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Short communication

A novel concept for the control of parasitic weeds by decomposing germination stimulants prior to action

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ABSTRACT

A new concept for controlling parasitic weeds is described. By decomposing germination stimulants prior to action no germination of seeds can take place anymore. Ethanol fractions of the strigolactone (SL) analogues viz., the standard synthetic analogues GR 24 and Nijmegen-1, and analogues derived from tetralone and coumarine, were added to an aqueous buffer with a pH ranging from 6 to 8 and the half lifes ($t_{1/2}$) of the hydrolysis were measured. Nijmegen-1 hydrolysed faster than GR 24 and the analogue from tetralone was the most stable one at all pH's. It was found that the aqueous solutions of either borax or thiourea rapidly decompose typical SL analogues, including GR 24 and Nijmegen-1, within an hour. The hydrolysis of SLs by borax was monitored with UV spectroscopy and for thiourea gas chromatography was used. This decomposition of SLs by either borax or thiourea in natural conditions would deprive the seeds of the parasitic weeds of the essential germination stimulants and as a consequence not allow them to germinate. Hence, conditions for an effective weed control are fulfilled.

1. Introduction

Parasitic weeds, especially *Striga* spp. and *Orobanche* spp., are increasingly becoming a threat to agricultural food production in many countries (Parker, 2009, 2012). These weeds attack a wide range of important food crops, thus directly affecting the food and nutrition security of humans and cattle in Africa, Asia and the Mediterranean region. Due to global warming induced changes in temperature and distribution of rainfall, these parasites have invaded new places like Southern Europe, thereby causing severe damage to important food crops (Grenz and Sauerborn, 2007). *Striga* and *Orobanche* are obligatory in nature, meaning that they entirely depend on the host plants for their nutrients and mineral needs. It is known for a long time that seeds of these parasites require stimulants present in host root exudates to germinate, ensuring that they germinate only in the presence of a host plant to which the germinated seed will attach itself in order to obtain essential ingredients for development. This is a naturally evolved mechanism for survival of these parasitic plants (Brown et al., 1949, 1951; Yoneyama et al., 2009; Xie et al., 2010). The first germination stimulant was isolated from root exudates of cotton (a non-host plant) by Cook et al. (1966). The structure of this stimulant, which was named strigol (**1**) (Fig. 1), was firmly secured twenty years later (Brooks et al., 1985). The isolation, identification and elucidation of natural germination stimulants, which collectively are named as strigolactones (SLs), is very difficult due to the fact that only minute amounts are exuded by plants, ca 15 pg per day per plant (Humphrey and Beale, 2006). At present, a whole series of natural SLs has been identified and characterized; typical examples are sorgolactone (**2**) (Fig. 1), orobanchol and solanacol (Yoneyama et al., 2009; Xie et al., 2010, 2013). The SLs invariably contain three annelated rings (the ABC scaffold) connected with a butenolide (D-ring) via an enol ether bridge (see structure of strigol (**1**)). Extensive structure–activity studies revealed that the bioactiphore of SLs resides in the CD part of the molecules (Mangnus and Zwanenburg, 1992; Zwanenburg et al., 2009; Zwanenburg and Pospíšil, 2013). This information allowed the design and synthesis of SL analogues with a much simpler structure than the natural SLs, but retaining the bioactivity (Nefkens et al., 1997; Zwanenburg et al., 2009; Zwanenburg and Pospíšil, 2013). The first series of SL analogues is the GR series described by Johnson et al. (1981). GR 24 (**3**) (Johnson et al., 1981; Malik et al., 2010) is most well-known and used as a standard in germination assays. Other successful examples are Nijmegen-1 (Nijm) (**4**) (Nefkens et al., 1997), a tetralone derived SL analogue Tet, (**5**) (Mwakaboko and Zwanenburg, 2011a) and a coumarine derived SL analogue Cou (**6**) (Mwakaboko and Zwanenburg, 2011b). (For the respective structures, see Fig. 1.)

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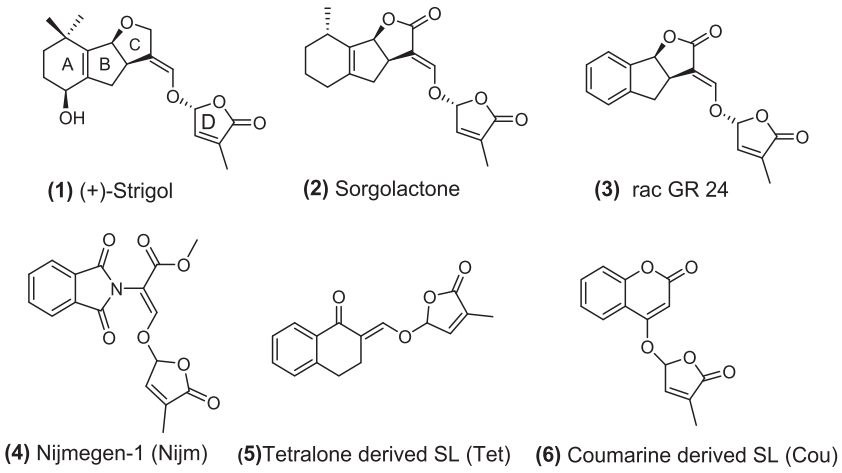

Fig. 1. Structures of some natural SLs and some synthetic SL analogues.

Several strategies for the control of these parasitic weeds have been considered; however none of them could provide fool-proof protection against their invasion and damage (Joel, 2000; Rubiales et al., 2009; Parker, 2009, 2012; Hearne, 2009; Rodenburg et al., 2010). A problematic aspect is that these weeds produce very large number of seeds that can remain viable in the soil for up to twenty years (Rubiales et al., 2009). An old control method of parasitic weeds is hand weeding, which is both labour intensive and ineffective. Crop rotation, the use of false hosts (trap crops) and catch crops can be effective to some extent, but require a strict agricultural regime (Hooper et al., 2009). Breeding of resistant crops requires exhaustive selection techniques; however unfortunately, the resistance disappears after a period of *ca.* five years (Pacureanu-Joita et al., 2009). Chemical control by herbicides can be effective in controlling the weeds, however, host plants may be affected as well and the method has been criticized for its environmental effects, especially when non-selective and highly toxic chemicals are used (Parker, 2009, 2012; Rubiales et al., 2009; Hearne, 2009).

It had long been realized (Johnson et al., 1976) that if a germination stimulant would be applied to the soil before the host crop is planted; the parasitic seeds would germinate in the absence of a host, but would not survive as the necessary attachment to the host plant is not possible. This method is commonly referred to as the "suicidal germination approach". The early successful attempts with GR 7 (this is GR 24 lacking the aromatic A-ring) to achieve suicidal germination were stopped due to lack of financial support and availability of sufficient amounts of stimulant. However, recently, SL analogues have successfully been used in pot experiments to induce suicidal germination (Kgosi et al., 2012). A successful suicidal germination in the field was reported using Nijmegen-1 as SL analogue in tobacco infested by *Orobanche cumana* (Zwanenburg et al., 2009). In these experiments the stimulant was formulated in an emulsion which prevents untimely hydrolysis of the stimulant and leaching down to lower soil layers.

It has been suggested by several authors that the limited stability of stimulants in soil would preclude their use in the suicidal germination approach (Babiker and Hamdoun, 1982). It was observed that the stability of GR compounds GR 24 and GR 7 was dependent of several soil factors mainly pH, with decreased stability in basic soils (Johnson et al., 1976; Babiker et al., 1988) and also from excess of moisture leading to decreased response of seeds to these SL analogues (Babiker et al., 1987).

The aim of this paper is to present a new concept for the control of parasitic weeds making use of the instability of germination stimulants under certain conditions.

2. Materials and methods

The germination stimulants viz., GR 24 (**3**), Nijm (**4**), Tet (**5**) and Cou (**6**) were prepared as described previously (Malik et al., 2010; Nefkens et al., 1997; Mwakaboko and Zwanenburg, 2011a, 2011b, respectively). Borax (Sodium tetraborate decahydrate) and thiourea were purchased from Sigma. Thin layer chromatography (TLC) was performed using Merck Silicagel 60F254 TLC plates and ethyl acetate/heptane 2:1 as eluent. UV spectra were recorded using a Jasco V630 spectrophotometer and for Gas–Liquid Chromatography (GLC) a Shimadzu 2010 Plus instrument was used. For the stability experiments, buffers were prepared from 5 mM ammonium acetate and adjusting the pH from 6 to 8 at 0.5 intervals by adding 0.5 M NaOH. The SL analogues were dissolved in ethanol (1 mM) and 50 μL were placed in a 20 ml vial, ethanol was removed in vacuo and buffer was added. Aliquots were taken at regular intervals (4 h, 1 d, 2 d, 1 w, 2 w) and analysed by Liquid Chromatography/Mass Spectrometry (LCMS). The $t_{1/2}$ values (Table 1) were determined from a plot of concentration vs time.

3. Results and discussion

We analysed the hydrolytic stability of some SL analogues at various pH values. It was found that at alkaline pH the stability of these analogues rapidly decreases. The results are collated in Table 1.

The results indicate that Tet (**5**) has the highest $t_{1/2}$ at all pH and is the most stable of the four SL analogues tested: 20 h even at pH 8.0. Cou (**6**) has a $t_{1/2}$ in the middle range and hydrolysis is of

Table 1
Stability of some SL analogues at various pH values.

Compounds/pH	Half-life ($t_{1/2}$ in h)				
	6.0	6.5	7.0	7.5	8.0
GR 24 (**3**)	140	120	100	42	5
Nijm (**4**)	35	22	10	<4	<4
Tet (**5**)	–	210	170	95	20
Cou (**6**)	90	75	45	13	<4