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Studies on the Flower Colours in the *Camellia*

On the Anthocyanin Constitution in *C. reticulata*, *C. saluenensis*, *C. pitardii*, *C. hongkongensis*, *C. rosaeflora* and *C. maliflora* and in the Cultivars Derived from Interspecific Hybridization

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Introduction

In the previous report[3] on the constitution of anthocyanins the authors demonstrated that the genus *Camellia* seemed to be divided into two groups, one with pigments of lower Rf values, and the other with those of higher Rf values, on the basis of the distribution of pigments on the two-dimensional thin layer chromatograms. The former group contained *C. japonica*, *C. japonica* subsp. *rusticana*, *C. sasanqua*, *C. hiemalis* and *C. vernalis*, whereas, the latter group contained *C. reticulata* and *C. saluenensis*. Moreover, the lower Rf group had two major distinct pigments, one specific to *C. japonica* and *C. japonica* subsp. *rusticana* and the other specific to *C. sasanqua* and *C. hiemalis*. The higher Rf group had four major distinct pigments significantly different from, and absent in, the lower Rf group. Two of these were specific to *C. reticulata* and the other two were specific to *C. saluenensis*. Therefore, in the pigment constitution some considerable and qualitative differences were recognized among the species analyzed.

In the previous report, however, the species dealt with were confined for the most part to the species of Japanese origin containing pigments of lower Rf values, and the detailed discussion concerning the species of higher Rf group was not given, because of the insufficiency of the available materials for analysis. Fortunately, a lot of materials other than those of Japanese origin have since been gathered. This paper deals with the results of the species of foreign origin and the garden hybrids derived from the interspecific hybridization, aiming at getting more comprehensive figures in the constitution of anthocyanins and some clues to the mode of pigment inheritance of the *Camellia*.

Materials and Methods

1. Plant material

Most of the cultivars of *C. reticulata* and interspecific hybrids were received from The Kurume Camellia Lovers' Association (Fukuoka), and the additional species of foreign origin were received from The Faculty of Agriculture, Kyushu University (Fukuoka) and The Faculty of Agriculture, Tokyo University of Agriculture and Technology (Tokyo).

Thirty-three cultivars of *C. reticulata*, 34 cultivars of interspecific hybrids, and 6 camellia species, *i.e.*, *C. saluenensis*, *C. pitardii* var. *pitardii*, *C. pitardii* var. *yunnanica*, *C. hongkongensis*, *C. maliflora* and *C. rosaeflora*, were used in this experiment.

The fresh petals collected were immersed in boiling water for approximately 10 to 20 seconds in

order to destroy the enzyme activity involved in the oxidation of the pigments. Then they were air-dried and stored in a desiccator.

2. Extraction and chromatography of anthocyanins

Extraction and purification of anthocyanins as well as the performing methods of two-dimensional thin layer chromatography, and the evaluation of constituent anthocyanins on the resultant chromatograms were carried out as previously described.[3] Total amount of anthocyanins was determined by a standard procedure.[1]

Results and Discussion

Fig. 1 is a stylized drawing of the chromatographic distribution of the anthocyanins found in this analysis, together with those found in the previous one[3] on *C. japonica* and *C. sasanqua*. The details of the distribution of these pigments in the various cultivars and species are presented in the appendix. Table 1 shows the summarized data for the species and interspecific hybrids prepared by dividing the sum total of the percentages of the respective pigments by the total number of the individuals examined.

Of these anthocyanins, spot 1, identical with cyanidin 3-monoglucoside, is specific to *C. japonica*, spot 5 is specific to *C. sasanqua*, spots 10 and 12 are specific to *C. saluenensis* and spots 11 and 13 are specific to *C. reticulata*.

1. *C. reticulata*

Although a small amount of anthocyanins of lower Rf values was frequently observed, all the cultivars of *C. reticulata* contained mainly the anthocyanins of higher Rf values, from spots 10 to 13. Major anthocyanins of these were spots 11 and 13, both of these were thus specific to this species.

As seen in the appendix and Fig. 2, the exceptions to this generalization were fixed to be cvs. 'Shot Silk' and 'Lila Naff', owing to the fact that both contained a fairly large amount of anthocyanins of lower Rf values, and the major anthocyanins of higher Rf values were spots 10 and 12, which were not specific to *C. reticulata*, but specific to *C. saluenensis*, therefore, these two cultivars

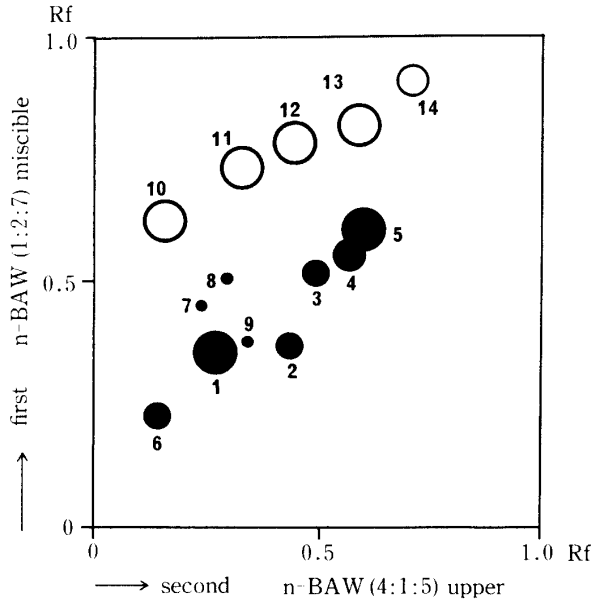

Fig. 1. Schematic representation of pigment-spots of anthocyanins in the *Camellia* appearing on chromatograms. Spot 1 is identical with cyanidin 3-monoglucoside.

Table 1. Summarized constitution of anthocyanins in the *Camellia*

Species or hybrids	Number of individuals or cultivars	Percentages of constituent anthocyanins*1													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
*japonica**2	101	65		4	7	24		+	+	+					
*sasanqua**2	42	15	1	14	3	62	5								
reticulata	33	3		+	+	2					7	32	8	48	
saluenensis	3	+				+	7				35	+	65	+	
hongkongensis	2	32	+			61	7								
pitardii var. *pitardii*	1	+									33		67		
pitardii var. *yunnanica*	1	+									3	25	5	67	
rosaeflora	1	20		+		26						23		31	
maliflora	1	31			5	64									
reticulata × *japonica*	8	10		3	2	6					9	22	12	36	
japonica × *reticulata*	3	45	1	5		7					14	11	11	6	
sasanqua × *reticulata*	4	9			2	2					18	16	21	32	
saluenensis × *japonica*	13	18		1	1	13					26	0	41	0	
saluenensis × *reticulata*	6	5				7					22	4	54	8	

*1 Numbers used are corresponding to the spot numbers represented in Fig. 1.
*2 From Sakata, Y. *et al.*[3]

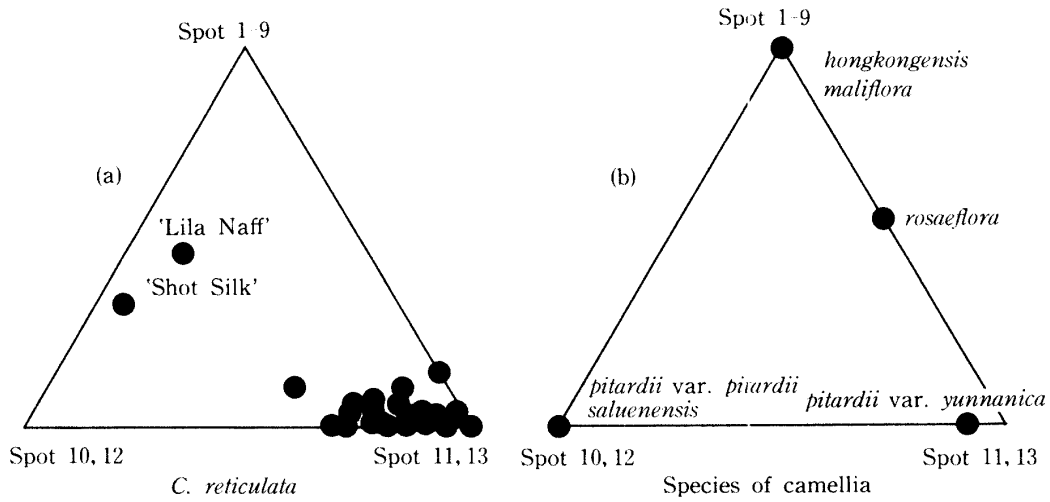

Fig. 2. The distribution of cultivars of *C. reticulta* (a) and other species of camellia (b) on the constitution of anthocyanins. Each corner of the triangle comprises 100% of each of the pigment-groups specific to *japonica* (spot 1–9), *saluenensis* (spot 10, 12) and *reticulata* (spot 11, 13), respectively.

might have a somewhat different origin from the common reticulatas.

Savige[4] pointed out that the wild form and most of the garden forms of *C. reticulata* exhibited various degrees of fertility and hybrids were easily obtained when crossed with *C. japonica*, *C. pitardii*, *C. saluenensis* and *C. sasanqua*. He also speculated that the reticulatas might be the result of an ancient cross infusion between the species of *C. pitardii* and *C. japonica*, with the Yunnan reticulata cultivars being developed from selected forms. If this happens to be the case, it might be probable that, concerning the constitution of anthocyanins in various cultivars of *C. reticulata*, the co-occurrence of a few pigments, *i.e.*, those specific to *reticulata* and the others specific to *japonica*

and *saluenensis*, might have happened as a result of an ancient gene flow from *C. japonica* and *C. pitardii* var. *pitardii* to *C. reticulata*, the latter containing *saluenensis*-specific pigments.

2. *C. saluenensis* and *C. pitardii*

As reported previously,[3] three individuals of the wild form of *C. saluenensis* contained pigments of higher Rf values. The major anthocyanins of this species were spots 10 and 12, both of these were specific to this species.

Two varieties of *C. pitardii*, *i.e.*, var. *pitardii* and var. *yunnanica*, showed the different constitutions of pigments as seen in the appendix. Although both varieties contained pigments of higher Rf values, major anthocyanins of the former were *saluenensis*-specific spots 10 and 12, and those of the latter were *reticulata*-specific spots 11 and 13, respectively. Savige[4] suggests that var. *pitardii* is diploid and very close to *C. saluenensis*, while var. *yunnanica* is a hexaploid and close to the wild form of *C. reticulata*. The different constitutions of anthocyanins in these two varieties revealed in this experiment, clearly supports his idea.

3. *C. hongkongensis*, *C. rosaeflora* and *C. maliflora*

As presented in Table 1, the constitution of anthocyanins in *C. hongkongensis* was very similar to that of *C. sasanqua*. Namely, it contained exclusively pigments of lower Rf values, and its main pigment was *sasanqua*-specific spot 5. In addition, it contained spot 6 which was identified as one of the derivatives of delphinidin characteristically occurring in the *sasanqua-hiemalis* group.[3,6] This was the first case in which delphinidin was found to have occurred in the species of Section Camellia. Together with *C. sasanqua* and *C. hiemalis*, classified in the Section Paracamellia, it might contribute to the breeding of true blue coloured cultivars.

The constitution of anthocyanins in *C. rosaeflora* belonging to the Section Theopsis was intermediate between the lower and the higher Rf groups, and the higher Rf pigments were similar to those of *C. reticulata*, in that these were exclusively spots 11 and 13 (Table 1). On the other hand, *C. maliflora* belonging also to the same Section contained exclusively pigments of lower Rf values, and its main anthocyanin was *sasanqua*-specific spot 5 (Table 1).

C. maliflora is very close to *C. rosaeflora* except for the morphological characteristics of their floral shape.[4] In 1958 Sealy[5] speculated that these two species might probably be the hybrids between species of the Section Theopsis and the Section Camellia, though it was difficult to suggest the exact species involved. If we accept his opinion, the following conclusion might be possible. Namely, with the common or the very closely related parent species of the Section Theopsis, *C. reticulata* might be involved in the case of *C. rosaeflora*, and one of the species with lower Rf pigments might be involved in the case of *C. maliflora*, as the other parent species from the Section Camellia.

4. Interspecific hybrids

As seen in the appendix and Table 1, the mode of pigment inheritance in the garden hybrids derived from the interspecific crosses among *C. japonica*, *C. sasanqua*, *C. reticulata* and *C. saluenensis* showed a considerable regularity.

In the crosses between the lower Rf and the higher Rf groups, the production of pigments of higher Rf values seemed to be dominant to that of those of lower Rf values, although the dominance was not complete. As presented in Fig. 3a–c, some exceptions of this mode of inheritance were cvs. 'Milo Rawell' and 'Fortyniner' (*reticulata*×*japonica*), cvs. 'Diamond Head' and 'Royalty' (*japonica*×*reticulata*), cv. 'Felice Harris' (*sasanqua*×*reticulata*) and cvs. 'Water Lily', 'Elegant Beauty' and 'Margaret Waterhouse' (*saluenensis*×*japonica*).

In the case of reciprocal crosses between *C. reticulata* and *C. japonica*, the latter containing

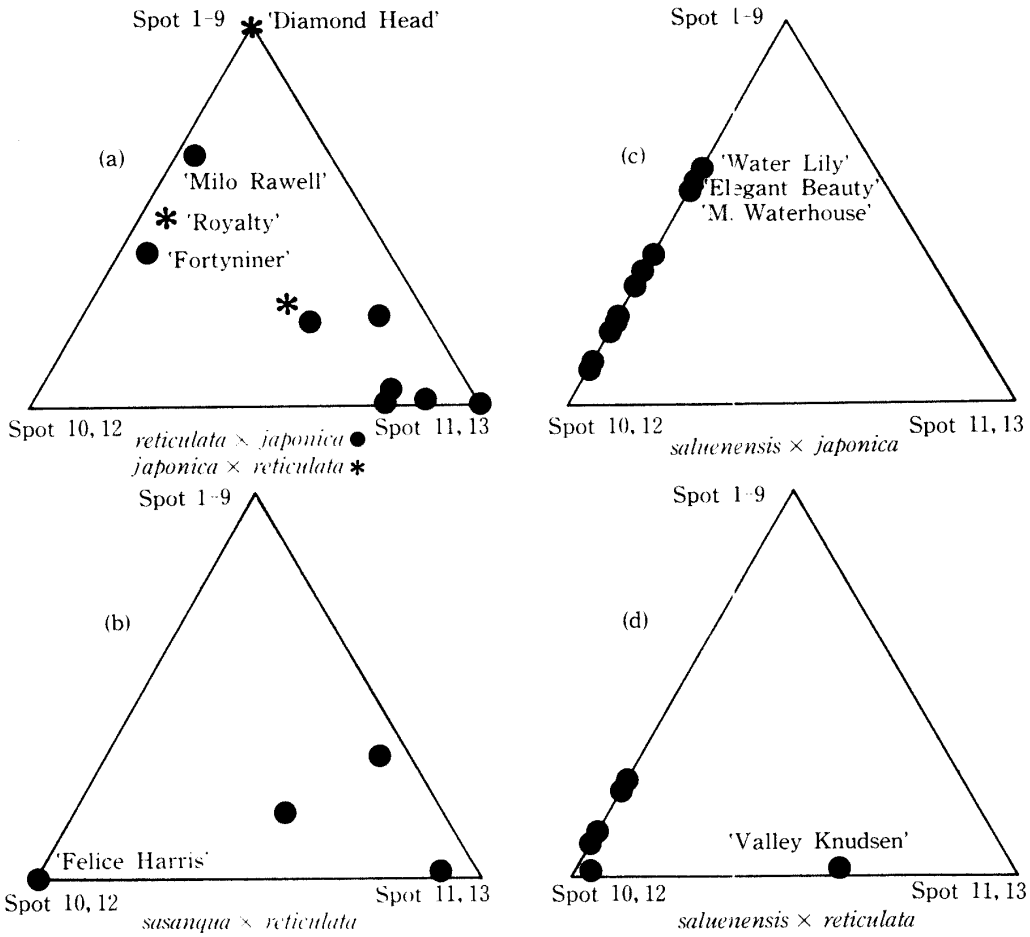

Fig. 3. The distribution of cultivars derived from *reticulata* × *japonica* or *japonica* × *reticulata* (a), *sasanqua* × *saluenensis* (b), *saluenensis* × *japonica* (c) and *saluenensis* × *reticulata* (d). Each corner of the triangle comprises 100% of the pigment-groups specific to *japonica* (spot 1–9), *saluenensis* (spot 10, 12) and *reticulata* (spot 11, 13), respectively.

exclusively spots 1 to 9, it is not clear why cvs. 'Milo Rawell', 'Fortyniner' and 'Royalty' contained larger amount of *saluenensis*- and *japonica*-specific pigments than *reticulata*-specific ones. It might be possible, however, that certain cultivars of *C. reticulata* containing a large amount of *saluenensis*-specific pigments were involved in the crosses as a parent cultivar.

On the other hand, cv. 'Diamond Head' differs morphologically from the other *C. japonica-reticulata* hybrids. It resembles *C. japonica* much more. Thus, it might not be a primary hybrid between *C. japonica* and *C. reticulata*.

Cv. 'Felice Harris', derived from the cross between *C. sasanqua* and *C. reticulata*, contained exclusively spots 10 and 12, specific to *C. saluenensis*. The cause of the similarity of this cultivar to *C. saluenensis* was not clear, but it might be possible that certain genotypes of *sasanqua* latently contained the genes and/or the genetic background favouring the production of spots 10 and 12, in place of *reticulata*-specific spots 11 and 13. Another possibility may be that certain *reticulata* with a large amount of *saluenensis*-specific pigments might have been involved in the cross.

In the case of cvs. 'Water Lily', 'Elegant Beauty' and 'Margaret Waterhouse', *japonica*-specific pigments rather predominated over *saluenensis*-specific ones. In this connection, using synthetic hybrids and back-crosses involving *C. japonica* and *C. saluenensis*, Parks *et al.*[2] showed that *japonica*-specific pigments occurred in low concentrations in the progenies of an F_1, but occurred in high

concentrations in those of the back-cross of an F_1 to *C. japonica*, therefore, the possibility still remains that these three cultivars might be derived from the backcross of an F_1 to *C. japonica*.

On the other hand, the crosses between the species with pigments of higher Rf groups showed regularity in the mode of inheritance of higher Rf anthocyanins (Fig. 3d). With an exception of cv. 'Valley Knudsen', which contained larger amount of *reticulata*-specific pigments than that of *saluenensis*-specific ones, all hybrids contained exclusively *saluenensis*-specific spots 10 and 12. Thus, the general conclusion might be drawn that the genes for the production of *saluenensis*-specific pigments are dominant to that for the production of *reticulata*-specific ones.

Summary

The present investigation was conducted to clarify the constitution of anthocyanins and pigment inheritance in the camellias of foreign origin and the garden hybrids derived from the interspecific hybridization.

Of the species of foreign origin, a group with pigments of higher Rf values contained *C. reticulata*, *C. saluenensis*, *C. pitardii* var. *pitardii* and *C. pitardii* var. *yunnanica*, and another with those of lower Rf values contained *C. hongkongensis* and *C. maliflora*. On the other hand, *C. rosaeflora* contained nearly equal amounts of the higher and the lower Rf pigments.

Of the pigments of higher Rf values, the major anthocyanins were spots 11 and 13 in *C. reticulata* and spots 10 and 12 in *C. saluenensis*, respectively. Two varieties of *C. pitardii*, *i.e.*, var. *pitardii* and var. *yunnanica*, showed a somewhat different constitutions of pigments. The former contained *saluenensis*-specific spots 10 and 12, and the latter contained *reticulata*-specific spots 11 and 13.

A main pigment was *sasanqua*-specific spot 5 in *C. hongkongensis*, and this species also contained one of the pigments of delphinidin derivatives. *C. maliflora* showed a pigment constitution quite similar to that of *C. sasanqua*, and *C. rosaeflora* contained nearly equal amounts of spots 1, 5 and *reticulata*-specific spots 11, 13.

The mode of pigment inheritance in the garden hybrids derived from the interspecific crosses among *C. japonica*, *C. sasanqua*, *C. reticulata* and *C. saluenensis* showed a considerable regularity. Namely, although the dominance was not complete, in the crosses between the lower Rf and the higher Rf groups, the production of pigments of higher Rf values was dominant to that of lower Rf values, and in the crosses between the higher Rf groups, the production of *saluenensis*-specific pigments was dominant to that of *reticulata*-specific ones.

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Appendix; Constitution of anthocyanins in the *Camellia*

Cultivar	Percentages of constituent anthocyanins*1														Total antho-cyanin*2
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
SECTION CAMELLIA															
C. reticulata															
Chrysanthemum Petal	3									5	86	3	3		0.05
William Hertrich										5	59	1	35		0.46
Noble Pearl	1									3	57	11	28		0.31
Wild Form	10		2		2						57		29		0.11
Cornelian	+									9	57	9	25		0.20
Red Emperor	1				1					10	54	6	28		0.31
Pagoda	6									17	48	7	22		0.46
Crimson Robe	5				3					7	46	3	36		0.31
Kohinor	1				2						45	5	47		0.15
Chang's Temple	3				+					13	44	8	32		0.54
open-pollinated (1)										30	42	+	28		0.18
Reticulata Leaf Spinel Pink										6	40	5	49		0.11
Eden Roc	1				+					10	39	17	38		0.08
Captain Rawes	1		2		2					6	37	7	45		0.20
Emily J. Box	7				1					7	33	5	47		0.20
Ellie Rubensohn	2									7	31	7	53		0.15
Crimson King	1				2					8	30	11	48		0.31
Tom Durrant	+									1	29	1	69		0.05
Ming's Temple	1									13	24	13	49		0.12
Janet Clark	1			+						5	22	6	66		0.38
Confucius	+				11					3	22	30	34		0.05
Arch of Triumph	+									4	20	5	71		0.31
open-pollinated (2)										13	19	5	62		0.12
Buddha	1									10	16	11	62		0.23
Mouchang											15		85		0.05
Butterfly Wings	+		+		3					1	15	1	80		0.08
Moutancha											12		88		0.05
open-pollinated (3)	1									11	12	2	74		0.12
Lion Head	1				+					11	12	4	72		0.31
Wild Silk	7				+					7	12	11	63		0.05
Early Peony										3	9	3	85		0.05
Shot Silk	1		6	9	14					10	8	52	+		0.11
Lila Naff	33				14					10	1	30	12		0.03
C. saluenensis															
wild form (1)										29	+	70	+		0.05
〃 (2)	1				+					35	+	65			0.12
〃 (3)	+				+					40	+	60			0.05
C. hongkongensis															
wild form	34				55	11									0.08
open-pollinated	29				66	5									0.12
C. pitardii															
var. *pitardii*	+									33		67			0.02
var. *yunnanica*	+									3	25	5	67		0.07

Appendix; (Continued)

Cultivar	Percentages of constituent anthocyanins*1														Total antho-cyanin*2
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
SECTION THEOPSIS															
C. rosaeflora	20		+		26						23		31		0.05
C. maliflora	31			5	64										0.10
HYBRID															
C. reticulata × japonica															
Arbutus Gum	1				+					6	46	5	42		0.12
Descanso Mist											34		61		0.15
Otto Hopfer	18		3		3					8	34	5	29		0.23
Howard Asper	1		1		2					5	23	12	55		0.03
John Taylor	1				+					16	21	4	58		0.38
Valentine Day	15		4		3					12	11	15	40		0.31
Milo Rowell	21		5	15	25					6	4	24			0.18
Fortyniner	22		7		14					19	2	32	4		0.54
C. japonica × reticulata															
Fire Chief	26									19	30	10	15		0.62
Royalty	24		14		13					23	2	22	2		0.46
Diamond Head	88	2	2		8										0.28
C. sasanqua × reticulata															
Dream Girl	23			7	3					3	35	5	24		0.08
Show Girl	1									6	16	3	74		0.12
Flower Girl	11		+		6					19	14	16	34		0.08
Felice Harris										42		58			0.03
C. saluenensis × japonica															
Debbie	+				10					20	+	70			0.15
E. G. Waterhouse	12				22					11		55			0.05
Galaxie	5		9		9					22		55			0.05
Lady Gowrie	12				7					29		52			0.11
Mary F. Taylor	19				5					24		52			0.03
Donation	3				6					44		47			0.08
Asahi	16			11	14					17		42			0.05
Anticipation	23				17					23		37			0.05
Bowen Briyant	12				18					34		36			0.08
Brigadoon	16				6					45		33			0.11
Elegant Beauty	47		8		3					21		21			0.23
Margaret Waterhouse	15				41					26		18			0.03
Water Lily	54				7					22		17			0.05
C. saluenensis × reticulata															
Valley Knudsen	1									15	21	15	48		0.11
Brian	+				1					35	3	61			0.12
Barbara Clark	10				16					26		48			0.08
Dr. Louis Pollizzi	7				16					17		60			0.05
Phyl Doak	3				9					17		71			0.05
Salutation	9									22		69			0.03

*1 Numbers used are corresponding to the spot numbers represented in Fig. 1.

*2 mg/100 mg of dry petal weight