The concentration of bioactive compounds in *Plantago lanceolata* is genotype specific

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Abstract

Plantain (*Plantago lanceolata*) is known to contain bioactive compounds including verbascoside (acteoside), aucubin and catalpol. Limited New Zealand data are available to quantify the concentrations of secondary plant compounds in cultivars of plantain. This experiment compared secondary plant compound concentrations for five cultivars or breeding lines of plantain and the botanical distribution of these compounds over a year. For all cultivars verbascoside concentration was greatest, aucubin intermediate and catalpol lowest. The concentration of catalpol and verbascoside in leaf tended to be greater for the cultivars 'Hercules' and 'Endurance' compared with 'Elite 2', 'PG742' and 'Tonic'. However, 'Hercules' and 'Endurance' tended to have lower concentrations of catalpol and verbascoside in scape (reproductive material from base to seedhead). The difference among cultivars in aucubin concentration was small. This experiment suggests that leaf concentration of catalpol and verbascoside is genotype specific.

Keywords: acteoside, botanical distribution, cultivar, leaf, plantain, scape, secondary compounds, seasonal distribution

Introduction

Incorporating plantain (*Plantago lanceolata*) into pastures has been identified as a potential mitigation strategy to reduce nitrogen (N) losses from livestock production systems (Totty *et al*. 2013; Box *et al.* 2017). Large reductions (>50%) in urinary N concentrations and estimated urinary N output have been observed for dairy cows grazing both pure (Box *et al.* 2017) and mixed (Totty *et al.* 2013) plantain swards. Some or all of these effects may be attributed to the presence of secondary plant compounds in plantain herbage. The most well-known secondary compounds present in plantain are the iridoid glycosides aucubin and catalpol, and the phenylpropanoid glycoside verbascoside (also known as acteoside) (Gardiner *et al.* 2016; Navarrete *et al.* 2016). Navarrete *et al.* (2016) showed secondary plant compounds (namely verbascoside) were responsible for reducing rumen ammonia (NH_3) production, a major source of urinary N. Further, plantain leaf extract has reduced soil N mineralisation (Dietz *et al.* 2013) and nitrous oxide (N_2O) losses by 70% (Gardiner *et al.* 2017). Dietz *et al*. (2013) applied extracted aucubin and plantain leaf material to soil which resulted in long-term low soil nitrate concentrations due to the inhibition of soil N mineralisation by aucubin. Gardiner *et al.* (2017) applied urine (700 kg N/ha) and either a plantain leaf extract or an aucubin solution (both containing 47 kg aucubin/ha) to soil and observed a reduction in N_2O emissions of 50 and 70%, respectively.

There are few data comparing the concentration of secondary compounds across seasons for New Zealand cultivars and breeding lines of plantain, and no current data to show the distribution of secondary compounds within plantain herbage. The objective of this study was to compare the seasonal concentration of secondary plant compounds in both the leaf and scape of five plantain cultivars/breeding lines.

Materials and methods

Experimental site and design

The experiment was conducted from January to December 2014 at Kimihia Research Centre Lincoln, Canterbury on a Templeton silt loam. The experiment was a randomised block design with five cultivar/breeding line treatments and four replicates. Plants were 12 months old at the beginning of the experiment. Irrigation was applied as spray irrigation after each cut from October to March.

Sample areas were single rows of approximately 20 plants. Rows were established from plants germinated in a glasshouse and transferred to the field at the 4-6 leaf stage. Monthly herbage samples were cut 2-3 cm above ground level with hand-shears (for those cultivars where sufficient herbage was available) and were sorted into leaf and scape (all reproductive material from base to the seedhead) before freezing in plastic zip-lock bags at -20ºC. Frozen samples were freeze-dried, ground through a 1 mm sieve and stored at 2°C in a dark temperature controlled room awaiting analysis.

Herbage analysis

Secondary plant compound concentration was determined by HPLC of an ethanol extraction of 0.1 g of freeze-dried plant material using the methods of Navarrete *et al.* (2016).

ISSN 2463-2872 (Print) ISSN 2463-2880 (Online)

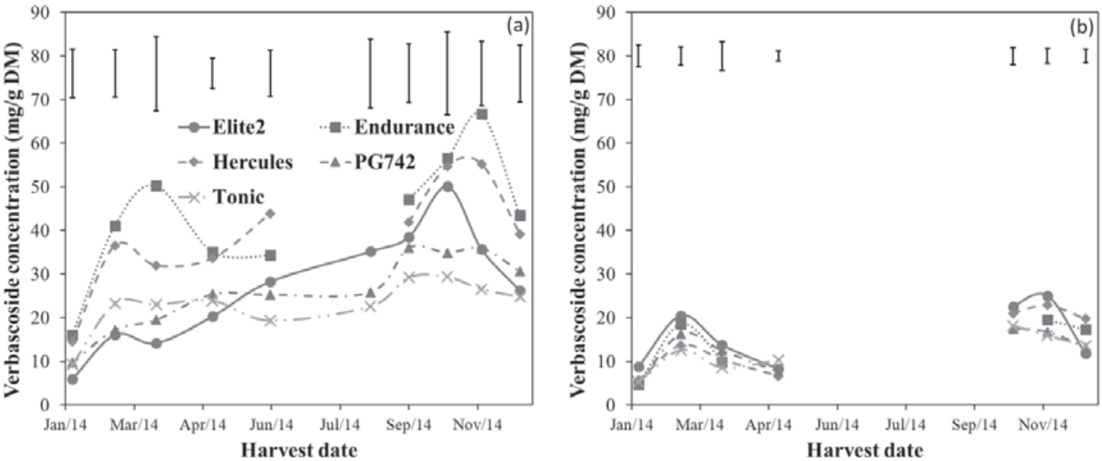

Figure 1 Verbascoside concentration (mg/g DM) in (a) leaves and (b) scape of five plantain cultivars from January to December 2014. Bars represent $LSD_{5\%}$. Breaks in the lines represent periods when material was not present or where herbage growth was insufficient for sampling.

Statistical analysis

Data were analysed by ANOVA for each harvest date using Genstat (VSN International Ltd., 2102) with the secondary compound as the fixed term and cultivar as the variable. Differences were considered significant at P<0.05. Means were separated by Fisher's protected least significant difference test ($LSD_{5\%}$).

Results

Distribution of compounds

The concentration of secondary plant compounds present in harvested material varied with cultivars, time of year and plant part (Figures 1-3). The average leaf concentration across cultivars was greatest for verbascoside (31.3 mg/g DM), intermediate for aucubin (10.6 mg/g DM) and lowest for catalpol (1.7 mg/g DM).

The concentration of verbascoside across cultivars tended to be higher in November than in other months (Figure 1a). The cultivars 'Hercules' and 'Endurance' occasionally had a greater concentration of verbascoside than 'Elite2', 'PG742' and 'Tonic' (Figure 1a). 'Endurance' tended to have the greatest average concentration of verbascoside in the leaf (43.4 mg/g DM, range 16.1-66.7), 'Hercules' intermediate (39.1 mg/g DM, range 14.5-55.2), and 'Elite2', 'PG742' and 'Tonic' the lowest (25.4 mg/g DM, range 6.0-50.1). Leaf verbascoside concentration in 'Hercules' and 'Endurance' showed a bi-modal distribution. Bi-modal peaks occurred in June for 'Hercules' (43.9 mg/g DM) and March for 'Endurance' (50.3 mg/g DM) and in November for both cultivars (55.2 and 66.7 mg/g DM, respectively) (Figure 1a). The effect of harvest date on verbascoside concentration was similar for 'Tonic' and 'PG742', generally increasing from January to November. The concentration of verbascoside in 'Elite2' gradually increased throughout the year, reaching a maximum of 50.1 mg/g DM in October then declining. The effect of harvest date on the concentration of verbascoside in the scape was less pronounced (Figure 1b).

For all cultivars the greatest concentration of aucubin in the leaf occurred in April (Figure 2a). Leaf concentration of aucubin for 'Hercules' and 'Endurance' was greater from late summer to autumn (February to April) than spring (September to November). The concentration of aucubin was similar (P>0.05) among cultivars in January, April, June, July and September. In February and March, 'Hercules' and 'Endurance' had a greater (P<0.05) concentration of aucubin than 'Tonic' and 'PG742' with 'Elite2' intermediate.

The concentration of catalpol in the leaf was low (>1 mg/g DM) at all harvests for 'Elite2', 'PG742' and 'Tonic' (Figure 3a). For most harvest dates, 'Hercules' and 'Endurance' had a greater (P<0.001) concentration of catalpol than 'Elite2', 'PG742' and 'Tonic'. The concentration of catalpol in the leaf of 'Hercules' and 'Endurance', was less than 6 mg/g DM between January and August. Peaks in the leaf concentration of catalpol for 'Hercules' and 'Endurance' occurred between September and December.

Botanical distribution of secondary plant compounds

The leaf of all cultivars contained more verbascoside than the scape when averaged across harvest dates. 'Elite2', 'PG742' and 'Tonic' had a greater proportion of verbascoside in the scape than 'Hercules' and 'Endurance'.

The botanical distribution of aucubin varied among cultivars. There was a similar concentration of aucubin

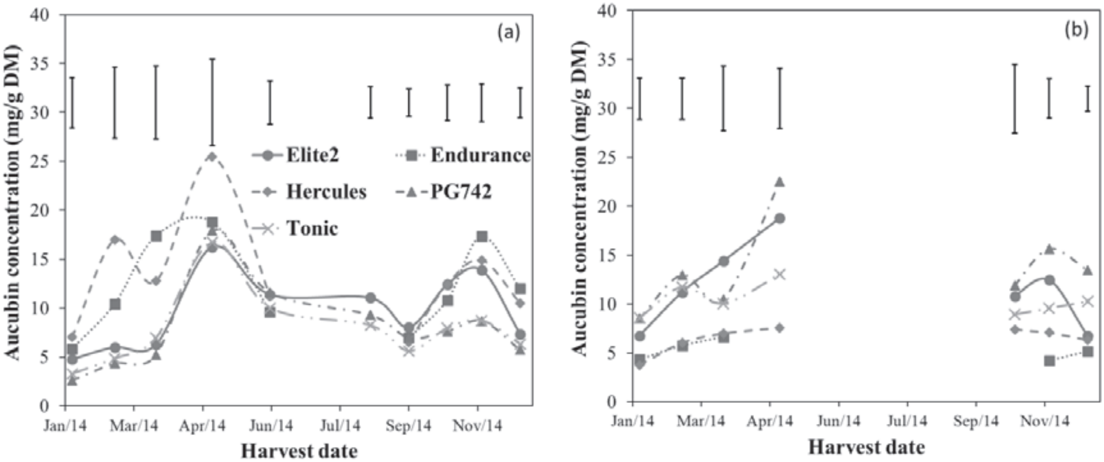

Figure 2 Aucubin concentration (mg/g DM) in (a) leaves and (b) scape of five plantain cultivars from 16 January to 8 December 2014. Bars represent $LSD_{5\%}$. Breaks in the lines represent periods when material was not present or where herbage growth was insufficient for sampling.

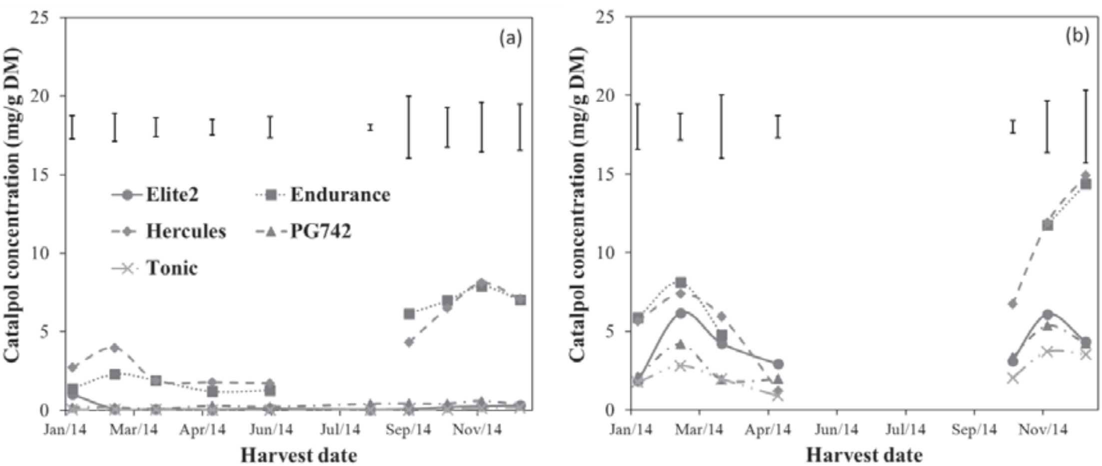

Figure 3 Catalpol concentration (mg/g DM) in (a) leaves and (b) scape of five plantain cultivars from 16 January to 8 December 2014. Bars represent $LSD_{5\%}$. Breaks in the lines represent periods when material was not present or where herbage growth was insufficient for sampling.

in the leaf and scape of 'Elite2', 'PG742' and 'Tonic'. Contrary to this, the average concentration of aucubin was greater in the leaf than the scape of 'Endurance' (43.4 and 14.0 mg/g DM, respectively) and 'Hercules' (39.1 and 14.7 mg/g DM, respectively).

Catalpol concentration was low (<0.5 mg/g DM) in the leaf of 'Elite2', 'PG742' and 'Tonic'. The concentration of catalpol in the scape of 'Elite2', 'PG742' and 'Tonic' was more than twice that of the leaf concentration. Overall, catalpol concentration was two time greater for 'Hercules' and 'Endurance' than for 'Elite2', 'PG742' and 'Tonic'.

Discussion

This experiment provided New Zealand data on the seasonal variation in concentrations of verbascoside, aucubin and catalpol in five cultivars/breeding lines of plantain and compared the botanical distribution (leaf and scape) of secondary compounds within each cultivar.

Secondary compound abundance

Secondary compound concentrations in plantain in this experiment varied with season, cultivar and plant part. However, regardless of cultivar, compound abundance was greatest for verbascoside, intermediate for aucubin and lowest for catalpol. This is consistent with previous New Zealand experiments which examined secondary compounds in 'Tonic' only (Navarrete *et al.* 2016; Box *et al.* 2017), but differed from the results of Tamura & Nishibe (2002) and Bowers *et al.* (1992) in Japan and America, respectively, suggesting significant genotype by environment interactions exist.

The concentrations of verbascoside in leaves were highly variable, with concentrations in the leaf ranging from 6.0 to 66.7 mg/g DM. This is comparable with the concentration of verbascoside in 'Tonic' (Navarrete *et al.* 2016) and for German cultivars (Dietz *et al.* 2013), but greater than concentrations reported by Al-Mamun *et al.* (2008). Concentrations of between 60 to 90 mg/g DM of verbascoside have been recorded in natural ecotypes of plantain (Fajer *et al.* 1992), and from 15 to 41 mg/g DM in the cultivar 'Tonic' (Tamura & Nishibe 2002), similar to the concentration reported here. These results suggest that opportunity exists to vary the concentration of verbascoside in plantain by phenotypic selection.

Averaged across all harvest dates, all cultivars contained from 7.9 to 13.1 mg/g DM aucubin in the leaf. This is substantially lower than the 40 mg/g DM reported by Bowers *et al.* (1992) in American and Japanese ecotypes, but comparable to concentrations in 'Lancelot' and 'Tonic' (Al-Mamun *et al.* 2008).

Catalpol concentration was consistently low (<1 mg/g DM) in the leaves of 'Elite2', 'PG742' and 'Tonic'. Nil detection of catalpol in 'Tonic' has been reported by Navarrete *et al.* (2016) and Al-Mamun *et al.* (2008); these findings could be a useful for distinguishing between cultivars. Tamura & Nishibe (2002) found no trace of catalpol in 'Tonic', but concentrations of between 10 and 20 mg/g DM in 'Lancelot'. Both 'PG742' and 'Elite2' are lines from the 'Tonic' population suggesting a degree of heritability for this trait. Naturalised ecotypes of plantain can contain up to 12.1 mg/g DM (Al-Mamun *et al.* 2008), which is greater than the observed concentrations in the leaves of all cultivars in the current experiment. Aucubin is an intermediate in the synthesis of catalpol (Damtoft 1994) and it is possible that selection for catalpol may have resulted in a reduction in aucubin concentration.

Cultivar differences

In the current study, 'Endurance' and 'Hercules' had greater average concentrations of verbascoside than 'Elite2', 'PG742' and 'Tonic' at most harvest dates. Concentrations of catalpol were much lower than verbascoside, particularly for 'Tonic' where catalpol remained below 5 mg/g DM for all harvests.

Cultivar effects for aucubin were only observed through autumn. Al-Mamun *et al.* (2008) also showed no difference in the aucubin concentration among 25 plantain ecotypes collected from northern Japan and two New Zealand cultivars ('Lancelot' and 'Tonic'). Similarly, Bowers *et al.* (1992) reported no differences in aucubin concentration between two plantain species collected in Rhode Island, USA, for early season harvested plantain herbage, but found differences between the genotypes later in the season when more growth occurred. In the current experiment the effect of harvest date on aucubin concentration was similar for 'PG742' and 'Tonic'. These cultivars exhibited smaller changes in aucubin concentrations throughout the year than did 'Hercules' or 'Endurance', which showed large seasonal fluctuations within the leaves. This stability in seasonal concentration of aucubin in 'Tonic' and its related cultivars makes aucubin a candidate for an intake marker of these types of plantain, if digestibility is predictable. However, a strong seasonal variation was recorded by Navarrete *et al.* (2016), therefore, further research may be required to better understand seasonal drivers behind aucubin production.

Seasonal distribution

Harvest date influenced the concentration of secondary compounds, particularly for verbascoside. The seasonal distribution of secondary compounds was similar among cultivars. The seasonal variation in the secondary compound concentrations in the current experiment may be due to the morphological state of the plantain. Younger leaves or more vulnerable tissues are often associated with greater concentrations of anti-herbivory properties (Rhoades & Cates 1976). For example, Bowers & Stamp (1992) found that new leaves of plantain have approximately twice the iridoid glycoside content of older leaves. Whilst it is not clear if (or how), the secondary compounds in plantain act as a defense against herbivory, the seasonal variation of leaf compounds in the current experiment, with large verbascoside peaks occurring in spring, suggests a possible mechanism to deter consumption by herbivores when leafy material is abundant.

Botanical distribution of compounds

Previous literature suggests the abundance of secondary compounds in plantain may be related to the morphological state of the plant (Bowers *et al.* 1992; Fajer *et al.* 1992; Tamura & Nishibe 2002). More specifically, it was suggested by Bowers *et al.* (1992b) that when plantain is in a reproductive state, there would likely be a decrease in leaf secondary compound concentrations due to partitioning of secondary compounds to scape. The increased catalpol concentration in scape may be a defence mechanism to protect reproductive tissue against herbivore predation (Pankoke *et al.* 2013).

In summer and autumn when scape was abundant, the concentration of catalpol in scape was at least twice that in the leaves, for all cultivars, regardless of harvest date. However, scape concentration of catalpol remained low (<6 mg/g DM) for 'Elite2', 'PG742' and 'Tonic'. Therefore, an increase in scape presence did not improve overall catalpol concentration present in plantain herbage for 'Elite2', 'PG742' and 'Tonic'. Concentration of catalpol in the scape of 'Hercules' and 'Endurance' peaked in December at 14.4 and 14.9 mg/g DM, respectively, when scape comprised 57 and 58% of total herbage DM (data not presented).

The concentration of aucubin in the leaf and scape was similar for 'Elite2', 'PG742' and 'Tonic'. Therefore, scape presence in these cultivars did not result in an increase or dilution of aucubin concentration. For 'Hercules' and 'Endurance', the concentration of aucubin in the scape tended to be lower than in the leaves. This may result in a dilution of the overall aucubin concentration in 'Endurance' and 'Hercules' herbage when scape is present. This experiment suggests that an increase in scape abundance does not equate to an overall increase in iridoid glycoside concentrations, but may result in dilution.

Concentrations of verbascoside in the leaf were lowest when scape presence increased (data not presented) and through the winter when growth was low for winter active varieties. Despite this, concentrations of verbascoside in the scape were on average half the concentration observed in the leaf, indicating partitioning of verbascoside to scape did not contribute to the reduction in leaf concentrations, but may contribute to an overall dilution of verbascoside. This was particularly true for cultivars with the highest leaf concentrations of verbascoside ('Hercules' and 'Endurance').

Conclusions

For all cultivars the average leaf concentration of compounds was greatest for verbascoside, intermediate for aucubin and lowest for catalpol. Of the test cultivars, the winter dormant 'Hercules' and 'Endurance' tended to have the greatest concentrations of secondary compounds. Results suggest that an increase in scape abundance does not equate to an overall increase in iridoid glycoside concentrations.

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