INSIGHTS

Desmodium species and associated biochemical traits for controlling *Striga* species: present and future prospects

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Summary

We discovered serendipitously a new and highly effective intervention against *Striga* spp., including *Striga hermonthica* (African witchweed), in cereals, which involves intercropping with cattle fodder legumes, *Desmodium* spp., including *D. uncinatum*. Although soil shading and additional nitrogen made some contribution to the reduction of *S. hermonthica* infestation, an allelopathic mechanism associated with the intercrop was a major factor. Root exudates of *D. uncinatum* contain novel flavonoid compounds, some of which stimulate germination of *S. hermonthica* and others dramatically inhibit its subsequent development, including radicle growth. *Desmodium* spp. have been developed as intercrops for both maize and sorghum and are now being evaluated for millet. From the experience with *Desmodium* spp., there is now the possibility for producing edible legumes suitable for intercropping with maize and other cereals to respond to a broader profile of farmer practices. These legumes would incorporate the powerful *S. hermonthica* controlling properties of *D. uncinatum* through feasible breeding programmes, with appropriate contributions from analytical chemistry to plant molecular genetics and biotechnology. In the longer term, it may also be possible to transfer genes associated with the allelopathic attributes to cereals themselves by heterologous gene expression, creating a new generation of parasitic weed-free cereals.

Keywords: *Striga hermonthica*, *Desmodium* spp., allelochemicals, control, biotechnology, witchweed, legume.

Introduction

Parasitic weeds in the *Striga* genus attack roots of cereal and legume crops in sub-Saharan Africa causing yield losses of over 50% (Parker, 1991). Various options to control *Striga* spp., such as crop resistance, have been developed (Lane *et al.*, 1997). Recently, maize varieties incorporating natural resistance to imidazolinone herbicides have also been developed (Gressel *et al.*, 2004; Vanlauwe *et al.*, 2008). Here we describe control of *Striga* spp. including *Striga hermonthica* (Del.) Benth. (Scrophulariaceae) (African witchweed), a devastating parasitic weed of cereals in Africa, by intercropping maize with fodder legumes, *Desmodium* spp., including *D. uncinatum* (Jacq.) (Fabaceae).

This was discovered serendipitously during the development of a 'push–pull' strategy for the control of Lepidopteran stem borers in maize in Kenya (Khan *et al.*, 2000). The 'push–pull' strategy involves planting highly attractive trap crops, such as Napier grass, *Pennisetum purpureum* K. Schumach., as border rows to attract and aggregate stem borer moths away from the central maize stand ('pull') and intercropping repellent plants such as *Desmodium* spp. between the rows of maize to repel ('push') the moths (Khan *et al.*, 2000; Cook *et al.*, 2007; Hassanali *et al.*, 2008). In field trials

in western Kenya, where *S. hermonthica* is highly prevalent, *D. uncinatum* and *D. intortum* (Mill.) were not only effective in controlling stem borers, but dramatically reduced infestations by this weed, leading to enhanced grain yields (Khan *et al.*, 2006a,b, 2008).

Allelochemicals-mediated mode of action

Although the phenomenon of allelopathy has been well recognised, its potential in weed control was only recently embraced and demonstrated (Olofsdotter *et al.*, 2002). Following our observations of suppression of *S. hermonthica* in intercrops involving *D. uncinatum* and *D. intortum*, we considered the possibility of useful allelopathic effect from these intercrops rather than the crop itself. A series of studies was initiated to investigate the mode of action. A number of mechanisms were proposed, including effects of increased available nitrogen in the soil, shading and an allelopathy associated with chemicals released from the *Desmodium* roots (Khan *et al.*, 2000, 2002). The effects of these factors were studied in field and screenhouse trials in western Kenya using *D. uncinatum* (Khan *et al.*, 2002). Field plots were established with maize intercropped with *D. uncinatum* with or without nitrogen, maize monocrop with or without nitrogen and maize monocrop with artificial ground shading of maize straw with or without nitrogen. *Striga hermonthica* seed levels in each plot, before and after each cropping season, were measured by elutriation. Nitrogen content in each plot, before and after each cropping season was measured. Although nitrogen, shading and the combined nitrogen and shading treatments suppressed *S. hermonthica* compared with the maize monocrop, a significantly greater reduction was achieved by the *D. uncinatum* intercrop and the combined *D. uncinatum* and nitrogen treatments, indicating an incremental effect of the intercrop through an allelopathic mode of action, in addition to contributions from the other two factors.

We confirmed the allelochemicals-mediated effect in a screenhouse experiment where the *D. uncinatum* plants were grown in pots on shelves and water dripping from their root systems irrigated maize planted in soil infested with *S. hermonthica* seeds. *Desmodium uncinatum* was planted with and without inoculation with the nitrogen-fixing bacterium, *Rhizobium* sp., to compare the effect of fixed nitrogen with that of the allelochemicals alone. Comparisons were made between maize plants irrigated by root eluates from *D. uncinatum*, with and without *Rhizobium* sp., and those irrigated by water passing through pots containing only autoclaved soil, with and without *Rhizobium* sp. There was a clear effect of the aqueous solution of chemicals eluting from pots in which *D. uncinatum* plants were growing on the number

of *S. hermonthica* that infested maize and that emerged from the soil, but no apparent effect from *Rhizobium* sp. (Khan *et al.*, 2002).

Aqueous samples of chemicals exuded by axenic *D. uncinatum* roots induced germination of *S. hermonthica* as effectively as the maize root exudates, indicating absence of a germination inhibitor (Khan *et al.*, 2002). However, radicals of germinated seeds exposed to root exudates from *D. uncinatum*, 24 and 48 h after germination were significantly shorter than of those exposed to maize exudates. Inhibition of radical growth was observed irrespective of whether the *D. uncinatum* had been grown in *S. hermonthica* infested or in uninfested clean soil. These observations led us to hypothesise that in addition to germination stimulants, there were additional factors affecting the growth and development of germinated *S. hermonthica* and that these prevented normal attachment to host plants. Some novel isoflavonoids were later isolated from root exudates of *D. uncinatum*, which demonstrated biological activities consistent with this hypothesis, e.g. uncinanone B, a germination stimulant and uncinanone C, a post-germination inhibitor (Tsanuo *et al.*, 2003).

Another key post-germination inhibitor recently characterised from the more polar fraction of *D. uncinatum* root exudates and solvent extracts was a di-*C*-glycosylflavone (Pickett *et al.*, 2007). Full chemical elucidation of other allelopathic agents is ongoing. The combined effect of germination stimulants and post-germination inhibitors represents a novel and highly efficient mechanism of suicidal germination of *S. hermonthica* seeds. It accounts for effective control of *S. hermonthica* in the field and provides a novel means of continual *in situ* reduction of the *S. hermonthica* seedbank in the soil, even in the presence of graminaceous host plants. In experimental maize-*Desmodium* plots in the field, the density of *S. hermonthica* seeds steadily decreased after every cropping season, while in maize monocrop plots it steadily rose (Fig. 1). Thus, *Desmodium*-based intercrops represent one of the very few examples of practical allelopathy at work and more than 10 000 small-scale farmers in eastern Africa are now using it for controlling *S. hermonthica*, in addition to its role in the 'push–pull' strategy for controlling stem borers (Khan *et al.*, 2008).

Exploiting allelopathic traits of *Desmodium*: ways forward

Many small-holder farmers in Africa, particularly those without livestock, prefer intercropping cereal crops with food legumes. Other than *Desmodium* spp., no legume, including cowpea (*Vigna unguiculata* L.), greengram (*Vigna radiate* L.), groundnut (*Arachis hypogaea* L.),
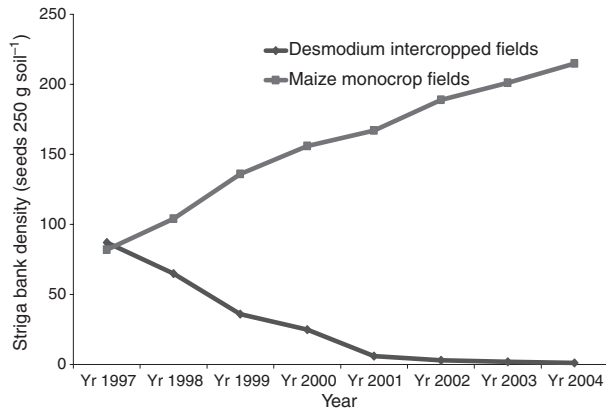
Fig. 1 Long-term effect of maize-*Desmodium* intercropping as compared with maize monocrop on *Striga hermonthica* seed density.

beans (*Phaseolus vulgaris* L.) and crotalaria (*Crotalaria ochroleuca* G. Don.), has demonstrated such potent suppression of *Striga* spp. (Khan *et al.*, 2007). We now sufficiently understand the mechanism by which *D. uncinatum* suppresses *S. hermonthica*, including the structural identity of some of the key mediating secondary metabolites involved, to explore new ways of developing or transferring relevant biochemical traits involved to edible legumes and possibly cereal crops themselves. This is expected to provide greater flexibility to farmers and to contribute even more to stabilising and improving cereal production in the poorest farming regions. Key approaches that could be explored are outlined below.

Breeding *Striga*-controlling traits in food legumes

It is unlikely that direct crosses could be made between *Desmodium* spp. and food legumes. However, certain edible legume cultivars may possess the same combination of allelopathic traits as *Desmodium*, but at insufficient levels. Comparative screening of a range of edible legumes, including West African species that have co-evolved with and show some resistance to *Striga gesnerioides*, may lead to the identification of food legumes with levels of *Desmodium*-type allelochemicals and associated *Striga*-controlling traits comparable with those of *Desmodium*. Conventional breeding programmes could then select and enhance the traits.

Genetic studies targeting germination stimulants

Root exudates of edible legumes generally show significant levels of *Striga* germination stimulation caused by strigolactones derived from the carotenoid pathway (Matusova *et al.*, 2005). Therefore, it may not be necessary to develop this trait further. However, direct quantitative comparison with *Desmodium* spp. needs to be undertaken to establish if this is sufficient for effective *Striga* control. Strigolactones have not been detected in *Desmodium* spp. so far. If the specific germination stimulants found in *D. uncinatum,* including the isoflavonoid uncinanone B, are found to be essential, then incorporation of these traits may involve transfer of associated genes into food legumes. Figure 2 outlines the biosynthetic scheme for the synthesis of bioactive constituents of the root exudates of *Desmodium*. Isoflavonoids, which arise *via* the isoflavone synthase (IFS), are expected to be present at sufficiently high levels in food legumes selected for breeding programmes. Thus, transformation of selected isoflavonoid targets to isoprenylates and their cyclisation to uncinanones would require transfer of specific genes, such as isoprenyl transferase, to food legumes.
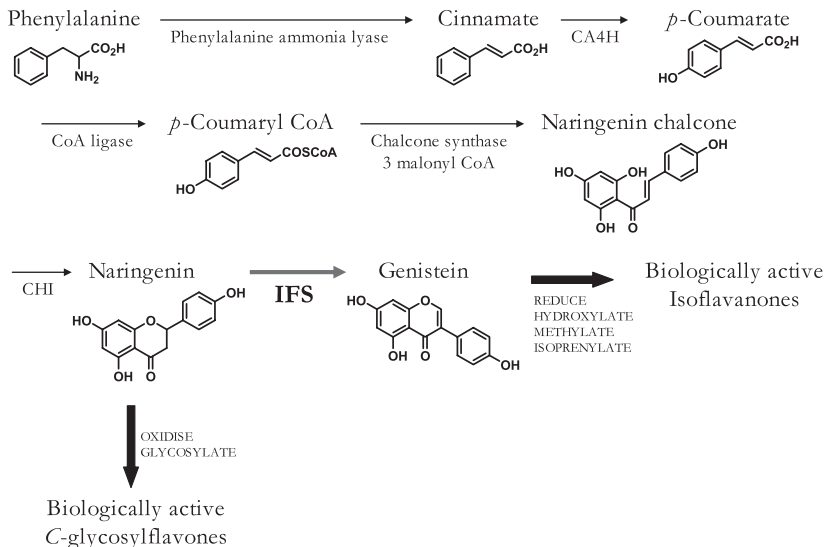

Fig. 2 Biosynthetic scheme for the synthesis of *C*-glycosylflavones in plants and biologically active isoflavanones in *Desmodium uncinatum.*

Genetic studies targeting post-germination inhibitors

Characterisation of new *Striga* inhibitory compounds from the polar fractions of *D. uncinatum*, other than di-*C*-glycosylflavone (Pickett *et al.*, 2007), may benefit from larger samples of roots and root exudates, so hairy root cultures that are being developed (J. Hamill, pers. comm.). The *C*-glycosylflavones being identified are formed from metabolite flavones prior to IFS in Fig. 2 and are likely to employ the potential precursors naringenin, naringenin chalcone or 2-hydroxynaringenin that are subsequently glycosylated. Thus, chemical studies would concentrate on identifying apigenin or mono-*C*-glycosylflavones vitexin or isomollupentin, demonstrating the presence of a *C*-glycosyltransferase, in cultivars of legumes that can be parents for breeding in, or upregulating, the complete *C*-glycosylation steps to the inhibitory di-*C*-glycosylflavones found in *Desmodium* spp. The incorporation of any of the metabolites, naringenin, naringenin chalcone, 2-hydroxynaringenin, apigenin, vitexin, or related mono-*C*-glycosylflavones derivatives in hairy root cultures of *Desmodium* spp. may help to establish the exact pathway. Transformation of these metabolites to post-germination inhibitors would open up the possibility of deploying existing biochemical and molecular genetic techniques to identify *C*-glycosyltransferase in *D. uncinatum*. Heterologous gene expression could then be attempted to incorporate these traits into edible legumes.

Locating the allelochemical genes in model and crop legume and cereal plants

Legumes with extensive EST (expressed sequence tag) libraries are readily available, particularly for *Lotus japonicus* L. and *Medicago truncatula* Gaerth. (barrel clover), and the full genomic sequence is accessible for *V. unguiculata* (Cowpea Genomics Initiative). These can be searched for signs of the chemistry relating to *Striga* spp. post-germination effects. If the appropriate chemistry is present, then this could greatly facilitate the identification of genes involved in the biosynthesis of other allelochemicals, which could be used to initiate breeding programmes or even heterologous transferral of target genes from *Desmodium* to other legume species. The prospect of transferring such genes into cereals can also be considered. Apigenin and vitexin are present in pearl millet, *Pennisetum* spp. and *Fonio* millet, *Digitaria exilis*, and were recently found in wheat. However, cereals may present a greater challenge compared with legumes because, in cereals, expression of the earlier parts of the inhibitory pathway is not directed to the roots, as in legumes. Nonetheless, there are a number of potentially useful

root-specific promoters that could be applied to addressing this problem.

Work on the post-germination inhibitory pathway could take two approaches. If there is evidence of *C*-glycosyltransferase activity, presence of the mono-*C*-glycosylflavones, EST databases for *L. japonicus* and *M. truncatula* and the *V. unguiculata* genome can be searched using sequence information from *O*-glycosyltransferases that are likely to share substantial sequence motifs with *C*-glycosyltransferases. The feasibility of using a proteomic approach for the identification of this genetic activity can also be assessed in *in vitro* bioassays for the glycosylation of flavonoid intermediates. The other approach will be to use sequence data from known *C*-glycosyltransferases acting on polyphenolic substrates, e.g. from *Streptomyces* species, to search the food legume databases. Evidence for the presence of *C*-glycosyltransferases in wheat and maize may also allow the associated genes to be identified, which could greatly facilitate the generation of cereal cultivars directly expressing *Striga* inhibitory allelochemicals.

Conclusions and future outlook

Although the control of *S. hermonthica* by *Desmodium* spp. was discovered by serendipity, the work to date demonstrates the practical value of allelopathic weed control, which can tackle even the most pernicious of weeds. However, understanding the mechanisms is essential to ensure that the use of *Desmodium* spp. as intercrops remains effective in the hands of smallholder farmers. This understanding also has the potential to allow exploitation of the allelopathic effects discovered for *Desmodium* in breeding programmes and in the creation of transgenic plants, not only for subsistence farming systems, but also potentially in agricultural systems of the developed world.

Acknowledgements

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