THE CYTOLOGY AND BREEDING BEHAVIOR OF RUBUS CANADENSIS L

DONALD LAIRD CRAIG

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THE CYTOLOGY AND BREEDING BEHAVIOR OF R. CANADENSIS L.

BY

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B.Sc.(Agr.), McGill University, 1947
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SECTION I

THE PROBLEM AND ITS IMPORTANCE

The problem being investigated is the cytology and breeding behavior of *Rubus canadensis*. This species is unusual in that it is a triploid and a facultative apomict. To date few attempts have been made to utilize it in *Rubus* breeding, and the knowledge of its breeding behavior is therefore very limited. There has also been no thorough study of the cytology of the species. In order to increase the present knowledge of this potentially useful species of *Rubus* an attempt was made to obtain further information on the following points:

1. Can reciprocal crosses be made between *R. canadensis* and other blackberries of known chromosome composition?

2. If true hybrids are produced, what is the chromosome number of the hybrids and the probable number in the male gametes?

3. What are the morphological and cytological characteristics of the hybrids?

4. If true hybrids are not produced, can the mode of reproduction be determined from a study of the development of seedlings obtained from self- or cross-pollinations?

5. What is the breeding behavior of true hybrids when inter-crossed and back-crossed to either parent?
6. What is the taxonomic status of *R. canadensis* in the light of the above findings?

Freedom from spines on the canes, winter hardiness of canes, an upright growth habit, flower productivity and fruit quality make *Rubus canadensis* a valuable species for plant breeding. Crosses between this species and other types of blackberries would afford an opportunity to transfer these desirable traits to a commercially acceptable blackberry. The freedom from spines is of particular value.

A collection of clones of *Rubus canadensis* from diverse geographical areas and habitats would permit a taxonomic and cytological evaluation of the species. Such a collection of clones when transferred to the same environment, would facilitate a study of genetical and morphological variation within the species.

A study of the breeding behavior of this species is complicated by the fact that it is a triploid which reproduces essentially as an apomict (Einset 11). The question arises as to whether its male and female gametes are at all functional and if so, with what regularity. A study of the breeding behavior would assist in determining the manner in which this species could be used to form true hybrids, and thus permit the transfer of some of its desirable characters to commercial varieties.
SECTION II
REVIEW OF LITERATURE

The economic importance of *Rubus* (Tourn.) L. as well as its taxonomic and genetic complexity has prompted many workers to utilize the genus for detailed studies.

Plant breeding for the production of named varieties has been carried out by Darrow, Williams and Waldo (10), Grubb (16), Pike (25), Slate and Klein (30), Waldo and Darrow (39), Waldo (40), Vaarama (37), Yeager (42) and Yeager and Meader (43).

Darrow, Williams and Waldo (10) made numerous crosses between eastern varieties of blackberries and western trailing blackberries. In comparison with Boysenberry and Youngberry the selections were not outstanding. They concluded that in making inter-group crosses, varieties with high chromosome numbers should be used as there is possibly a differential survival rate between seedlings of higher and lower chromosome number.

Grubb (16) reporting on raspberry breeding at East Malling lists the desirable traits possessed by different varieties. Details on fruit characteristics are given on selfed progenies of a number of commercial varieties and the genetic segregation for different plant characteristics are recorded.
The validity of hybrids produced by Yeager (42) from a cross between the diploid Taylor red raspberry and the octoploid *Rubus chamaemorus* is substantiated by the results obtained by Pike (25) in a further study of its breeding behavior.

Slate and Klein (30) produced 3588 blackberry seedlings from crosses between varieties and selections. In the production of these seedlings they reported the occasional occurrence of partial or complete failure of the berries to develop normally in certain years. This irregularity was not attributed to lack of pollination or chromosome irregularities. The degree of sterility varied between plants of the same clone and canes of the same plant. It was their opinion that selections expressing this characteristic should be avoided in making crosses.

The origin of the Logan and Mammoth blackberries was questionable. Waldo and Darrow (39) made crosses between an octoploid trailing blackberry and a tetraploid red raspberry and illustrated that this was their probable origin.

An extensive breeding program involving the Logan and western trailing blackberries, eastern upright blackberries and European blackberries permitted Waldo (40) to evaluate parents for transmission of fruit flavour, colour, size, leaf spot resistance, hardiness, plant vigour and sex to their offspring.

Vaarama (35) utilized the northern species *Rubus arcticus* in crosses with *R. idaeus*. Hybrids were produced
but nothing of outstanding commercial value resulted from the cross.

Yeager (42) crossed the following species with raspberry and blackberry varieties: Rubus pumila, R. morifolius, R. chamisoura, R. odoratus, R. idaeus, R. arcticus and R. canadensis. Selections from crosses between the diploid Taylor red raspberry and the octoploid R. chamisoura have been introduced.

The cytology of Rubus has been investigated by Britton and Hull (4), Crane (7), Einset (11, 12), Fisher, Darrow and Waldo (14), Darrow and Waldo (9), Heslop-Harrison (18), Hull and Britton (19), Thomas (34), Vaarama (35, 38) and Yarnell (41).

Britton and Hull's (4) cytological study of root-tip squashes from the hexaploid seedlings of selfed Eldorado showed many cells with fewer than 42 chromosomes. This instability was attributed to the mechanism causing the chromosomes of a single cell to group into two plates, each with its own spindle.

The reproductive behavior of a number of species and varieties of Rubus was investigated by Crane (7). He found that diploid forms always behave sexually but occasionally an unreduced germ cell takes part in fertilization giving rise to polyploid forms.

He also found that reproduction in polyploid forms may be entirely sexual, entirely non-sexual or partly sexual and partly non-sexual. The species used as male in
cross pollination was found to influence the degree to which apomixis was developed. The occurrence of segregation in non-sexual offspring necessitates test crosses, and cytological and embryological studies in order to determine the precise mode of reproduction.

Einset (12) studied a number of species in the subgenus Eubatus with the object of assisting in the evaluation of taxonomic units founded on field observations. Root tip chromosomes were counted and chromosome behavior at meiosis studied. The study of chromosome behavior at meiosis was for the purpose of adding to the understanding of natural relationships of plant forms. Einset (12) states that the pairing of chromosomes in early meiotic stages and the assortment of the chromosomes to the spores could assist in determining the probable origin of forms such as hybrids, autopolyploids and allopolyploids.

Einset (12) reported the number (21) of chromosomes in root tips of R. canadensis but he did not study the species' meiotic chromosomes although he studied other triploid forms.

Apomixis in American polyploid blackberries has been studied by Einset (11). From this study of 24 different seed parents representing 5 different sections of the Eubati, Einset concluded that 80 to 100 per cent of the seedlings produced by the individuals were apomictic in origin. This was true regardless of the type of pollination that occurred. These seedlings were apparently the result of pseudogamous development from unreduced eggs. He noted
that hybrid seedlings resulted infrequently after fertilization of reduced or unreduced eggs.

Fisher, Darrow and Waldo (14) studied the chromosome numbers in several blackberries. Wild selections with 42, 56 and 48 somatic chromosomes were reported in addition to named varieties and selections ranging from 14 to 63 somatic chromosomes. The high degree of polyploidy has little effect on fertility.

Pseudogamy in blackberries was studied by Darrow and Waldo (9). From this study they concluded that European blackberries are normally pseudogamous and American blackberries are not.

Prior to 1940 somatic chromosome counts had been made on less than one-quarter of the British Rubus flora. In 1941 Heslop-Harrison (18) undertook a study to expand the knowledge of the cytology of the genus in an attempt to contribute to the understanding of the origin of the British Rubus flora. Chromosome determinations were made on 80 new taxa and a remarkable agreement was found between British and Swedish Moriferi veri. In Britain 90.7 per cent of the taxa are tetraploid. Euploid chromosome numbers were found in every individual that was examined. Aneuploidy was therefore assumed to be of rare occurrence in the subgenus Eubatus.

Hull and Britton (19) made a very thorough cytological study on the development of colchicine-induced and natural polyploid breeding lines in the genus Rubus. Colchiploid Rubi were found almost invariably to be of chimeral nature.
The parts of the plants affected by colchicine could be propagated thus establishing polyploid lines for breeding purposes. Fertile colchiploid individuals were selected at the tetraploid and hexaploid level. The functioning of unreduced, fertilized eggs occasionally accomplished what was not achieved through colchiploidy.

Unstable plants were found above the hexaploid level. In unstable plants below this level split divisions produced cells apparently unable to reproduce themselves. The propagation of unstable plants by root cuttings resulted in plants with widely differing phenotypes. Propagation of unstable plants by leaf-bud cuttings resulted in plants phenotypically identical to the cane from which they were taken.

Thomas (34) combined a somatic and meiotic study with an embryological study of Rubus in order to determine apomorphic modes of reproduction. He concluded that within the embryo sac a fusion of haploid nuclei, the presence of two differentiated eggs and one synergid or the presence of the undifferentiated nuclei at the micropylar end account for the segregation of characters among asexual progeny observed by Crane. Thomas correlated a completely apomorphic mode of reproduction with a transition from allopolyploidy to autopollploidy.

Vaaaram (35) in a cytological study of 5 Rubus species and 3 hybrids, reported that all 5 species contained one pair of chromosomes with satellites. In the hybrids he
observed only one chromosome with a satellite. He concluded on the basis of chromosome morphology that *R. cassina* and *R. saxatilis* are probably of hybrid origin. He also reported irregular meiosis in pollen mother cells of the 21 chromosome hybrid between *R. cassina* and *R. idaeus*. Four to seven bivalents were observed at metaphase I, and the lagging univalents at first and second divisions were assumed to form small pollen grains.

In a later study, Vaarama (38) observed microsporogenesis in hybrids between high ploidy Pacific *Rubia Ursina* types and the tetraploid eastern trailing blackberry Macatawa. The hybrids were characterized by an irregular meiosis which was apparently not correlated with oddness or evenness of polyploidy. The observations suggested that chromosomes of all different sets present in the genomes are homologous enough for pairing in all combinations. Thus Vaarama suggests that this is evidence that differentiation between the chromosomes of various *Rubus* genomes has taken place mainly through gene mutations and minor structural changes. Where conditions for preferential pairing are favourable, the existing differences become effective.

Yarnell (41) reported that chromosome behavior can be an important factor in berry breeding. The natural chromosome doubling of a hybrid between a diploid wild dewberry and a diploid red raspberry resulted in fertile seedlings from an almost sterile *F*₁ hybrid. The fertile seedlings were called
Studies of the embryology of the genus have been conducted by Berger (3), Christen (6), Custafsson (15), Kerr (22), Pratt and Einset (26) and Pratt, Einset and Clausen (27).

Berger (3) made a comparative embryological study between sexual, diplosporic and aposporic hybrids of *Rubus caesius* and sexual and partially apomictic species of *Rubus*. All of the species studied belong to the section *Moriferi* of the subgenus Eubatus.

Christen (6) discusses the occurrence of apospory and diplospory in various species of European Rubi. He concludes that unreduced egg cells capable of fertilization probably occur in *Rubus* and crosses may therefore produce three types of hybrid offspring resulting respectively from non-fertilized and unreduced eggs, the fertilization of haploid eggs, and the fertilization of unreduced eggs.

Custafsson (15) states that the mechanism of pseudogamy occurring in *Rubus* is not completely known. The unreduced gametophyte is formed by apospory and the egg cell develops without external stimulus. Pseudogamy differs from apomixis by having the fused polar nuclei fertilized. The European *Moriferi veri* have developed a special system of equilibrium characterized by two features, the disappearance of apomixis in hybridization and its recovery by segregation. The individual biotypes are thus able to acquire a faculty of variation but at the same time maintain their quality of
constancy.

Seed development in blackberries was studied by Kerr (22). In the course of his study, Kerr noted that both polar nuclei and egg are fertilized in syngamic seed formation and that only the polar nuclei are fertilized in pseudogamic seed development. In the blackberry, fertilization occurs a day after pollination. In pseudogamic species the development of several gametophytes per ovule was also observed.

Embryo sac development in a number of North American blackberry species was studied by Pratt and Einset (26). *R. canadensis* was included among the species studied. The young ovules of all the *Rubi* studied had a sub-epidermal layer of cells each of which divided periclinally to form a parietal cell and a sporogenous cell. The epidermal cells and parietal cells divided repeatedly as the ovule grew. It was noted that several sporogenous cells could become megaspore mother cells.

In *R. canadensis*, Pratt and Einset (26) distinguished three to six sporogenous cells and one to five megaspore mother cells in 38 per cent of the lower ovules. Late stages of meiosis with lagging chromosomes were also observed and tetrads appeared in 12 per cent of the lower ovules of these buds. The development of one-nucleate embryo sac from a megaspore was clearly observed as were aposporic embryo sacs. Chalaza cells, sporogenous cells, sporogenous daughter cells and parietal cells initiated
the aposporic sacs. The authors state that the best criterion for the origin of aposporus sacs is the presence or absence of three degenerating megaspores at the micropylar end of the one nucleate embryo sac.

The pattern of the sac development was found to be irregular in some instances. In one sac, an egg, two synergids, two antipodals, and three nuclei in contact in the centre of the cell were observed. The origin of the extra polar nucleus could not be determined. The other extreme in sac arrangement was one in which there were numerous nuclei at either end.

Two or three sacs per ovule were found in 32 per cent of the lower ovules at anthesis and only 39 per cent of the ovules at anthesis had an apparently viable egg. Non functional ovules showed arrested sporogenous cells, immature or abnormal sacs, degeneration of the egg, breakdown of the sac and disintegration of the nucellus.

The other polyploid forms of Rubus studied by Pratt and Einset (26) showed a pattern of facultative apospory similar to *R. canadensis*. The development of the embryo sac in the diploid *R. alleghenensis* was quite regular although aposporic sacs were infrequently observed.

Pratt, Einset and Clausen (27) found the pattern of facultative apospory in a triploid *R. idaeus* to be similar to that found in triploid blackberries. The chromosome numbers of the offspring suggested that unreduced eggs functioned parthenogenetically or following fertilization.
Reduced eggs of various chromosome numbers functioned after fertilization and possibly parthenogenetically.

Although the embryology and mode of reproduction of *Rubus canadensis* have been studied by Einset (11) and Pratt and Einset (26), a comprehensive survey of the literature does not indicate that this species had been previously used for a detailed study of its breeding behavior. The one exception was Hedrick's (17) report that it was being used at Geneva, N. Y. in crosses with cultivated varieties with the hope that out of the progeny a thornless type would appear. Correspondence (December, 1958) with the New York Fruit Testing Station, Geneva, N. Y., indicated that nothing of value came from these crosses.

Fernald (13) in the Eighth Edition of Grays Manual of Botany classified *Rubus canadensis* in the following manner:

**Species**  
*R. canadensis* L.

**Subgenus** V  
*Rubatus* Focke

**Genus**  
*Rubus* L.

**Tribe**  
*RUBRÆE Dumort

**Family**  
*ROSACEAE*

His description of *R. canadensis* was as follows:

"*R. canadensis* L. (Canadian), Smooth B. - Canes smooth and without (or with exceptional and very remote) prickles, 0.5 - 3 m. high, erect or high-arching; primocane - leaves with smooth petioles or exceptionally with 1 or 2 small prickles; leaflets (3-) 5 (-7), ovate or ovate lanceolate, long - acuminate, glabrous on both surfaces, the median and
terminal ones long - petiolulate, the terminal with broadly rounded to cordate base; floricanes strongly ascending (unless lodged by heavy snow); primary inflorescences elongated racemes of numerous (up to 25) flowers, standing well above the basal foliaceous bracts; most bracts reduced and stipuliform, the rachis minutely pilose; petals 1.2 - 2 cm. long, 6 - 12 mm. broad; fruit globose or thimble-shaped - 12 mm. long, dryish or in some colonies juicy and of rich quality. (R. Millsapughii Britt.; R. Randii (Bailey) Rydb., the smallest extreme). Thickets and clearings, Nfld. to W. Ont. and Minn., s to N.S., N.E., Pa., upland to n. Ga. and Tenn. Fl. June, July; fr. Aug., early Sept."

In this classification of Rubus, 195 species were included in the sub-genus Rubatus. The sub-genus was then further divided into 12 sections. R. canadensis was one of 6 species assigned to Section 12, Canadenses Bailey. The species within this section were differentiated principally on the presence or absence of prickles, absence of or scarcity of pubescence on leaves, number of prickles per decimeter of cane and on the formation of the inflorescence.

Common names for R. canadensis include Smooth Blackberry (13), Thornless Blackberry (1) and Mountain Blackberry (17).

The species has a biennial growth habit which results in the production of primocanes the first year which persist and become floricanes the next. The primocanes originate from adventitious buds located in the base of the plant and in the roots. Following fruiting the two year old floricanes
die. Clones of *R. canadensis* vary in their degree of fertility although they produce abundant conspicuous white flowers. The quality of the fruit is also variable.

*R. canadensis* could possibly be confused with *R. pensylvanicus* Poir. in that it too may be nearly spineless, and the two species have a similar geographic range (13). However, the presence of pubescence on the underside of the leaves of *R. pensylvanicus* clearly distinguishes it from *R. canadensis*.

A common habitat for *R. canadensis* is the partially shaded areas along wood roads, highways and on the banks of streams, but it may also be found in open areas such as old pasture land or burnt over woodland. This species prefers a well drained soil abundant in organic matter.

The presence of 21 somatic chromosomes (Einset, 12) is another distinguishing characteristic of *R. canadensis* although there are other *Rubus* species having this triploid chromosome number.

The somatic chromosome number of the members of the sub-genus *Rubatus* varies from 14 to 84. This is in marked contrast to the almost uniform number of 14 possessed by all members of the sub-genus *Idaeobatus* (Fisher, Darrow, Waldo, 14).

In the sub-genus *Rubatus*, Bailey (1) described 17 species under Section 8, Canadenses. In the same section Fernald (13) recognized 6 species and Hedrick (17) recognized 4 species. Roland's (29) Flora of Nova Scotia includes only the species
that L. H. Bailey considered indigenous to the Province. These include *R. canadensis*.

In 1897 L. H. Bailey made a visit to the Linnaean Herbarium, London, and while there examined the Linnaean specimen of *R. canadensis*. He recognized it to be equivalent to *R. millspaughii* Britt. which he regarded as of general distribution over the northeastern United States. Later investigations by Bailey and other taxonomists verified this conclusion, and thus *R. millspaughii* was relegated to the position of a synonym (13) of *R. canadensis*.

Although *R. canadensis* is confined to North America, Gustafsson (15) considered it to be closely related to some of the European blackberries. The relationship is to Rubi Suberecti which is one of Sudres' classes of Moriferi, the true brambles (32).

Species belonging to the sub-genus Eubatus are abundant in North America and Europe but the richest centre of development is South America. No members of this sub-genus are found in the regions around the Pacific and Indian Oceans (Beijerinck, 2).

The confusion that exists in the American sub-genus Eubatus is evident from the obvious lack of agreement by those who have attempted a taxonomic classification. Such a classification is further complicated by the fact that at the present time evolution is occurring within the sub-genus. Man is speeding this process by his advances into undeveloped areas, by his construction and destruction, by fires, and by transplanting species from widely separated areas. This has
resulted in a great commingling of types producing numerous variants many of which breed true as apomictic types, thus establishing new species in the biological sense. It is fairly certain that the ancestors of the present Rubus complex are mostly extinct (Stebbins, 33).

The fact that the biological concept of a species as a genetically isolated population does not apply to all "species" of Rubus further complicates the taxonomy. The free interchange of genes that should occur between members of the "biological species" is in many cases prevented by their apomictic type of reproduction; nevertheless it is possible to recognize widely ranging and morphologically distinct types that satisfy the most conservative botanist.

Insufficient and improper deductions from experimental work conducted within the sub-genus Rubus has added further confusion. Darrow and Waldo (9) reported that "European blackberries differ from American blackberries in that many, if not all, may reproduce the parent exactly when grown from seed while no American varieties have been known to do this." Einset (11) proved that this was not the case, but on the contrary many members from different sections of American Rubus do behave as apomicts.

Petersen's (24) report that apomixis did not occur in the blackberries of New England led Gustafsson to conclude (Gustafsson 1942) that the origin of European polyploid blackberries could not have been in North America because
the closely related American species were all sexual. We now know that Peitersen was not aware of facultative apomixis and the possible variation among apomicts, thus he concluded that all species were sexual.

The action of environment on morphological characters has also been the subject of experimental investigation. Beijerinck (2) illustrated the extremes that the taxonomist can encounter. He mentions the fact that types of brambles that normally have an upright growth habit may become prostrate when grown in moist humus rich in nitrogen, and that plants grown in dense brushwood will be greatly elongated in comparison to similar types growing in unshaded areas. Beijerinck (2) states that "the way in which trichomes and emergences are distributed on the turion and the character of these appendages are to be considered distinguishing marks of much greater importance and it is on these characters that the subdivision of the Moriferi is chiefly based."

Brown (5) concluded from his study that most morphological characters depended more on geographic distribution than on presumed chromosome set constitution, although certain characters such as leaf incision and glandulation were directly correlated with chromosome number groups.

Although Stebbins (33) acknowledged that the data presented by Brainerd and Peitersen was far too scanty, he was of the opinion that the seedling segregation they
obtained from hybrids suggested that many of the species recognized by systematists were unstable hybrids or hybrid derivatives.

Thus we find that the sub-genus Eubatus provides material for a great deal of experimental investigation. Cooperative between taxonomists, plant breeders and cytologists can do much toward bringing order out of chaos as has already been shown by Einset (12), Brown (5), Stebbins (33), and others. There is still much to be done. It is hoped that the present study of a single species will contribute to a better understanding of the problem.
SECTION III
MATERIALS AND METHODS

The objective of this study was to collect clonal material of Rubus canadensis for a study of its cytology and breeding behavior. This clonal material was also to afford a study of clonal variation for such characteristics as fertility, fruit quality, cane spininess, hardiness and vigor. An attempt was to be made to study microsporogenesis. The findings from this study were to be substantiated by a cytological study of somatic chromosome numbers resulting from self pollinations and cross pollinations between R. canadensis and other blackberries.

Clones.

Clonal propagation material of Rubus canadensis was collected in 1955 from the following places: Pittsburgh, N. H., Simms Stream, N. H., Stafford, N. H., Franconia, N. H., Franconia Notch, N. H., Packers Falls, N. H. and Durham, N. H. The above areas range from the extreme northwestern to the lower southeasterly area of the state. The north to the south distance is approximately 170 miles. The least distance between any two collection points was 16 miles, the greatest distance 80 miles. Additional clonal material was secured from Lubec, Maine; Grand Manan Island in the Bay of Fundy and at Chester Basin, Nova Scotia. The clones were designated as follows:
Clone 3 Simms Stream, N. H. #2
Clone 4 Packers Falls, N. H. D.1
Clone 6 Lubec, Maine
Clone 8 Simms Stream, N. H. #3
Clone 9 Franconia, N. H. #5
Clone 10 Simms Stream, N. H. Extra vigorous
Clone 12 Pittsburgh, N. H. #5
Clone 13 Franconia Notch, N. H.
Clone 14 Simms Stream, N. H. #1
Clone 15 Franconia, N. H. #4
Clone 17 Hodgdon, Durham, N. H.
Clone 19 Grand Manan Island
Clone 22 Chester Basin, N. S.

The plant material was assembled and planted at the Research Station, Kentville, N. S. In addition to the plants in the field, propagations from each clone were planted in pots for use in the greenhouse. Herbarium specimens were made of each clone and deposited in the Research Station herbarium, Kentville, N. S.

Floricanes from the clonal material was evaluated in April, 1959, for winter hardiness, degree of spininess, cane height and diameter. These measurements were based on the five tallest canes from each clone. The canes were cut off at ground level prior to taking measurements.

Crosses made.

In March, 1955, a plant of R. canadensis which was
growing in the University greenhouse at Durham, N. H., was used in crosses with other blackberries and for a study of self-fertility. All pollinations were made at anthesis.

The seeds resulting from the above crosses were sent by mail to Kentville, N. S., at which place they were stratified and germinated. These and subsequent seedlings were potted up and grown in the greenhouse during the cool weather. In the summer the potted plants were grown out of doors and in the fall, after the canes had matured, the plants were placed in a 32° to 40°F. storage room. In the early spring these plants were removed from storage and forced into growth in the greenhouse.

Reciprocal crosses were made in 1957 between a clone of **R. canadensis** and the Lowden, the Hedrick, and the Bailey blackberries. When the fruits were mature, the hybrid seed was cultured using the culture methods outlined by Randolph and Randolph (28). To prepare the seed for culture, the endocarp of the fruit was removed under sterile conditions. The seed was then transferred to the culture medium contained in capped glass bottles. In some cases the seed coat was removed and the naked embryo cultured. The cultured seeds were placed under continuous fluorescent light. The resulting seedlings were transferred to a sterilized soil medium as soon as they formed true leaves.

A tetraploid seedling from the 1955 crosses flowered in the greenhouse in 1958. The flowers were self-pollinated
and the resulting seed cultured.

A tetraploid seedling from the 1957 crosses flowered in the greenhouse in 1959. The flowers were self-pollinated and backcrossed to the two parents *R. canadensis* and Hedrick.

Fertility study.

To determine self and cross-fertility Clone 17 which was growing in the New Hampshire greenhouse was self-pollinated in 1955. In 1956 Clones 6, 10, 12 and 17 were self-pollinated in the Kentville greenhouse and Clones 3, 4, 8, 10 and 12 were cross-pollinated with pollen from Clone 6. In August, 1957, fruit set was determined on Clones 3, 4, 6, 8, 9, 10 and 12 which were growing in the field at Kentville. At flowering time the racemes were bagged with finely woven cheese cloth and a count of the fruit set was made to determine any variation between clones with respect to self- and cross-fertility.

Root tip chromosome investigations.

All somatic chromosome determinations were made on root tips which were fixed in CRAF (21) and stained in Crystal Violet or from root tip smears using a modification of Belling’s aceto-carmine technique (8). In 1956 root tips from clones grown from the 13 collections mentioned above were fixed in CRAF and stained with Crystal Violet.

In 1957 the root tips from seedlings resulting from crosses made in 1955 at New Hampshire were collected,
stained and examined for chromosome numbers. A detailed 
study on a single plant of this progeny was carried out 
because this plant appeared to be mitotically unstable. 

The chromosomes in the root tips of seedlings from 
the 1957 crosses were counted in 1958. The chromosomes 
in the root tips of the seedlings resulting from the self-
pollination of an F₁ hybrid between Maple Grove and 
R. canadensis were counted in 1959.

Microsporogenesis.

For a study of microsporogenesis, anthers from 
unopened buds from clone 6 were placed in a drop of aceto-
carmine and smeared under a coverslip. The slides were 
briefly heated and then examined under the research micro-
scope.

Seedling morphology.

In 1959 the seedlings from the reciprocal crosses made 
in 1957 were studied for variations in leaf morphology. 
The fourth leaf down from the terminal leaf was removed 
from a primocane of each seedling. The length, width, thick-
ness and number of serrations were recorded for each terminal 
leaflet. Length was length of blade from apex to petiole, 
width was taken at the widest point of the blade and 
thickness on the mid-vein at the point where basal lateral 
veins join the mid-vein.
SECTION IV

RESULTS

Clones.

The morphological variation that exists between clones of *R. canadensis* is exemplified by the measurements recorded in Table 1. A comparison of floricane heights shows that the

Table 1. *Rubus canadensis* clonal differences in cane height, cane diameter, spininess and susceptibility to winter injury. 1959

<table>
<thead>
<tr>
<th>Clone</th>
<th>Floricane height</th>
<th>Basal diameter</th>
<th>Basal 12&quot;</th>
<th>Top 24&quot;</th>
<th>Winter injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>63</td>
<td>0.44</td>
<td>2-11</td>
<td>9-11</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>0.38</td>
<td>2-9</td>
<td>2-4</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>69</td>
<td>0.50</td>
<td>6-17</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>46</td>
<td>0.44</td>
<td>3-32</td>
<td>5-9</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>53</td>
<td>0.38</td>
<td>0-1</td>
<td>0-1</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>59</td>
<td>0.50</td>
<td>16-47</td>
<td>3-10</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>45</td>
<td>0.44</td>
<td>12-21</td>
<td>2-7</td>
<td>5</td>
</tr>
<tr>
<td>14</td>
<td>50</td>
<td>0.38</td>
<td>0-2</td>
<td>0-1</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>43</td>
<td>0.44</td>
<td>0-2</td>
<td>0-1</td>
<td>4</td>
</tr>
<tr>
<td>17</td>
<td>53</td>
<td>0.44</td>
<td>7-33</td>
<td>0-1</td>
<td>4</td>
</tr>
</tbody>
</table>

Note: Measurements are the average of the five tallest canes per clone. Winter injury rated on the basis of 1 to 5. 5 indicates no injury, 4 slight injury to tips of canes.
ten clones varied from 43 to 69 inches and the basal diameter varied from 0.38 to 0.50 inches. The number of spines on the basal portion and top portion of the floricanes also varied. Clone 9 had one or less spines per decimeter both on the basal and top portions of the cane while Clone 10 had 16-47 spines per decimeter on the basal 12 inches of the floricanes and 3-10 on the top 24 inches, and Clone 6 had 6-17 spines per decimeter on the basal 12 inches and no spines on the top portion of the cane. The other 7 clones were intermediate to these extremes.

The number of suckers per clone varied considerably. Clone 17 produced numerous suckers and a quantity of good floricans whereas Clone 10 produced a number of good floricans but very few suckers. Other clones lay between these extremes.

The variation in hardiness did not appear to be significant. All clones with the exception of Clone 12 were slightly injured during the winter 1958-59. This injury extended from 1-4 inches on the floricanes tips.

Crosses made.

The results of crosses made in 1955 in the University greenhouse, Durham, N. H. are recorded in Table 2. Crosses between Snyder, Maple Grove, Geneva 32 and Geneva 33 with R. canadenlis as the male parent resulted in fruit set on 90 of the 296 flowers pollinated. Fifteen of the 90 flowers produced whole fruits, 75 flowers produced from 1 to 10
drupelets. Seed collected from the crosses produced 21 plants.

Table 2. Results of crosses made in the University greenhouse, Durham, N. H. 1955

<table>
<thead>
<tr>
<th>Cross</th>
<th>Number of flowers pollinated</th>
<th>Number of flowers setting fruit</th>
<th>Number of whole fruits</th>
<th>Number with 1-10 drupelets only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snyder x Clone 17</td>
<td>107</td>
<td>28</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Maple Grove x Clone 17</td>
<td>106</td>
<td>48</td>
<td>4</td>
<td>44</td>
</tr>
<tr>
<td>Geneva 33 x Clone 17</td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Geneva 32 x Clone 17</td>
<td>72</td>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Clone 17 selfed</td>
<td>160</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

The reciprocal crosses made in 1957 between the Lowden, Hedrick and Bailey tetraploid blackberries and the triploid R. canadensis produced 79 seedlings (Table 3). These seedlings resulted from the pollination of 697 flowers which produced 1004 seeds (Table 4). These seeds were cultured and grown to mature plants (Figs. 14-16). The crosses between Clone 15 x Lowden, Hedrick and Bailey resulted in 13.2 per cent of the seed having no embryo development. The reciprocal cross showed 22 per cent of the seeds having no embryos and 21 per cent with partially aborted embryos. From 852 seeds cultured for seedlings, 111 or 13 per cent produced plants from which root tips could be collected (Table 3).
Table 3. Results from the culture of *Rubus* embryos.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Number of seeds</th>
<th>Normal embryos</th>
<th>Aborted embryos</th>
<th>Number of embryos cultured</th>
<th>Number of seedlings produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone 10</td>
<td>58</td>
<td>53</td>
<td>5</td>
<td>53</td>
<td>-</td>
</tr>
<tr>
<td>Clone 14</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Clone 15</td>
<td>46</td>
<td>41</td>
<td>5</td>
<td>34</td>
<td>5</td>
</tr>
<tr>
<td>Clone 8</td>
<td>87</td>
<td>83</td>
<td>4</td>
<td>74</td>
<td>25</td>
</tr>
<tr>
<td>Clone 12</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Clone 15 x Hedrick</td>
<td>119</td>
<td>100</td>
<td>19</td>
<td>94</td>
<td>14</td>
</tr>
<tr>
<td>Clone 15 x Lowden</td>
<td>111</td>
<td>101</td>
<td>10</td>
<td>97</td>
<td>30</td>
</tr>
<tr>
<td>Clone 15 x Bailey</td>
<td>47</td>
<td>41</td>
<td>3</td>
<td>35</td>
<td>6</td>
</tr>
<tr>
<td>Hedrick x Clone 15</td>
<td>277</td>
<td>231</td>
<td>46</td>
<td>199</td>
<td>14</td>
</tr>
<tr>
<td>Lowden x Clone 15</td>
<td>172</td>
<td>128</td>
<td>44</td>
<td>102</td>
<td>5</td>
</tr>
<tr>
<td>Bailey x Clone 15</td>
<td>278</td>
<td>237</td>
<td>41</td>
<td>143</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 5. Seed set following reciprocal crosses with *Rubus canadensis*.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Number of flowers pollinated</th>
<th>Number of seeds produced</th>
<th>Seeds per pollination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone 15 x Lowden</td>
<td>30</td>
<td>111</td>
<td>3.7</td>
</tr>
<tr>
<td>Clone 15 x Hedrick</td>
<td>31</td>
<td>119</td>
<td>3.5</td>
</tr>
<tr>
<td>Clone 15 x Bailey</td>
<td>29</td>
<td>47</td>
<td>1.6</td>
</tr>
<tr>
<td>Lowden x Clone 15</td>
<td>145</td>
<td>172</td>
<td>1.2</td>
</tr>
<tr>
<td>Hedrick x Clone 15</td>
<td>94</td>
<td>277</td>
<td>2.9</td>
</tr>
<tr>
<td>Bailey x Clone 15</td>
<td>368</td>
<td>278</td>
<td>0.8</td>
</tr>
</tbody>
</table>

A tetraploid seedling resulting from a 1955 cross between Maple Grove and *R. canadensis* (Table 2) flowered in 1958. The flowers of the tetraploid plant were self-pollinated and the resultant seed cultured to force embryo development. Twenty-two seedlings were produced from this cross.

A seedling from a cross between Hedrick and *R. canadensis* flowered in 1959. This seedling was used for selfing and back crossing to the parents. The results of these crosses are shown in Table 5. Ten self-pollinated flowers of the F₁ hybrid produced 10 whole berries. Twenty flowers of Hedrick pollinated with pollen from the F₁ hybrid produced 18 whole berries and 2 partial berries and 20 *R. canadensis* flowers pollinated with F₁ hybrid pollen produced 16 partial berries ranging from 1 to 10 drupelets.
Table 5. The results of selfing and backcrossing on \( F_1 \) hybrid (Hedrick x \( R. \) canadensis). 1959

<table>
<thead>
<tr>
<th>Cross</th>
<th>Number of flowers pollinated</th>
<th>Number of flowers setting fruit</th>
<th>Number of whole berries</th>
<th>Number with 1-10 drupelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>( F_1 ) (Hedrick x ( R. ) canadensis) selfed</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Hedrick x ( F_1 ) (Hedrick x ( R. ) canadensis)</td>
<td>20</td>
<td>20</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>( R. ) canadensis x ( F_1 ) (Hedrick x ( R. ) canadensis)</td>
<td>20</td>
<td>16</td>
<td>0</td>
<td>16</td>
</tr>
</tbody>
</table>

Fertility study.

The results of the tests to establish the degree of self- and cross-fertility in \( R. \) canadensis are shown in Tables 6, 7 and 8. Clones 6, 10, 12 and 17 when self-pollinated in the greenhouse in 1956 produced an average of 0.7, 7.0, 7.7 and 3.0 drupelets respectively (Table 6).

Table 6. Fruit set following selfing of greenhouse grown clones of \( R. \) canadensis. 1956

<table>
<thead>
<tr>
<th>Clone number</th>
<th>Number of flowers selfed</th>
<th>Number of complete fruits formed</th>
<th>Number of drupelets formed</th>
<th>Average number of drupelets per flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>17</td>
<td>0</td>
<td>52</td>
<td>3.0</td>
</tr>
<tr>
<td>12</td>
<td>19</td>
<td>0</td>
<td>147</td>
<td>7.7</td>
</tr>
<tr>
<td>10</td>
<td>125</td>
<td>0</td>
<td>879</td>
<td>7.0</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>0</td>
<td>24</td>
<td>0.7</td>
</tr>
</tbody>
</table>
Table 7. Fruit set in the greenhouse following cross pollination with Clone 6 of B. canadensis, 1956

<table>
<thead>
<tr>
<th>Clone number</th>
<th>Number of flowers pollinated</th>
<th>Number of complete fruits formed</th>
<th>Number of drupelets formed</th>
<th>Average number of drupelets per flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>3x6</td>
<td>26</td>
<td>0</td>
<td>20</td>
<td>0.8</td>
</tr>
<tr>
<td>8x6</td>
<td>21</td>
<td>0</td>
<td>13</td>
<td>0.6</td>
</tr>
<tr>
<td>12x6</td>
<td>13</td>
<td>0</td>
<td>8</td>
<td>0.6</td>
</tr>
<tr>
<td>4x6</td>
<td>17</td>
<td>0</td>
<td>6</td>
<td>0.4</td>
</tr>
<tr>
<td>10x6</td>
<td>29</td>
<td>0</td>
<td>46</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Clone 6 used as the pollen parent in crosses with Clones 3, 4, 8, 10 and 12 in the greenhouse in 1956 produced 0.8, 0.4, 0.6, 1.6 and 0.6 drupelets per flower respectively (Table 7).

Clones 3, 4, 6, 8, 9, 10 and 12 when self-pollinated and given the opportunity to cross pollinate in the field in 1957 gave consistently higher fruit set when open pollinated (Table 8). The one exception was Clone 4 in which case fruit set following open pollination was about equal to selfing. Open pollinated Clone 3 produced 90 per cent entire fruit in comparison to Clone 9 which produced 0 per cent entire fruit. When self-pollinated only Clones 3 and 4 produced entire fruit. Clone 3 produced 6 per cent entire fruits, Clone 4, 24 per cent.

The crosses made in 1957 of Clone 15 x the commercial varieties Lowden, Hedrick and Bailey respectively produced an average of 3.0 seeds per pollinated flower (Table 4) while the
Table 8. Fruit set following selfing and open pollination under field conditions, 1957

<table>
<thead>
<tr>
<th>Clone number</th>
<th>Number of drupelets per fruit</th>
<th>Entire fruit</th>
<th>Per cent with entire fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td>#3 selfed</td>
<td>22 27 2 0 3</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>#3 open pollinated</td>
<td>- - 4 6 90 100</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>#4 selfed</td>
<td>18 12 3 7 15 55 27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#4 open pollinated</td>
<td>16 11 2 9 12 50 24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#6 selfed</td>
<td>26 4 2 0 0 32 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#6 open pollinated</td>
<td>20 22 13 32 25 112 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#8 selfed</td>
<td>31 42 3 2 0 78 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#8 open pollinated</td>
<td>0 5 19 9 12 45 27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#9 selfed</td>
<td>10 1 - - - 11 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#9 open pollinated</td>
<td>1 18 10 1 1 30 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#10 selfed</td>
<td>13 34 12 5 - 64 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#10 open pollinated</td>
<td>0 1 3 6 18 28 64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#12 selfed</td>
<td>26 26 1 - - 53 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#12 open pollinated</td>
<td>fertility of fruiting branches varied from fully fertile to completely sterile</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
reciprocal produced an average of 1.2 seeds per pollinated flower.

Root tip chromosome investigations.

Permanent slides of root tips of the thirteen clones showed that all the clones were triploids (3N=21).

The root tip chromosome counts of the seedlings resulting from crosses made in 1955 are shown in Table 9. The crosses

Table 9. Root tip chromosome counts of seedlings from crosses made in University greenhouse, Durham, N. H. 1955.

<table>
<thead>
<tr>
<th>Cross</th>
<th>2N*</th>
<th>3n</th>
<th>4n</th>
<th>5n</th>
<th>Unstable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snyder x Clone 17</td>
<td></td>
<td>1</td>
<td>6</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Maple Grove x Clone 17</td>
<td></td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Geneva 32 x Clone 17</td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* N refers to the basic chromosome number, which in this species is 7.

Snyder, Maple Grove, Geneva 32 x *R. canadensis* produced 2 triploids, 16 tetraploids, 1 pentaploid and 2 unstable seedlings.

A seedling with unstable chromosome numbers from the Maple Grove x *R. canadensis* cross was the subject of a further detailed study. The vegetative appearance of this plant in contrast with 3N, 4N and 5N sister seedlings is shown in Figs. 4-7.

A total of 71 chromosome counts were made on the cells.
of root tip smears of the unstable plant. The distribution of chromosomes present in the various cells is shown in Fig. 1. Camera-lucida drawings made of a number of these cells are shown in Figs. 8-10. A wide variation in chromosome number was observed even in different cells of the same root tip. Counts such as 17, 29, 33 and 37 somatic chromosomes were noted in the same root tip.

In this unstable plant there was a higher frequency of aneuploid chromosome numbers than of polyploid multiples of the haploid chromosome number. Cross sections of embedded material did not reveal specific chromosome numbers for specific regions of the root tip. Longitudinal sections of the same root tips showed normal cell division with regular movements of the chromosomes during anaphase.

The results of root tip chromosome counts (Figs. 11-13) made on seedlings of reciprocal crosses made in 1957 between _R. canadensis_ and the commercial blackberry varieties are recorded in Table 10. The cross in which _R. canadensis_ was the female parent resulted in 32 triploid seedlings and 18 pentaploid seedlings. Crosses in which _R. canadensis_ was the male parent produced 3 diploids, 3 triploids and 23 tetraploids.

The chromosomes in the root tips of 17 of the 22 seedlings resulting from a self-pollination of one of the tetraploid seedlings from the 1955 cross between Maple Grove and _R. canadensis_ were counted in 1959. Three of these seedlings were diploids, 14 were tetraploids.
Table 10. Root tip chromosome counts of seedlings from reciprocal crosses. 1957

<table>
<thead>
<tr>
<th>Cross</th>
<th>2N</th>
<th>3N</th>
<th>4N</th>
<th>5N</th>
<th>Unstable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone 15 x Lowden</td>
<td>-</td>
<td>17</td>
<td>-</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>Lowden x Clone 15</td>
<td>1</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Clone 15 x Hedrick</td>
<td>-</td>
<td>11</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Hedrick x Clone 15</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Clone 15 x Bailey</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Bailey x Clone 15</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Clone 15 is a triploid (3N). Hedrick, Bailey and Lowden are tetraploids (4N).

The distribution of seedlings having different root tip chromosome numbers are shown in Table 11. These seedlings resulted from 4N x 3N crosses and 3N x 4N crosses. This table shows that when the tetraploid commercial blackberries were the female parent 76 per cent of the seedlings were tetraploid, 10 per cent were triploid, 6 per cent were diploid, 4 per cent unstable and 2 per cent pentaploid.

Table 11. Distribution of somatic chromosome numbers from reciprocal crosses made in 1955 and 1957.

<table>
<thead>
<tr>
<th>Cross</th>
<th>2N</th>
<th>3N</th>
<th>4N</th>
<th>5N</th>
<th>Unstable</th>
</tr>
</thead>
<tbody>
<tr>
<td>4N x 3N</td>
<td>3</td>
<td>5</td>
<td>39</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3N x 4N</td>
<td>0</td>
<td>32</td>
<td>0</td>
<td>18</td>
<td>0</td>
</tr>
</tbody>
</table>
When *B. canadensis* was the female parent 64 per cent of the seedlings were triploid and 36 per cent pentaploid.

**Microsporogenesis.**

An examination of *B. canadensis* microsporocytes showed that meiotic chromosome pairing at metaphase I was very irregular and that there was no one typical arrangement. The meiotic chromosomes were very small and difficult to analyse. Univalents, bivalents and trivalents were distinguished on the basis of size and it appeared that in some cases, larger associations than 3 chromosomes were present. Metaphase I sporocytes were observed that appeared to have as few as 2 and as many as 11 configurations. Several sporocytes were found in which chromosome associations could be identified with a fair degree of certainty. These had such configurations as 1 trivalent, 8 bivalents and 2 univalents (Figs. 17, 29); 3 trivalents, 6 bivalents (Figs. 18, 30); 6 trivalents, 3 univalents (Figs. 19, 31). Other sporocytes were observed in which asynapsis had occurred, resulting in 21 unpaired chromosomes (Fig. 26). The sporocytes within any given locule varied in meiotic configurations.

Anaphase I sporocytes were observed in which chromosome movement appeared to be regular. Other sporocytes were observed in which anaphase I chromosomes were lagging (Fig. 20). Similar observations were made at telophase I (Fig. 25) and anaphase II (Figs. 23, 24). The configurations at metaphase II were also irregular (Figs. 21, 22). Sporocytes with
chromosome numbers ranging from 5 to 14 were observed although 5 or 6 were more common.

Meiosis resulted in the formation of many normal appearing tetrads although small spores were observed in 5 to 10 per cent of them (Fig. 27). The pollen grains were variable in size. Three different size grains could be clearly distinguished in addition to many aborted grains (Fig. 28).

Seedling morphology.

The variation in leaf morphology that existed in the seedlings of various ploidy and parents involved in the 1957 crosses is shown in Figs. 32-37 and in Table 12. The figures and the table point out the variations between seedlings of different somatic chromosome number and the variation between seedlings of the same chromosome number.

Seedlings with 28 somatic chromosomes had the longest (average 13.1 cm.) and widest (average 8.6 cm.) terminal leaflets. This group of seedlings also had the thickest leaves (average 1.6 mm.) and the largest number of leaf serrations (average 117) (Table 12). Morphologically the tetraploids showed a great range of variability and no seedling was identical to the tetraploid parent.

Seedlings with 14 somatic chromosomes were easily identified by their long narrow leaflets (Fig. 37). The seedlings with 35 chromosomes could be identified by their more uniform leaf measurements, their compactness of the leaflet
Table 12. Leaf measurements of seedlings resulting from reciprocal crosses between *R. canadensis* and the Lowden, Hedrick and Bailey blackberry.

<table>
<thead>
<tr>
<th>Seedling chromosome number</th>
<th>Number of plants measured</th>
<th>Length of terminal leaflet</th>
<th>Width of terminal leaflet</th>
<th>Thickness of terminal leaflet</th>
<th>Number of serrations on terminal leaflet</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>3</td>
<td>11.9 cm.</td>
<td>6.1 cm.</td>
<td>1.38 mm.</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.8-13.0</td>
<td>5.5-6.5</td>
<td>1.16-1.79</td>
<td>84-113</td>
</tr>
<tr>
<td>21</td>
<td>2</td>
<td>12.1 cm.</td>
<td>8.9 cm.</td>
<td>1.59 mm.</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.0-12.2</td>
<td>8.4-9.5</td>
<td>1.41-1.77</td>
<td>52-123</td>
</tr>
<tr>
<td>28</td>
<td>21</td>
<td>13.1 cm.</td>
<td>8.6 cm.</td>
<td>1.67 mm.</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.0-16.2</td>
<td>6.2-12.1</td>
<td>1.14-2.45</td>
<td>61-148</td>
</tr>
</tbody>
</table>

*Lowden, Hedrick, Bailey x R. canadensis*

<table>
<thead>
<tr>
<th>Seedling chromosome number</th>
<th>Number of plants measured</th>
<th>Length of terminal leaflet</th>
<th>Width of terminal leaflet</th>
<th>Thickness of terminal leaflet</th>
<th>Number of serrations on terminal leaflet</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>6</td>
<td>9.1 cm.</td>
<td>5.4 cm.</td>
<td>1.18 mm.</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.3-12.6</td>
<td>4.8-7.0</td>
<td>1.00-1.49</td>
<td>69-89</td>
</tr>
<tr>
<td>35</td>
<td>11</td>
<td>9.9 cm.</td>
<td>7.3 cm.</td>
<td>1.40 mm.</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.2-12.3</td>
<td>5.9-9.7</td>
<td>1.18-1.67</td>
<td>90-134</td>
</tr>
</tbody>
</table>
arrangement and by the fact that they were the only hybrid
seedlings with glabrous upper and lower leaf surfaces.

The seedlings with 21 chromosomes resulting from *R.
canadensis* as the male parent could not be identified on the
basis of morphological traits. They resembled the seedlings
with 28 chromosomes. In contrast, the seedlings with 21
chromosomes resulting from *R. canadensis* as the female parent
were easily identified because they appeared to be identical
to the female parent.

The 22 seedlings resulting from the self-pollination of
the F₁ hybrid between Maple Grove and *R. canadensis* were
observed for morphological differences. The tetraploids
were found to be quite variable. Leaf color, shape and
serration indicated considerable genetical variation. In
contrast, the three diploids appeared to be identical to
one another and to *R. canadensis*. This resemblance to *R.
canadensis* indicates that most or all of the chromosomes
contributed by the male gamete in the formation of the
original hybrid functioned to form the female gametes that
produced these seedlings. It would appear that fertilization
did not occur and the reduced egg became functional.

The leaves of the diploid seedling were narrow, dark
green and shiny in contrast to the tetraploid seedling
leaves which were broad, bluish green and dull. The leaf
apex in both types of seedlings was acuminate to long
accuminate.

The diploid primocanes were considerably more slender
than the tetraploid seedling primocanes and were practically free of spines. Spines on the primocanes of the tetraploid seedlings varied from 0 to 12 per decimeter of cane. Four of the tetraploid seedlings were as free of spines as the original R. canadensis.
SECTION V

DISCUSSION OF RESULTS

The validity of species designation for *Rubus canadensis* is supported by the ease with which it can be identified in its natural habitat. Distinguishing morphological characters were found to be clear cut and unmodified over a wide geographical range. The fact that all clones which were collected and identified as *R. canadensis* had on cytological examination, a somatic chromosome count of 21, lends further support to its acceptance as a species.

A study of clonal fertility, cane vigor, degree of spininess, winter hardiness and ability to reproduce by means of suckers showed that some variation does exist within the species. These important characteristics should be considered prior to selecting plants of this species for use in a breeding program. It is possible to select individual clones that possess good fertility and fruit quality, relative freedom from spines, a vigorous growth habit and the ability to produce numerous suckers. These characteristics make *R. canadensis* a valuable species for breeding purposes.

In the first attempt to hybridize *R. canadensis* in 1955 with the Snyder, Maple Grove and Geneva 32 blackberries, true hybrids must be concluded to have formed in the case of the two triploids, the pentaploid and the two unstables that were produced. The majority of the seedlings were, however,
tetraploid. The possibility of non-reduction of the female gametophyte must be considered before designating these tetraploid seedlings as true hybrids. An analysis of the chromosome counts made on root tips, and the plant morphology of the seedlings resulting from the self-pollination of one of these tetraploid seedlings indicates that the tetraploids were hybrids. This conclusion is based mainly on the presence of the three diploid seedlings which bore a close resemblance to R. canadensis. This indicated that most or all of the chromosomes contributed by the male in the formation of the original hybrid functioned to form the female gamete that produced these seedlings. It would appear that fertilization did not occur and the reduced egg in the F₁ tetraploid hybrid became functional.

The great variation between the F₂ tetraploid seedlings would also rule out reproduction by apomixis, and substantiates the above conclusion. In addition to the canadensis-like diploids, four of the tetraploid seedlings were virtually free of spines and in this respect resembled R. canadensis. It is assumed that the freedom from spines was transmitted by R. canadensis. This is further evidence to support the theory that hybridization occurred. The production of spine free tetraploid seedlings fulfilled one of the basic objectives of this study. These seedlings have not fruited and if they themselves are not commercially acceptable then they are of value for use in additional crosses for the
production of a spine free blackberry.

One also concludes from an analysis of the somatic chromosome constitution of the seedlings from the 1955 crosses that *R. canadensis* forms functional male gametes with varying chromosome number. In the tetraploid x triploid cross, it appeared that *R. canadensis* contributed male gametes having 7, 14 and 21 chromosomes. The variation observed in the size of pollen grains substantiates this conclusion. Hull and Britton (19) also assumed the variation in pollen grain noted in one of their polyploid seedlings was due to varying chromosome number. The somatic chromosomes in the root tips of these seedlings were carefully counted and the failure to find aneuploid seedlings could be attributed to the relatively small number of seedlings that grew to maturity from this cross.

The somatic chromosome number (Table 10) of the seedlings resulting from reciprocal crosses made in 1957 between *R. canadensis* and the Lowden, Hedrick and Bailey blackberries again demonstrated the difference that occurs between male and female gamete formation. The presence of triploid, tetraploid and pentaploid seedlings in the tetraploid-triploid cross indicates that microsporogenesis does occur with some regularity. In contrast, meiosis in the ovules of *R. canadensis* appeared to result only in unreduced gametes. The crosses between *R. canadensis* as the female parent and tetraploid blackberries resulted in triploid and pentaploid seedlings. The triploids were identical to the
female parent and were assumed to be apomicts. The penta-
ploids were true hybrids, indicating that this clone of
*R. canadensis* behaved as a facultative apomict. The
morphological differences between the seedlings and parents
are illustrated in Figs. 32-37.

Although there was considerable difference in the
morphology of the seedlings having the same chromosome
number and between seedlings of a different chromosome
number, the morphology of the leaves could be used to
separate some of the seedlings into groups of similar ploidy.
The diploids from crosses in which *R. canadensis* was the
male parent and the triploids and pentaploids from crosses
in which *R. canadensis* was the female parent were easily
separated on this basis. The triploids and tetraploids
from the latter cross could only be separated by a somatic
chromosome count. The lack of any resemblance to either
parent by many of the tetraploid seedlings supports the
conclusion that true hybrids have been formed.

One of the F₁ hybrids from a Hedrick × *R. canadensis* cross
which flowered in 1959 was found to be self-sterile and to
back-cross to either parent. Fully formed berries resulted
from the self-pollination and the back-cross to Hedrick.
The partially formed berries which resulted from the back-
cross to *R. canadensis* were typical of the fruit set that
results when this particular clone is self- or cross-
pollinated.

The self-pollinations of *R. canadensis* resulted in
triploid seedlings only. To date, no one has reported an aneuploid or a polyploid form of this species. On selfing, this species apparently reproduces only as an apomict. The somatic chromosome counts of seedlings resulting from self-pollination suggested that the seedlings did not result from the union of male and female gametes.

Aneuploid seedlings were not found in the populations resulting from reciprocal crosses between the triploid and tetraploid blackberries. Einset (11) found very few aneuploid blackberry seedlings from cross- and self-pollinations and Kerr (22) postulated that the low percentage of aneuploids was due in part to the failure of these seeds to germinate. Hull and Britton (19) reported the occurrence of numerous aneuploids from their blackberry crosses and considered it unusual.

It was noted that many of the cultured embryos resulting from the *R. canadensis* crosses failed to grow to a point where true leaves formed, although a small root and green cotyledons were apparent. Numerous albino seedlings were also noted. It is possible that a number of these seedlings were aneuploids.

The discovery of seedlings possessing somatic cells of unstable chromosome number is not unusual. Hull and Britton (19) reported mitotic instability in *Rubus* and stated that somatic instability is of practical significance to the plant breeder because of the replacement of the biennial canes by a root sucker making it difficult to maintain a
desired breeding line. There is also danger of introducing an unstable seedling which will become altered at a later date.

In addition to Rubus, mitotic instability has been reported in Ribes nigrum L. (36), Allium Cepa L. (20) and Hymenocallis (31).

The origin of somatic instability has not been satisfactorily explained. Huskins (20) noted in root-tip squashes that the chromosomes of Allium Cepa were capable of separating as entire chromosomes or individual chromatids and that these two processes were carried on simultaneously in the same nuclei. He also found that the spindle mechanism and astral rays were of secondary importance. Snead (31) concluded that chromosome variation in Helianthemum was due to a split spindle mechanism which was associated with a failure of cell walls to form during the mitosis which preceded meiosis.

The Ribes nigrum chromosome variants studied by Vaarama (36) in the meristems of young ovules and root tips were found to have numerous spindle abnormalities. One cell exhibited two spindles, which gave rise to a diminished chromosome number, and similar changes were thought to cause the varying chromosome number.

In their study of mitotic instability in root-tip squashes of Rubus, Britton and Hull (4) observed that in some instances, there was a distinct grouping of chromosomes into two or more groups during prophase, followed by the
formation of two or more spindles during metaphase. This "split metaphase" condition was characterized by the chromosomes in an individual cell becoming divided into two groups, each of which had its own spindle. Four groups of chromatids were formed at anaphase which resulted in at least three daughter cells. In some cells, however, there was no grouping of chromosomes, and only one spindle was observed. Where unequal chromosome numbers were found, it was thought that there had been an incomplete separation of chromosomes resulting in unequal numbers of chromosomes moving to the poles during anaphase.

In the present study, no evidence was found to support the occurrence of more than one spindle per cell. Only single metaphase plates were found. The great variation of chromosome numbers within a single root tip suggested that perhaps there had been an unequal distribution of chromosomes at anaphase. This variability might be attributed to the orientation of the chromosomes on the spindle. In some cells, one or more chromosomes were observed some distance from the main group. When the cell plate formed, the chromosomes that were unattached to the spindle became included in one of the resulting daughter cells. The smear technique used for this study prevented a study of the spindle, thus one could not draw conclusions on the part it played.

When sections of root tips cut longitudinally were examined, using Hematoxylin stain which shows the spindle,
no abnormalities were noted. On the basis of the 115 sections of the five root tips examined, there did not appear to be any evidence to support the idea of instability due to disturbances in the spindle mechanism or due to lagging chromosomes at anaphase. Nevertheless, it was felt that the examination of sections was too limited to discard the idea entirely. In addition, there was no indication of a particular chromosome number for any one section of the root, and therefore there was no evidence of chimeras in the tissue.

One possible explanation for the instability lies in the possibility of an aneuploid zygote which could conceivably occur in this tetraploid-triploid cross. Abnormalities could occur in the early divisions of the zygote. A continuation of such unequal divisions would lead to minor variations within sectors, but these could not be detected with the material studied.

Although the present study included a more intensive investigation of a single aneuploid plant than those previously studied, the results do not appear to support clearly either the idea of a chimaeral formation of aneuploid cells, or instability due to spindle abnormalities, although either or both of these appear to be the more probable causes.

The value of the seed culture technique for blackberries is not fully apparent from Table 3. Observations made at a later date on material not pertaining to this study
indicate that this is an excellent method for handling valuable material. Embryos which received a three month after ripening period at temperatures of 35 to 40°F, germinated nearly 100 per cent. The embryos reported in Table 3 did not receive this after ripening treatment. This method of germinating blackberry seed produces seedlings a year sooner than the method of sowing seeds in pots or flats and allowing nature to take its course.

A study of cross and self-fertility in *R. canadensis* provided additional information on the mode of reproduction of this species. The four self-pollinated clones noted in Table 6 were of variable fertility. The data on fruit set indicated that the pollen from Clone 6 was less functional than that of the other three clones. Einset (11) reported that seed development in *R. canadensis* was pseudogamous, thus the endosperm nucleus must be fertilized in order for apomictic development to occur. The data in Tables 4 to 7 show that fruit set is also dependent on the type of male gamete present. Table 8 provides further evidence of the variation that exists between clones. With but one exception, all clones produced the largest quantity of fruit when given an opportunity to cross-fertilize. Clone 3 and Clone 10 produced the largest number of entire fruits. One concludes that these more fertile clones would have the greatest value for plant breeding purposes. The variation in fertility between clones implies a genetical difference, while the difference in fertility between flowers on the
same plant suggests the influence of other factors.

Slate (30) reported that sterility in blackberries varied from season to season, from clone to clone and from cane to cane within a clone. He doubted that this variation was due to chromosome behavior or improper pollination because first test seedlings were not affected. It was noted only when the selections were moved into variety plantings for testing purposes. A similar condition was noted in Clone 12 in 1957 but in 1958 this variation in its fertility was not apparent.

Even if all clones of *R. canadensis* are assumed to be of apomictic origin, considerable morphological and genetical variation between clones could be expected. Crane (7) found that segregation occurred amongst the apomictic progeny of certain crosses in *Rubus*. Thomas (34) reported pronounced segregation in *R. vitifolius* and attributed it to chromosome crossing over followed by parthenogenetic development from a restitution nucleus. Whether *R. vitifolius* behaved as an apomict depended upon the ploidy of the pollen parent.

In a discussion of apomixis in *Rubus*, Stebbins (34) pointed out that various types of chromosomal and genetic changes can occur within apomicts. One type occurs through the pairing of chromosomes followed by crossing over in the megaspore mother cells with subsequent restoration of the diploid chromosome number in the egg through the formation of restitution nuclei. Mutations and aneuploidy will also cause variations between apomicts. The observed variation
In fertility and between morphological characteristics could be attributed to one or more of these factors.

Microsporogenesis of *R. canadensis* has been the subject of a very limited study. Longley (22) made a brief reference to it in his cytological studies in the genus *Rubus*.

He observed seven lagging chromosomes at anaphase I, but does not refer to any other stage. He concluded after studying several triploid forms of *Rubus* that 10 bivalents form at metaphase I and that due to lagging chromosomes, daughter nuclei with varying chromosome numbers are formed.

Einset (12) and Vaarama (36) did not include *R. canadensis* in their studies of microsporogenesis, although they describe other triploid forms in some detail.

In his study of three triploid forms of *Rubus*, Einset (12) concluded that at meiosis, variable numbers of trivalents, bivalents and univalents were formed. He observed that at anaphase I trivalents normally disjoin, two chromosomes going to one pole and one to the other. He also noted that univalents were present, lagged and were lost to the microspore nuclei in many instances. Einset (12) states "the gametes of triploids have variable numbers of chromosomes; theoretically they may range from the n number (7 in *Rubus*) to the 2N number (14)."

This study of meiosis in *R. canadensis* lends support to the conclusions drawn from the breeding behavior of the species. Somatic chromosome counts of seedlings from crosses in which *R. canadensis* was the male parent indicated
that functional 7, 14 and 21 chromosome gametes were produced. The examinations of the various stages has shown that 7, 14 and 21 chromosomes were present in the sporocytes. The sporocytes with 21 chromosomes occur because of asynapsis. The sporocytes with 7 and 14 chromosomes are the result of meiotic divisions.

A theoretical distribution of chromosomes based on the assumption that 7 chromosomes go to each pole and the other 7 move to the poles at random would produce less than 2 per cent of the sporocytes with 7 or 14 chromosomes. The occurrence of 8 or more pairs of synapsed chromosomes would indicate that an even lower percentage could be expected. The fact that functional gametes with 7, 14 and 21 chromosomes were produced suggests that gametes containing an unbalanced number of chromosomes abort as is evident by an examination of pollen grains (Fig. 28) or they fail to compete with the balanced gametes at the time of pollen germination and tube growth.

The presence of metaphase I sporocytes with 2, 14 or 5 chromosome configurations is difficult to explain. These configurations were not large so were not considered due to multivalent associations. The cell walls were intact, thus the mechanical eliminations of chromosomes due to the technique used in preparing the slides was considered unlikely. A degeneration of chromatic material appears to have taken place.

The results of this study of microsporogenesis in R.
canadensis are in close agreement with the results reported by Einset (12) and Vaarama (36) for other triploid forms of Rubus.

This study has shown that the apomictic triploid R. canadensis can be crossed with tetraploid commercial blackberries to form true hybrids that are fertile. There are no hardy spineless commercial blackberries at present and since R. canadensis is both hardy and spineless it can be considered very valuable as a breeding parent.
SUMMARY

Clones of *Rubus canadensis* were collected from widely separated geographical areas and were established at the Research Station, Kentville, N. S., for a study of their cytology and breeding behavior.

The clones were found to vary in cane height, cane diameter, plant vigor, degree of spininess and fertility. As a female parent, *R. canadensis* produced triploid and pentaploid seedlings. As a male parent it produced diploid, triploid, tetraploid and pentaploid seedlings as well as some seedlings with an unstable chromosome number. An F₂ generation of a tetraploid hybrid seedling produced spineless diploid seedlings, spiny tetraploid seedlings and spineless tetraploid seedlings.

Chromosome numbers in the root tip cells of an unstable seedling were found to vary from 15-44. Within a single root tip the chromosome number was extremely variable but no spindle abnormalities were noted.

At microsporogenesis, sporocytes with varying meiotic configurations were observed including some in which asynapsis had occurred. Pollen grains were variable in size and approximately 70 per cent aborted. Chromosome numbers of hybrid seedlings indicated that sporocytes with 7, 14 and 21 chromosomes were produced. The failure of some of the cultured seed to grow suggested the possibility that some
aneuploids may also have been produced.

The results of this study indicate that the very desirable characteristics of the facultative apomictic _Rubus canadensis_ can be transferred to cultivated blackberries through a proper breeding procedure.
BIBLIOGRAPHY


THE DISTRIBUTION OF CHROMOSOME NUMBERS
IN THE UNSTABLE SEEDLING
Figure 2-3. Somatic chromosomes in root tip cells of the Maple Grove and *R. canadensis* blackberry.

Fig. 2 - Photomicrograph of chromosomes of the tetraploid blackberry

Maple Grove (4N=28) x 8000.

Fig. 3 - Photomicrograph of chromosomes of the triploid blackberry *Rubus canadensis* L. (3N=21) x 8300.
Figures 4-7. Photographs showing morphological variation between sister seedlings from a Maple Grove x R. canadensis cross.

Fig. 4 - Seedling in which the unstable chromosome counts were found.

Fig. 5 - A triploid seedling (3N=21).

Fig. 6 - A tetraploid seedling (4N=28).

Fig. 7 - A pentaploid seedling (5N=35).
Figures 8-10. Metaphase plates of smear preparations of the root tip of the unstable plants of the cross Maple Grove x R. canadensis.
Fig. 8 - 41 chromosomes (x 2900).
Fig. 9 - 25 chromosomes (x 4860).
Fig. 10 - 31 chromosomes (x 4860).

Figures 11-13. Metaphase plates of sections of root tips from seedlings of a tetraploid x triploid (Lowden x R. canadensis) blackberry cross and from a triploid x tetraploid cross (R. canadensis x Lowden).
Fig. 11 - Somatic chromosomes of a diploid seedling (Lowden x R. canadensis) in which 2N=14 (x 2000).
Fig. 12 - Somatic chromosomes of a triploid seedling (R. canadensis x Lowden) in which 3N=21 (x 2000).
Fig. 13 - Somatic chromosomes of a pentaploid seedling (R. canadensis x Lowden) in which 5N=35 (x 2000).
Figures 14-16. Stages in the embryo culture of blackberry seed.

Fig. 14 - Continuous illumination of embryos growing in nutrient medium.

Fig. 15 - Close-up of seedlings growing in nutrient medium.

Fig. 16 - Seedlings transferred from nutrient medium to sterilized soil to which nutrients were added.

Fig. 17 - Metaphase I sporocyte showing 9 of the 11 configurations present. Configurations considered to be 1 trivalent, 8 bivalents, and 2 univalents (x 2000).

Fig. 18 - Metaphase I sporocyte showing 7 of the 9 configurations present. Configurations considered to be 3 trivalents and 6 bivalents (x 2000).

Fig. 19 - Metaphase I sporocyte showing 9 of the 9 configurations present. Configurations considered to be 6 trivalents and 3 univalents (x 2000).

Fig. 20 - Anaphase I sporocyte showing chromosomes lagging between polar groups (x 2000).

Fig. 21 - Sporocytes showing different metaphase II configurations and asynapsis (x 800).

Fig. 22 - Metaphase II sporocytes showing irregular configurations (x 2000).

Fig. 23 - Anaphase II sporocyte showing irregular position of chromosomes in one of the two groups within the sporocyte (x 2000).

Fig. 24 - Anaphase II sporocytes showing chromosomes lagging between polar groups (x 2000).

Fig. 25 - Telophase I sporocyte showing 3 lagging chromosomes scattered in cytoplasm between the polar groups (x 2000).
Fig. 26 - A sporocyte in which asynapsis has occurred (x 2000).

Fig. 27 - Two normal tetrads and one abnormal tetrad in which there is a small spore (x 800).

Fig. 28 - Collapsed and mature pollen grains. The mature pollen grains vary in size (x 2000).

Fig. 29 - Metaphase I sporocyte showing the 11 configurations present. Same cell as Fig. 17. Configurations considered to be $1^{iii}$, $8^{ii}$, $2^1$ (x 6600).

Fig. 30 - Metaphase I sporocyte showing the 9 configurations present. Same cell as Fig. 18. Configurations considered to be $3^{iii}$ and $6^{ii}$ (x 6600).

Fig. 31 - Metaphase I showing 9 of the 9 configurations present. Same cell as Fig. 19. Configurations considered to be $6^{iii}$ and $3^1$ (x 6600).
Figure 29

Figure 30

Figure 31
Figures 32-37. Morphological variation in leaves from Rubus plants of different somatic chromosome number.

Fig. 32 - Triploid Rubus canadensis (2N=21).

Fig. 33 - Tetraploid blackberry variety Lowden (2N=28).

Fig. 34 - Pentaploid seedling from Lowden x R. canadensis cross (2N=35).

Fig. 35 - Tetraploid seedling from Lowden x R. canadensis cross (2N=28).

Fig. 36 - Triploid seedling from Lowden x R. canadensis cross (2N=21).

Fig. 37 - Diploid seedling from Lowden x R. canadensis cross (2N=14).