Who's who in the fleabane (*Conyza* spp.) family?

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Summary Flaxleaf fleabane (*Conyza bonariensis* (L.) Cronquist) has become a problem weed throughout the entire cropping belt of south-eastern Australia. One of the barriers for effective control of *C. bonariensis* is the morphological confusions with other fleabane species. This study used the scanning electron microscopic technique to investigate the differences in trichome and stomatal densities between *C. bonariensis* and tall fleabane (*C. sumatrensis* (Retz.) E.Walker). Results showed that *C. bonariensis* had significantly higher trichome densities than *C. sumatrensis*. The trichome densities in *C. bonariensis* ranged from 67.2 to 221.9 trichomes mm^{-2} on the adaxial leaf surface and from 74.0 to 168.1 trichomes mm^{-2} on the abaxial leaf surface, while the trichome densities in *C. sumatrensis* ranged from 40.3 to 147.9 trichomes mm^{-2} on the adaxial surface and from 53.8 to 134.5 trichomes mm^{-2} on the abaxial surface. There were no significant differences in trichome densities between the adaxial and abaxial surfaces in both species. For the stomatal density, *C. bonariensis* had an average 314.3 ± 12.5 stomata mm^{-2} on the adaxial surface, which was significantly higher than that on the abaxial surface (237.4 ± 9.1 stomata mm^{-2}). However, *C. sumatrensis* had a lower adaxial stomatal density (217.7 ± 8.5 stomata mm^{-2}) compared to the abaxial surface (275.1 ± 15.3 stomata mm^{-2}). Both the trichomes and stomata can influence the herbicide control of this weed, with the high trichome numbers potentially restricting herbicide uptake and the high stomatal numbers facilitating the uptake. The restrictive trichome barrier should be overcome to increase the leaf penetrability and herbicide uptake, thereby improving foliar herbicide efficacy on *C. bonariensis*.

Keywords *Conyza bonariensis*, *C. sumatrensis*, trichomes, stomata, herbicides.

INTRODUCTION

Flaxleaf fleabane (*Conyza bonariensis*) is native to South America. Its introduction into Australia can be dated back to the 1840s (Wu 2007). *Conyza bonariensis* has now become a problem weed throughout the entire cropping belt of south-eastern Australia. It was originally confined to roadsides and waste lands, but it is now commonly seen invading cropping and pasture lands.

The following eight fleabane species are naturalised in Australia: *Conyza aegyptiaca* (L.) Aiton, *C. bilbaoana* J.Remy, *C. bonariensis*, *C. canadensis* (L.) Cronquist var. *canadensis*, *C. leucantha* (D.Don) Ludlow & P.H.Raven, *C. parva* (syn. *C. canadensis* var. *pussila* (Nutt.) Cronquist), *C. primulifolia* (Lam.) Cuatrec. & Lourteig (syn. *C. chilensis* Spreng.) and *C. sumatrensis* (syn. *C. albida* Willd. ex Spreng.). Among these species, *C. bonariensis* and *C. sumatrensis* are the most common species, with *C. bonariensis* being the most widespread. One of the barriers for effective control of *C. bonariensis* is the confusions with other fleabane species, as many *Conyza* species display overlapping morphological traits. Molecular techniques such as DNA barcoding, have been successfully employed to delineate some *Conyza* species (Alpen *et al.* 2014).

This study used scanning electron microscopy to compare the trichome and stomatal densities between *C. bonariensis* and *C. sumatrensis*. The results will have significant implications in the effective management of *Conyza* spp. as different species could respond differently to herbicide applications due to the varied trichome and stomatal densities.

MATERIALS AND METHODS

Two mature plants of each flaxleaf fleabane (*C. bonariensis*) and tall fleabane (*C. sumatrensis*) at flowering stage were collected from Charles Sturt University campus (CSU) (S35.06751, E147.350779) and Estella (S35.074087, E147.349684), Wagga Wagga, New South Wales in March 2013. The plants were cut at the soil surface, placed in a zip lock plastic bag and kept in an insulated container for transport. The collected plant materials were then pressed and dried for scanning electron microscopy (SEM) examination.

Trichome and stomatal densities assessed by SEM

Ten mature leaves were randomly chosen from the middle part of the stem of each plant. For each leaf,

small areas (3 mm × 5 mm) were cut from the adaxial and abaxial surfaces, adhered to 12 mm carbon tabs (ProSciTech, Australia) and observed by SEM (JEOL JCM 5000 NeoScope). The trichome and stomatal densities were counted from a single SEM image, 0.149 mm^2 for trichome and 0.038 for stomata per leaf. Means were separated by Fisher's LSD at the 5% level using Genstat 14th edition.

RESULTS

Stomatal and trichome densities in *C. bonariensis*

Trichome densities in *C. bonariensis* ranged from 67.2 to 221.9 trichomes mm^{-2} on the adaxial surface and from 74.0 to 168.1 trichomes mm^{-2} on the abaxial leaf surface (Table 1). There were no significant differences in trichome densities between the adaxial and abaxial surfaces. Similarly, CSU and Estella samples of *C. bonariensis* did not differ significantly in trichome densities on the adaxial leaf surface. However, CSU sample had a higher ($P = 0.017$) abaxial trichome density (144.9 ± 5.8 trichomes mm^{-2}) than the Estella sample (Table 1).

Stomatal densities in *C. bonariensis* ranged from 234.7 to 417.2 stomata mm^{-2} and from 182.5 to 312.9 stomata mm^{-2} on the adaxial and abaxial leaf surfaces, respectively (Table 2). The adaxial leaf surface had an average stomatal density of 314.3 ± 12.5 stomata mm^{-2}, which was significantly higher than the stomatal density on the abaxial surface (237.4 ± 9.1 stomata mm^{-2}, $P < 0.001$). Adaxial stomatal density also differed between the two *C. bonariensis* samples ($P = 0.033$). The adaxial stomatal density in the CSU sample (286.8 ± 11.5 stomata mm^{-2}) was lower than that in Estella sample (339.0 ± 18.6 stomata mm^{-2}). By contrast, the two *C. bonariensis* samples had similar abaxial stomatal densities (Table 2).

Stomatal and trichome densities in *C. sumatrensis*

Trichome densities in *C. sumatrensis* ranged from 40.3 to 147.9 trichomes mm^{-2} and from 53.8 to 134.5 trichomes mm^{-2} on the adaxial and abaxial leaf surfaces, respectively (Table 3). On average, there were 92.1 trichomes mm^{-2} on the adaxial surface, which was similar to the trichome densities on the abaxial surface (87.7 trichomes mm^{-2}). The *C. sumatrensis* sample collected from CSU had significantly higher trichome densities on both adaxial and abaxial leaf surfaces, when compared to the Estella sample.

The two *C. sumatrensis* samples had stomatal densities ranging from 156.5 to 268.8 stomata mm^{-2} on the adaxial leaf surface and from 182.5 to 391.1 stomata mm^{-2} on the abaxial leaf surface (Table 4). *C. sumatrensis* had a lower ($P = 0.002$) adaxial stomatal density (217.7 ± 8.5 stomata mm^{-2}) compared to the stomatal density of 275.1 ± 15.3 stomata mm^{-2} on the abaxial leaf surface (Table 3). In addition, abaxial stomatal density was higher ($P < 0.001$) in the CSU sample (325.9 ± 17.9 stomata mm^{-2}) than the Estella sample (224.2 ± 9.7 stomata mm^{-2}). However, the two samples did not differ significantly in adaxial stomatal densities (Table 4).

Table 1. Trichome densities (trichomes mm^{-2}) in *C. bonariensis*.

Sample	Mean ± SE	Min	Max	P
	Adaxial leaf surface			
CSU	144.9 ± 14.5	67.2	221.9	0.197
Estella	123.0 ± 8.3	67.2	154.6	
	Abaxial leaf surface			
CSU	144.9 ± 5.8	121.0	168.1	0.017
Estella	119.0 ± 7.6	74.0	147.9	

Table 2. Stomatal densities (stomata mm^{-2}) in *C. bonariensis*.

Sample	Mean ± SE	Min	Max	P
	Adaxial leaf surface			
CSU	286.8 ± 11.5	234.7	339.0	0.033
Estella	339.0 ± 18.6	260.8	417.2	
	Abaxial leaf surface			
CSU	226.0 ± 8.7	182.5	260.8	0.245
Estella	247.7 ± 15.2	182.5	312.9	

Table 3. Trichome densities (trichomes mm^{-2}) in *C. sumatrensis*.

Sample	Mean ± SE	Min	Max	P
	Adaxial leaf surface			
CSU	116.3 ± 8.1	74.0	147.9	<0.001
Estella	67.9 ± 5.3	40.3	94.1	
	Abaxial leaf surface			
CSU	100.9 ± 8.3	67.2	134.5	0.013
Estella	74.6 ±4.5	53.8	100.9	

Table 4. Stomatal densities (stomata mm^{-2}) in *C. sumatrensis.*

Sample	Mean ± SE	Min	Max	P
	Adaxial leaf surface			
CSU	229.5 ± 9.4	182.5	260.8	0.175
Estella	206.0 ± 13.7	156.5	286.8	
	Abaxial leaf surface			
CSU	325.9 ± 17.9	234.7	391.1	< 0.001
Estella	224.2 ± 9.7	182.5	260.8	

Comparison of trichome densities between species
Both samples of *C. bonariensis* had significantly higher trichome densities on both adaxial and abaxial leaf surfaces than the samples of *C. sumatrensis*. For example, *C. bonariensis* from CSU had adaxial and abaxial trichome densities of 144.9 ± 14.5 and 144.9 ± 5.8 trichomes mm^{-2}, while the *C. sumatrensis* sample from CSU had 116.3 ± 8.1 and 100.9 ± 8.3 trichomes mm^{-2} on adaxial and abaxial surfaces, respectively (Figure 1).

Comparison of stomatal densities between species
The stomatal densities on the adaxial leaf surface tend to be higher in the *C. bonariensis* samples than in the *C. sumatrensis* samples, with significant differences between the *C. bonariensis* and *C. sumatrensis* samples collected in Estella (Figure 1). However, the abaxial stomatal density was significantly lower ($P < 0.001$) in the CSU *C. bonariensis* sample (226.0 ± 8.7 stomata mm^{-2}) than in the *C. sumatrensis* sample (325.9 ± 17.9 stomata mm^{-2}). There were no significant differences in abaxial stomatal density between the *C. bonariensis* and *C. sumatrensis* samples collected in Estella (Figure 2).

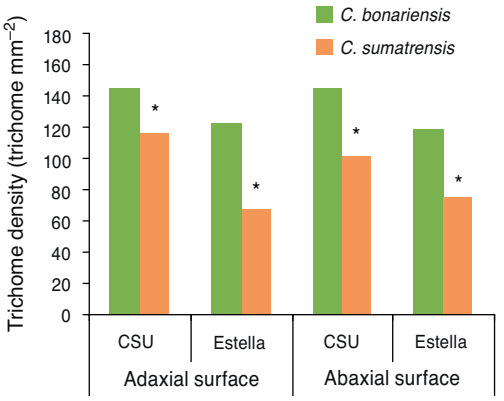

Figure 1. Comparison of trichome densities (trichomes mm^{-2}) between species (standard error bars shown). * indicates significant differences between the two fleabane.

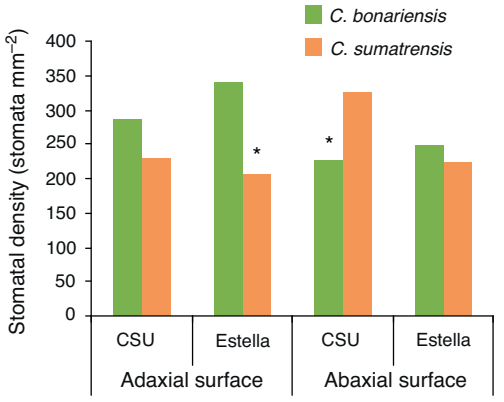

Figure 2. Comparison of stomatal densities (stomata mm^{-2}) between species (standard error bars shown). * indicates significant differences between the two fleabane.

DISCUSSION

Conyza bonariensis is often called hairy fleabane, which is reflected in the presence of dense trichomes on both leaf surfaces. It has trichome densities of 133 trichomes mm^{-2} on both adaxial and abaxial leaf surfaces in the present study, which were much higher than those reported previously (Procopio *et al*. 2003). They estimated that the trichome density in *C. bonariensis* from Brazil was 26.20 trichomes mm^{-2} on the adaxial surface and 35.40 trichomes mm^{-2} on the abaxial surface, respectively.

Individual fleabane plants may differ in trichome and stomatal densities, which was in agreement with Zhu *et al.* (2013), who found that the summer perennial weed silverleaf nightshade (*Solanum elaeagnifolium* Cav.) had different trichome and stomatal densities between individual plants, depending on the climatic and growing conditions. More research is needed to include samples from geographically diverse areas so that a better understanding between the trichome and stomatal densities and climatic conditions could be established.

Conyza bonariensis had higher trichome densities than *C. sumatrensis*, which could partly explain the differential sensitivities of the two *Conyza* species to herbicide applications. The high density of trichomes in *C. bonariensis* could contribute to the natural tolerance of this weed to herbicide applications (Wu *et al*. 2008). In addition, the varied levels of trichome densities between *C. bonariensis* plants could contribute to the inconsistent and unsatisfactory herbicide control frequently reported by many growers.

On the other hand, this study also revealed that the stomata densities in *C. bonariensis* were 313 stomata mm^{-2} on the adaxial leaves and 237 stomata mm^{-2} on the abaxial leaves, although lower stomatal densities have been reported by Procopio *et al*. (2003).

Trichomes and stomata can both influence the chemical control of *C. bonariensis*. Dense trichomes

are barriers for herbicide uptake due to their hydrophobic nature, droplet interception and the creation of air pockets (Brewer *et al.* 1991, Burrows *et al.* 2013), while the high stomatal numbers could facilitate the herbicide adsorption via the stomatal pores and the guard cells (Wanamarta and Penner 1989, Burkhardt *et al*. 2012, Burrows *et al.* 2013). Further research could be directed to break the trichome barrier and identify effective adjuvants to increase the leaf penetrability, thereby improving foliar herbicide efficacy on *C. bonariensis*. The potential difficulties in the uptake of foliar herbicides also suggest that root absorbed residual herbicides should be used in conjunction with the foliar applied herbicides to improve control of *C. bonariensis* (Wu *et al*. 2007).

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