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Isolation and identification of *Desmodium* root exudates from drought tolerant species used as intercrops against *Striga hermonthica*

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ABSTRACT

Plants from the genus *Desmodium*, in particular *D. uncinatum*, are used on sub-Saharan small-holder farms as intercrops to inhibit parasitism of cereal crops by *Striga hermonthica* and *Striga asiatica via* an allelopathic mechanism. The search for *Desmodium* species which are adapted to more arid conditions, and which show resilience to increased drought stress, previously identified *D. intortum*, *D. incanum* and *D. ramosissimum* as potential drought tolerant intercrops. Their potential as intercrops was assessed for resource poor areas of rain-fed cereal production where drought conditions can persist through normal meteorological activity, or where drought may have increasing impact through climate change. The chemical composition of the root exudates were characterised and the whole exudate biological activity was shown to be active in pot experiments for inhibition of *Striga* parasitism on maize. Furthermore, rain fed plot experiments showed the drought tolerant *Desmodium* intercrops to be effective for *Striga* inhibition. This work demonstrates the allelopathic nature of the new drought tolerant intercrops through activity of root exudates and the major compounds seen in the exudates are characterised as being *C*-glycosylflavonoid. In young plants, the exudates show large qualitative differences but as the plants mature, there is a high degree of convergence of the *C*-glycosylflavonoid exudate chemical profile amongst active *Desmodium* intercrops that confers biological activity. This defines the material for examining the mechanism for *Striga* inhibition.

1. Introduction

In subsistence farming regions of sub-Saharan Africa (SSA), where parasitic weeds of the *Striga* genus devastate subsistence cereal crops, a successful intervention called push–pull technology effectively inhibits *Striga* damage and has been adopted by more than 96,000 farmers (http://www.push-pull.net/, Fischler, 2010). The technology utilises forage legumes in the *Desmodium* genus (Fabaceae) as an intercrop which provides the key chemical components for inhibiting development of *Striga* in the field. The mechanism by which this occurs has been shown to be caused by allelopathic root exudates from *Desmodium* that both stimulate the germination of *Striga*, (Khan et al., 2002) but more importantly inhibit the subsequent development of the germinated seed, (Tsanuo et al., 2003; Hooper et al., 2010) so that in the field, almost no parasitism is observed (Khan et al., 2010; Pickett et al., 2010). The technology blends in with the cultural practise of mixed cropping and has been taken up by farmers introduced to it through their peers in farmer-led groups (Khan et al., 2008, 2014; Murage et al., 2011). It provides additional benefits to insect pest (cereal stemborer) and weed control by providing fodder for zero-grazed livestock while improving soil quality through organic carbon and fixed N (Khan et al., 2008a; Midega et al., 2013). In order to expand the technology into areas that are either more arid or under threat of increased drought through climate change, new species of *Desmodium* must be identified that are resilient to drought and which can also provide the chemistry that confers inhibition of *Striga*. Once identified, the intercrop should protect drought tolerant cereal crops such as sorghum or new drought tolerant maize varieties against *Striga*, thereby increasing food security for small-holder farmers. In addition, the further characterisation of active root exudate chemistry provides the basis for identifying the enzymic and genetic basis for allelochemical production *in planta* (Hooper et al., 2009; Khan et al., 2008b).

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In our screening for drought tolerance, three *Desmodium* species were originally identified as showing potential as they survived extended periods of drought when grown in African screen-house conditions. They are *Desmodium intortum* (Mill.) Urb., *Desmodium incanum* (G. Mey.) DC. and *Desmodium ramosissimum* G. Don. We hypothesise that taxonomically related *Desmodium* species may produce similar root exudate chemistry to the *Desmodium* species known to be effective in the field (*Desmodium uncinatum* (Jacq.) DC.), and so be potential intercrops for inhibition of *Striga* parasitism of subsistence cereals in more arid agronomic environments,. In order to investigate their potential for *Striga* inhibition, the root exudates of these plants were collected and analysed by HPLC and liquid chromatography-mass spectrometry (LCMS) to determine whether the same *Striga* inhibiting chemistry previously identified from *D.* uncinatum (Hooper et al., 2010) was present and which could be attributed to inhibition of *Striga*. In addition, by performing screen-house pot experiments, the drought tolerant *Desmodium* plant root exudates were tested against *Striga hermonthica* on maize, to demonstrate *Striga* inhibiting biological activity. The drought tolerant *Desmodium* species were then tested as *Striga* inhibiting intercrops for sorghum in rain fed plot experiments under field conditions in Western Kenya.

2. Results and discussion

2.1. Striga inhibition by drought tolerant Desmodium species root exudates

In screen house experiments, the parasitism of maize by *S. hermonthica* was inhibited strongly by irrigation with the root exudates of all the tested *Desmodium* species compared with irrigation through soil alone (Fig. 1). The mean number of *Striga* plants emerged on root exudate treated maize differed among treatments with distilled water control (Fig. 1; $F_{5,107} = 98.23$, $P < 0.001$) showing the root exudates for all tested *Desmodium* species were effective in inhibiting *Striga* parasitism.

2.2. Striga inhibition by drought tolerant Desmodium species in demonstration plots

The four species of Desmodium for which exudates were studied, were grown as intercrops with drought tolerant sorghum (the early maturing commercial hybrid Gadam Hamam) in rain fed plots on the icipe Thomas Odhiambo Campus on the shores of Lake Victoria. The performance of the plots after the short and rainy seasons of 2014 were assessed (Fig. 2). All the drought tolerant *Desmodium* species tested showed significant *Striga* inhibiting properties in the field, as they did in the pot experiments, with no significant differences to the performance of *D. uncinatum* the intercrop now used widely in farmer fields.

2.3. Exudate analysis

The root exudates of *D. uncinatum*, *D. intortum* and *D. incanum* were collected one month after the transfer of plants into hydroponic solution and analysed by HPLC (Fig. 3). The seedlings were transferred to the hydroponic solution at two weeks after germination from seeds. The structure of the major flavonoid peaks in these initial exudates were identified by HPLC co-elution with known standards, isolated from extracting leaf or root tissue of conspecific plants which were fully characterised by nuclear magnetic resonance (NMR) spectroscopic and electrospray ionisation mass spectrometry (ESIMS) analysis (Fig. 3). The major components seen in the *C*-glycosylflavonoid (CGF) region of *D. uncinatum* are isoschaftoside (**5**), vitexin (**8**) and a broad signal for 2″-*O*-glucosylvitexin (**7**). In the case of *D. intortum*, the major components are vicenin-2 (**2**) and isoschaftoside (**5**). The component isoschaftoside (**5**) has been reported previously as being able to interfere with *Striga* development post-germination (Hooper et al., 2010). However, analysis of the exudate from *D. incanum* revealed that it comprised a number of CGFs structures, of which isoschaftoside was only a minor component. All three species were found to produce isoschaftoside (**5**) in their exudate in

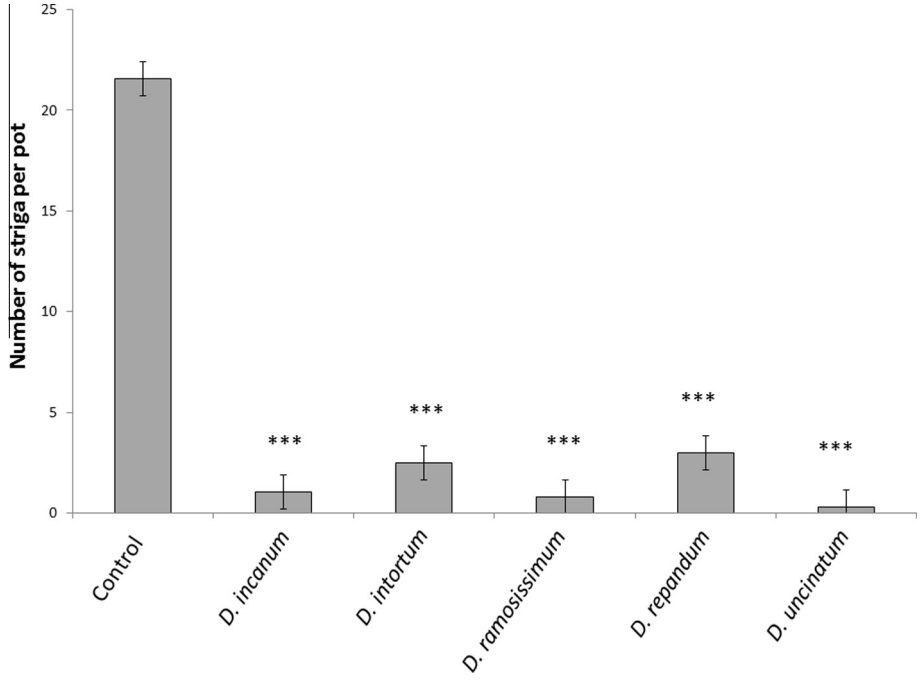

Fig. 1. Mean (±SE) number of *S. hermonthica* per pot treated with *Desmodium* root exudates ($n = 18$). Asterisks indicate significant difference ($P < 0.001$) from the control treatment.