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Commentary

Appropriateness of biotechnology to African agriculture: *Striga* and maize as paradigms

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Introduction

The witchweeds *Striga hermonthica* and *S. asiatica* decimate maize, millet, sorghum, and upland rice throughout sub-Sahara Africa where, according to FAO studies, over 100 M people lose half their crop production to this flowering, root-attaching parasite (Berner et al., 1995). *Striga* spp. are a major reason that maize yield in the 1.2 million ha sown in sub-Sahara Africa has dropped from near the world average of 4.2 T/ha in the last few decades to the present 1.3 T/ha (FAO, 2001). Although crop rotation, especially with introduced intercrops or rotational crops (Carsky et al., 2000; Khan et al., 2000; Oswald and Ransom, 2001), organic and inorganic fertilizers (Combari et al., 1990; Mumera and Below, 1993; Gacheru and Rao, 2001) can partially allay the problem, no control measure has been developed that subsistence farmers find within their financial means, or that fit well into their traditional cropping systems. Moreover, these measures require several seasons of repeated use before they begin to produce yield benefits (Ransom, 2000). Thus, despite widespread extension efforts, they have not been and are unlikely to be widely adopted, as they are not what the farmers consider 'appropriate' for their needs of providing sufficient food for their families on small, intensively cultivated holdings.

Striga can be controlled by foliar applications of existing herbicides after the flower stalk has emerged, requiring spray equipment and too much herbicide, and would be ineffective for the current season. In addition to draining photosynthate, minerals and water (Press and Graves, 1995), *Striga hermonthica* does most of its damage to maize in Africa through phytotoxins before the weed emerges from the soil (Gurney et al., 1995). Spray applications of most herbicides would kill intercropped legumes, which are planted by many subsistence farmers in an effort to reduce risk and increase the dietary intake of protein that would otherwise come from maize alone.

Subsistence farmers in Kenya and elsewhere cultivate maize with judiciously used, small inputs of fungicide and insecticide seed dressings, and weeks later, apply a few granules of insecticide into the whorl of maize leaves to control stem borers. We reasoned that small amounts of herbicide could control the parasitic *Striga* while it is still underground, before the weed damages the crop (Abayo et al., 1996, 1998; Kanampiu et al., 2001). African farmers adopt new maize varieties and technologies having perceived value, including hybrid maize, as borne out in economic studies of fertilizer adoption in African subsistence agriculture (McCown et al., 1992).

Maize is a very important part of the human caloric intake throughout sub-Sahara Africa, and the situation of production, consumption and parasitic weed constraints is similar to that throughout the region. Kenya has an average per capita maize consumption of 103 kilograms per year of which 91% is used for food

(Pingali and Pandey, 2001). Maize yield and production in Kenya are 1.7 tons/ha and 2.7 million tons, respectively, and are on the decline, making Kenya a net importer of 427 000 tons of maize. In Kenya alone, there are over 80 000 ha of land severely infected with *Striga hermonthica*. Doubling yields from 1 ton would produce enough maize to provide an additional 400 000 people with their current average annual maize consumption. The areas badly affected by *Striga* are also the areas where many of the poorest people live with the highest percentage of maize in their diet. Farmers who no longer lose their maize to *Striga* can be expected to put more input into weeding, and to invest in some fertilizer. They will certainly see the benefit of buying herbicide-coated seed each season, and can easily achieve 3.5 T/ha. Maize imports can be reduced, and the cost of distribution cut down.

Table 1. Increase in yield of maize using 'in-seedo' treatment with herbicides

Lightly or moderately infested sites			Heavily infested sites		
control	treated		control	treated	
Tons grain/ha		# sites	Tons grain/ha		# sites
1.0	3.5	21	2.2	3.7	17

The infestations of *Striga hermonthica* and *Striga asiatica* on the 38 sites located in 4 different countries measured in 8 different seasons where farmers' sites were low/moderately (naturally) infested, and on-station were heavily (artificially) infested in each season (with each season's data counted as a site), and the average yields presented. It is not possible to have the equivalent of hand weeded controls in farmers' field experiments with *Striga*, as the damage is done before *Striga* emergence, and thus the use of infestation levels as a comparison point. The data for imazapyr and pyrithiobac seed treatments are bulked at all concentrations used.

Proof of concept that biotechnology can provide the answers to *Striga*

Some transgenic (Joel et al., 1995) and tissue culture derived (Abayo et al., 1996, 1998; Berner et al., 1997) herbicide-resistant crops enable the early control of parasitic weeds before or during attachment to the host. The specific genes confer herbicide resistance through a modification of the binding site such that the herbicide does not bind to its target in the crop (Newhouse et al., 1991). Such herbicides are exuded from crop roots and kill attached *Striga* as well as its seeds in the soil before they germinate (Kanampiu et al., 2002).

We (Abayo et al., 1996, 1998; Kanampiu et al., 2001) and others (Berner et al., 1997) have developed methods of coating small amounts of acetolactate synthase inhibiting herbicides on biotechnologically produced (non-transgenic) imidazolinone-resistant (IR) maize seed. These methods considerably lower the amount of herbicide required to control *Striga* on a per hectare basis, but the herbicide concentration is very high in the vicinity of the seed, necessitating the high level of resistance conferred by such target-site mutations in ALS. The case has also been made that herbicide-resistant crops would preclude the common practice of intercropping cereals with grain legumes, reducing dietary protein intake and availability. Our studies show that seed-applied herbicide has no effect on intercropped cowpeas (*Vigna unguiculata* (L.) Walp.), yellow gram (*Vigna* spp.) (Figure 1), or field beans (*Phaseolus vulgaris* L.) (Kanampiu et al., 2002) if the legume is sown more than 12 cm from the treated maize seed. Since such legumes are typically sown equidistant between maize hills within the row (i.e. 30 cm from each hill), or between the maize rows (spaced at 75 cm), the technique poses negligible risk to the intercrop.

Imazapyr and pyrithiobac, which gave the best results, inhibit only acetolactate synthase (ALS), an enzyme not present in mammals. They have been exhaustively tested and no toxicological problems have been found. They are vastly less toxic than betacyfluthrin, the insecticide used for stem borer control which has an oral LD_{50} (rats) of 500 mg kg^{-1} (Tomlin, 1994) *versus* >5000 mg kg^{-1}, (i.e. immeasurable) for imazapyr (Gagne et al., 1991), and 1000–5000 mg kg^{-1} for pyrithiobac (Tomlin, 1994). The herbicides dissipate from the soil well before the next planting season, without any ill effect to subsequent crops. Maize yield from treated seed is more than doubled when *Striga* infestations are moderate, the yield benefit can be almost infinite when the *Striga* infestations are severe as there is near total crop loss without seed treatment. The results of multi-field trials in four countries are summarized in Table 1.

Lightly or moderately infested farmers' rainfed fields have low inherent soil fertility while the heavily infested irrigated experiment-station fields were fertilized annually, explaining why the grain yield in untreated controls was 1.0 tons/ha in low/moderately-infested farmer sites as compared to 2.2 tons/ha in heavily infested field station sites.

The on-farm yields increased on average from 1.0 to 3.5 tons/ha, by only investing US$ 4 in herbicides. There would have to be an additional expenditure of

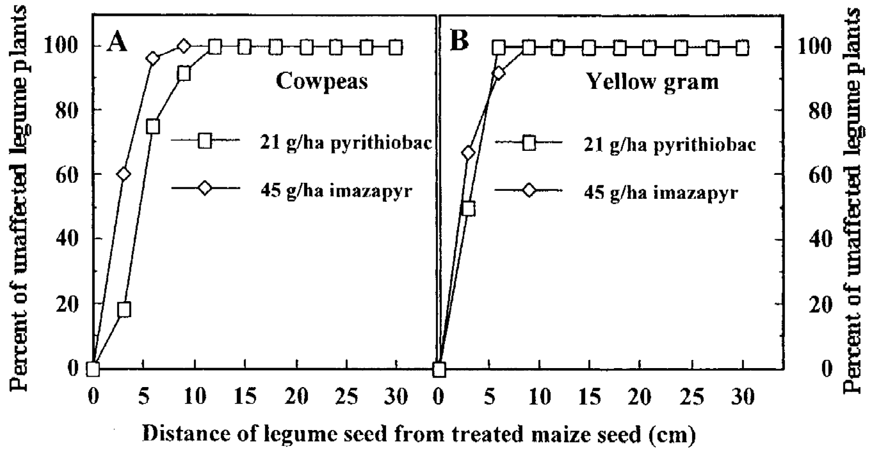

Figure 1. Lack of effect to normally-spaced intercropped legumes from herbicide treatment to seeds of herbicide resistant maize. The data are presented as the average number of completely unaffected (healthy) yellow gram and cowpeas. The maize variety used is a synthetic, open-pollinated imidazolinone-resistant (IR) variety bred by backcrossing Pioneer hybrid 3245IR into local varieties (Kanampiu et al., 2002). The Pioneer hybrid 3245IR, originally derived from a tissue culture selection (Newhouse et al., 1991), bears a tryptophan 552 to leucine mutation in the acetolactate synthase gene (Bernasconi et al., 1995). Imazapyr was applied at 0.84 mg per seed and pyrithiobac at 0.4 mg per seed, with a commercial fungicide insecticide seed dust. These rates are equivalent to 45 and 21 g per hectare of pure herbicide, respectively, and are more than double the dose that we recommend for use for seed treatments. The rates were chosen to accentuate any potential risk to the intercropped legumes from over-application of herbicide. The field experiment shown (one of three in successive seasons, all with similar results) was conducted during March–August 1999 at the Kenya Agricultural Research Institute (KARI) National Sugar Research Center at Kibos, in western Kenya. The field site was heavily infested with *Striga hermonthica*. The experimental design was a randomized complete block with three replications. The normal spacing between legume and maize is 30 cm.

$29 for open pollinated or hybrid ALS resistant seed and $77 for fertilizer per hectare, and the herbicide would be applied by the supplier together with the insecticide+fungicide seed dressing. Currently the local price of maize is $142/ton. Therefore, farmers can realize a net of more than $300 per hectare from the yield increase, an approximately tenfold increase on investment in a single 5 month season. The benefits from this technology are high and once adopted, it can ease undernourishment within the grower families, with some extra maize for sale.

Appropriateness of this technology

We therefore propose that this biotechnology is not only appropriate for African agriculture, but as opposed to the developed world, it is more appropriate for African agriculture where hunger is common (Pearce, 2000) and yield losses impinge directly on human caloric intake. In Kenya, 91% of maize produced is directly consumed by humans (CIMMYT, 1999) while maize is mainly livestock feed in the developed countries. The single dominant gene can be easily backcrossed into local varieties by conventional breeding methods, as we have done from the initial USA corn-belt material.

Other appropriate technologies for *Striga* and for maize cultivation

Bt genes

More than 50% of the maize area in developing countries faces serious problems of insect infestation. In Kenya alone, farmers estimate crop losses due to stem borers at 15% of their ultimate harvest, in a country where many people live on less than US $1 a day. Infestations of these pests can decimate individual maize fields – depriving a rural family of vital income and a year's supply of their main food source.

Various strains of commonly found *Bacillus thuringiensis* (Bt) produce protein crystals containing one or more δ-endotoxins during sporulation. The δ-endotoxins bind to specific receptors in the insect mid-gut after ingestion, leading to insect death. Each Bt protein is active in only one or a few insect species, making them far more selective than most chemical insecticides. Bt sprays have been used for many years to control several insect pests.

The genes coding for various Bt proteins were among the first to be introduced into agriculturally important cotton and maize. North American farmers planted nearly 8 million hectares of transgenic Bt crops in 1999. The area is expected to increase globally, as other countries begin to approve these crops. Controversial concerns over Bt crops range from a general dislike of genetic engineering, to issues related to insect resistance management and increased profits to the companies that commercialize the crops. The concerns surrounding the release of Bt crops have not been realized, and farmers have seen increased yields, with lower insecticide use, which is needed more in many developing countries than in the developed ones.

One of the Bt genes that have been introduced into maize and commercially released in the USA, *cry1ab*, is effective against some of the African insect pests, as are some proteins produced by other *cry* genes (CIMMYT, unpublished data). These natural insecticide genes could be introduced into local open-pollinated as well as in hybrid maize varieties. Farmers prefer their inputs being in the form they are most used to – the seed, and not insecticides. Many farmers are currently not using insecticides due to costs, and thus would immediately see their yields increase. Many others who now can afford limited amounts of chemicals would see increased yields and incomes, with perhaps most important, decreased health risks due to the decreased pesticide use. These benefits must clearly outweigh the slight risks that any new technology brings.

Transposon transmitted suicide genes for Striga

It has been proposed that it may be possible using genetic engineering to debilitate weeds leading to self control, for *Striga* (Gressel and Levy, 2000) and for other cross-pollinated weeds where there are no closely-related crop, nor wild or weedy species (Gressel, 2002). This concept is an adaptation of a proposal for controlling insects with the TAC-TIC model: "Transposons with Armed Cassettes for Targeted Insect Control". The insects are transformed with assisted-suicide (inducible) genes ('*kev*' (Kevorkian) genes), which if activated, debilitate the host (Pfeifer and Grigliatti, 1996; Grigliatti et al., 2001).

Adapting this would entail developing transgenic *Striga* with high copy number transposons carrying *kev* genes, i.e. deleterious transposons (DTs), which will quickly spread the gene to field populations because *S. hermonthica* is an obligate outcrosser requiring exogenous pollination (Aigbokhan et al., 1998). The antisense constructs for *Striga* could be introduced within the Ac transposon. The recently mapped gene for haustorial recognition and attachment (Stranger et al., 1999) could be included in a *kev* construct (in the antisense direction or for suppressive overexpression); the ideal inducer would be one emanating from maize roots (Gressel and Levy, 2000).

Many targets can be considered where the genes are known, e.g. in primary biosynthesis pathways that are targets for herbicides (Gressel and Levy, 2000). Plants expressing such an antisense gene will react as if treated by a herbicide when the *kev* inducer is activated. Tandem constructs of more than one gene could add to the versatility of the approach; e.g. the addition of genes abolishing secondary dormancy might assist transposon spread as well as facilitate a more rapid depletion of the seed bank (Gressel and Levy, 2000).

Biocontrol

Various mycoherbicidal organisms (Abbasher et al., 1995; Marley et al., 1999; Ciotola et al., 2000), bacteria (Berner et al., 1999; Miche et al., 2000) and arthropods (Smith et al., 1993; Pronier et al., 1998) that attack *Striga* might serve as biocontrol agents, protecting crops from damage. None have yet fully proven themselves in the field, and there are good scientific/ecological reasons to doubt that a single organism can be cost-effectively applied to provide the level of control needed in field crop situations. The organisms might have the needed effectiveness if they are transgenically-enhanced with virulence genes, as has been generally described in Vurro et al. (2001), although failsafe mechanisms must be considered to prevent off-target movement or gene introgression (Gressel, 2001). Transgenes encoding hormone overproduction conferring hypervirulence have been successfully introduced into fungi controlling a related parasite (Cohen et al., 2002).

Concluding remarks

We advise that extreme caution be used in accepting designations of what is appropriate to African cropping systems from those with a non-scientific agenda, and from those who have little awareness of the needs or the agricultural ecosystems in question. Conversely, crop protection solutions rarely last forever in agriculture; pest organisms typically evolve mechanisms to circumvent all control tactics, whether chemical or otherwise. One should expect that *Striga* will evolve resistance to herbicides as well as to rotational, trap, or catch crops, or to biocontrol agents. The best one can do is to devise strategies to delay the inevitable. In the case of seed treatment with herbicides, it has been proposed that farmers should rogue the five resistant plants per hectare that models suggest will appear each year, to extend the life of this technology (Gressel et al., 1996). If the seed treatment technology is integrated with hand rouging of any *Striga* that appears, and/or crop rotation, the duration of utility of all the techniques should be enhanced.

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