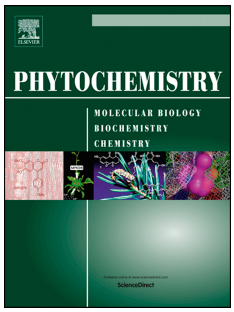

Grayanotoxin I variation across tissues and species of *Rhododendron* suggest pollinator-herbivore defence trade-offs

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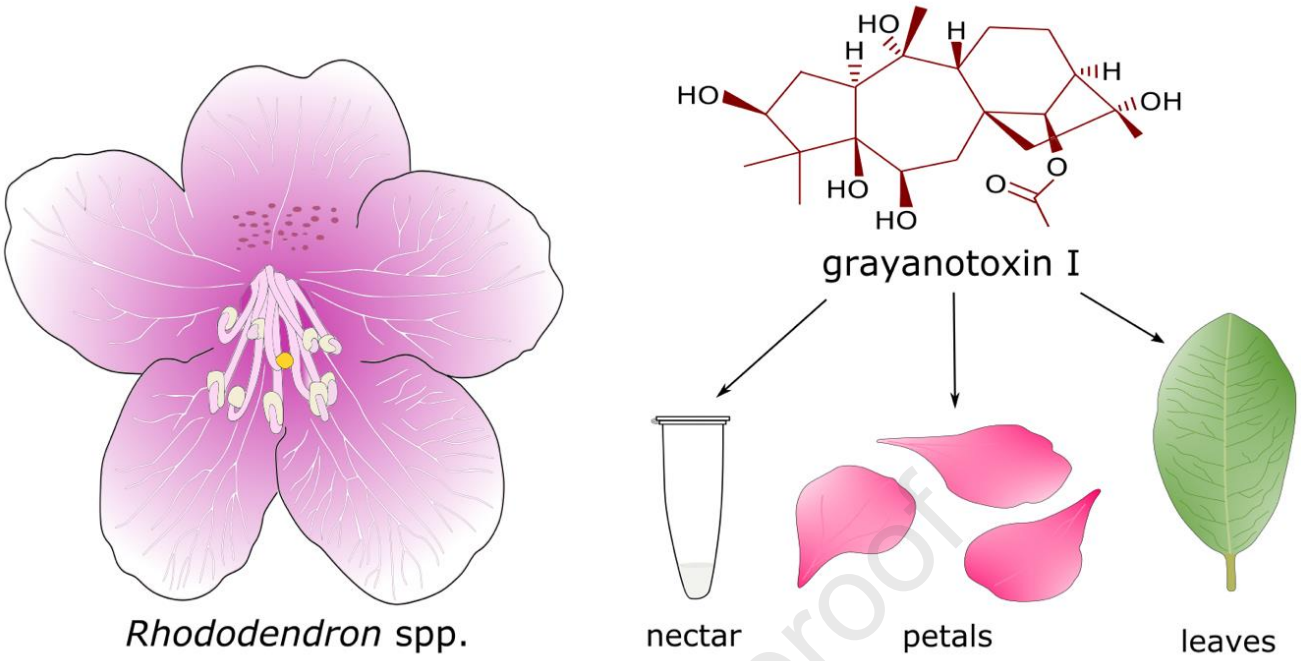

Grayanotoxin I (GTX I) was isolated from the nectar, petals, and leaves of seven *Rhododendron* species. GTX I was present in all species and variability in toxin concentration was found both within and between species. GTX I concentrations were positively correlated between leaves and petals, as well as leaves and nectar, demonstrating potential phenotypic linkage.

Grayanotoxin I variation across tissues and species of *Rhododendron* suggest pollinator-herbivore defence trade-offs.

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Abstract

Grayanotoxin I (GTX I) is a major toxin in leaves of *Rhododendron* species, where it provides a defence against insect and vertebrate herbivores. Surprisingly, it is also present in *R. ponticum* nectar, and this can hold important implications for plant-pollinator mutualisms. However, knowledge of GTX I distributions across the genus *Rhododendron* and in different plant materials is currently limited, despite the important ecological function of this toxin. Here we characterise GTX I expression in the leaves, petals, and nectar of seven *Rhododendron* species. Our results indicated interspecific variation in GTX I concentration across all species. GTX I concentrations were consistently higher in leaves compared to petals and nectar. Our findings provide preliminary evidence for phenotypic correlation between GTX I concentrations in defensive tissues (leaves and petals) and floral rewards (nectar), suggesting that *Rhododendron* species may commonly experience functional trade-offs between herbivore defence and pollinator attraction.

Keywords

Rhododendron, Ericaceae, plant defence, functional trade-offs, phenotypic correlation, nectar chemicals, grayanotoxin.

1. Introduction

Plant specialised metabolites provide an important defence against invertebrate herbivores (Klocke et al. 1991, Schoonhoven et al. 2006, Xiao et al. 2012, Barlow et al. 2017). Within pollen, for example, these chemicals likely protect the male gametes (Pacini and Hesse 2005, Dobson et al. 2000). Nectar is a floral reward for mutualists that mediates interactions with pollinators and herbivores, both of which can exert selection pressures on the diversity and abundance of floral chemicals (Berenbaum et al. 1986, Mauricio and Rausher 1997, Schiestl et al. 2011, Agrawal et al. 2012, Huber et al. 2016, Palmer-Young et al. 2019, Kessler and Halitschke 2009, Stevenson 2020). Consequently, evolutionary trade-offs may occur in the composition and concentrations of plant specialised metabolites within nectar.

Given that nectar rewards pollinators, the secretion of toxins into nectar that could harm or deter mutualists may seem paradoxical. However, nectar toxins can provide protection from nectar robbers and other floral larcenists (Stephenson 1982, Irwin et al. 2004); as well as preventing the growth of microorganisms which would otherwise significantly alter nectar

chemistry (Adler 2000, Rivest and Forrest 2020, Vannette 2020). Nectar specialised metabolites may prevent ineffective pollinators from depleting nectar rewards. As such, they may be a beneficial ecological filter ensuring greater nectar resources for more efficient pollinators that are not susceptible to these toxins (Adler 2000, Irwin et al. 2014, Tiedeken et al. 2016). Some potential benefits of nectar specialised metabolites for pollinators have also been reported, including reduced gut pathogen load (Manson et al. 2010, Koch et al. 2019), enhanced memory of floral signals (Wright et al. 2013), and increased visitation rates to flowers (Singaravelan et al. 2005). Plants may incur a net fitness cost if the occurrence of specialised metabolites in nectar is not adaptive, but instead results from physiological constraints (Adler 2000). If toxins are produced in leaves and petals as a chemical defence to herbivory, 'leakage' of these toxins into nectar could be a pleiotropic consequence (Adler 2000). Causality is difficult to determine because detailed physiological understanding of nectar production and secretion in different species is currently lacking, complicated by variation between taxa in the source tissue of nectar specialised metabolites and the complex multi-stage nectar production process (Nepi 2007, Stevenson et al. 2016, Roy et al. 2017). Phenotypic correlations of plant specialised metabolites have been reported between, for example, nectar and leaves of *Asclepias* (Manson et al. 2012), and nectar and petaloid sepals of *Aconitum* (Barlow et al. 2017). This indicates that pleiotropic constraints could have a role in the expression of specialised metabolites in nectar of these species (Smith 2016, Junker et al. 2017). However, specialised metabolites have also been found exclusively in either nectar, pollen, or leaves (Kessler and Baldwin 2007, Marlin et al. 2014, Stevenson et al. 2016). Palmer-Young et al. (2019) investigated floral chemistry of thirty one species across diverse taxa and only thirty four percent of compounds were found in both pollen and nectar. These data suggest a capacity for tissue-specific regulation of plant specialised metabolites.

We investigated toxin levels within the flowers and leaves of *Rhododendron* (Ericaceae) species. The genus *Rhododendron* contains approximately a thousand species that are distributed across the Northern hemisphere and within Southeast Asia (Chamberlain et al. 1996, Stevenson 2020). Plant toxicity in *Rhododendron* is determined by the ent-kaurane diterpenoids grayanotoxin I (GTX I) and grayanotoxin III (GTX III) (Qiang et al. 2011, Egan et al. 2016). These compounds are restricted to the Ericaceae and have been reported in several *Rhododendron* species including *R. japonicum* A.Gray (Koda et al. 2016, Fukumoto 1993), *R. ponticum* L. (Egan et al. 2016), *R. simsii* Planch. (Scott-Brown et al. 2016) and *R. molle* (Blume) G.Don (Li et al. 2015). GTXs are neurotoxins that provide an important plant chemical defence by binding to animal sodium channel receptors and inhibiting them (Qiang et al. 2011, Li et al. 2013). GTX I was found to be toxic and repellent to thrips (*Heliothrips haemorrhoidalis*), a herbivore that targets *Rhododendron* (Scott-Brown et al. 2016). Other grayanoid diterpenes

have been shown to deter or harm cabbage white larvae (*Pieris rapae*) (Zhong et al. 2006) and Colorado potato beetles (*Leptionotarsa decemlineata*) (Klocke et al. 1991).

Grayanotoxins are present in honey derived from *R. ponticum* nectar (Onat et al. 1991, von Malottki and Wiechmann 1996) and have recently been extracted directly from nectar samples (Tiedeken et al. 2014, Egan et al. 2016). Typically, nectar toxins are found in trace amounts compared with vegetative tissue (Palmer-Young et al. 2019), but nectar GTX I concentrations in *R. ponticum* occurred at a concentration that was a similar order of magnitude to that found in leaf and twig sample extracts (all calculated from dry weight) (Wong et al. 2002, Hough et al. 2010, Egan et al. 2016). Nectar GTX I levels in the native range of *R. ponticum* were at concentrations high enough to kill pollinating insects such as solitary bee species and honeybees, although *Bombus terrestris* were reportedly tolerant (Tiedeken et al. 2014, Tiedeken et al. 2016). The exclusion of certain medium-sized floral visitors, due to GTX I in *R. ponticum* nectar, could be maladaptive. These pollinators may be efficient pollen vectors and animal pollinators are required for optimal seed production (Stout 2007, Egan et al. 2016). Egan et al. (2022) found phenotypic correlations between GTX I levels in the leaves and corolla, and leaves and nectar, of *R. ponticum*. In the *R. ponticum* native range only, positive selection on GTX I levels in leaves indirectly led to positive total selection on nectar and corolla toxin levels. Whereas corolla and leaf GTX I levels were selectively neutral in the non-native range, while nectar GTX I levels were under negative selection - thought to be pollinator mediated. As such, in the non-native range of *R. ponticum* GTX I is selectively allocated, enabling reduced toxin concentrations within nectar without compromising chemical defence in leaves.

The impact of nectar toxins on pollinators and herbivores can be dose-dependent (Tadmor-Melamed et al. 2004, Lerch-Henning and Nicolson 2013, Manson et al. 2013). As such, investigating the intraspecific and interspecific variation in nectar GTX I levels in *Rhododendron* provides a first step towards understanding the ecological effects of this toxic nectar on plant-pollinator mutualisms (Egan et al. 2016). Here we conduct a quantitative characterisation of GTX I in the nectar, petals, and leaves of seven *Rhododendron* species sampled in a botanical garden. Several individuals were sampled from each species enabling investigation of within-species variation. We examined whether there was a phenotypic correlation between GTX I concentrations in vegetative and reward tissue, providing insight into whether toxic nectar could result from pleiotropy. Ultimately, this research provides an important preliminary investigation into the qualitative and quantitative GTX I phenotypes of several *Rhododendron* species.

2. Results and Discussion

GTX I levels were quantified in the leaves, petals, and nectar of seven *Rhododendron* species: *R. augustinii* Hemsl. (n = 11), *R. campanulatum* D. Don. (n = 9), *R. decorum* Franch. (n = 6), *R. degronianum* Carriere. (n = 11), *R. pseudochrysanthum* Hayata. (n = 8), *R. rubiginosum* (n = 9) Franch and *R. yunnanense* Franch (n = 8).

2.1 All *Rhododendron* species investigated produced GTX I in leaves, petals, and nectar

GTX I occurred more frequently within leaves compared to nectar ($z = 2.56$, $p = 0.03$). GTX I was present at detectable levels in the leaf sample extracts of 60% of individuals, 48% of petal sample extracts, and 42% of nectar samples (Figure 1a).

Every study species produced GTX I in nectar, petals, and leaves. GTX I was only present in the nectar sample extracts of a single *R. augustinii* and *R. rubiginosum* at quantifiable levels; a second individual of each species had trace amounts of GTX I in nectar samples. GTX I occurred, at quantifiable levels, in the leaf sample extracts of four *R. rubiginosum* plants but only one *R. augustinii* plant. In contrast, GTX I occurred in the petal sample extracts of every *R. degronianum* individual (n = 11), in addition to the leaf sample extracts of every *R. degronianum* and *R. pseudochrysanthum* (n = 8) plant. In the majority of species 1 – 2 individuals had trace levels of GTX I in sample extracts, that is GTX I was detected but at levels too low to quantify (Figure S5, Table S2). We consider these trace readings as zeroes for our subsequent analyses.

GTX I may have been detected in additional samples if a higher volume of nectar had been collected. However, we know with high confidence other cases where nectar GTX I is absent, for example, Egan (2015) found species investigated within *Rhododendron* section Vireya had no nectar GTX I present. There are also known GTX I polymorphisms previously reported even within species, including *R. ponticum* where 18% of plants in the introduced range lacked GTX I in nectar (Egan et al. 2016). This may indicate either a genetic mechanism whereby GTX I production is 'switched off' or a mutation affecting biosynthesis.

Within each species, the frequency of GTX I occurrence was largely consistent across leaf, petal, and nectar samples (Figure 1b). Species explained much of the variation in GTX I occurrence in leaf ($\chi^2 = 43.58$, df = 55, $p < 0.001$), petal ($\chi^2 = 31.18$, df = 55, $p < 0.001$), and nectar tissue ($\chi^2 = 19.10$, df = 55, $p = 0.004$). There is some support for interspecific differences in leaf, petal, and nectar GTX I occurrence. Comparing each species' estimated mean GTX I occurrence in LMM analyses produced some significant differences, but in subsequent

pairwise analyses significance was not detected (Figure 1b). As such, further investigation into interspecific differences using a larger sample size is required.

2.2 GTX I concentrations were higher in the leaves compared to petals and nectar

Significantly higher concentrations of GTX I were recorded in sample extracts from leaves (mean ± SE, 1793 mg/kg ± 331 (w/v)) compared to petals (230 mg/kg ± 41 (w/v)) (t = 7.10, df = 46, p < 0.001) and nectar (123 mg/l ± 48 (v/v)) (t = -3.73, df = 46, p < 0.001) (Figure 1c). These differences in concentration between plant materials were consistent across all species (Figure 1d). While nectar samples were fresh, leaf and petal samples were freeze-dried and a correction was applied (see Section 4.3) so that the final GTX I concentrations in leaf and petal sample extractions were given relative to fresh weight. However, given differences in extraction efficiencies between fresh and dried material, the leaf and petal vs nectar sample extract concentrations may not share direct equivalence due to the experimental procedure. As such, our comparison between nectar concentrations and those in the sample extracts of leaves and petals is tentative. Only young leaves were sampled, which often contain higher concentrations of defensive metabolites (Hatcher 1990, Leiss et al. 2009, Wiggins et al. 2016). An investigation into GTX I concentrations in *R. simsii* found that young leaves contained higher levels of GTX I than mature leaves and this was associated with resistance to insect herbivory (Scott-Brown et al. 2016). Within some species investigated here, there was high variability in toxin concentration, for example, *R. campanulatum* leaf sample extracts (2217 mg/kg ± 1043, n = 7 (w/v)).

Species explained much of the variation in GTX I concentration ($F_{4, 43}$ = 5.85, p < 0.001). *R. degronianum* sample extracts had the highest GTX I concentration, which was significantly higher than *R. campanulatum* (t = -3.38, df = 31, p = 0.016), *R. decorum* (t = -4.00, df = 31, p = 0.003), *R. pseudochrysanthum* (t = 3.26, df = 31, p = 0.021) and *R. rubiginosum* (t = 4.18, df = 31, p = 0.002) concentrations (Figure 1d). Within this analysis, the nectar GTX I concentrations were largely within a range (30 – 1010 µM (v/v)) that has known effects on specific pollinators using artificial nectar in a laboratory setting, only four nectar sample extracts had concentrations below 30 µM. Concentrations of 1100 µM were previously shown to be toxic to honeybees (*Apis mellifera*) and a solitary bee (*Andrena scotica*) (Tiedeken et al. 2016), and at concentrations of 100 µM honeybee motility was adversely impacted (Oliver et al. 2015). Bumblebees (*Bombus terrestris*) were not susceptible to GTX I at these concentrations (Tiedeken et al. 2016) which may provide a selective advantage for preferred pollinators.

2.3 Leaf GTX I concentrations were positively correlated with petal and nectar GTX I concentrations

The GTX I concentrations in leaf sample extracts and nectar within individuals had a marginally significant positive association ($t = 2.06$, $df = 40$, $p = 0.046$). Leaf and petal sample extracts had GTX I concentrations that were also positively correlated ($t = 5.12$, $df = 40$, $p < 0.001$) (Figure 2a). This phenotypic correlation between nectar, as a floral reward, and leaves implies that the presence of GTX I in nectar could be maladaptive, or that adaptation has occurred through evolution from an initial non-adaptive role (Armbruster et al. 1997). Egan et al. (2022) also found phenotypic correlations between the leaf and petal, and leaf and nectar GTX I concentrations of *R. ponticum*, but only within its native range. In non-native Irish populations there was uncoupling between *R. ponticum* nectar GTX I concentrations and those of leaf sample extracts, with some individuals lacking GTX I in nectar. We found that occurrence of GTX I in nectar and petals did not always coincide with GTX I occurrence in leaf sample extracts (Figure 2b), despite positive correlations between GTX I concentrations implying phenotypic linkage. This uncoupling occurred across species with GTX I present in nectar but not leaf sample extracts of 3 individuals. Interestingly, these 3 plants also had the lowest nectar GTX I concentrations recorded. GTX I occurred in the leaf sample extracts but not the nectar of 1 – 3 individuals of every species (except for *R. yunnanense*, which only had GTX I present in a single individual). Why this occurred within these subsets of individuals remains unclear. Overcoming linkage in this way may enable the maintenance of leaf chemical defence despite reduced toxin levels in nectar.

2.4 *Rhododendron* plant size may influence the occurrence of GTX I

When zero values were excluded in tests for phenotypic correlation, smaller plants had higher leaf sample extract GTX I concentrations in the models comparing leaf with petal ($t = 4.48$, $p < 0.001$) and leaf with nectar ($t = 2.68$, $p = 0.011$). Size may alter resource allocation strategies, as environmental stressors can have different effects depending on plant size (Boege et al. 2005). Herbivory, for example, can be particularly detrimental to juvenile plants resulting in greater investment in defensive specialised metabolites (Bryant and Julkunan-Totto 1995). Scott-Brown et al. (2016) found that young leaves had the highest concentrations of grayanotoxin I in glasshouse grown *R. simsii*, with concentrations decreasing in progressively older leaves. While Egan et al. (2022) found that in wild populations of *R. ponticum* older leaves contained significantly more GTX I than younger leaves. In both studies there was an inverse relationship between GTX I concentrations and the herbivore population size – for *R. simsii* the thrip *Heliothrips haemorrhoidalis* and for *R. ponticum* the black vine weevil *Otiorhynchus sulcatus*. No significant relationship was detected between plant size and GTX I concentration in the models including all samples. As such, sampling across a developmental time course of different plant tissues, along with larger sample sizes, would provide greater insight into the relationship between plant size and toxin levels.

3. Conclusion

All *Rhododendron* species investigated produced GTX I in leaves, petals, and nectar likely as part of a defence mechanism against herbivores. The occurrence of GTX I in nectar may also mediate plant-pollinator interactions. The marked variation in GTX I occurrence between species is possibly due to differences in defensive strategies. Future studies could also incorporate interspecific differences in physical deterrents against herbivory to investigate this. High intraspecific variability in toxin levels was apparent, but GTX I concentrations were consistently lower in nectar and petals compared to leaves. High leaf GTX I concentrations may have an important adaptive value in minimising vegetative tissue damage. Our preliminary evidence that smaller *Rhododendron* plants expressed higher levels of GTX I suggests that plant size may influence GTX I resource allocation or could indicate potential trade-off between growth and toxin production. Positive correlations between GTX I concentrations in vegetative and floral tissues were consistent with the hypothesis that GTX I occurrence in nectar may have originated from pleiotropic constraints. However, not all individuals across species produced GTX I in nectar when it was present within leaves and vice versa, suggesting the potential for uncoupling of toxin expression between these plant materials. To our knowledge, this is the first characterisation of GTX I distribution across these *Rhododendron* species. We also provided an initial insight into linkage between leaf and nectar, and leaf and petal, chemical phenotypes. How defensive strategies differ between species and how plant-pollinator relationships vary in different ecological contexts are exciting questions for future *Rhododendron* research.

4. Experimental

4.1 General experimental procedures

Plant GPS coordinates were collected using a Garmin etrex handheld GPS (WGS-84 datum). Liquid chromatography-mass spectrometry (LC-MS) analyses of sample extracts were completed using a Waters Alliance LC and ZQ MS detector (LC model 2695). The source temperature was 80^{o}C and gas flow rates for desolvation was 250 l/hr and for cone 50 l/hr. The injection volume was 10 µl onto a Phenomenex Luna C18(2) column (150 x 3.0 mm inner diameter, 5 µm particle size) kept at 30^{o}C. The gradient elution had a mobile phase of (A) methanol, (B) water and (C) 1% formic acid in acetonitrile (A = 0%, B = 90%, C = 10% at 0 min; gradient until: A = 90%, B = 0%, C = 10% at 20 min; plateau for 10 mins so: A = 90%, B = 0%, C = 10% at 30 min; A = 0%, B = 90%, C = 10% 31 min). Flow rate was 0.5 ml/min and detection used negative mode electrospray MS. The MS was in scan mode from 125 – 1200

amu in negative mode and dwell time was 0.1 sec. All data analysis and figures were completed in R version 3.2.1 (R Core Team 2015). Figures were made using the package ggplot2 (Wickham 2009). Statistical modelling was competed in the R packages nlme (Pinheiro et al. 2014), lme4 (Bates et al. 2014), multcomp (Hothorn et al. 2008), and MuMIn (Barton 2013).

4.2 Collection of plant material

Samples were taken from plants of the following species: *Rhododendron augustinii* (Ericaceae), *R. campanulatum*, *R. decorum*, *R. degronianum*, *R. pseudochrysanthum*, *R. rubiginosum*, *R. yunnanense*. Plants were sampled from Wakehurst Place, West Sussex (National Grid Reference: TQ331306; Latitude: 51.0689° N, Longitude: 0.0872° W) between 28th April – 7th June 2016, as the flowering time varied between plants. Samples were collected between 13:00 – 18:00. *Rhododendron* species were selected first using the Kew Living Collections Database, which enabled clonal specimen to be excluded and gave the number of living specimen. The selected species had 10 or more labelled (non-clonal) individuals identified within the field. Nectar and petals were sampled from 6 – 12 flowers per individual along with the leaf closest in proximity to each flower. Nectar was taken with a capillary tube (≥ 8 µl per plant). These samples were pooled to give one sample of each plant material per individual. To standardise the sampling procedure plants were sectioned into four axes (based on compass bearings) and, where possible, a subset of mature flowers in β-phase (as defined by Mejías et al. 2002) closest to these axes were sampled. After collection, samples were stored at -20°C. Plant height and area was approximated; for area an elliptical circumference was calculated by measuring plant width and length.

4.3 Chemical analysis

The fresh weight of each sample was measured before petal and leaf samples were freeze dried at -40°C. Petal and leaf samples were ground by hand, a standardised weight per sample contributed towards one pooled petal sample and one pooled leaf sample per individual.1 ml of 50% methanol was added to 10 mg of ground sample, extracts were incubated at room temperature for 8 hr and vortexed after 10 min, 4 hr, and 8 hr. The samples were centrifuged at 11000 xg for 2 min. Nectar samples were centrifuged and then mixed with 100 µl 50% methanol, vortexed, and centrifuged. Sample extracts of all plant materials were stored at -20°C.

Purified GTX I was isolated from an *R. ponticum* specimen by Tiedeken et al. (2016) to create the GTX I standard that was used in our analyses, through a methanol extraction with dried *R. ponticum* flowers (100g). GTX I was extracted and isolated 14 times and in total 1.4 kg of

R. ponticum flowers yielded approximately 400 mg GTX I (extraction procedure detailed in Tiedeken et al. 2016). The standard was used for a dilution series that produced a calibration curve (1 – 1000 mg/l) and enabled GTX I concentrations to be calculated from LC-MS peak areas for each sample.

LC-MS analysis results were filtered to measure GTX I concentrations, using m/z 411 extracted ion chromatograms and the GTX I peak formation time (c.a. 8.5 min). The GTX I concentrations in petal and leaf sample extracts were calculated using dry weight and then these values were corrected to give GTX I concentrations relative to fresh weight. As such, final GTX I concentrations reported in leaf and petal sample extracts were given relative to the overall fresh weight. This enabled tentative comparisons with the GTX I concentrations in nectar samples which were extracted from fresh material. Where we report absence of GTX I we cannot rule-out the possibility that if more plant material had been collected GTX I would have been detected.

4.4 Data analysis

Plant size could not be determined within the field, but height and area of plants was approximated. A principal component analysis was conducted to combine these factors in PCs representing components of plant size.

A GLMM was used to test whether plant materials and plant size influence GTX I occurrence (presence/ absence). The model was performed with a 'logit' link function and binomial errors and was fitted by maximum likelihood (Laplace Approximation merMod), with individual nested within species added as a random effect.

To test for an effect of species and plant size on the occurrence of GTX I, three GLMs were conducted considering GTX I occurrence in either leaves, petals, or nectar. These analyses were performed as an alternative to a GLMM model including all plant materials that failed to converge due to low replicate numbers.

An LMM was used to test whether species and plant material affected GTX I concentration, given that GTX I was present. The model was fitted by restricted maximum likelihood (REML) and individual was added as a random effect. Samples where GTX I was not detected were excluded along with species where toxin was expressed in ≤ 4 individuals: *R. augustinii* (n = 4) and *R. yunnanense* (n = 3). The response variable was $\log_{10}$ transformed.

LMMs fitted by restricted maximum likelihood (REML) were used to test for an effect of GTX I concentration in leaves on either GTX I concentration in petals or nectar. This enabled GTX I concentrations to be compared between plant materials within an individual. Species was added as a random effect and individuals with no GTX I detected in any plant material were

excluded. The petal and nectar model response variables were transformed by ^0.25 and ^0.3 respectively.

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Figures

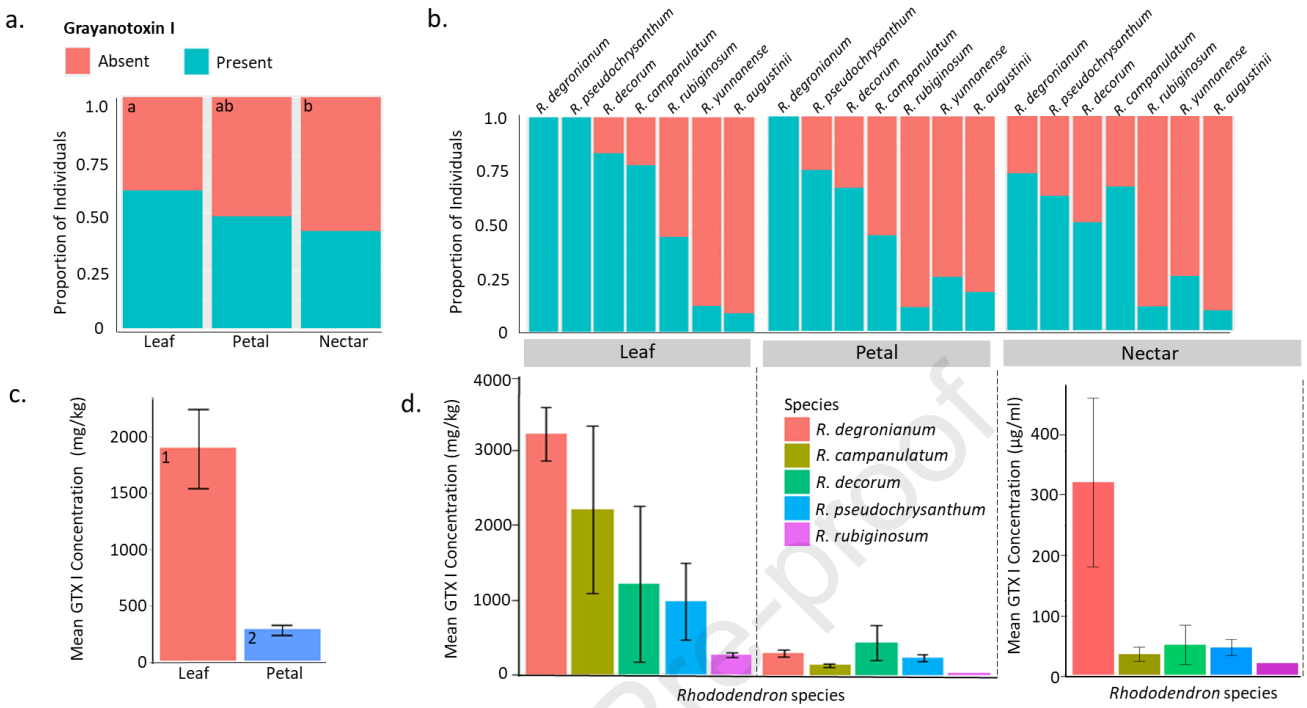

Figure 1. The distribution of GTX I in different plant materials and species of *Rhododendron*. a) The proportion of individuals within which GTX I is absent (red) or present (blue) in leaf, petal, and nectar sample extracts. b) GTX I occurrence in different plant material and *Rhododendron* species. c) The mean GTX I concentration (mg/kg) in leaf and petal sample extracts (w/v). d) GTX I concentrations in different plant material sample extracts (nectar concentrations in µg/ml (v/v)) and *Rhododendron* species. Note that the Y axis scales are 10-times higher for leaves and petals than for nectar sample extracts. Species with ≤ 4 individuals producing GTX I (*R. augustinii* and *R. yunnanense*) were excluded. Error bars represent ± SE. In a and c if the bars do not share a number or letter the data is significantly different.

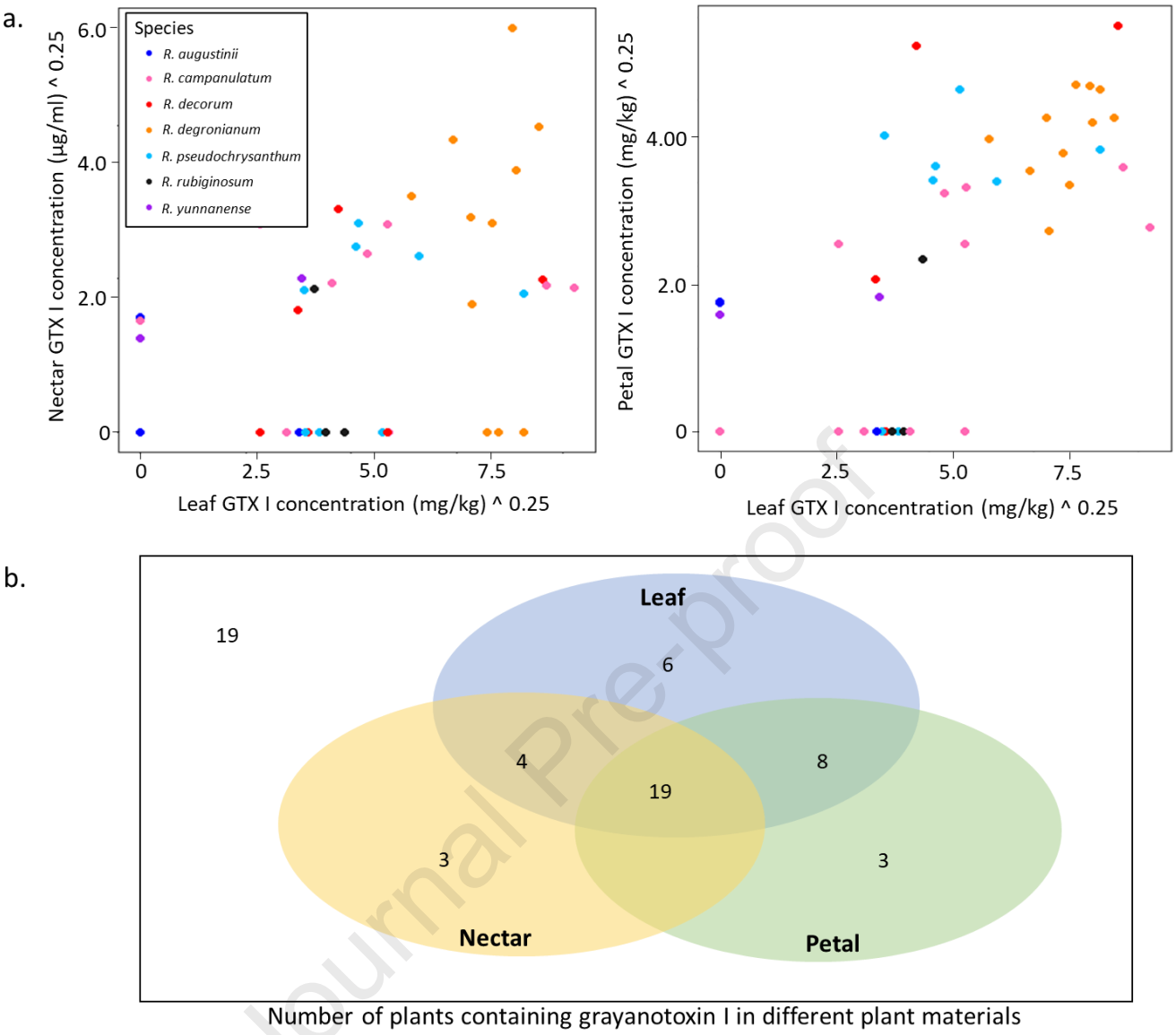

Figure 2. Relationships between GTX I concentration and occurrence in different plant materials within an individual. a) GTX I concentration (data transformed by ^0.25) in leaf sample extracts (w/v) and nectar samples (v/v) (top left) and leaf and petal sample extracts (w/v) (top right). Each data point represents an individual and is colour coded according to species. Individuals with no GTX I detected in any tissue type were excluded. b) Venn diagram of GTX I occurrence in leaf, petal, and nectar. Numbers represent the total number of plants within each category and position within the diagram corresponds to which plant materials contained GTX I.

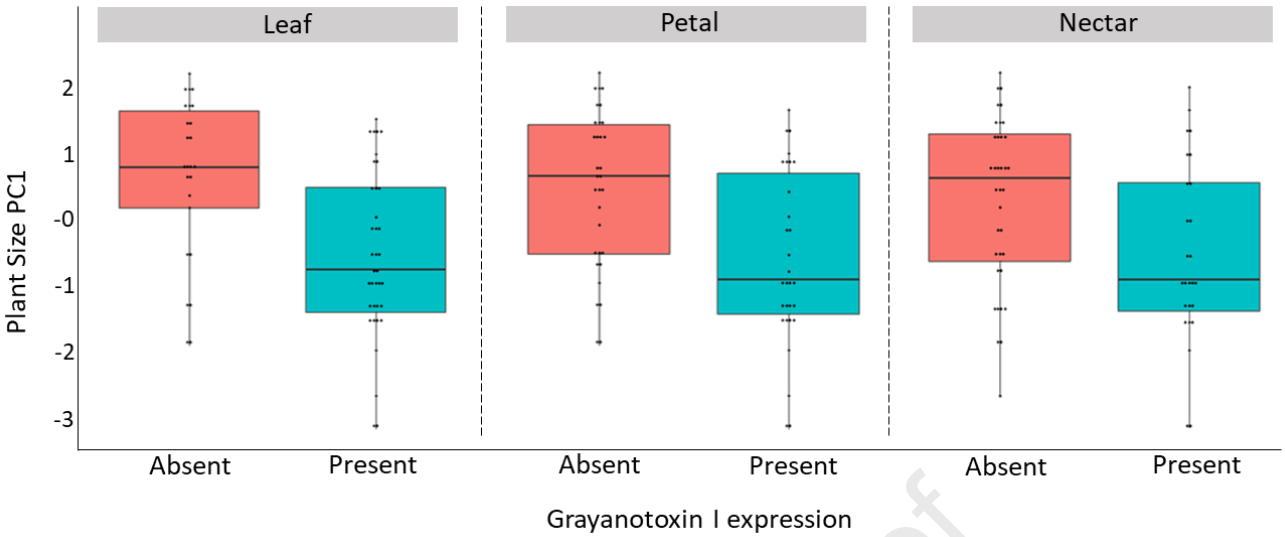

Figure 3. Boxplots demonstrating the relationship between plant size and occurrence of GTX I in different plant material sample extracts (leaf, petal, and nectar). Plant size is represented by the first principal component (explaining 87% of the variance) of a PCA combining plant height and area. The black line in each box indicates the median value and the whiskers 25/75% quantile +/- 1.5 * interquartile range, respectively.

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- Grayanotoxin I (GTX I) is present in the nectar of multiple *Rhododendron* species.
- Phenotypic correlation occurs between GTX I concentrations in leaves and nectar.
- There is high interspecific variation in GTX I concentrations.
- GTX I concentrations were significantly higher in leaves than nectar or petals.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: