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A priori risk assessment of breaking of genetically engineered resistance in maize by the parasitic weed *Striga hermonthica*

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1. Introduction

Development of resistance against pesticides or breaking of host resistance by parasites is very common. In fact development of resistance against pesticides is considered nearly inevitable over a longer period of time (e.g. Urech *et al.*, 1997), just like the evolutionary arms race between pathogens and host plants will give rise to new, virulent strains (Crute *et al.*, 1997). Genetic modification however can introduce completely new mechanisms of resistance into plants, which are thought to have very little risk of resistance development. Examples of these new resistance mechanisms are the use of plantibodies (*in planta* expression of animal-derived antibodies against pests or pathogens), triggering of toxin genes by pest or pathogen-derived signals and activation of plant disease responses by introduction of pathogen-derived genes (Ohl *et al.*, 1997; Kawchuk & Prufer, 1999; Shen *et al.*, 2000). The expectation is that breaking of novel resistance mechanisms will be virtually impossible, if these mechanisms disrupt a basic aspect of the life cycle of the pathogen. This notion seems logical, but has not been tested yet, because crops with novel resistance mechanisms are not grown at large scale at the moment. The only crops with genetically modified resistance that are used at larger scale are maize and cotton with the insecticidal *cry*-genes derived from the bacterium *Bacillus thuringiensis* (Bt). In this case however the mechanism is not new and resistance against Bt- based conventional insecticides has already developed. This has evoked a heated public debate and a great interest of the registration boards of the countries where Bt-transgenic crops were admitted, Australia and the USA. Although not technically required by law, resistant management plans were a major issue in the registration process for both the US Environmental Protection Agency (EPA) and the Australian National Registration Authority (Roush, 1997).

In the present study a risk assessment is made of a strategy of genetic modification of maize against the plant parasitic weed *Striga hermonthica*. Since no direct data on the risk of resistance breaking are available, the study is based on general *a priori* risk assessment schemes and models for pesticides, on experience with durability of conventional resistance and on ecological and physiological data on the host-pathogen interaction.

Several species of the root- parasitic genus *Striga* (Scrophulariaceae) cause major crop losses in the tropical areas of Africa and Asia. On poor soils *Striga* is a major production constraint in many crops. Biological and economical factors make *Striga* very hard to control. Biological characteristics making control of *Striga* difficult are the low damage threshold, the longevity of the seeds and the high reproductive capacity of the weed (Parker & Riches, 1993; Gbèhounou, 1998). Economic factors hindering control of *Striga* are the lack of resources of the subsistence and smallholder farmers that suffer mainly from the weed, which excludes the use of chemical control. Furthermore, the amount of labor demanded by non-chemical control methods like hand-pulling make these options impractical in many cases (Reichmann *et al.*, 1995; Ransom, 2000). Resistant crop varieties seem a good option to control *Striga* problems, since there is no need for additional labor or chemical input requirement.

Striga has a complicated life cycle, which depends for several processes on chemical signals of its host (Parker & Riches, 1993). One of the key processes triggered by the host plant is germination of the *Striga* seeds. Without the plant signal, seeds will not germinate which would disrupt the link between the life cycle of the parasite and the host. This would effectively solve the problems with *Striga*. (Joel, 2000). The strategy of disturbing the chemical communication between *Striga* and its host is one of the avenues that is exploited in the multinational research project 'Improved Striga control in Maize and Sorghum' funded by the EU INCO research program. In this project a collection of transposon mutants of maize will be screened. Maize lines that show reduced stimulation of germination of *S. hermonthica* seeds are thought to have mutations in the genes encoding for or regulating the pathway of the production of the germination-stimulating signal molecules. These lines are promising starting points for genetic analysis of resistance or for breeding programs of *Striga*-resistant maize varieties.

The set-up of this literature study is as follows: first the (chemical) ecology and physiology of the parasite-host relation is reviewed. Then risk assessment and resistance management in analogous situations is considered and the applicability to the question of this study is addressed. Situations considered analogous to the problem of resistance breaking by *Striga* are: occurrence of resistance against herbicides, durability of resistance against *Striga* in existing host plant varieties, and resistance management in genetically modified crops producing the insecticidal *Bacillus thuringiensis* toxins. In conclusion, the question of resistance breaking by *Striga* will be considered in the light of the ecological data and the information from analogous situations, as far as applicable.

2. Ecology of the *Striga* – host interaction

Three species of *Striga* cause major agricultural problems in Africa and Asia. The species *S. hermonthica* (Del.) Benth. and *S. asiatica* (L.) Kuntze parasitize wild grasses and cereal crops. *S. gesnerioides* (Willd) Vatke parasitizes dicots from various families, such as the Agavaceae, Convulvulaceae, Euphorbiaceae and Fabaceae. The main agricultural host for *S. gesnerioides* is cowpea (*Vigna unguiculata*) (Parker & Riches, 1993). Major crops parasitized by *S. hermonthica* several types of millet (*Pennisetum, Eleusine* and *Panicum* spp.) sorghum (*Sorghum bicolor*) rice (*Oryza sativa*), sugarcane (*Saccharum officinarum*) and maize (*Zea mays*). *Striga* species can cause very high damage, sometimes resulting in complete crop failure and farmers may be forced to give up fields with heavy infestations (Musselman, 1980; Parker & Riches, 1993). Annual yield losses in Africa due to *Striga* are indicated to be in the range of 1 to 12 billion US dollars (Gbèhounou, 1998).

Striga plants are root parasites that take up assimilates and water from their hosts through haustorial connections. *S. hermonthica* possesses photosynthetic ability, but derives about 35 % of its organic carbon from its host (Parker & Riches, 1993). Removal of carbon explains only about 20 % of the yield reduction of the host. Disturbance of water relations and hormone balance resulting in disruption of host carbon fixation and possibly production of toxic compounds account for the larger part of *Striga* damage to its host (Berner *et al.*, 1995; Press *et al.*, 1996; Frost *et al.*, 1997).

The life cycle of *Striga* is closely related to its host and several chemical cues from the host are needed to trigger various developmental stages of the weed (Fig. 1).

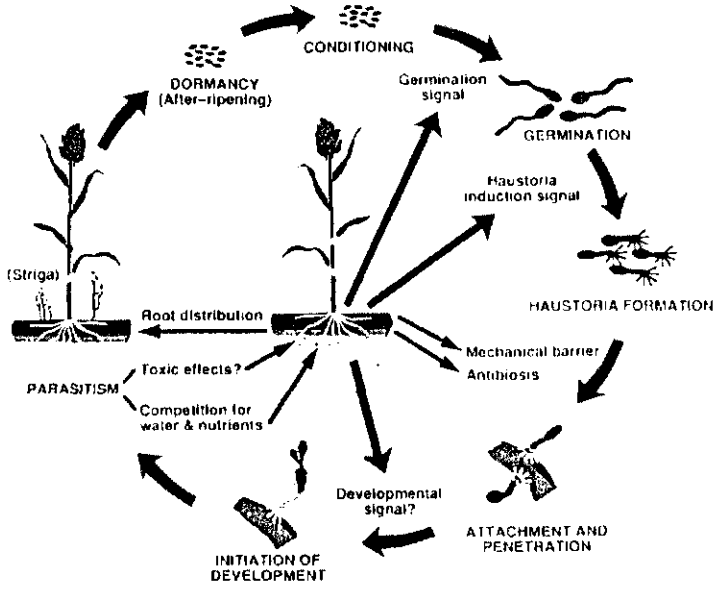

Figure 1. Life cycle of Striga.

Striga seeds are very small and can only reach the host plant root if they germinate within 2 - 4 millimeters of the root (Ramaiah *et al.*, 1991). This implies that the signal from the plant roots must either be very weak, immobile or unstable in soil (or a combination of these). A different chemical signal is needed to induce haustorium formation on the roots. After the formation of the haustorium, *Striga*

penetrates the host root and forms a xylem connection to the host (Parker & Riches, 1993; Arnaud *et al.*, 1999). After successful penetration of the host root *Striga* emerges 2 – 3 weeks after germination and produces up to 450,000 new seeds 6 – 10 weeks after germination (Eplee, 1992; Parker & Riches, 1993; Berner *et al.*, 1997).

Seed longevity

Striga seeds may survive prolonged periods and still remain infective. Survival times up to 14 years are reported, (Bebawi *et al.* 1984; Eplee, 1992) but under moist field conditions considerable seed decay occurs within one year (Pieterse *et al.*, 1996; Gbèhounou, 1998; Van Ast *et al.*, 2001). The newer data indicate that crop rotation and introduction of resistant varieties can reduce the population size of *Striga* but due to the longevity of the seeds, the extreme high reproduction rate and the low damage threshold, it may take several years before a significant effect is reached (Gbèhounou, 1998) .

Induction of germination

Striga seeds germinate under influence of chemical plant signals, provided the seed is in a physiological stage in which it is receptive to the plant signal. To become receptive, new seeds must pass through a dormancy phase and a conditioning phase at high temperature and moisture conditions. If the seed is not triggered to germinate after the conditioning it may become quiescent again upon drying or enter a stage of secondary dormancy if the wet conditions continue ('wet dormancy') (Parker & Riches, 1993; Berner *et al.*, 1997).

Not only host plants produce germination stimulants. The first identified germination stimulant, strigol, was isolated from the root exudate of the non-host cotton (Cook *et al.*, 1966) and germination stimulating compounds have been described from exudates of other non-hosts (e.g. Ma *et al.*, 1996). There is a debate in the literature whether the group of strigolactones (Butler, 1995) or dihydrosorgoleone is the main compound inducing germination in the field. A series of publications focuses on dihydrosorgoleone (Chang *et al.*, 1986; Boone *et al.*, 1995; Fate & Lynn, 1996; Erickson *et al.*, 2001). The role of dihydrosorgoleone seems questionable however, since this compound is not found in all hosts of *Striga*, whereas strigolactones have been found in all major *Striga* hosts. Furthermore the relation between resistance of plant varieties and their production of dihydrosorgoleone is much less clear than the relation between the production of strigolactones and plant resistance (Hess *et al.*, 1992; Olivier & Leroux, 1992). The concentration by which strigolactones induce germination is in the range of 10^{-11} to 10^{-9} M, while dihydrosorgoleone is active at concentrations of 10^{-6} M. Yet recently Erickson *et al.* (2001) pointed out that in sorghum the production of dihydrosorgoleone is much higher than that of the strigolactones and they claim that the conclusions of Hess *et al.* (1992) are flawed by methodological problems. However the proposed chemical scheme explaining the similarity in activity of dihydrosorgoleone and the strigolactones is invalid (Boone *et al.*, 1995, Wigchert & Zwanenburg, 1999). It seems that the arguments to support the role of strigolactones as the most important communication compound between *Striga* and its hosts are convincing. It must be pointed out though that the concept of *Striga* control through transposon mutants lacking production of germination stimulants does not hinge on the identity of the actual germination stimulant, as long as the mutant lines are tested in a bioassay, rather than for the production of certain chemicals. In maize, the target plant of this study, dihydrosorgoleone was not found while strigolactones are produced (Siame *et al.*, 1993).

In the rest of this study, the strigolactones will be considered the main agent of induction of germination of *Striga*, because of the above mentioned reasons.

Generally host plants produce more than one germination stimulating compound, though one of these usually is the most important (Siame *et al.*, 1993).

The strigolactones belong to the biochemical group of sesquiterpenes. Sesquiterpenes are a diverse group of secondary plant metabolites. They have no known role in the basic metabolism of the plant but some sesquiterpene lactones show activity against other plants (allelopathy) and micro-organisms. Some bitter compounds in plants belong to this group (e.g. Jisaka, 1993; Wedge *et al.*, 1998; Macias *et al.*, 1999).

Mechanism of germination stimulation

The key process of induction of germination in *Striga* seeds is ethylene production. When the seeds are in the right physiological condition ethylene and compounds that stimulate ethylene production will trigger germination (Logan & Stewart, 1991; Gabbar *et al.*, 1993; Babiker *et al.*, 2000). *Striga* seeds show production of ethylene, when they germinate and natural and synthetic germination stimulants induce ethylene production in *Striga* (Harren *et al.*, 1994; Thuring *et al.*, 1994). Both germination and ethylene production are inhibited by aminoethoxyvinyl glycine (AVG), an inhibitor of ethylene biosynthesis (Logan & Stewart, 1991) and germination is inhibited by 2,5-norbornadiene, an inhibitor of ethylene action (Gabbar *et al.*, 1993). Addition of an ethylene biosynthesis intermediate, 1-amino-cyclopropane-1-carboxylic acid (ACC), was found to override the inhibitory effect of AVG (Gabbar *et al.*, 1993). These findings convincingly show the key role of ethylene synthesis in the germination of *Striga*. Apart from the strigolactones and a number of synthetic strigol analogues (Krantz *et al.*, 1996; Nefkens *et al.*, 1997; Thuring *et al.*, 1997) numerous synthetic and natural compounds stimulate germination in *Striga* seeds. These include several growth hormones: kinetins, zeatin, and ethylene. Other chemicals that induce germination are scopletin, inositol, dihydrosorgoleone, sodium hypochlorite and jasmonates (Worsham, 1987; Igbinnosa & Okonkwo, 1992; Yoneyama *et al.*, 1998; Erickson *et al.*, 2001). The concentration in which these alternative germination stimulants are active is at least 1000 times higher than that of the strigolactones. This indicates that the mechanism of action of the non-strigolactone stimulants is fundamentally different from that of the strigolactones. An attractive hypothesis is that these compounds influence the ethylene biosynthesis through different mechanisms including possibly wound response reactions (Gabbar *et al.*, 1993). This would explain the large array of structurally unrelated compounds that can induce germination in *Striga*.

Based on the extremely low concentration of strigolactones needed to stimulate germination in *Striga* and the fact that only synthetic analogues with exactly the same stereochemical configuration as the natural compounds show biological activity the hypothesis was posed that there is a specific receptor for strigolactones in *Striga* (Wigchert & Zwanenburg, 1999; Zwanenburg & Reizelman, 2001).

Spontaneous germination

Striga seeds may germinate spontaneously (without plant signal) under some circumstances (Pavlista *et al.*, 1979; Okonkwo, 1991; Maas, 2001). Data about the factors inducing spontaneous hatch and its relevance under field condition are lacking, but under laboratory conditions spontaneous germination can occur in up to 81 % in some seed batches (Maas, 2001). Presumably the removal of seed coat contained germination inhibitors contributes to spontaneous germination (Maas, 2001). The percentage of spontaneous hatch differs strongly between different batches, even of the same *Striga* species. A correlation with age was found, but age alone could not explain the variation observed between seed batches (Okonkwo, 1991; Maas, 2001).

3. A priori risk assessment of resistance breaking

The analysis of the risk of resistance breaking by *Striga* consists of two compounds: the first compound is the risk of the occurrence of resistance breaking *per se*. The second compound is the question what threat (hypothetically) resistant *Striga* would pose and which factors influence that risk.

One basic question in the *a priori* risk assessment of genetically engineered resistance is whether resistance breaking can occur at all. For the best studied case, the use of *Bacillus thuringiensis* (Bt) *cry*-genes against insects, the outcome of this question is obvious, since resistance against the Bt toxins has already occurred in conventional application of the insecticide. In other cases the answer to the question is far from obvious. Novel resistance mechanisms may pose an insurmountable hurdle for the adaptive capacity of the target pest, in which case resistance breaking will never occur. Of course this can only be proven by long term and large-scale use of the genetically modified resistant varieties, and these data are not available. However an approximation analysis can be made, based on the ecological and physiological data on the pathogen – host interaction and by comparison to analogous systems.

Chance of resistance breaking by *Striga*

One of the main factors determining the chance of resistance breaking is the variability of the target species. *S. hermonthica* is quite variable in many characteristics (Parker & Riches, 1993) and it has developed host races that may have distinct morphotypes, to which no formal taxonomical status is given (Olivier *et al.* 1998; Mohamed *et al.*, 2001). *S. hermonthica* can form hybrids with *S. aspera* that can successfully backcross with either parent. This means that both species may serve as gene reservoir for each other, since both species are sympatric in West Africa and are strictly allogamous with common pollinators (Aigbokhan *et al.*, 1998; Aigbokhan *et al.*, 2000). The response of *S. hermonthica* to germination stimulants is quite variable. *S. hermonthica* populations do not react equally strongly to root exudates from several host plants and the effect of the synthetic germination stimulant GR 24 is also variable (Gbèhounou, 1998). The reaction to germination stimulants is related to the host plant from which the different *Striga* populations originated (Parker & Reid, 1979; Gbèhounou, 1998). These data show that there is a significant plasticity in the mechanism of germination control in *S. hermonthica* and in its genetic basis.

Transposon mutants of maize showing reduced stimulation of *Striga* germination in a bioassay can have four types of mutations: 1. blockage of a biosynthesis step in sesquiterpene lactone production; 2. changes in a modification step of the sesquiterpene lactone; 3. changes in excretion; 4. changes in the regulation of the germination stimulant biosynthesis pathway. The first possibility would give rise to a phenotype without any sesquiterpene lactone production. The second mutation type would result in production of a different sesquiterpene lactone and the third and fourth possibility would yield a phenotype with reduced or increased production of the original sesquiterpene lactone. Increased production of germination stimulant may also inhibit *Striga* germination (Joel, 2000). All these mechanisms would result in a reduced or completely inhibited germination when *Striga* seeds are exposed to the root exudate of such a mutant line in a bioassay. It is clear that these different possibilities give rise to different potential mechanisms of resistance breaking by *Striga*.

The likelihood of resistance breaking if the production of sesquiterpene lactones in the host is completely blocked seems very low. Although *Striga* seeds react to a large range of other chemical (see above) the possibility that one of these compounds will take over the role of germination signal seems unlikely. The chance of the *Striga* germling to reach the host plants depends not only on germination

being triggered, but also on germination being triggered only within the close vicinity of the plant root (see above). Therefore the germination signal from the host root has to have a characteristic distribution in soil. To enable successful penetration of the host plant an alternative germination signal must have a similar combination of stability and mobility in soil and a similar dose-response relation as the strigolactones have. The chance of both these conditions being met seems very small. Yet because of the existence of naturally occurring alternative germination stimulants, the chance of *Striga* adapting to a host that does not produce strigolactones is not zero.

The likelihood of *S. hermonthica* adapting to a mutant host with a modified sesquiterpene lactone (mutation type 2) seems relatively high, because the significant plasticity in the mechanism of germination control in *S. hermonthica.* Adaptation of *S. hermonthica* to new hosts in Africa is considered a recent event, from evolutionary perspective (Parker & Riches, 1993; Olivier *et al.* 1998). This indicates that mutations in the genetic basis of germination stimulation are quite frequent or that the genetic basis of germination stimulation is diverse. Indeed *S. hermonthica* was able to adapt itself to maize as a new host within several years after the introduction of maize in northern Cameroon (Parker & Riches, 1993).

Low rate of production of strigolactones will probably mean that a plant is resistant to *Striga* as a relation between amount of strigol and resistance level was found in sorghum (Hess *et al.*, 1992; Weerasuriya *et al.*, 1993). It is not clear whether *Striga* can adapt to different quantities of stimulation germination from its host, since the triggering of germination has to occur within a limited distance from the host root. Yet since there will be a large random variation in the distance in which a host root will pass a *Striga* seed in the field, it seems likely that some seeds will receive the proper dose of germination stimulant, as long as the production of stimulant is not extremely low. This situation would give rise to a partial resistance, meaning that still some *Striga* would develop and reproduce. The chances of resistance breaking of the *Striga* population on a partially resistant host are bigger than on a totally resistant variety, because the *Striga* population would exist much longer. Only at low levels of partially resistance the selection pressure for virulence on the *Striga* population would be low, but in that case the effectiveness of the host resistance is questionable.

The situation of the risk of resistance breaking of genetically modified maize lines can be compared to the introduction of conventional resistant varieties. Most data are available on *S. asiatica* on resistant sorghum and on *S. gesnerioides* on resistant cowpea (Parker & Riches, 1993; Lane *et al.*, 1997). The resistance of cowpea against *S. gesnerioides* is not based on absence of germination stimulation (Lane *et al.*, 1993; Lane *et al.*, 1996). The resistance in cowpea seems to be stable since at least 1987, when resistant varieties were passed out to farmers (Lane *et al.*, 1997). Resistance in sorghum to *S. asiatica* is based on lack of germination stimulation in several sorghum varieties (Hess *et al.*, 1992; Weerasuriya *et al.*, 1993). In the 1930's and 1940's breeding of sorghum for resistance against *S. asiatica* was started in South Africa and India (Ramaiah, 1987; Parker & Riches 1993). Some of the varieties developed in this early work show reduced or no germination stimulation. The resistant variety Framida, that is still in use (Haussmann *et al.*, 2001), is thought to have originated from the work in South Africa. This shows that resistance based on reduced germination stimulant production may hold for at least 50 years. In general, the *S. asiatica* resistant varieties of sorghum show lower levels of resistance against *S. hermonthica* or inconsistent resistance in some regions (Parker & Riches, 1993). Probably S. *hermonthica* is more virulent on sorghum than *S. asiatica,* and a higher level of resistance is necessary to get agronomically satisfactory results. Because of this, the conclusion that resistance against *S. asiatica* in sorghum based on low germination stimulant production is very stable cannot be expanded to *S. hermonthica* without reservations.

The occurrence of spontaneous germination in *Striga* (see above) deserves attention in this framework. It seems likely that *Striga* seeds germinating without a host plant signal will not result in a contact with a root and therefore to the death of the germling. Even if a spontaneously germinating seed would find a host root, develop and produce progeny, there would be no increased virulence in that progeny. A selection pressure towards increased spontaneous germination by plants not producing germination

stimulants seems unlikely, since this would upset the connection of the parasite to its host and would strongly impair the fitness of the resulting population. Therefore spontaneous germination does not pose a special risk of resistance breaking.

In conclusion the risk of resistance breaking to non-strigolactone producing varieties seems very low, the risk in varieties that produce modified strigolactones seems high and the risk for varieties that produce low amounts of strigolactones seems low to moderate.

One way of reducing the risk of resistance breaking is to take the variability of *S. hermonthica* into account and test promising new host varieties against a large collection of *S. hermonthica* originating from several hosts and against *S. aspera*, that can function as a source of virulence genes for *S. hermonthica.*

Factors influencing risks posed by (hypothetically) resistant *Striga*

Friesen *et al.* (2000) list seven generally recommended management strategies for attenuating herbicide resistance: 1. rotate herbicides; 2. use herbicide mixtures; 3. follow label directions; 4. include non-selective herbicides as rotational compounds; employ 5. economic thresholds and 6. cultural weed control; 7. monitor changes in the weed populations. Most of these recommendations are not applicable to the system of resistance braking *Striga* on resistant maize lines. Herbicide use is often impossible due to economic constraints of the smallholder farmers in sub-Saharan Africa, and cultural control measures will not be routinely applied if a resistant maize line is efficient. The only realistic preventive option of this list would be to monitor the weed population, which in practice would mean monitor resistant maize fields and eradicate resistance breaking *Striga* by any means available.

The most widely accepted theory on the development of herbicide tolerance is that in a very small portion of a weed population resistance against the herbicide is already present (Franetovich, 1995). This view means that resistance development is inevitable on the long run (Urech *et al.*, 1997; Friesen *et al.*, 2000). The rate of resistance developments then relates to the intensity of resistance selection (Martinez-Ghersha *et al.*, 1997). The selection pressure of a herbicide is directly proportional to the efficacy of the herbicide (Wrubel & Gressel, 1994; Froud-Williams, 1995). Predictions based on the Gressel & Segel (1990) model for development of herbicide resistance suggest that the main variables determining the rate of herbicide resistance development are : 1. intensity of selection pressure; 2. seedbank dynamics, where resistance is more likely to developing those species that have a relatively short seedbank life; and 3. the relative fitness of the resistant weed. The model of Maxwell *et al.* (1990) indicates that the two most important factors influencing the rate of herbicide resistance development are 1. relative fitness of the resistant weed, compared to susceptible weeds as well as the crop and 2. gene flow between susceptible and resistant weed plants.

When translating these factors to the case in this study, the selection pressure of any useful mutant line should be high, since the level of *Striga* that can be tolerated is very low (Parker & Riches, 1993). The seedbank dynamics of *Striga* are strongly dependent on soil conditions, mainly soil moisture (Gbèhounou, 1998). *S. hermonthica* is obligatory allelogamous, which ensures a relatively rapid gene flow through the population.

The relative fitness of herbicide resistant weed varieties is perhaps the most important criterion determining their spread (Kremer, 1998). Basically there is no reason to assume that resistance-breaking *Striga* would show a reduced fitness *per se*, since the signal perception and transduction events that lead to germination are most likely unimportant in the rest of the lifecycle of *Striga.* However, resistance breaking would mean that the signal perception in the *Striga* variety is changed. Therefore it seems likely that resistance breaking *Striga* lines are less fit on conventional hosts that still produce the normal type and amount of germination stimulant.

A generalised scheme to determine risks of development of resistance against pesticides was proposed by Rotteveel *et al.* (1997). (See annex 1). When applying this scheme to the risk of resistance breaking by *Striga* key factors determining the outcome of the analysis are:

1. Is the system based on one or more active ingredients? Since there is only one mode of action in the system *Striga* – resistant maize variety the answer is one ingredient only.
2. Is the crop grown continuously or in rotation. Given the wide variety of cropping systems applied in sub-Saharan Africa, both possibilities will occur.
3. Is integrated control applied or is pesticide application the only control measure? Translated to the question of this study the question would be whether the resistant maize variety is the only control measure used for *Striga*. At this moment the question cannot be answered, since much would depend on the efficacy of the maize variety and of the fact whether other non-resistant plants in the rotation are attacked. If maize is the only susceptible crop and the mutant variety would be effective in suppressing *S. hermonthica* damage, it seems logical that farmers will not apply additional control measures. If other crops in the rotation suffer *Striga* problems or if the resistance of the mutant variety is only partial, additional control measures may be applied.
4. Is the problem to be controlled a pest in one crop only or in many crops of the rotation? Again, both possibilities will occur, due to the diversity of cropping systems in sub-Saharan Africa.

The conclusion of the application of the *a priori* risk analysis scheme of Rotteveel *et al.* (1997) is that the risk of resistance spread is moderate to high, depending mostly on crop rotation and integrated control measures. However, it must be pointed out that this conclusion is based on the supposition that resistance-breaking *Striga* has the same fitness as other *Striga*.

Probably the best analysed case of resistance management is the use of cotton and maize plants carrying the *Bacillus thuringiensis* (Bt) *cry* genes. Since target pests are continuously exposed to the toxins, critics of this technology fear a rapid increase in resistant pests, which would endanger the use of the Bt toxins in conventional spray applications. Four strategies are considered for managing resistance to Bt-transgenic crops: 1. refuges of non-transgenic host plants in which susceptible insects can develop without exposure to Bt toxins; 2. moderate expression of toxin allowing some susceptible insects to survive; 3. different toxins deployed in different host varieties in a mosaic in the same area; and 4. use of varieties where each plant has a mixture of toxins (Tabashnik, 1994; Roush, 1997; Gould, 1998).

Adapting this scheme to the control of resistance breaking *Striga* the recommendations would be 1. to leave refuges of non-transgenic host plants; 2. use of partially resistant transgenic plants; 3. using different lines of transgenic maize having different types of mutations; and 4. using transgenic lines combining different types of mutations. The first strategy could be applied by mixing seeds of genetically modified and conventional maize lines (Roush, 1997). Another possibility is to include non-resistant *S. hermonthica* hosts in rotation with resistant maize. The latter option is more attractive, because that will be common practice in many cases already. The use of partial resistance is also attractive, provided that the level of the partial resistance is high enough to control the *Striga* population efficiently. This may be problematic, since *Striga* has a high reproductive capacity. The use of mosaics of genetically modified plants is considered the worst option for resistance management in insects pests (Roush, 1989, 1993). This conclusion seems to hold for management of resistant *Striga* too. The use of multiple mutant traits (e.g. low production of an altered type of germination stimulant) would be a very effective strategy, but it is questionable whether favourable mutant lines with different mechanisms will be available. If one mutant line is available that shows good *Striga* control, it is not obvious that the time-consuming process of making crossings with other mutant lines will be undertaken, for the sake of preventing spread of a hypothetical resistance-breaking *Striga* variety.

4. Conclusions and discussion

The risk of breaking transgenic resistance in maize by *Striga* has two aspects. The first aspect is the chance of the resistance breaking *per se* and the second aspect is the risk posed by (hypothetical) resistant *Striga*.

The risk of resistance breaking *per se* depends mostly on the type of transgenic maize. If the transgenic line would produce no germination stimulants (strigolactones) at all, the risk of resistance breaking is very low, but not zero, because of the numerous alternative germination stimulants. If the transgenic plants produce a different type of strigolactone, the chances of resistance breaking are high, because of the great plasticity of germination signal reception in *Striga*. If the resistance in the transgenic line is based on reduced or increased amounts of germination stimulant excreted, the risk of resistance breaking is low to moderate. The risk of resistance breaking can be reduced by incorporating a wide range of *Striga hermonthica* varieties, originating from different regions and host plants in the bioassay of potentially resistant hosts. Inclusion of *S. aspera* at some stage in the screenings would be advisable, since this species can serve as gene pool for *S. hermonthica*.

The risk of spread of hypothetical resistance breaking *Striga* varieties depends strongly the crop rotation, the deployment of alternative control measures and the fitness of resistance breaking *Striga*. The high reproductive capacity and the obligate allelogamous reproduction strategy of *S. hermonthica* are factors increasing the risk of spread of resistance breaking varieties. However it seems likely that resistance breaking *Striga* has a reduced fitness in normal crops, since the chemical communication between conventional hosts and resistance breaking *Striga* will be disturbed.

Measures to reduce the risk of spread of resistance breaking *Striga* are crop rotation, selection of a partially resistant variety, and incorporation of more than one resistance mechanism in the same variety. An additional measure should be to monitor the fields with resistant maize and eradicate any resistance breaking *Striga*.

The worst case scenario in controlling resistance breaking *Striga* populations would be that such a variety would spread over the area where the genetically modified maize variety is grown, rendering the resistance useless. A major difference with other discussions on genetically engineered resistance against insects or herbicides is that there seem to be no other effects of the spread of a resistance breaking variety than the loss of the resistant host. Resistance breaking *Striga* would have no ecological advantage over the conventional type, outside the range where the resistant maize is grown. *S. hermonthica*, the main target of the project, is restricted to agricultural systems in Africa, so spread of resistance breaking varieties into natural biotopes is unlikely. There is no risk of other control strategies or pesticides loosing their effectiveness, as is feared in the case of Bt-transgenic maize and cotton. The effect of breaking of the transgenic resistance seems similar to the effect of breaking of resistance in a conventional resistant host variety. No further ecological or agricultural damage is expected in this case.

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Appendix I.

A priory risk assessment scheme for pesticide resistance by Rotteveel *et al.*, 1997

1. a. product contains one active ingredient 2
   b. product contains more than one active ingredient 3
2. a. the active ingredient in use for more than 30 years without evidence of resistance ..... **risk negligible**
   b. resistance known, or active ingredient in use for less than 30 years 4
3. a. mixture satisfies mixing criteria ..... **risk negligible**
   b. mixture does not satisfy mixing criteria 2
4. a. crop grown continuously 5
   b. crop grown in rotation 15
5. a. major pest 6
   b. minor pest ..... **risk low**
6. a. integrated control 14
   b. chemical control only 7
7. a. all active ingredients in use to control the pest in the crop concerned belong to one resistance group 8
   b. registered active ingredients belong to more than one resistance group 11
8. a. resistance known in the field, from laboratory data or from active ingredients belonging to the same resistance group 9
   b. resistance not known, mode of action known 10
   c. novel compound, resistance and mode of action unknown ..... **risk unknown**
9. a. persistent active ingredient with specific mode of action, or active ingredient applied frequently ..... **risk very high**
   b. non-persistent active ingredient with specific mode of action, or active ingredient not applied frequently ..... **risk high**
   c. active ingredient with a non-specific mode of action ..... **risk moderate**
10. a. persistent active ingredient with specific mode of action or active ingredient applied frequently ..... **risk high**
    b. non-persistent active ingredient with specific mode of action of active ingredient not applied frequently ..... **risk high**
    c. active ingredient with a non-specific mode of action ..... **risk moderate**
11. a. resistance known in the field, from laboratory data to active ingredients belonging to the same resistance group 12
    b. resistance not known, mode of action known 13
    c. novel compound; resistance and mode of action unknown ..... **risk unknown**
12. a. persistent active ingredient with specific mode of action or active ingredient applied frequently ..... **risk high**
    b. non-persistent active ingredient with specific mode of action or active ingredient not applied frequently .. **risk moderate**
    c. active ingredient with non-specific mode of action ..... **risk moderate**
13. a. persistent active ingredient with specific mode of action or active ingredient applied frequently ..... **risk high**
    b. non-persistent active ingredient with specific mode of action or active ingredient not applied frequently .. **risk moderate**
    c. active ingredient with non-specific mode of action ..... **risk low**
14. a. active ingredient is an important control factor in the integrated control system; persistent in nature and has a specific mode of action ..... **risk moderate**
    b. other compounds ..... **risk low**
15. a. minor pest in one crop only, no volunteer plants in succeeding crops ..... **risk negligible**
    b. minor pest in all crops ..... **risk low**
    c. major pest in one crop only 16
    d. major pest ion all crops of the rotation concerned 6
16. a. chemical control only 17
    b. integrated control ..... **risk low**

17. a. all registered active ingredients currently in use to control the pest in the crop belong to one resistance group 18
    b. registered active ingredients belong to more than one resistance group ..... **risk low**
18. a. resistance known in the field, from laboratory data or to active ingredients belonging to the same resistance group 19
    b. resistance not known, mode of action known 20
    c. novel compound; resistance and mode of action unknown ..... **risk unknown**
19. a. persistent active ingredient with specific mode of action or active ingredient applied frequently ..... **risk high**
    b. non-persistent active ingredient with specific mode of action or active ingredient not applied frequently... **risk moderate**
    c. active ingredient with non-specific mode of action ..... **risk low**
20. a. persistent active ingredient with specific mode of action or active ingredient applied frequently ..... **risk moderate**
    b. other active ingredients ..... **risk low**