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LEAF FLAVONOID AND OTHER PHENOLIC GLYCOSIDES AS INDICATORS OF PARENTAGE IN SIX ORNAMENTAL *FUCHSIA* SPECIES AND THEIR HYBRIDS

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Abstract—In a leaf flavonoid analysis of six *Fuchsia* species and seven *Fuchsia* hybrids, flavonols were found to be abundant in all taxa except *F. procumbens*. Flavone glycosides were found in only three species: luteolin 7-glucoside in *F. splendens*; and luteolin and apigenin 7-glucuronides and 7-glucuronidesulphates, tricin 7-glucuronidesulphate and diosmetin 7-glucuronide from one or both of the New Zealand species *F. procumbens* and *F. excorticata*. Luteolin 7-glucuronidesulphate is reported for the first time. Other less common phenolics identified include the flavanone, eriodictyol 7-glucoside from *F. excorticata*, a galloylglucose from *F. triphylla*, and a galloylglucosesulphate present in all taxa. Eight of the flavonoid glycosides proved useful as marker substances for particular *Fuchsia* species: quercetin 3-rhamnoside, 3-glucuronide and 3-rutinoside for *F. fulgens*; quercetin and kaempferol 3-galactosides for *F. boliviana* var. *luxurians*; diosmetin 7-glucuronide for *F. excorticata* and apigenin 7-glucuronide and 7-glucuronidesulphate for *F. procumbens*. The chemical results on the hybrids support the view that the cultivar 'Mary' is a hybrid of *F. boliviana* var. *luxurians* and *F. triphylla* and that both *F. fulgens* and *F. triphylla* are involved as parents of the cultivars 'Koralle' and 'Traudchen Bondstedt'.

INTRODUCTION

Flavonoid pigments have been successfully used in several plant groups, notably in the genus *Baptisia* [1], for the purpose of identifying the parental origin of natural hybrids [2], but they have rarely been used in solving similar problems among cultivated plants. The present study of leaf flavonoids in garden *Fuchsia* (Onagraceae) was aimed at seeking objective indicators of parentage among these valuable ornamental plants.

The many garden forms of *Fuchsia* are known to be of complex hybrid origin, involving originally the two species, *F. magellanica* and *F. fulgens*. Genetic material from *F. boliviana*, *F. regia* and *F. triphylla* was introduced into the hybrids at a later stage [3]. Unfortunately, documentation concerning the origin and breeding of garden *Fuchsia* is almost completely lacking. Furthermore, the usual procedures for determining the hybrid origin of plants are of limited value with these particular taxa. Morphological measurements are restricted in value by the fact that most of the putative parental species show considerable infraspecific variability in both leaf and floral characteristics. Cytologically, the genus *Fuchsia* is difficult because the chromosomes are very small; the only readily accessible data on the garden hybrids are of their chromosome numbers and, hence, ploidy levels.

Previous studies of the floral anthocyanins in *Fuchsia* indicated that additive inheritance of the pigments occurred in the hybrids [4] and that both the petal anthocyanins and the leaf flavonoids might be of value in confirming the parental origin of some of the garden forms. The present detailed study of leaf flavonoids in *F. triphylla* and related species and hybrids was undertaken in order to see if hybrids could be positively identified in this way.

Fuchsia triphylla, a species endemic to Haiti and Santo Domingo, was the first fuchsia to be brought into cultivation, in *ca* 1739. It was then lost to cultivation until 1873, when Thomas Hogg sent seed to New York and living plants were sent from there to Europe. Since then, over 40 *triphylla* hybrids have been raised, one of the earliest being *Fuchsia* cv. 'Mary', probably a cross of *F. triphylla* and *F. boliviana* var. *luxurians*. *Fuchsia fulgens* and *F. splendens* have also been used in crosses with *F. triphylla* but the exact parentage of many such hybrids is still uncertain. In 1976, Wright [3] raised the first of a new line of *triphylla* hybrids, when he crossed *F. triphylla*, which is in the section of the genus, *Fuchsia* [5], with the New Zealand species *F. procumbens* (section *Skinnera*), with the production of *Fuchsia* cv. 'Whiteknights Ruby'.

The present paper describes a detailed investigation of leaf flavonoids in the four key species in section *Fuchsia*, i.e *F. triphylla*, *F. boliviana*, *F. splendens* and *F. fulgens*, and in some of their hybrids. Two New Zealand species, *F. procumbens* and *F. excorticata* (both section *Skinnera* [6]) and hybrids between them and between *F. procumbens* and *F. triphylla* were also studied. A later paper will describe similar studies on other hybrids. This represents the first extended chemical study of leaf flavonoids in the genus, although the flavonoid pattern in the Onagraceae as a whole is now well documented (see, e.g. ref. [7]).

RESULTS

The results of a leaf flavonoid analysis of six *Fuchsia* species and seven hybrids are presented in Table 1.

Table 1. The leaf flavonoid and other phenolic glycosides of some *Fuchsia* species and their hybrids

Species or hybrid	Flavonol glycosides*																			Other glycosides		
	Quercetin							Kaempferol				Flavone glycosides										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
F. boliviana Carr. var. *luxurians* Johnston	+	+	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	+	–
F. triphylla L.	+†	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
F. boliviana Carr var. *luxurians* Johnston × *F. triphylla* L. (3C29 and 3C32)	+†	+‡	+	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	+	+	–
F. 'Mary'	+†	+‡	+	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	+	+	–
F. procumbens R. Cunn.	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	–	+	–
F. 'Whiteknights Ruby' (*F. triphylla* × *F. procumbens*) (20C 7)	+†	+‡	+	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	+	+	–
F. splendens Zuccarini	+	+	–	+	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	+	–
F. splendens Zuccarini (100C105) × *F. boliviana* Carr var. *luxurians* Johnston	+†	–‡	–	+	–	(+)	+	+†	–‡	–	–	+	–	–	–	–	–	–	–	+	–
F. fulgens De Candolle	+	–	+	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	+	–
F. 'Koralle'	+†	–	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	+	+	–
F. 'Traudchen Bondstedt'	+†	–	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	+	+	–
F. excorticata L.	+	+	+	+	–	+	–	+	+	–	–	–	+	+	–	–	+	+	–	+	+
F. excorticata L. × *F. procumbens* R. Cunn.	+	+	+	+	–	trace	–	+	+	+	+	–	+	+	+	–	+	–	–	+	+

*Quercetin glycosides: **1** = 3-glucoside, **2** = 3-galactoside, **3** = 3-rhamnoside, **4** = 3-arabinoside, **5** = 3-glucuronide, **6** = 3-rutinoside, **7** = 3-rhamnosylgalactoside; kaempferol glycosides: **8** = 3-glucoside, **9** = 3-galactoside, **10** = 3-rhamnoside, **11** = 3-arabinoside; flavone glycosides: **12** = luteolin 7-glucoside, **13** = luteolin 7-glucuronide, **14** = luteolin 7-glucuronidesulphate, **15** = apigenin 7-glucuronide, **16** = apigenin 7-glucuronidesulphate, **17** = tricin 7-glucuronidesulphate, **18** = diosmetin 7-glucuronide; other glycosides: **19** = a galloylglucose (R_f 35 in BAW and 25 in 15% acetic acid), **20** = a galloylglucosesulphate (R_f 15 in BAW and 53 in 15% acetic acid), **21** = eridictyol 7-glucoside.

†A diglucoside is also present.

‡A digalactoside may also be present.

Flavonols were the most frequent constituents, present in all taxa surveyed except *F. procumbens*. By contrast, flavone glycosides were found only in *F. splendens*, *F. procumbens*, *F. excorticata* and their progeny. Luteolin 7-glucoside occurred only in *F. splendens* and in its hybrid with *F. boliviana* var. *luxurians*. The flavone glycosides characterized in the New Zealand taxa were: luteolin and apigenin 7-glucuronides and 7-glucuronidesulphates, tricin 7-glucuronidesulphate and diosmetin 7-glucuronide. This represents the first report of luteolin 7-glucuronidesulphate in nature. Tricin 7-glucuronidesulphate has been isolated only once before, from *Cyperus polystachyos* (Cyperaceae) [8].

Other less common phenolic compounds isolated from the genus include the flavanone, eriodictyol 7-glucoside, from *F. excorticata* and its hybrid with *F. procumbens* and a partially characterized galloylglucose (R_f 30 in BAW and 29 in 15% acetic acid), which was found to be a good chemical marker for *F. triphylla* and for five of its hybrids: 3C29, 3C32, 'Mary', 'Koralle' and 'Traudchen Bondstedt'. Another gallic acid derivative (R_f 13 in BAW and 55 in 15% acetic acid) was found in all the species and hybrids examined. It gave gallic acid, glucose and an intermediate on 40 min acid hydrolysis and was cationic, since it was electrophoretically mobile at pH 2.2 but could not be further characterized. Of the various phenolics identified in *Fuchsia* leaves, 12 proved to be useful marker compounds for particular species (Table 2). *F. excorticata* was especially well separated from *F. procumbens* by the presence of seven flavonol glycosides as well as by having

Table 2. Marker glycosides for *Fuchsia* species

Species	Markers
F. boliviana var. *luxurians*	Quercetin and kaempferol 3-galactosides
F. fulgens	Quercetin 3-rhamnoside, 3-glucuronide and 3-rutinoside
F. splendens	Luteolin 7-glucoside and quercetin 3-rhamnosylgalactoside
F. triphylla	Galloylglucose derivative
F. excorticata	Diosmetin 7-glucuronide and eriodictyol 7-glucoside
F. procumbens	Apigenin 7-glucuronide and 7-glucuronide sulphate

eriodictyol 7-glucoside and diosmetin 7-glucuronide. In general, the species markers could be detected in all seven hybrids studied (Table 1); however, these plants also produced extra flavonoids not present in their respective parents. Thus, from 'Mary' and 3C29 (*F. boliviana* var. *luxurians* × *F. triphylla*) quercetin 3-arabinoside, a 3-diglucoside and 3-digalactoside were isolated. In the hybrid *F. splendens* × *F. boliviana* var. *luxurians* quercetin and kaempferol 3-diglucosides and 3-digalactosides were isolated, but the corresponding 3-monoglucosides and 3-monogalactosides present in the parents could not be detected in the hybrid. A quercetin 3-diglucoside was also found in 'Koralle' and 'Traudchen Bondstedt' and these hybrids also produced quercetin 3-rhamnosylgalactoside in addition to quercetin 3-rutinoside. In the hybrid, *F. excorticata* × *F. procumbens* the ability to synthesize two parent compounds: apigenin 7-glucuronidesulphate and diosmetin 7-glucuronide was apparently repressed but two new 'hybrid' glycosides, kaempferol 3-rhamnoside and 3-α-L-pyranosylarabinoside were characterized. Similarly, the hybrid between *F. procumbens* and *F. triphylla* produced a quercetin 3-diglucoside and digalactoside and was apparently unable to synthesize apigenin 7-glucuronide and apigenin and tricin 7-glucuronidesulphates, which are characteristic of *F. procumbens*.

With the above exceptions, the four hybrids of known parentage inherited in additive fashion the flavonoids of the respective parents. The cultivar 'Mary', thought to be a hybrid between *F. boliviana* var. *luxurians* and *F. triphylla*, was identical in flavonoid pattern to a recent cross between these two species (3C29 and 3C32). The other two *triphylla* hybrids examined, 'Koralle' and 'Traudchen Bondstedt', had identical flavonoid profiles in which the galloylglucose marker for *F. triphylla* and the three quercetin glycoside markers of *F. fulgens* (Table 2) were all clearly evident. This confirms the suggestion of Wright [9] that both these species may be involved as parents to these hybrids. *F.* 'Koralle' and *F.* 'Traudchen Bondstedt' are two of six *triphylla* hybrids raised by Bondstedt in 1905 [3].

DISCUSSION

The present results indicate that leaf flavonoids are potentially useful tools in the identification of *Fuchsia* species and in determining the origin of their hybrids. All six *Fuchsia* species studied have distinct leaf flavonoid profiles (Table 2). As these chemical markers can be seen to be inherited by the hybrids of known parents, it is clearly feasible to use them as indicators of parentage in hybrids of uncertain origin. It is, thus, possible to speculate from the present data that *F. triphylla* and *F. fulgens* must be both involved as parents of the cultivars 'Koralle' and 'Traudchen Bondstedt' and that 'Mary' is a result of a cross between *F. boliviana* var. *luxurians* and *F. triphylla*. In a preliminary survey of 12 *triphylla* hybrids [Fronczyk, J. H., unpublished results] the galloylglucose 'marker' for *F. triphylla* was present on the 2D paper chromatograms of all the plants and it was possible to assume the involvement of *F. fulgens* and *F. boliviana* var. *luxurians* in a number of them. Unfortunately, it is not possible to distinguish other marker substances in the hybrids simply from a 2D chromatogram. The large number of flavonoid glycosides present means that isolation and purification of individual glycosides is essential before positive identification can be made, especially in the case of flavonol glycosides. Some of these glycoside pairs, e.g. quercetin 3-glucoside and 3-galactoside, kaempferol 3-glucoside and 3-galactoside, and quercetin 3-rhamnoside and kaempferol 3-glucoside remain as inseparable mixtures even after three or four chromatographic purifications. They could be distinguished only by means of R_f data and acid hydrolysis to aglycone and sugar. Thus, the flavonoid analysis of *Fuchsia* hybrids is a very time consuming operation. However, it is likely that HPLC could eventually be used to facilitate flavonoid identifications; this technique has already been used to separate mixtures of closely related flavonol glycosides present in Poinsettia sepals [10].

The fact that occasionally parental compounds do not appear in the hybrids examined is not really unexpected, in view of the complex mixture of related glycosides present. Undoubtedly, the quantitative balance of flavonoid synthesis could well be upset by hybridization so that the production of a particular component in one or other species is lowered to an amount below the level of ready detection. On the other hand, the production of new glycosides in the hybrids is more unusual, though it has been recorded before [2]. It could be due in this case to gene interactions changing the specificities of the enzymes involved in flavonol glycoside synthesis. Detailed studies of the enzymes involved would be needed to substantiate this idea. Fortunately, the minor discrepancies from the additive profile expected in the hybrids examined do not prevent the flavonoids from providing valuable chemical markers for hybrid identification in *Fuchsia* cultivars.

EXPERIMENTAL

Plant material. Verified plants of *Fuchsia boliviana* Carr var. *luxurians* Johnston, *F. triphylla* L., *F. splendens* Zuccarini, *F. fulgens* De Candolle, *F.* 'Koralle', *F.* 'Traudchen Bondstedt' and *F.* 'Mary' were received from Mr. J. O. Wright, Botany Department, University of Reading, who also supplied material of his own *Fuchsia* hybrids: *F. boliviana* var. *luxurians* × *F. triphylla* (3C29 and 3C32), *F.* 'Whiteknights Ruby' (*F. triphylla* × *F. procumbens*) and *F. splendens* (100 C105) × *F. boliviana* var. *luxurians*. Voucher specimens of all these plants are deposited at RNG. Dried leaf material of *F. excorticata* L., *F. procumbens* R. Cunn. and a hybrid between these two species made by Mrs. D. H. Percy, were received from Dr. Eric Godley, Botany Division, DSIR, Private Bag, Christchurch, New Zealand. Voucher specimens of these three plants are deposited at CHR. Two further accessions of *F. procumbens* and *F. excortica*, supplied by

Table 3. R_f data (× 100) of some new and related glycosides from *Fuchsia* species*

Glycoside	BAW	15% HOAc	H_2O	CAW (1:1)	PhOH	Electrophoretic mobility†
Quercetin						
3-Rhamnosylgalactoside	55	52	23	26	35	—
3-Rutinoside	55	52	23	26	35	—
3-Glucuronide	54	33	60	12	15	—
Luteolin						
7-glucuronide	19	08	47	52	42	—
7-glucuronidesulphate	10	22	78	22	25	0.42
Tricin						
7-glucuronidesulphate	13	30	78	30	47	0.65
Apigenin						
7-glucuronidesulphate	17	46	84	46	—	0.68
Galloyglucose derivatives						
(1) Galloylglucose						
(*triphylla*)	30	29	06	0	—	—
(2) Galloylglucosesulphate	13	55	79	0	—	0.73
1-Galloylglucose	30	46	05	0	—	—

*Solvent key: BAW = *n*-butaryl–acetic acid–water (4:1:5, top layer); CAW (1:1) = $CHCl_3$–HOAc chloroform–acetic acid (1:1, saturated with water); PhOH = PhOH–H_2O (4:1).

†Mobilities relative to quercetin 3-sulphate as 1.00 measured in acetic acid–formic acid buffer, pH 2.2, at 400 V for 2 hr.

Mr. J. O. Wright, were also examined; they had identical flavonoid profiles to the original samples.

Identification of leaf flavonoids. Flavonoid glycosides were isolated from 80% methanolic leaf extracts either by PPC in BAW, 15% HOAc, H_2O and sometimes BEW or, for small quantities of leaf, by cutting out appropriate spots from 2D paper chromatograms run in BAW and 15% HOAc. 2D paper chromatograms on No. 1 paper in BAW and 15% HOAc were run for all species and hybrids for comparative purposes. After purification, known glycosides were characterized on the basis of R_f, UV spectral analysis, acid hydrolysis to aglycone and sugar and, where possible, by direct comparison with authentic markers. R_f data for the new or unusual glycosides are given in Table 3. Flavonoid sulphates were detected by paper electrophoresis at pH 2.2 (HOAc–HCO_2H buffer) for 2 hr at 400 V.

Identification of quercetin 3-rhamnosylgalactoside from F. splendens. The glycoside was isolated from an 80% methanolic extract as above. It co-chromatographed with rutin in BAW, 15% HOAc, PhOH and H_2O and had the same UV spectral properties. Acid hydrolysis gave quercetin, galactose and rhamnose. H_2O_2 oxidation gave a disaccharide with similar R_G values to rutinose in BAW, BEW, PhOH and BBPW (run 48 hr) and a similar R_G value on paper electrophoresis in borate buffer at pH 10.

Identification of luteolin 7-glucuronidesulphate from F. procumbens. The glycoside was isolated from an 80% methanolic leaf extract by PPC in BAW and 15% HOAc and finally purified by electrophoresis on 3MM paper at pH 2.2 (HOAc–HCO_2H buffer) for 2 hr. R_f data are given in Table 3. UV λ_{max}^{MeOH} nm: 255, 268′, 350; + NaOAc 255, 268′, 360, 405′; + H_3BO_3 260, 268′, 380. Acid hydrolysis gave luteolin, luteolin 7-glucuronide and glucuronic acid after 45 min. Sulphatase oxidation at 37°, pH 5.0 acetate buffer, gave a trace of luteolin and some luteolin 7-glucuronide after 1 hr. When the reaction was left overnight only luteolin was found because of some glucuronidase activity in the sulphatase preparation.

Identification of eriodictyol 7-glucoside from F. excorticata. The glycoside was isolated from an 80% methanolic leaf extract by PPC in BAW, 15% HOAc and H_2O. It gave eriodictyol [co-PC in BAW, CAW (2:1), H_2O and 15% HOAc] and glucose on acid hydrolysis and co-chromatographed with eriodictyol 7-glucoside in BAW, BEW, H_2O and 15% HOAc, R_f data are given in Table 3. UV λ_{max}^{MeOH} nm: 285, 325; + NaOH 287, 365.

Partial identification of a galloylglucose from F. triphylla. The glycoside was isolated from an 80% methanolic leaf extract by PPC in BAW and 15% HOAc. UV λ_{max}^{MeOH} nm: 278; + NaOH 340. Acid hydrolysis gave gallic acid and glucose. The glycoside did not co-chromatograph with 1-galloylglucose. R_f data are given in Table 3; it appears to be different in R_f from the three galloylglucose esters recently reported in leaves of unspecified *Fuchsia* material by Haddock *et al.* [11], but no direct comparison was possible.

Partial identification of a galloylglucosesulphate from F. boliviana *var.* luxurians. The glycoside was isolated from an 80% methanolic leaf extract by PPC in BAW and 15% HOAc. UV λ_{max}^{MeOH} nm: 270; + NaOH 335. Acid hydrolysis gave gallic acid, glucose and an intermediate. R_f and electrophoretic data are given in Table 3.

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