogelii* on plants from treated seeds. There were significant differences between plants from treated and untreated seeds for all of the parameters measured except host dry weight and root dry weight (Table 1). However, host above-ground dry weight from plants exposed to *S. gesnerioides* was 100% greater for plants treated with imazaquin than from untreated seeds. There was a 50% increase in host above-ground dry weight due to seed treatment with plants grown in association with *A. vogelii.* Cowpea flowering was delayed by up to 7 days as a result of seed treatment; due to a delay in flowering, maturity would also have been delayed, although this parameter was not measured in this experiment.

Effects of imazaquin rates. There were no significant differences between the two isolates of *S. gesnerioides* or between the two isolates of *A. vogelii* for any of the parameters measured. Data for the two isolates of each parasite were subsequently pooled for further analysis. Results of the effect of seed treatment on parasite attachment are presented in Figure 1. On cultivar IT84s 2246-4, the number of attached and emerged *S. gesnerioides* plants was greater than the number of *A. vogelii* for both untreated and treated seeds. Both parasites were inhibited by cowpea seed treatment with imazaquin. An average of 25.0 and 2.8 emerged *S. gesnerioides* and *A. vogelii* plants, respectively, per host plant were found on untreated infested controls. An average of 4.8 and 0.4 emerged *S. gesnerioides* and *A. vogelii* plants, respectively, per host plant were found on plants from treated seeds. Of these, 90% of the emerged *S. gesnerioides* and 100% of the emerged *A. vogelii* were found on plants from the 1.8 mg a.i./ml imazaquin treatment. Unemerged parasites attached to plant roots from treated seeds were generally very small and frequently deformed.

The effects of seed treatments on average host seed weight are presented in Figure 2. The decrease in seed weight at the highest imazaquin concentration was due to delayed flowering and subsequent seed set attributable to the chemical treatment.

The earliest cowpea flowering observed occurred on some plants from untreated seeds in uninfested pots at 34 DAP; the mean flowering time for these pots was 35.8 DAP. There were no significant overall differences in average flowering day attributable to *S. gesnerioides* or *A. vogelii* or isolates of these parasites (Table 2). Delays in flowering attributable to seed treatment fit the linear regression: (Flowering day = 35.7

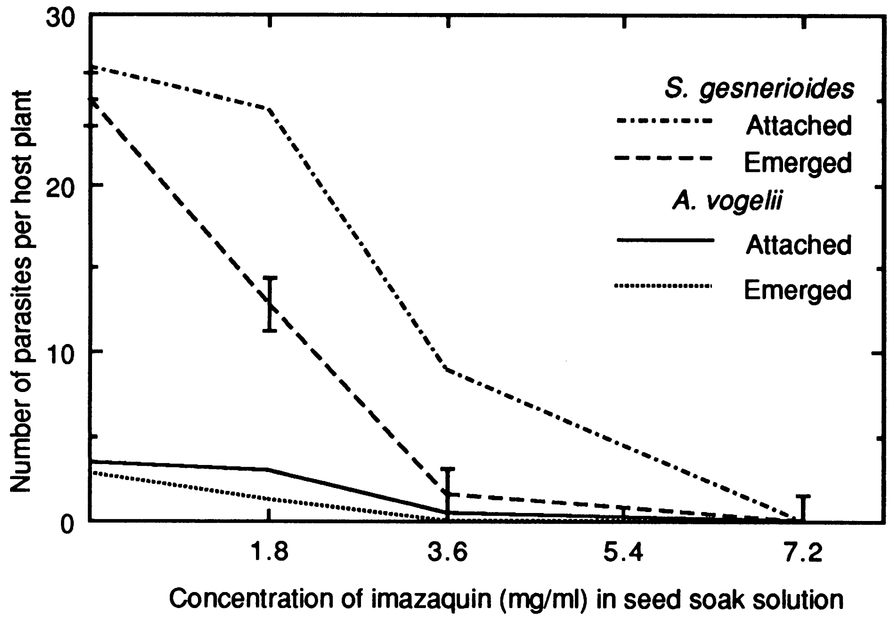

Fig. 1. Attached (below ground and emerged) and emerged *Striga gesnerioides* and *Alectra vogelii* on cowpea plants (cv. IT84s 2246-4) from seeds soaked for 5 min in aqueous imazaquin solutions and planted in pots infested with 3,000 germinable seeds of either parasite. Data are pooled from two isolates of each parasite. Vertical bars represent standard errors of the means.

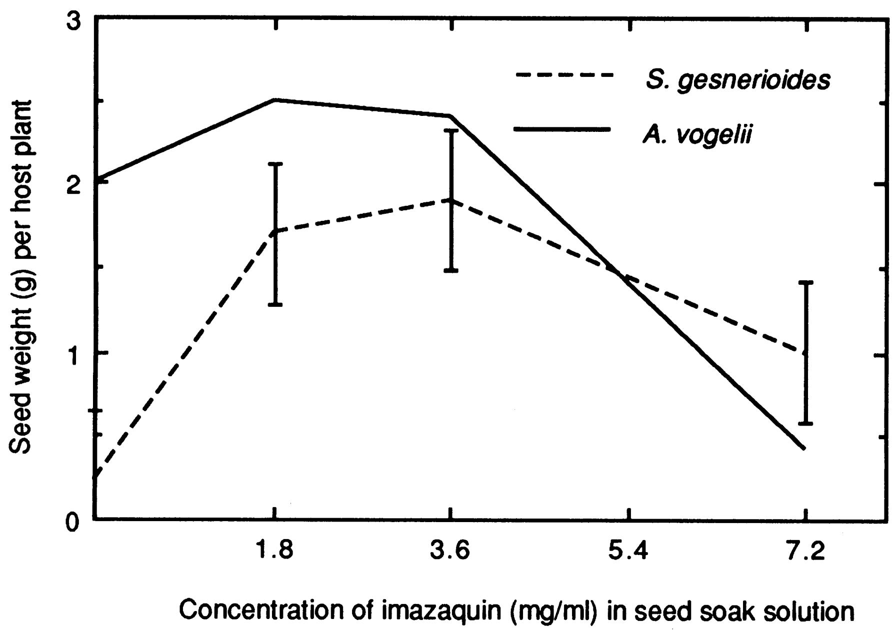

Fig. 2. Mean seed weights of cowpea (cv. IT84s 2246-4) plants from seeds soaked for 5 min in aqueous imazaquin solutions and planted in pots infested with 3,000 germinable seeds of either *Striga gesnerioides* or *Alectra vogelii.* Cowpea plants were harvested 63 days after planting. Mean seed weight of an untreated, uninfested control was 2.7 ± 0.3 g. Vertical bars represent standard errors of the means.

+ 16.7*[mg a.i./ml], R^2 = 0.55, as a proportion of the total sum of squares). The average effect was a 2.9-day delay in flowering for each increase in imazaquin concentration of 1.8 mg a.i./ml.

The effects of cowpea seed treatments on host plant dry weight are presented in Figure 3. There was no significant reduction in dry matter at the highest imazaquin concentrations, indicating that the only deleterious effects of imazaquin seed treatment in this experiment were delays in flowering.

Effects of seed treatment exposure duration. There was no significant reduction in percent seedling emergence with a 5-min soak time compared to untreated controls when concentrations of 1.8, 3.6, and 5.4 mg a.i./ml were used (Fig. 4). Longer soak times at these concentrations reduced seedling emergence and resulted in more severe herbicide damage symptoms (stunting and interveinal chlorosis) than the 5-min soak. Only one emerged *S. gesnerioides* plant was found on any of the plants from treated seeds (5.4 mg a.i./ml, 15-min soak). This parasite had just broken the soil surface at the time of harvest. An average of 18.6 emerged parasites per host plant was found on untreated infested controls. There were no significant differences in total numbers of attached *S. gesnerioides* between the soak durations (Fig. 5). However, longer soak times at the higher imazaquin concentrations produced significant reductions in total host dry weight compared with that measured in the uninfested control (Fig. 6).

DISCUSSION

To our knowledge, this is the first report of the successful use of a commercial herbicide formulation as a seed treatment for seed plant parasite control. Successful attachments, those that survived through harvest, of both *S. gesnerioides* and *A. vogelii* were reduced below those of the infested, untreated control at all seed treatment imazaquin rates in all of the experiments. Microscopic examination of the host root–parasite interface in vitro and the presence of some successful attachments on plant roots from treated seeds indicate that postattachment parasite mortality is the mechanism of control. This is encouraging because imazaquin seed treatments would not only protect the host but also lead to the decline of parasite levels in the soil. An additional treatment of cowpea seeds with a material very efficient in stimulating germination of these parasite seeds should augment this effect, because more parasite seeds would germinate and subsequently be killed by the imazaquin treatment after attachment.

Reductions in successful attachments were generally more consistent at rates in excess of 1.8 mg a.i./ml. However, prolonging the seed soak duration at a

Table 2. Mean flowering times (DAP) for cowpea plants (cv. IT84s 2246-4) from seeds treated[a] with different rates of imazaquin and grown in pots infested with 3,000 germinable seeds of isolates of either *Striga gesnerioides* or *Alectra vogelii*[b]

Parasite	Concentration of imazaquin (mg a.i./ml) in seed soak solution 0	1.8	3.6	7.2	Mean
S. gesnerioides	36.7	38.2	40.8	44.7	39.2
A. vogelii	36.3	40.0	39.3	47.7	39.9
Probability of difference	0.85	0.36	0.45	0.14	0.46
Overall mean	36.5	39.1	40.1	46.2	

[a] Seeds were treated by soaking for 5 min in the respective solutions and air-drying overnight on the day prior to planting.

[b] Data for two isolates of each parasite were pooled. Average flowering time of an uninfested control at 0 imazaquin was 35.8 DAP, which was not significantly different from average flowering times of any parasite isolate infestation at the 0 imazaquin rate.

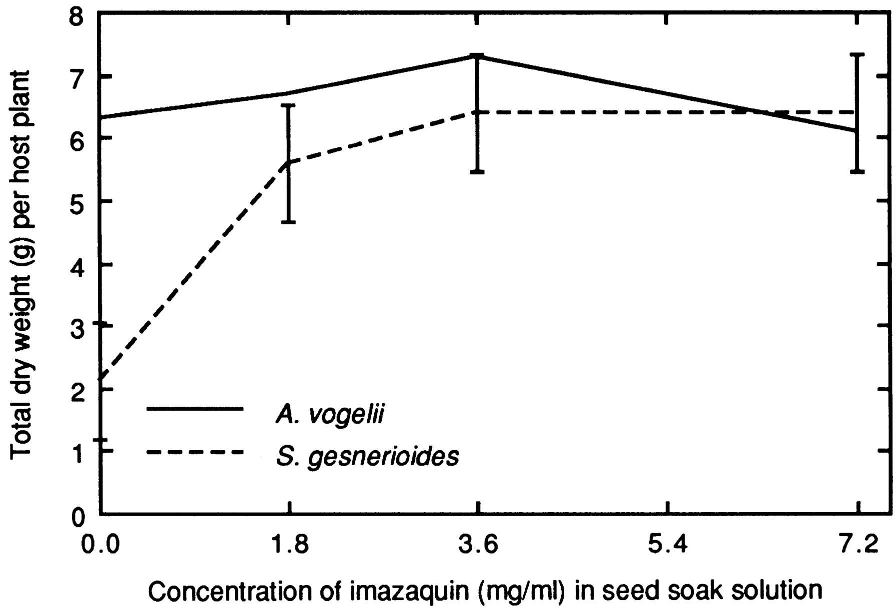

Fig. 3. Mean total (above ground and below ground) dry weights of cowpea (cv. IT84s 2246-4) from seeds soaked for 5 min in aqueous imazaquin solutions and planted in pots infested with 3,000 germinable seeds of either *Striga gesnerioides* or *Alectra vogelii.* Cowpea plants were harvested 63 days after planting. Mean total dry weight of an untreated, uninfested control was 8.0 ± 0.9 g. Vertical bars represent standard errors of the means.

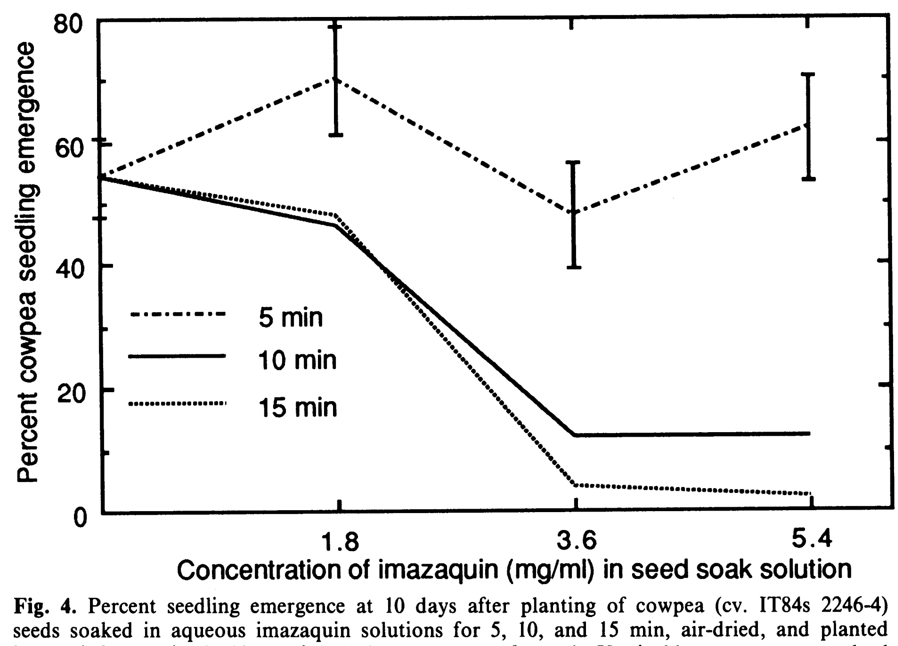

Fig. 4. Percent seedling emergence at 10 days after planting of cowpea (cv. IT84s 2246-4) seeds soaked in aqueous imazaquin solutions for 5, 10, and 15 min, air-dried, and planted in pots infested with 3,000 germinable *Striga gesnerioides* seeds. Vertical bars represent standard errors of the means.

1.8 mg/ml imazaquin rate may increase the efficacy of parasite control without increasing the cost of treatment. Calculated from the derived regression of imbibition on seed soak time, the treatment of 30 kg (a normal seeding rate per hectare in Africa) of cowpea seed for 5, 10, and 15 min with a 3.6 mg/ml imazaquin solution would require 10.02, 12.72, and 15.42 L of solution, respectively. These soak times with a 3.6 mg/ml imazaquin solution would require 200.4, 254.4, and 308.4 ml, respectively, of the commercial formulation. At a cost of $25/L of the commercial product, this seed treatment usage pattern results in a herbicide cost of $5.01, $6.36, and $7.71/ha. A recent purchase of 1 L of Scepter from a local Nigerian source cost $15/L or the equivalent of $3.00, $3.81, and $4.63/ha for seed treatment, depending on soak time. Halving the rate to 1.8 mg a.i./ml while prolonging the seed soak duration to 15 min would reduce costs to $2.31/ha, assuming equal imbibition at the lower rate. The low concentration at longer soak times also makes the seed treatment more robust for African farmers who would not be precise in timing of treatment durations; treatment duration was shown to be important at higher application rates of imazaquin. The low cost at the 1.8 mg/ml rate combined with flexibility of soak times makes this an ideal *S. gesnerioides* and *A. vogelii* control measure for African farmers.

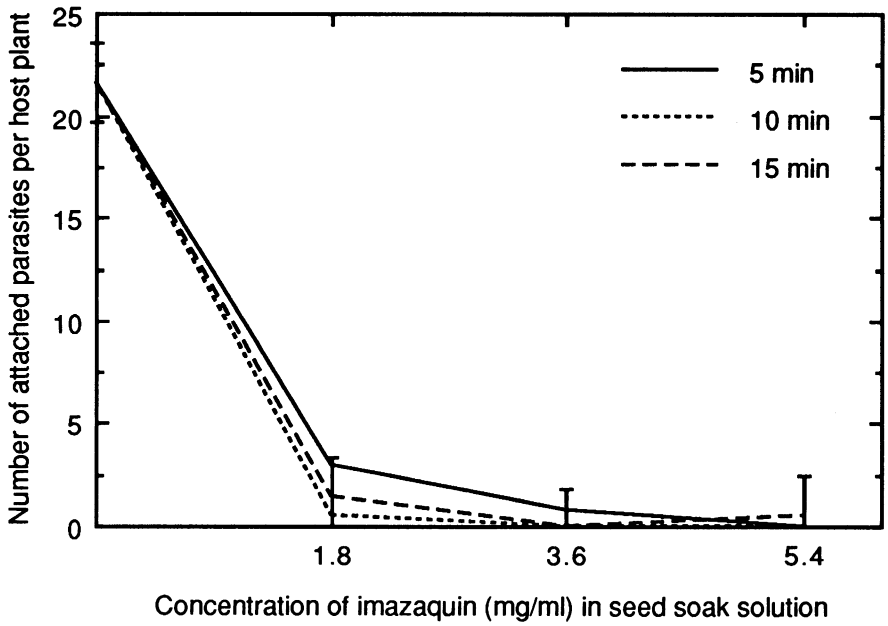

Fig. 5. Mean numbers of attached (below ground and emerged) *Striga gesnerioides* on 55-day-old cowpea (cv. IT84s 2246-4) plants from seeds soaked in aqueous imazaquin solutions for 5, 10, and 15 min, air-dried, and planted in pots infested with 3,000 parasite seeds.

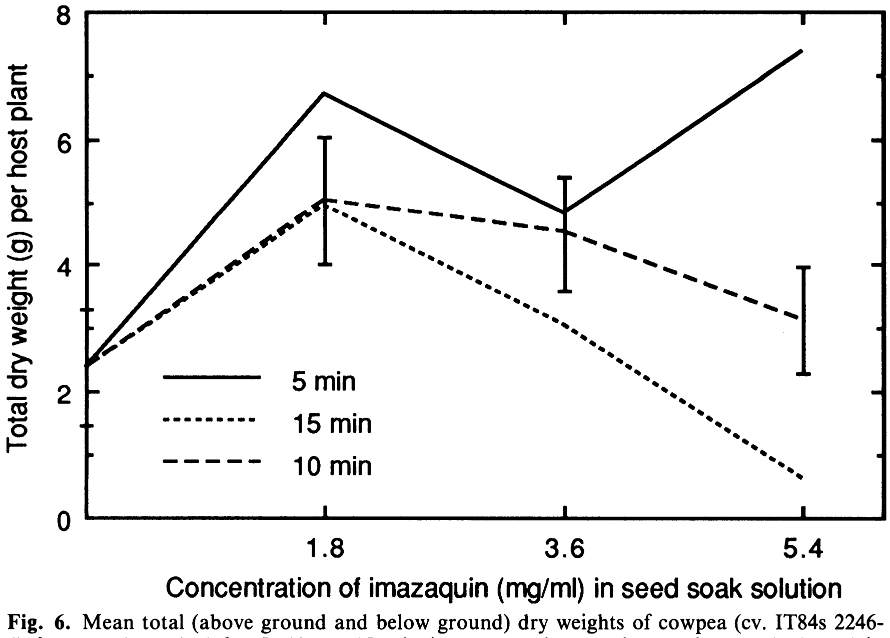

Fig. 6. Mean total (above ground and below ground) dry weights of cowpea (cv. IT84s 2246-4) from seeds soaked for 5, 10, or 15 min in aqueous imazaquin solutions and planted in pots infested with 3,000 germinable seeds of *Striga gesnerioides.* Mean total dry weight of an untreated, uninfested control was 6.3 ± 0.5 g. Vertical bars represent standard errors of the means.

In general, a 5-min seed soak in imazaquin solutions of 3.6–7.2 mg a.i./ml concentrations was adequate to significantly reduce successful parasite attachment. However, increasing concentrations led to delays in flowering, and presumably maturity, and would increase costs of treatment. Prolongation of seed soak durations beyond 5 min, at rates higher than 1.8 mg a.i./ml, significantly decreased seedling emergence and host plant dry weight. As higher seed treatment rates led to greater reductions in parasite attachment, the higher rates could be used in combination with increased cowpea seeding rate if the in-field parasite infestation was high and a quick reduction in parasite populations was desired. Use of this practice would depend on the priorities of the farmer, his precision in timing of seed soak durations, and his economic situation.

Seed soak durations of 5 min at all imazaquin concentrations produced substantial increases in host plant dry weight over that of the untreated infested control. We are currently conducting trials under both controlled field conditions and African farmer management. Results of these trials will help us tailor efficacious and economic seed treatment recommendations.

Use of Scepter for weed control was designed for pre- and postemergent spray treatments. The formulation of the material was appropriately made for this use. In these experiments, we showed that the commercial formulation could also be effectively used for parasite control as a seed treatment. Modification of the commercial formulation for this purpose might lead to better parasite control with less host damage at higher rates. Other modifications of the herbicide class and product formulation may be achievable to protect other crops against parasites such as *Striga hermonthica* and *Cuscuta* and *Orobanche* spp.

Because imidazolinones are potent inhibitors of acetolactate synthase, with resultant blockage of amino acid biosynthesis, this class of chemicals may have a role as a research tool in studying host–parasite interactions. Development of chemicals that inhibit biosynthesis of single amino acids, critical for parasite survival, may lead to increasingly elegant, economic, and environmentally innocuous parasite controls.

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