Tephrosia vogelii: a pesticide of the future for African farming

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INTRODUCTION

Global population is expected to reach 9 billion by 2050, increasing pressure on food production (Godfray *et al.*, 2010). Constraintson crop production areparticularly acute in Africa where population growth is greatest and where 80% of all food is produced by small holder farmers on farm sizes of less than one hectare (Stevenson and Belmain, 2016). Crop losses caused by pests and diseases are two major barriers to agriculture that will have major impacts on global food security (Poppy *et al.*, 2014). They are particularly pertinent to small holders who consider insect pests to be the most significant problem over which they can have some control. Current pest control technologies are dependent on synthetic pesticides, but small holders in Africa may overlook them due to cost (Sola *et al.*, 2014) or poor efficacy (Midega *et al.*, 2016). Despite relatively lower use in Africa compared to other parts of the world, synthetic pesticides in Africa have well-documented negative impacts on users due to a poor regard for health and safety of applicators and widespread misuse that increases residues, putting consumers at risk. Negative environmental impacts against beneficial insects are also common, with over and under application exacerbating the development of insecticide resistance. For all these reasons, less harmful and simpler alternatives need to be sought (de Bon *et al.*, 2014; Stevenson and Belmain, 2016).

Figure 1. Bruchid beetles are major pests of stored legumes in sub-Saharan Africa

Alternatives exist for small holder farmers in Africa including biological control agents and botanically active substances (Moshi and Matoju, 2017). Indeed the use of pesticidal plants has been a major component of pest management in sub-Saharan Africa for generations, and their use continues to this day (Kamanula *et al.*, 2011). One of their great advantages is they can be propagated locally and self-harvested (Grzywacz *et al.*, 2014; Belmain and Stevenson, 2001) so can circumvent the distribution limitations of the commercial sector which can be severely impacted in more remote locations. However, this potentially exposes farmers to hazardous materials as some species may be toxic and their use is often substandard.

PESTICIDAL PLANTS AS ALTERNATIVES TO SYNTHETIC PESTICIDES IN AFRICA.

Over the past decade our work has concentrated on optimising the use and propagation of pesticidal plants in Africa for small holder farmers. We provided evidence for plant species previously unknown as botanically active against pests. These include two indigenous trees; *Securidaca longepedunculata* Fresen. (Polygalaceae) and *Zanha africana* (Radlk.) Exell (Sapindaceae) for which the root bark has been used locally by farmers in sub-Saharan Africa to protect legumes from damage by beetles (Bruchidae) (Stevenson *et al.*, 2016; Stevenson *et al.*, 2009) (**Figure 1**). Root bark, as a natural resource, may be fine for medicines, for which very small quantities are required. However, and despite our success in propagating these two species (Anjarwalla *et al.*, 2016), root bark is not sustainable for large scale interventions and field applications.

To address this issue we have focussed on other species that can be grown rapidly and at scale and for which the botanically active chemicals are widespread in the plant making their use more sustainable and practical (Stevenson and Belmain, 2016). Of these perhaps the species with greatest potential is *Tephrosia vogelii* Hook f. (Leguminosae) (**Figure 2**). We encountered how popular this species was in Eastern Africa through farmer surveys (Kamanula *et al*., 2011). Farmers reported using crushed leaves to admix with grain to protect it from beetles. However, this highlighted some problems straight away that required phytochemical analysis to resolve and emphasised the many pitfalls of using botanically active substances in pest management without robust underpinning with scientific analysis.

THE CHEMISTRY AND BIOLOGICAL ACTIVITY OF *Tephrosia vogelii.*

Background literature indicated that *T. vogelii* biosynthesised rotenoids (isoflavonoids) which were already well-known for their insecticidal properties and therefore likely explained their use in pest control. Our subsequent chemical analysis of the species bore this out and identified several isoflavonoids (Stevenson *et al*., 2012) of which deguelin and tephrosin were the most abundant and further showed that deguelin was the most important compound due to its higher levels of bioactivity against insects (Belmain *et al*., 2012). We also identified several other less abundant rotenoids that partly contributed to activity including rotenone, sarcolobine and α-toxicarol (**Figure 3**).

However, our survey revealed that, in Malawi at least, some farmers reported that the *Tephrosia* they were using simply did not work. To confound this problem a second species, *Tephrosia candida* (Roxb.) DC., was also claimed to be in circulation among farmers through having been promoted for soil improvement outreach programmes implemented by the World Agroforestry Centre (ICRAF). Farmers claimed this second species was difficult to physically distinguish from *T. vogelii* and so was being similarly used for insect pest control. Our immediate suspicions were that the material which didn't work was T. candida with a potentially different metabolome to that which we had established in *T. vogelii.* So we set about collecting both species for comparative chemical analysis.

Figure 2. *Tephrosia vogelii* is pesticidal and used by small holders in Malawi.

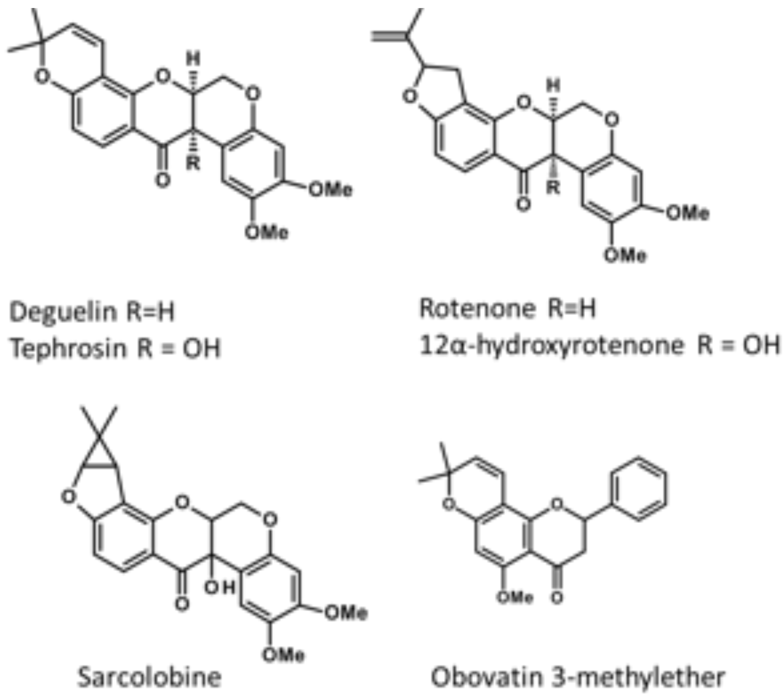

Figure 3. Rotenoids and flavanones from *Tephrosia vogelii* hemotypes.

However, a botanical assessment using traditional morphological analyses with comparison to authentic herbarium specimens along with ITS sequence data of the plant materials at the Royal Botanic Gardens, Kew (Stevenson *et al.*, 2012) revealed that what had been promoted in Malawi as *T. candida* was in fact a different provenance of *T. vogelii*. This highlighted that research on and use of wild plant species must have robust botanical underpinning.

So to explain the lack of insecticidal activity in *Tephrosia* we collected *T. vogelii* from 12 different locations across Malawi in the regions where we had conducted the farmer survey. Our analysis showed that the chemistry of the species fell into two distinct chemotypes. Chemotype 1 was characterised by rotenoids (**Figure 3**) whereas chemotype 2 contained no rotenoids at all. Instead, this second chemotype was characterised by several previously unreported prenylated flavanones, along with the known flavanone obovatin 5-methyl ether and a flavone, Z-tephrostachin (**Figure 3**). The rotenoid biosynthetic pathway appeared to be completely absent from chemotype 2. Biological evaluation of these flavonoids and comparison to the rotenoids and crude extract of *T. vogelii* chemotype 1 confirmed that the absence of rotenoids did explain the ineffectiveness of plant materials used by some farmers in our survey.

Surprisingly the occurrence of the ineffective material was widespread. Around 25% of the material we collected was chemotype 2 indicating that 1 in 4 farmers was using *T. vogelii* plant material to protect stored grains that was ineffective. This poor efficiency could completely undermine any confidence farmers might have in alternatives to synthetic pesticides and must be avoided in outreach programmes through robust chemical assessment of the plant materials. Inconsistency in the chemistry and thus efficacy of plant material is a primary limitation of its successful uptake (Isman, 2008) and efforts to prevent this should be a high priority.

Not only did our chemical analysis explain why farmers were not achieving the pesticidal benefits, but also allowed us to develop a method to select the correct chemotype for propagation and distribution through outreach networks. Where farmers grow *T. vogelii* in fallows to improve soil quality they can ensure this is the correct chemotype for use as a plant pesticide through chemical analysis. We have established a laboratory at Mzuzu University chemistry department with an HPLC to provide this service to extension workers and farmers.

Knowing the chemistry underpinning the botanical activity has also allowed us to look more at other aspects of optimising its use. For example, *T. vogelii* is effective not only in stored products but also in field application (Mkenda *et al.*, 2015). Farmers traditionally make cold water extracts of the leaves and spray this on their crops. This achieves very poor efficiency of extraction as the rotenoids are poorly soluble in water. However, using comparative chemical analysis we showed that the use of surfactants in the extraction process increased the extraction of rotenoids even into water (Stevenson *et al.*, 2012). Now our farmers have adopted this practise and use locally available liquid soaps to improve the efficacy of their plant materials. The inclusion of surfactants at this early stage also improves spreading when sprayed on to the target. We have also used our chemical analysis to understand more about the seasonal variation of the chemicals and identified the best time of year to harvest *T. vogelii* to obtain the highestyields of active ingredient (Belmain *et al.* 2012).

FUTURE APPLICATIONS AND SAFETY

With the growing demand for effective pest control technologies that are environmentally benign and less toxic than synthetic chemicals currently available in Africa Tephrosia might present a viable option. It is easily propagated in large quantities and even improves soil quality so could have multiple benefits. However the active components are rotenoids which are no longer registered for use in Europe even though they are still listed as acceptable in organic food production. If rotenoids get into water ways they are at least as toxic as pyrethroids to fish so their use must be regulated and monitored. However, the active ingredients have oral LC50's that are similar to caffeine. Indeed, estimated ingestion of 300-500mg/kg in humans is required to cause potential fatal outcomes, and based on the concentrations we found in *Tephrosia* (1000ppm), an average human would need to consume more that 20kg of dry leaf material to reach lethal amounts. By inhalation, however, exposure is more dangerous; so if extracts of *Tephrosia* are sprayed in fields, extreme caution must be taken to avoid respiratory exposure. One other limitation of *Tephrosia* is that the active rotenoids are UV labile. However, this means that they are generally less persistent and, therefore, less harmful to beneficial insects and less likely to leave residues on treated field crops. Our recent work has shown that this could be considered one of the great advantages of using pesticidal plants as they can help promote ecosystem services and more agro-ecologically sustainable crop production (Amoabeng *et al.*, 2013; Mkenda *et al.*, 2015).

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