20. Saniewska, M. and J. Pieniazek. 1972. Mobilization of carbohydrate reserves in apple shoots by auxin and gibberellin. Bul. Acad. Pol. Sci. Ser. Sci. Biol. 20:501–507.
21. Sinaska, I., M. M. Grochowska, and S. Lewak. 1972. Changes in the endogenous gibberellins contents in immature apple seeds. Bul. Acad. Pol. Sci., Cl. II Ser. Sci. Biol.
22. Sitton, B. G. 1931. A preliminary report on the relation of foliage to filling of the pecan. Nat. Pecan Assoc. Bul. 30:120–125.
23. Sitton, B. G. 1933. Some of the influences of foliage on set and filling of the pecan. Nat. Pecan Assoc. Bul. 32:89–90.
24. Sparks, D. 1979. Physiology-site, growth, flowering, fruiting, and nutrition. p. 211–239. In: R. A. Jaynes (ed.). Nut tree culture in North America. North. Nut Growers Assoc.
25. Sparks, D. and C. E. Brack. 1970. Effect of removing leaves and fruit on return bloom and fruit set of the 'Stuart' pecan. Proc. Southeastern Pecan Grow. Assoc. 63:69–71.
26. Thor, C. J. B. and C. L. Smith. 1935. A physiological study of seasonal changes in the composition of the pecan during fruit development. J. Agr. Res. 50:97–121.
27. Worley, R. E. 1971. Effects of defoliation date on yield, quality, nutlet set, and foliage regrowth for pecan. HortScience 6:446–447.
28. Worley, R. E. 1979a. Pecan yield, quality, nutlet set, and spring growth as a response to time of fall defoliation. J. Amer. Soc. Hort. Sci. 104:192–194.
29. Worley, R. E. 1979b. Fall defoliation date and seasonal carbohydrate concentration of pecan wood tissue. J. Amer. Soc. Hort. Sci. 104:195–199.
30. Worley, R. E. and S. A. Harmon. 1969. Effect of fungicides on nutlet set of pecans. HortScience 4:127–128.
31. Zipf, R. E. and A. L. Waldo. 1952. Spectrophotometric analysis of carbohydrates and study of anthrone reagent. J. Lab. Clin. Med. 39:497–502.

J. Amer. Soc. Hort. Sci. 106(6):761–764. 1981.

Gene Dose Effects on Cane Thorn Density and Cotyledonary Gland Number in Tetraploid Blackberries[1]

Gary C. Pavlis[2] and J. N. Moore[3]
Department of Horticulture, University of Arkansas, Fayetteville, AR 72701

Additional index words. blackberry breeding, thornless, *Rubus*

Abstract. **Fourteen tetraploid seedling populations of blackberry (*Rubus* sp.), representing quadruplex, (*TTTT*), triplex (*TTTt*), duplex (*TTtt*), simplex (*Tttt*), and nulliplex (*tttt*) genotypes for the major gene conferring thorniness, were evaluated for segregation of cane thorn density and cotyledonary gland number. Comparisons of seedling distribution curves, means and variances of segregating and non-segregating populations did not show a gene dose effect on gland and thorn occurrence. Inheritance of cotyledonary glands and cane thorns in blackberry was qualitative with the density of glands and thorns apparently controlled by several modifying genes.**

Thornlessness in cultivated blackberries (*Rubus* subgenus *Eubatus*) is a desired goal of most blackberry breeding programs. Although the existence of a thornless character in *Rubus* was reported as early as 1629 (11), only recently have commercial cultivars been developed through controlled breeding. Blackberry breeders in the 19th and early 20th centuries often obtained only thorny seedlings in advanced generations of thornless parents. It is now known that most of the thornless sports available were periclinal chimeras in which the gametes were derived from genetically thorny tissue (3).

The most valuable source of genetic thornlessness at the tetraploid level has been the British cultivar 'Merton Thornless'. It is the progenitor of several commercial thornless cultivars including 'Thornfree', 'Smoothstem', 'Black Satin', and 'Dirksen Thornless' (1, 13). Thornlessness of 'Merton Thornless' and its derivatives is conditioned by a single gene with the thornless condition expressed in the homozygous recessive state (12). Crosses of thornless with a homozygous dominant thorny cultivar produce a 35:1 thorny to thornless ratio in the F_2 generation.

Selection for thornless blackberries in segregating populations was made much more efficient by the discovery of Crane and Darlington (2) that thorny seedlings possessed glands along the edges of the cotyledons while thornless seedlings had glabrous cotyledons. Thus, thorny segregates can be eliminated shortly after germination.

In the F_2 generation of a cross of a homozygous thorny clone (*TTTT*) and a thornless clone (*tttt*), 5 genotypes should be produced in the ratio 1 *TTTT*: 8 *TTTt*: 18 *TTtt*: 8 *Ttt*: 1 *tttt*, assuming random chromosome segregation. While the homozygous recessive genotype can be readily identified by the lack of cotyledonary glands, no method is known to separate the various thorny genotypes except by a test cross. Identification and use of simplex and duplex individuals would enhance breeding progress since their use in breeding would give a greater proportion of thornless segregates than the conventional 35:1 ratio obtained from using a quadruplex parent.

Scott et al. (12) observed that thorny segregates obtained from F_2 populations of 'Merton Thornless' crossed with thorny genotypes varied in degree of thorniness. We have observed that cotyledons of thorny segregates vary in number of marginal glands. The purpose of this study was to test the hypothesis that cane thorn density and cotyledonary gland number are associated characters and that both vary in frequency due to an additive gene dose effect at the locus for thorniness.

[1]Received for publication April 18, 1981. Published with approval of the Director, Arkansas Agricultural Experiment Station.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[2]Formerly, Research Assistant in Horticulture. Present address: Department of Horticulture, Rutgers University, New Brunswick, NJ 08903.
[3]Professor of Horticulture.

Materials and Methods

Crosses were made among Arkansas breeding lines to produce populations representing all possible combinations of parental genotypes for the major gene conferring thorniness (Table 1). Seeds were scarified for 3 hr in concentrated sulfuric acid and stratified for 4 months at 4.5°C (9) prior to germination in a greenhouse. At about 2 weeks of age, seedlings were transplanted into peat pots and individually labeled as to family of origin and seedling number within the family. This identification was maintained throughout the greenhouse and field phases of the experiment.

Shortly after transplanting, 1 cotyledon was removed from each seedling, placed on a black stage of a 10× microscope, and the number of glands on the periphery determined. This was done for 14 seedling families comprising a total of 3504 seedlings (Table 1).

In the spring of 1977, at 2 months of age, the seedlings were field planted at a spacing of 0.61 × 3.05m. The design was a randomized block design with 4 replications of each seedling population. In the dormant period following the second growing season, a thorn count was made on one erect cane of each seedling. A vernier caliper was set at 7 mm and moved down the cane until it could go no farther. The cane was cut at this point and at a point 30.5 cm above the bottom cut. The number of thorns on each standardized sample was counted and this count paired with the cotyledonary gland count of the same plant.

A SAS univariate program was run which gave a frequency distribution of the gland and thorn counts for each seedling family. The means, variance, SD, CV, and a distribution curve were also obtained from this program. In addition, SAS bivariate programs were run to determine correlation coefficients between gland and thorn counts. A Student's *t*-test was performed on the mean counts of the seedling families to test for significant differences among them and an F test was run on the variance of segregating and non-segregating progenies to determine if they differed statistically.

Results and Discussion

The seedling progenies exhibited a broad range of variation both in the number of glands on the cotyledons and the number of thorns on the canes. A direct relationship between glabrous cotyledons and thornless canes has been noted in *Rubus* by many workers (4, 5, 6, 7, 8). However, when thornless segregates are omitted from the calculations, correlation coefficients are greatly reduced and most are not significant (Table 1). Only the seedling families with nulliplex, simplex and duplex segregates show significance, but at low values. Thus, we conclude that while cotyledonary glands and cane thorns are associated in a qualitative manner, no quantitative relationship exists in other than nulliplex segregates.

The hypothesis that variation in gland and thorn number was due to an additive gene dose effect was placed in doubt by the seedling distribution curves of cotyledonary gland counts of the seedling families. Under this hypothesis, seedling families such as 7604 and 76115 would be expected to segregate into 3 classes; quadruplex, triplex, and duplex, with the distribution curve showing 3 distinct peaks. However, the curves for 7604, 76115 and all other segregating families very closely resemble the curves of non-segregating families, such as 7620 and 76105, and thus fail to support the additive gene hypothesis (Fig. 1). If segregation classes existed, they were obscured by excessive overlaying of the class curves, making it impossible to accurately place an individual into a particular class. Similar curves were obtained for cane thorn distributions (not shown).

Another attempt to separate segregates into distinct classes was made by analyzing mean gland and thorn counts of the seedling families (Table 2). We theorized that the non-segregating families which produced all quadruplex or all duplex seedlings could be used to determine the expected means for these 2 classes. We expected the quadruplex family means to be greater than the means of the duplex families. The nulliplex means would always be zero. Thus, the simplex and triplex expected means could be calculated from the known means of the other classes. When a Stu-

Table 1. Correlation coefficients (r) for the relationship between cotyledonary gland number and cane thorn number in blackberry seedlings.

Progeny	Parental combination[z]	No. of seedlings	Correlation coefficient (r)[y]	
			Including 0 counts	Omitting 0 counts
7601	TTTT x TTTT	120	.000	No 0 counts
7602		120	-.110	No 0 counts
7615		479	.121	No 0 counts
7620		300	-.014	No 0 counts
7604	TTTT x TTtt	300	.136	No 0 counts
76115		60	.030	No 0 counts
76105	TTTT x tttt	298	.000	No 0 counts
7625	TTtt x TTtt	87	.101	.101
7628		420	.235*	.134*
7629		120	.224*	.115
7630		300	.247*	.189*
7695	TTtt x tttt	300	.705*	.381*
7696		300	.833*	.310*
76104		300	.442*	.246*

[z]Allelic complement with respect to genes conferring thorniness.
[y]Correlation coefficients significant at the 5% level are noted by *.

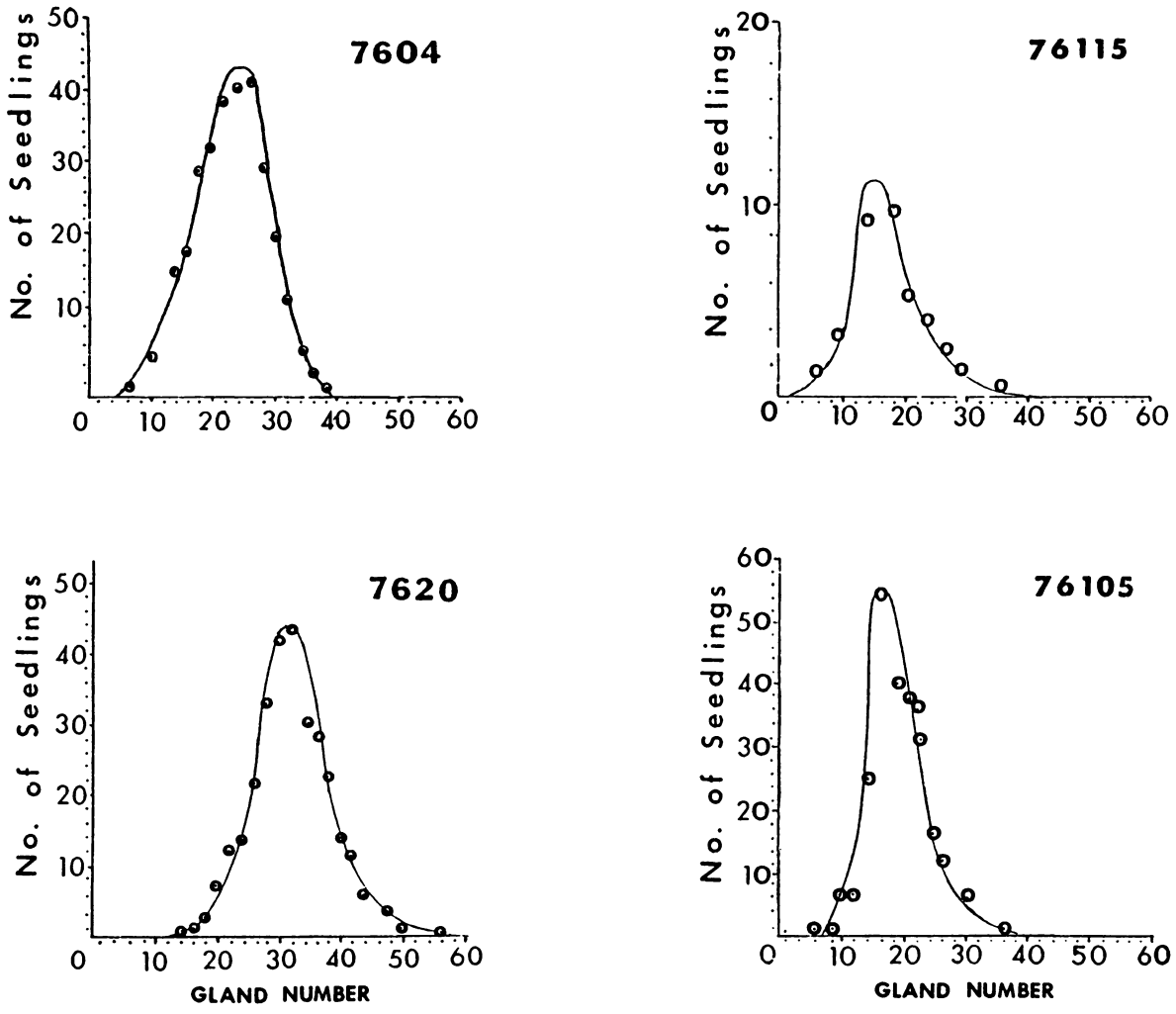

Fig. 1. Representative seedling distribution curves for cotyledonary gland number in segregating (7604, 76115) and non-segregating (7620, 76105) blackberry seedling populations.

Table 2. Range and mean of counts of glands and thorns of seedling blackberry families.

Progeny	Parental combination[z]	Expected segregation	Cotyledonary glands			Cane thorns		
			Minimum	Mean	Maximum	Minimum	Mean	Maximum
7601	TTTT x TTTT		14	25.9	46	12	43.9	104
7602		100% TTTT	9	23.9	36	9	45.3	93
7615			10	29.3	59	3	38.4	138
7620			14	31.7	64	16	52.9	161
	TTTT x TTtt	16.6% TTTT						
7604		66.6% TTTt	6	23.6	40	3	40.2	108
76115		16.6% TTtt	5	18.5	36	6	28.5	62
76105	TTTT x tttt	100% TTtt	3	19.4	38	4	36.5	100
7625	TTtt x TTtt	2.7% TTTT	0	25.8	50	0	46.4	122
7628		22.2% TTTt	0	19.8	46	0	38.4	146
7629		50.0% TTtt	0	23.9	43	0	49.7	91
7630		22.2% Tttt	0	21.3	47	0	28.3	87
		2.7% tttt						
7695	TTtt x tttt	16.6% TTtt	0	12.5	47	0	21.9	147
7696		66.6% Tttt	0	8.7	35	0	18.3	95
76104		16.6% tttt	0	18.7	53	0	44.9	332

[z]Genotype with respect to genes controlling hair and thorn occurrence.

Table 3. Phenotypic variance of segregating and non-segregating blackberry progenies with respect to genes for cotyledonary glands and cane thorns.

Segregating progenies	Degrees of freedom	Cotyledonary glands		Cane thorns	
		s^2	F value[z]	s^2	F value[z]
7604	299	34.5	0.89	312.6	0.98
7625	86	101.7	2.62*	294.3	0.92
7628	419	64.0	1.65*	364.9	1.14
7629	119	60.2	1.55*	393.5	1.23
7630	299	59.7	1.54*	231.2	0.72
7695	299	111.6	2.88*	459.6	1.43*
7696	299	98.9	2.55*	465.8	1.45*
76104	299	93.8	2.42*	876.8	2.74*
76115	59	47.9	1.24	173.4	0.54
Non-segregating progenies[y]	1312	38.8		320.4	

[z]F value is the ratio of variance of segregating progeny divided by pooled variance of non-segregating progenies. * denotes significance at 5% level.
[y]Pooled variances of progenies 7601, 7602, 7615, 7620, and 76105.

dent's *t*-test was calculated, however, it showed that the mean values of the quadruplex seedling families 7601, 7602, 7615 and 7620 were not statistically different from the duplex seedling family 76105, which is further evidence against an additive gene dose model.

The variances of gland and thorn counts of non-segregating families were pooled to determine an overall variance for the non-segregating families. These variances were compared to the variances of each of the segregating families by using an F test (Table 3). The results are inconsistent among families with most showing significant, but low F values for gland number, while most are not significant for thorn number. If allelic gene action was primarily additive, we would expect the variances of segregating families to be significant and consistently higher than those of non-segregating populations. The lack of consistency and low magnitude of the differences in variance between segregating and non-segregating families found in this study does not support an additive gene dose effect.

The data obtained in this study suggest that the inheritance of cotyledonary glands and cane thorns in blackberry is qualitative with the density of glands and thorns under the control of a number of modifying genes. A single dominant gene conferring thorniness in a tetraploid species, with variation in thorn number conditioned by modifiers, would give the type of segregation data obtained in this study. The frequency distribution curves would be expected to be similar to those in Fig. 1, in the presence of environmental variability. Furthermore, the means and ranges of all families would be similar, which also agrees with our results. The relatively high variances for gland and thorn number in progenies not segregating for the major gene for thorniness is likely due to one or more modifying factors. Thus the genetic model in tetraploid blackberries may be similar to that in cotton in which leaf hairs are inherited monogenically while hair density is affected by modifying genes (10).

Literature Cited

1. Brooks, R. M. and H. P. Olmo. 1974. Register of new fruit and nut varieties list 29. HortScience 9:437–441.
2. Crane, M. B. and C. D. Darlington. 1927. The origin of new forms in *Rubus*. Genetics. 9:241–278.
3. Darrow, G. M. 1955. Nature of thornless blackberry sports. Fruit Var. Hort. Dig. 10:14–15.
4. Haskill, G. 1961. Seedling morphology in applied genetics and plant breeding. Bot. Rev. 27:382–421.
5. Haskill, G. and J. Hill. 1961. Segregation in tetraploid blackberries. Heredity 16:354–358.
6. Keep, E., V. H. Knight, and J. H. Parker. 1976. Raspberry genetic investigations. Rpt. East Malling Res. Sta. for 1975. p. 100.
7. Knight, R. L. and E. Keep. 1964. Soft fruit breeding. Rpt. East Malling Res. Sta. for 1963. p. 158–160.
8. Lewis, D. 1939. Genetical studies in cultivated raspberries. I. Inheritance and linkage. Genetics 38:367–379.
9. Moore, J. N., G. R. Brown, and C. Lundergan. 1974. Effect of duration of acid scarification on endocarp thickness and seedling emergence of blackberries. HortScience 9:204–205.
10. Muttuthamby, S., M. Aslam, and M. A. Kahn. 1969. Inheritance of leaf hairiness in *Gossypium hirsutum* L. cotton and its relationship with Jassid resistance. Euphytica 18:435–439.
11. Parkinson, J. 1629. Paradisi in sole Paradisus Terrestris. London.
12. Scott, D. H., G. M. Darrow, and D. P. Ink. 1957. 'Merton Thornless' as a parent in breeding thornless blackberries. Proc. Amer. Soc. Hort. Sci. 69:268–277.
13. Scott, D. H. and D. P. Ink. 1966. Origination of Smoothstem and Thornfree blackberry varieties. Fruit Var. Hort. Dig. 20:31–33.