

COMPATIBILITY AND EMERGOLOGICAL STUDIES
OF SPECIES CROSSES IN THE AZALEAS

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INTRODUCTION

Azaleas, at present one of the most popular ornamental plants, are members of Rhododendron subgenus Anthodendron Endl. Of the four sections in the subgenus, section Tsutsutsi G. Don is the most important since not only the azaleas used for greenhouse forcing but also those that are grown in the majority of the gardens in the Middle Atlantic States and the Deep South are hybrid derivatives of this section. These species are native to the Orient exclusively, twenty-three being described in Wilson and Rehder's monograph of the subgenus (26). From these, hundreds of named varieties have been obtained through interspecific hybridization and breeders are continually attempting to improve on the older races by extending the range of climatic adaptability as well as to select for variations in flower type.

The completely deciduous azaleas comprise the remaining three sections, Pentanthera G. Don, Sciadorhodion Rehder and Rhodora G. Don. Of these, section Pentanthera approaches section Tsutsutsi in importance, for here are found the species and their horticultural varieties that are sufficiently hardy to withstand winters where other azaleas, especially those in the section Tsutsutsi, are likely to be winter-killed. Hybrids in the section Pentanthera have resulted from numerous interspecific crosses involving species from North America, the Orient and Eurasia and are collectively referred to as the Ghent and the Mollis hybrids. Unfortunately, their distribution is limited by the fact that they are difficult to propagate vegetatively as well as not being suitably adapted to areas where high summer temperatures and sustained dry periods prevail. The azaleas in the sections Sciadorhodion and Rhodora are of limited ornamental

value and cultivated only as true species, hybrids which do exist considered to be only of academic, not horticultural, value.

Since hybrids derived from intersectional crosses, particularly involving sections Tsutsutsi and Pentanthera, might increase the color range of the flowers, the climatic adaptability and alter plant habit, most azalea breeders have attempted such crosses at one time or another, usually without success. Only one existing azalea race, "Vuykiana" (25), is described as resulting from such a breeding program. The seedlings of the several crosses that were involved are described as being more vigorous but otherwise resembling the mother plants, all of which were species from the section Tsutsutsi. Pollen parents in every cross were hybrids from the section Pentanthera but were perfectly compatible with species from the section Tsutsutsi. Lee, et al.(9), suggested that, on the basis of such a similarity to the seed parents, these azaleas might be apomictic rather than sexual in origin. Wilson and Rehder (26) described several intersectional hybrids between species in the sections Pentanthera and Rhodora but these are evidently no longer in cultivation. Notes on crosses between the subgenera Anthodendron Endl. and Eurhododendron Endl. were also compiled by Wilson and Rehder. Although the progeny of such crosses are mostly sterile, they are in limited cultivation by means of vegetative propagation and are commonly known as "azaleodendrons."

Even though genetic or physiological irregularities in the progeny of intersectional crosses are common knowledge, there are few discussions of the problem in print. Noguchi (13) observed the behavior of forty-six intersectional Rhododendron crosses involving species native to the Orient. Complete failure to obtain viable seed or seedlings that exhibited varying degrees of chlorophyll defects resulted from the crosses between sections Tsutsutsi and Pentanthera. Eventually these offspring died,

none developing beyond the seedling stage. Bowers (1), Morrison (9) and Sax (20) also noted that progeny with chlorophyll deficiencies were obtained from crosses of an intersectional nature and that the seedlings would not survive.

Sax (19) and Nakamura (12) studied the cytology of Rhododendron with emphasis on chromosome numbers for various species, mostly azaleas. All species examined were diploids, $2n=26$, with the exception of two azaleas, R. calendulaceum Torrey and R. canadense Torrey, which were tetraploids, native to North America. On the basis of pairing of the chromosomes of known hybrids, Sax concluded that there was no cytological basis for incompatibility. He considered that, although the species utilized had been "separated geographically for millions of years," their genetic makeup must be very similar to permit them to function normally as interspecific hybrids.

The morphology and embryology of the order Ericales, to which Rhododendron belongs, has been studied and utilized by several workers to provide information on interordinal relationships as well as to formulate a system of classification within the order. Copeland (4,5), Chou (3), Cox (6), Kavaljian (8) and Palser (14,15) have provided many of the details of floral morphology and comparative anatomy for the Ericales and some of the studies considered the development of the seed. Unfortunately, none of these efforts were directed toward Rhododendron and developmental stages between the mature ovule and the mature seed have not been described, although Pelsitrot (16) reported on the mature seeds of R. molle G. Don and R. japonicum G. Don. Palser (15) conducted studies of a developmental nature on the megagametophyte in the Andromedeae and also described the early endosperm development and embryogeny for Cassiope

hypnoides G. Don (14), but none of the studies were in relation to breeding behavior. Maheshwari (11) summarized the standard stages of Erican embryology, listing twelve "well-marked embryological features" that are presumed to occur throughout the order. With respect to pollen tube development and events leading to fertilization in Rhododendron, information is sparse. Bowers (1) stated in a footnote that pollen tubes required twenty-four hours to attain the length of the style of R. maximum L. and noted certain details of self-incompatibility in that species attributed to pollen tube failure, but no similar data are available for any azaleas.

It is the purpose of this thesis to:

1. Obtain, study and describe progeny of intersectional crosses involving sections Tsutsutsi, Pentanthera and Sciadorhodion.
2. Repeat certain previous crosses as a basis of comparison for previously unreported combinations.
3. Ascertain at what stage of development failure occurs if hybrids are not derived from the attempted crosses.

MATERIALS AND METHODS

The Rhododendron collection at the U. S. Plant Introduction Garden, Glenn Dale, Maryland, contains representatives of all four sections of Rhododendron subgenus Anthodendron Endl. Thirteen species¹ were used to make intrasectional² and intersectional crosses in this study. Those species used from the section Pentanthera, native to the United States, are part of the collection made in the wild by C. O. Erlanson in 1936, then Botanist, Division of Plant Exploration and Introduction, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture. The exotic species used are either seed or plant introductions, authenticated as to origin and identity in the records of the Division of Plant Exploration and Introduction. The following species were used:

Rhododendron section Tsutsutsi G. Don

<u>Rhododendron</u>	<u>indicum</u>	Sweet
<u>R.</u>	<u>linearifolium</u>	var. <u>macrosepalum</u> Makino
<u>R.</u>	<u>mucronatum</u>	G. Don
<u>R.</u>	<u>obtusum</u>	var. <u>kaempferi</u> Wilson

¹ Nomenclature follows that established by Wilson and Rehder (26).

² Throughout this paper "intrasectional" will refer to species crosses within the section; "intersectional" refers to species crosses between different sections; "pollination" indicates the actual application of pollen to a stigma; and "cross" implies the attempt to hybridize between two species.

Rhododendron section Pentanthera G. Don

<u>R.</u>	<u>alabamense</u> Rehder
<u>R.</u>	<u>atlanticum</u> Rehder
<u>R.</u>	<u>canescens</u> Sweet
<u>R.</u>	<u>japonicum</u> Suringar
<u>R.</u>	<u>luteum</u> Sweet
<u>R.</u>	<u>molle</u> G. Don

Rhododendron section Sciadorhodion Rehder and Wilson

<u>R.</u>	<u>reticulatum</u> G. Don
<u>R.</u>	<u>schlippenbachii</u> Maximowicz
<u>R.</u>	<u>weyrichii</u> Maximowicz

Since style length differences might be regarded as a reason for incompatibility, ten styles of each species were measured from stigmatic end to the top of the ovary and the average length noted, particularly in relation to the reciprocal crossability of species.

During the outdoor flowering period and in the greenhouse, 1948-52, flowers were emasculated prior to anthesis and the corolla of each flower completely removed. All adjacent buds and open flowers not emasculated were discarded. According to Morrison (9) observations over many years have indicated that bagging of azaleas is not needed, especially if the corolla is removed. However, additional protection was provided against natural cross-pollination when materials were to be used for embryological studies by placing a #6 sipping straw down over the pistil and peduncle. When the stigmas were receptive, intraspecific, intrasectional and intersectional pollinations were made. One hundred and eight crosses were attempted, of which sixty-three were intersectional (Table 1). From five to fifteen pollinations were made for each cross, intrasectional crosses being limited to five pollinations in order that a large number of intersectional pollinations could be made.

TABLE 1. Number of azalea crosses and pollinations in relation to the different sections involved.

Sectional Parentage*	No. Crosses	No. Pollinations
<u>Tsutsutsi</u> x <u>Tsutsutsi</u>	16	80
<u>Tsutsutsi</u> x <u>Pentanthera</u>	20	260
<u>Tsutsutsi</u> x <u>Sciadorhodion</u>	4	20
<u>Pentanthera</u> x <u>Pentanthera</u>	24	120
<u>Pentanthera</u> x <u>Tsutsutsi</u>	20	255
<u>Pentanthera</u> x <u>Sciadorhodion</u>	6	30
<u>Sciadorhodion</u> x <u>Sciadorhodion</u>	5	25
<u>Sciadorhodion</u> x <u>Tsutsutsi</u>	7	35
<u>Sciadorhodion</u> x <u>Pentanthera</u>	6	30
	108	855

*Actual species crosses shown in Text Figure 2.

Capsules that developed to maturity were collected in October and the seeds sown the following February in sphagnum moss. The intrasectional crosses in Tsutsutsi were made in 1948 and 1951, those in the section Pentanthera in 1949-1952, and those in the section Sciadorhodion in 1950-52.

Some intersectional crosses were made each year from 1948 to 1952. The crosses that were made annually depended on the availability of both pollen and pistils, which was governed by bud survival following adverse winter conditions. Intrasectional progeny in sections Pentathera and Sciadorhodion, as well as progeny from all intersectional crosses, have been grown for further observations. Other seedlings were discarded shortly after germination was noted.

Since seedlings with chlorophyll deficiencies were obtained from some intersectional crosses, observations were made in relation to their survival and subsequent development. In order to determine if such seedlings could

survive to maturity, albino progeny of two intersectional crosses were sprayed with 10% sucrose and 0.5% ferrous sulphate solutions. Other albino seedlings were grafted onto green seedlings of the same age, using a sharpened, knifelike needle, performing the operation under a 7x dissecting microscope. Grafted seedlings were placed in petri dishes on sterilized sphagnum moss at 72 degrees Fahrenheit.

Almost complete failure to obtain mature capsules following intersectional pollinations indicated the need to examine the stages immediately following pollination, particularly comparing them with normal development. For this study, R. japonicum and R. mucronatum were used. Intraspecific crosses and the reciprocal crosses were made with these species and five to seven pistils were collected at anthesis and at a predetermined schedule of time following pollinations. Pistils were collected at 24 hourly intervals from anthesis up to 144 hours; at 48 hourly intervals from 144 hours to 336 hours; and finally, 96 hourly intervals from 336 hours to 576 hours.

Pistils were killed in CRAF solution, dehydrated in an ethyl-alcohol tertiary-butyl alcohol series and embedded in paraffin. Sections were cut at 12 microns and stained with Paragon hematoxylon, counter-stained with fast green. Observations were made with 10x and 45x dry objectives and 60x and 90x apochromatic oil immersion objectives, with 10x and 20x compensating oculars.

RESULTS

BREEDING BEHAVIOR

a. Observations on style length

The measurements to determine the variations in style lengths of individual species of azaleas showed that considerable difference occurred between species (Text Figure 1), particularly in sections Tsutsutsi and Sciadorhodion but to a lesser extent in Pentanthera.

TEXT FIGURE 1

Average Style Length Of Some Azalea Species

	Length (mm.)
R.reficulatum	29.5
R.schlippenbachii	36.1
R.weyrichii	41.2
R.alabamense	43.3
R.atlanticum	46.5
R.canescens	45.2
R.japonicum	46.1
R.luteum	52.8
R.molle	45.6
R.indicum	30.8
R.linearifolium macrosepalum	48.1
R.mucronatum	47.3
R.obtusum kaempferi	34.7

Such variations might be presumed to be a limiting factor in species compatibility, but, when the results of the intrasectional crosses are examined, these differences no longer have any value. For example, R. indicum

with a style averaging 30.8 mm. in length is perfectly compatible with R. mucronatum (ave. style length - 47.3 mm.), irrespective of which species is used as the seed parent. Styler length was used, however, as one basis for selecting R. mucronatum and R. japonicum for embryological studies since both have approximately the same style length.

b. Range of species compatibility

The results from crossing a wide range of species both within and between sections showed that species crossability is determined on a sectional basis (Text Figure 2). Out of forty-six intrasectional crosses, only one did not produce normal seedlings, R. weyrichii x R. reticulatum, but it will be noted from Text Figure 2 that when the cross was repeated, the seedlings were normal. No crosses of an intrasectional nature in sections Tsutsutsi and Pentanthera produced abnormal seedlings. Text Figure 2 also shows that only five intersectional crosses produced viable seed, all of which resulted in abnormal seedlings of one kind or another. It will further be observed from Text Figure 2 that the only successful crosses between sections involved Tsutsutsi and Pentanthera, those intersectional crosses attempted with section Scidorhodion failing in every attempt. Of the successful crosses between sections Tsutsutsi and Pentanthera, the number of capsules that resulted and the kinds of seedlings derived are shown in Table 2. Only the cross R. obtusum var. kaempferi x R. japonicum produced seedlings that were either chlorophyll deficient or normal green, all other crosses resulted in entirely ^{chlorophyll} deficient seedlings or entirely normal seedlings.

TABLE 2

Results of intersectional azalea crosses on the basis of capsules producing viable seed and seedling cotyledon types.

Parentage	Pollinations Capsules	Seedling Types (Cotyledon color)
<i>R. canescens</i> x <i>R. mucronatum</i>	15/3	white-pale green
<i>R. japonicum</i> x <i>R. obtusum kaempferi</i>	15/2	white-yellow
<i>R. mucronatum</i> x <i>R. japonicum</i>	10/1	white
<i>R. obtusum kaempferi</i> x <i>R. japonicum</i>	10/3	white; green
<i>R. linearifolium macrosepalum</i> x <i>R. luteum</i>	10/3	green

TEXT FIGURE 2

Results From Interspecific Azalea Crosses

	SECTION Sciadorhodion			SECTION Pentanthera						SECTION Tsutsutsi			
	<i>R. reticulatum</i>	<i>R. schlippenbachii</i>	<i>R. weyrichii</i>	<i>R. alabamense</i>	<i>R. atlanticum</i>	<i>R. canescens</i>	<i>R. japonicum</i>	<i>R. luteum</i>	<i>R. molle</i>	<i>R. indicum</i>	<i>R. mucronatum</i>	<i>R. obtusum kaempferi</i>	<i>R. linearifolium macrosepalum</i>
○ = no seed set													
● = normal seedlings	●	●	●	●	●	●	●	●	●	●	●	●	●
◐ = abnormal, died													
◑ = abnormal, lived													
♀ = apomictic?													
<i>R. reticulatum</i>	●	●	◐						○				
<i>R. schlippenbachii</i>		●		○	○	○		○		○	○	○	○
<i>R. weyrichii</i>	●		●						○				
<i>R. alabamense</i>		○		●	●	●	●	●		○	○	○	○
<i>R. atlanticum</i>		○		●	●	●	●	●		○	○	○	○
<i>R. canescens</i>		○		●	●	●	●	●		○	○	○	○
<i>R. japonicum</i>				●	●	●	●			○	●	♀	○
<i>R. luteum</i>	○			●	●	●		●		○	○	○	♀
<i>R. molle</i>	○		○						●				
<i>R. indicum</i>		○	○	○	○	○	○	○		●	●	●	●
<i>R. mucronatum</i>		○	○	○	○	◐	○	○		●	●	●	●
<i>R. obtusum kaempferi</i>		○		○	○	○	●	○		●	●	●	●
<i>R. linearifolium macrosepalum</i>		○	○	○	○	○	○	○		●	●	●	●

c. Behavior of abnormal seedlings

Only R. weyrichii x R. reticulatum, a new hybrid in the section Sciadorhodion, produced seedlings that were abnormal other than in relation to chlorophyll development. These seedlings were entirely green after emergence but shortly after the first true leaves appeared exhibited a severe dying back of the shoots. This was followed by new growth from the base of the plant that developed to a greater height than had the previous shoots only to suddenly repeat the necrosis of leaves and twigs. Seedlings from all other intrasectional crosses grew normally under identical cultural conditions. Although the defective seedlings are now three years old, the sequence of development followed by collapse of the stems has continued.

The intersectional crosses which actually produced viable seeds resulted in two kinds of abnormalities (Table 2). Either seedlings were deficient with respect to chlorophyll development or they were normally green but, on flowering, showed no phenotypic basis for being considered sexual in origin. The cross R. canescens x R. mucronatum is a new hybrid combination and the only one that produced chlorophyll defective seedlings that lived. The three capsules obtained from this cross produced over two hundred seedlings that ranged in color from white to pale green. Of these, all died except twenty-one which continued to exhibit abnormalities as they grew. These were also seedlings which were least chlorotic in appearance. As the seedlings developed, growth from the terminal bud ranged from white to pink, frequently with a green midrib to each leaf. (Fig. 1). In addition, white shoots developed at the base of the plants. Generally, white shoots, either from the terminal or the base of the plant, turned green during the summer or shriveled and died. Consequently, when

the seedlings had completed their annual development, they were always green. However, although the old leaves remained green during the winter, new shoots that were produced in the spring were again white or pink in all seedlings except two; these are discussed separately.

Although deviations from normal green color was the most striking defect, displayed in some manner by all but one seedling, other defects were noted. The one seedling which was entirely green after the first chlorosis disappeared has remained stunted to the extent that during three years of development, it has produced only a rosette of leaves and the plant is less than one inch across and almost prostrate.

Another seedling of the cross R. canescens x R. mucronatum formed a flower bud which developed to the point of anthesis but then aborted. When dissected, the four flowers had eight to ten stamens and pollen tetrads were found in the anthers. Since the seed parent is from the section Pentanthera which (as the name indicates) is characterized by having only five stamens throughout the section while the pollen parent is described as having eight to ten stamens, the probability of a sexual hybrid is excellent. Furthermore, the leaves were semi-persistent during the winter and characterized by a pubescence that was intermediate between the two parents. Even this seedling, although perfectly green the previous year, produced typical white shoots at the base of the plant in the spring of 1953 (Fig. 2).

Two crosses produced seedlings that died while in the cotyledon stage, R. japonicum x R. obtusum var. kaempferi and R. mucronatum x R. japonicum. In these combination, seedlings were either white or yellow. Both lots of seedlings were sprayed with sucrose and iron sulphate but no beneficial effects were noted. Grafting onto seedlings of R. mucronatum

also failed but this was possibly due to inadequate technique even though it had been possible to join the cut ends of the seedlings together.

Of the crosses that produced green seedlings, R. obtusum var. kaempferi x R. mucronatum also gave an extremely high percentage of defective seedlings. Only five seedlings, presumably apomictic, survived out of a total initial progeny of two hundred or more seedlings. The green seedlings developed at a normal rate and showed no deformities. On flowering, there was no evidence of influence of the pollen parent and the foliage was persistent during the winter, which is typical of the seed parent. From the cross, R. linearifolium x R. luteum, only green seedlings were obtained and at no time suggested paternal influence. The only peculiarity of this particular cross was that the capsules had dried and split in early August and, when sown immediately, resulted in perhaps three hundred seedlings. All other capsules on the plant, not hand pollinated, were entirely green at that time.

If the number of capsules with viable seeds from the cross R. canescens x R. mucronatum is compared with the number of pollinations, it shows that only 20% "took" (Table 2). Normally, from fifteen pollinations, some three thousand or more seedlings might be expected but from this particular cross, which represents the most successful attempt at intersectional hybridization between Tsutsutsi and Pentanthera to date, only twenty-one seedlings survived beyond the cotyledon stage, reducing the survival rate to approximately 0.7% of normal expectations.

In addition to the limited number of crosses that did produce viable seed, a number of intersectional crosses developed partially mature capsules (Fig. 3). These were dry and generally dehisced by midsummer, those from the cross R. mucronatum x R. japonicum beginning to split in late

July (Fig. 3). Other capsules did not enlarge but remained on the plant most of the summer, such as those from R. japonicum x R. mucronatum (Fig. 3). Crosses that involved the section Sciadorhodion generally resulted in immediate shriveling of the pistil and abscission shortly thereafter.

EMBRYOLOGICAL STUDIES

a. General organography

The pistil of R. japonicum consists of a five-lobed stigma with clefts between each of the lobes (Fig. 4) that merge into an open channel traversing the length of the style and leading through passageways directly to each locule of the ovary. Each of the five locules of the ovary contains about fifty or sixty anatropous ovules, borne on the outer surface of a massive placenta which extends from a central column deeply into the cavity of the locule, thus placentation is essentially axile. R. mucronatum did not vary noticeably except that the passageways of the ovary were irregular in outline while those of R. japonicum were straight, smooth channels.

The ovule of both species is unitegmic, consisting of an epidermal layer clearly differentiated by the depth of staining, due to tanniferous materials reported contained in the cells. Four rows of loosely arranged cells form the body of the integument and these are inwardly contained by an endothelial layer made up of cells oriented at right angles to the long axis of the ovule (Fig. 5). The endothelium surrounds the chalazal end and the main body of the embryo sac but disappears as it reaches the micropylar end. The nucellus has disorganized prior to maturation of the embryo sac which therefore comes in direct contact with the endothelium.

The megagametophyte (Fig. 5) is a typical eight-nucleate, seven-celled body in which the egg apparatus lies in the somewhat constricted

micropylar end of the embryo sac with the egg and the synergids arranged in the form of an inverted T. The polar nuclei are located at the mid-section of the cavity, lying side by side and unfused. The antipodals are arranged at the chalazal bend of the ovule, generally in the form of a T. The embryo sac is thus a narrow, lineate body comprising the greater part of the ovule in a lengthwise manner.

b. Events following intraspecific pollination

The events following intraspecific pollination with R. japonicum and R. mucronatum are similar in detail but will be discussed separately, except for the initial, general observations.

Pollen of Rhododendron consists of tetrads held together by viscous, non-cellular threads. When pollen was applied to the stigma, the germinating pollen tubes passed through the clefts of the stigma and proceeded down the hollow style and into the ^{lum} of the ovary (Figs. 4,6). In R. japonicum x R. japonicum, pollen tubes were found in the passageways of ovaries examined at 96 hours after pollination and consistently thereafter. At 576 hours after pollination, pollen tubes showed no visible evidence of disintegration although the gametes presumably had been discharged considerably earlier. The number of pollen tubes increased up to 144 hours following pollination since styles did vary in length (from 42 mm. to about 50 mm.). Penetration of the ovule is porogamous and as a result of the pollen tube entrance into the micropyle, that end of the embryo sac was darkly stained, presumably due to the remnants of the pollen tube and contents (Fig. 6). Fertilization was considerably obscured but one or both synergids were destroyed in the process and secondary fusion could be observed in considerable detail, leading to the formation of the primary endosperm nucleus. The polar nuclei, unfused prior to fertilization (Fig.7), merged contents while in the resting stage; a fusion body was observed that

included two nucleoli and the immersed sperm (Fig. 8). Subsequently, the developing endosperm mother cell was observed to have acquired a third nucleolus, that contributed by the male nucleus (Figs. 9,10), and finally emerged with a single, large nucleolus as a result of the fusion of the three individual nucleoli (Fig. 11). Syngamy and triple fusion were completed in most ovules prior to 192 hours after pollination.

After fertilization, the zygote undergoes no immediate divisions, the endosperm begins to divide immediately and the antipodals remain intact until the endosperm has divided several times. Since observations relating to the mode of initial endosperm division is at variance with earlier reports for other Ericales, results are described in detail.

In R. japonicum, the endosperm formation may be divided into two main series of events, the first division and the second paired set of divisions unaccompanied by intervening wall formation but rather by appearance of wall after the quartet of nuclei have assumed a linear formation; subsequent divisions of the cellular type were observed, in which wall formation followed each of the divisions of the nuclei.

The pair of nuclei resulting from the division of the primary endosperm nucleus divided again immediately. On the basis of observations made here, the micropylar member of this pair probably divided first (Figs. 12,13), if the size of the three nuclei observed in Figure 13 is of significance. The three resulting nuclei were observed in a linear arrangement at first but, by the time four nuclei were evident, a diamond-shaped or box-like arrangement had been assumed (Figs. 14,15). No intervening wall formation could be noted and it would have been difficult to account for in view of the arrangement of this quartet of nuclei. At 240 hours following pollination, the endosperm nuclei were found in various

stages of migration that must have occurred in order to attain the typical linear arrangement of the endosperm (Figs. 16,17). Finally, the nuclei were observed as late as 288 hours extended in a linear arrangement from the chalazal end of the embryo sac to the micropylar and walls partitioning the nuclei were evident at right angles to the long axis of the ovule (Figs. 18,19). Subsequent endosperm divisions clearly showed walls, oriented both laterally and longitudinally (Figs. 21,24). At the most advanced stage of development observed (576 hours after pollination), the endosperm consisted of two or more cells at the micropylar end of the embryo sac and a comparable group at the chalazal end, about as long as wide while those endosperm cells at the center of the embryo sac were usually long and narrow, oriented parallel to the main axis (Fig. 21).

The embryo, meanwhile, had made only limited growth and at 576 hours consisted of an elongate body extending out of the micropylar constriction into the main body of the ovule cavity. Some divisions had occurred but developmental details could not be described other than that the first division of the zygote was probably transverse, on the basis of the arrangement of the nuclei (Fig. 20) and occurred about 240 hours after pollination.

Other features concurrent with growth of the embryo and endosperm that distinguished the fertilized ovule were the well developed epidermis and a widening of the cavity of the ovule, crushing the main body of the integument and the assumption of the typical "winged" appearance of the mature seed. Seeds observed in the proper orientation were strongly flattened and elongate.

Rhododendron mucronatum followed a similar pattern of development but details were less apparent due to difficulty involved in sectioning.

The ovary of this species is covered with appressed setose hairs that greatly retard penetration of fixative. Pollen tubes were found in the ovaries between 96 and 144 hours after pollination and the early divisions of the endosperm resulted in the same box-like arrangement of nuclei (Figs. 22,23) that was noted in R. japonicum. Endosperm nuclei were later observed in a linear arrangement followed by the typical cellular type of development (Fig. 24). In Figure 24, three micropylar endosperm cells can clearly be noted as well as the elongate cells of the main body. Evidently, the divisions of the nuclei at the micropylar end occurs prior to that in the center of the cavity. Although no developmental material was suitable to study the embryo, capsules of this species, as well as others, were collected in mid-August, artificially dried and ^{the seed} sown immediately. Cotyledons of the emerging seedlings were completely differentiated yet abnormally small, and it is evident that development of the embryo takes place fairly rapidly subsequent to 576 hours after pollination.

The results of the intraspecific pollinations of R. japonicum and R. mucronatum were conclusively similar both as to rate of development and mode. Normal pollen tube growth followed by fertilization and growth response was observed in all ovaries examined. In both species, early endosperm development followed the nuclear type of development up to the four-nucleate stage and all later divisions were typically cellular.

c. Events following intersectional pollination

When pistils following the pollinations of R. japonicum x R. mucronatum and R. mucronatum x R. japonicum were examined, pollen had germinated and pollen tubes were found in the styles. When ovaries of R. japonicum were examined up to 576 hours after pollination, no pollen tubes had entered the ^{the} lum/ and were not found in the basal portion of the styles. Average pene-

tration was about half the length of the style and the farthest that pollen tube penetration was noted in this species was 25 mm. from the stigma. A total of 38 ovaries was examined without finding evidence of fertilization. Ovules in such instances showed no development, the egg remained intact and the polar nuclei were always unfused. None of the ovules showed the developmental changes associated with fertilization such as increase in size and intensification of staining of both ovules and ovarian tissue. Ovaries collected at 96 and 336 hours following pollination are shown in Figures 25 and 26.

Pistils of R. mucronatum following intersectional pollinations were similar in that 28 ovaries showed no pollen tube penetration and the ovules remained undeveloped, the polar nuclei unfused and the egg intact (Fig. 27). At 432 hours after pollination, one ovary was observed with pollen tubes in the passageways. Ovules from this material were observed in two conditions; either they were typical of other unfertilized ovules or development was advanced to a stage comparable to that in normal circumstances (Fig. 28). It will be observed that the ovule figured was well along in development, the endosperm having initiated cellular division. Since earlier instances of pollen tube penetration were not found, the developmental pattern of embryo and endosperm could not be determined. When this observation is compared with results of breeding attempts, it will be noted that this cross (R. mucronatum x R. japonicum) produced viable seed. Failure to find pollen tubes in the ovaries following the pollination of R. japonicum x R. mucronatum coincides with the negative results obtained for breeding attempts. Failure to find any appreciable development of the ovules and ovary even though pollen tubes were present in the styles agrees with the observation (Fig. 3) that when the cross R. japonicum x R. mucronatum was attempted, the ovaries did not enlarge.

DISCUSSION

The multitude of azalea varieties that have been developed in the past century indicate the ease with which species in the same taxonomic sections hybridize, at least when the two large sections Tsutsutsi and Pentanthera are considered. The other two sections, Sciadorhodion and Rhodora, include relatively few species and these have resulted in few hybrids. The cross, R. weyrichii x R. reticulatum and its reciprocal reported in this investigation, may be the first successful attempt to hybridize in the section Sciadorhodion. However, it should be recalled that this cross also gave rise to defective seedlings. Of added evidence is the fact that R. schlippenbachii, also in the section Sciadorhodion, has never been successfully used in any interspecific cross, although it is perfectly self-fertile. Morrison (9) obtained negative results when he attempted to cross R. schlippenbachii with R. reticulatum and, at this writing, no hybrids between R. schlippenbachii and R. weyrichii have been reported.

The entire mass of evidence from hybridization experiments substantiates the taxonomic and morphological observations utilized to assign species to the sections Tsutsutsi and Pentanthera. In the section Sciadorhodion, on the other hand, the magnitude of species alliances is of a different order at least with regard to crossability. Furthermore, Sciadorhodion is a relatively new section and includes species previously placed in the section Tsutsutsi [Wilson and Rehder (26)]. Bowers (1) regards Sciadorhodion as more closely allied to Rhodora than to the other sections, but species crosses of a Sciadorhodion-Rhodora complex have not been sufficiently studied to make any valid deductions.

Cytological studies shed no light on the subject other than to confirm the general stability of the entire genus Rhododendron. Except for

the two tetraploids, R. calendulaceum and R. canadense, all species studied by Sax (19) were diploid. Furthermore, crosses between Asiatic and North American species in the section Pentanthera were made with such ease when the geographical barrier was artificially overcome that a general conclusion may be drawn with respect to the similarity of genetic make-up.

Considering this positive intrasectional compatibility, one might have anticipated only minor difficulties when crosses were attempted between azalea sections, especially since the known hybrids between azaleas and broad-leaved Rhododendrons are actually subgeneric crosses and have been in cultivation for many years. Between the two most valuable sections, Tsutsutsi and Pentanthera, only the race "Vuykiana" has been reported although Lee (9) and Skinner (23) have regarded this race as apomictic on the basis of appearance and the backcrossing experiments reported by Skinner. Furthermore, the few normal green seedlings obtained here from the cross R. obtusum var. kaempferi x R. japonicum showed no pollen parent influence even though they were a part of a progeny that was practically all chlorophyll deficient.

Although species from the section Sciadorhodion have been used in intersectional crosses, it will be noted that no seed formed irrespective of whether they were used as pollen or seed parents in this investigation. Noguchi (13), however, did obtain seedlings from the cross R. schlippenbachii x R. japonicum although they died shortly after emergence.

The evidence, reviewed above, indicates that the seedlings representing the cross R. canescens x R. mucronatum are the only valid sexual hybrids between sections Tsutsutsi and Pentanthera, and even these seedlings are deficient to such a degree that their full value has not been realized even at the age of three years.

BARRIERS TO INTERSECTIONAL HYBRIDIZATION

Several barriers to intersectional hybridization were noted in the present investigation and may be classed as: those affecting development of normal seedlings; and those that prevent normal seed production.

a. Abnormal seedlings as barriers to intersectional hybridization

The most striking defect that occurred in seedlings following intersectional crosses is chlorophyll deficiency. In every attempt reported, this behavior has been observed [Bowers (1), Morrison (9), Noguchi (13), and Sax (20)]. Defective seedlings were also obtained in the present study. These usually died soon after they emerged except for those normal green seedlings which were considered to be apomictic and, until the present paper, no study has reported survival.

Riley (18) and Sharp (22) have summarized the status of chlorophyll inheritance, particularly in relation to chlorophyll deficiencies. Either a direct contact of various genes, probably all recessive or non-Mendelian inheritance, may account for albino seedlings. Many genes for albinism are known in corn and this type of inheritance is probably responsible for the albino and virescent azalea seedlings, since the distinction between normal green and deficient growth is quite sharp, no leaves having a mosaic pattern of green and white as a non-Mendelian might account for.

Dwarf progeny also occurred following intersectional hybridization. One seedling that was entirely green, although three years old, has grown no larger than normal seedlings several weeks old. In seedlings that are chlorotic, stunted growth could be expected but in the green seedling dwarfing is probably the result of genetic incompatibility.

Judging from the results obtained here, normally developing, green seedlings from intersectional azalea crosses can arbitrarily be considered as apomictic while those marked by chlorophyll defects and dwarf habit

can be classed as sexual in origin. Final determination depends on the appearance of the flowers and the results of backcrossing to both parents successfully.

b. Failure to obtain viable seed as a barrier to intersectional hybridization

Following intersectional pollination, a low number of crosses produced viable seed and in many crosses the capsules did not even mature. Failure of pollen tubes to enter the ovary is the main limiting factor, and only in one instance were pollen tubes found to have overcome the antagonism that prevented normal pollen tube growth.

In a study of the kinds of sterilities in plants, Sears (21) recognized three classes of incompatibilities: (1) failure of pollen to germinate; (2) failure of pollen tubes to reach the ovary; and (3) normal growth of pollen tubes followed by fertilization but ovule abortion. In this work, Sears was considering self-incompatibility, but the same criteria should also apply to cross-incompatibility. The basis for failure of the pollen tubes to grow into the ovary was regarded as a matter of inhibition rather than a lack of sufficient stimulus to effect normal growth, and was accounted for by an antagonistic substance in the style. Sears found conditions comparable to those observed here in the cross R. japonicum x R. mucronatum where apparently normally growing pollen tubes ceased to develop abruptly. He observed in Nemesia strumosa Benth. pollen tubes of an incompatible type grew as fast in the styles as compatible ones for the first three-fourths of the length and underwent a sudden decline in growth rate, finally stopping short of the top of the ovary. A parallel situation to the observations made here with the cross R. mucronatum x R. japonicum where some pollen tubes had entered the ovary, effecting fertilization as

soon as did normal pollen tubes, was described by Sears in Tolmiea menziesii Torr. and Gray. In that plant, pollen tubes were strongly inhibited in the lower part of the style but some were able to proceed into the ovary and effected fertilization without delay. In such an occurrence, the number of fertilized ovules would considerably affect future ovary development, i.e., if insufficient ovules were fertilized, the ovary would probably absciss, whereas, if due to numerous pollen tubes entering the ovary a sufficient number of ovules were fertilized, the ovary would be stimulated to develop. Presumably such a condition would be most likely to occur in ovaries that contained numerous potential seeds as does Rhododendron. Unfortunately, Sears did not make any concrete suggestions as to the nature of the antagonistic substance. The physiology text by Stiles (24) discussed the same problem and reported that several kinds of materials will influence pollen tube growth, namely, salts of heavy metals, alkaloids and organic acids. In this relation, Rappleve (17) attempted to stimulate pollen tube growth in several species of Lilium with boron but obtained negative results. The present study offers no solution to the problem but merely corroborates the Sears' theory that the antagonistic substance may at times completely inhibit pollen tube growth but in some instances does not delay fertilization appreciably. Such a system would account for both the partial development observed in some capsules as well as the low seed yield obtained from intersectional azalea crosses.

Summarizing the discussion to this point, it has been observed that a series of barriers prevent extensive intersectional hybridization in azalea and the most effective are: (a) gamete isolation in the form of pollen tube-style antagonism; and (b) defective seedlings that fail to live. Other barriers are effective to a lesser extent, such as geographic isola-

tion and polyploidy. One intersectional cross produced defective seedlings that grew despite a marked chlorophyll deficiency that existed in the emerged seedlings. Of these, one plant formed a flower bud which aborted but had immature flower characters that occur only in the pollen parent. Other defective seedlings which were entirely white did not survive even though nutrients were applied and grafting onto green seedlings attempted. Green seedlings were also obtained from intersectional crosses but showed no pollen parent influence when they flowered. These may be regarded as apomictic. As an expedient to obtain further breeding material, mass pollinations involving intersectional combinations from the sections Tsutsutsi and Pentanthera are suggested but relying on those Pentanthera species native to North America, since it will be recalled that all intersectional combinations involving entirely Asiatic species have failed both here and in previous attempts while the cross involving R. canescens, a native American species, produced seedlings that survived.

EMBRYOLOGICAL STUDIES OF RHODODENDRON

a. Normal development

At anthesis, the embryo sac of R. japonicum and R. mucronatum is a typical eight-nucleate, seven-celled body in which the polar nuclei are unfused. Fertilization is effected in both species subsequent to ninety-six hours and, on the basis of style length, this time interval will probably hold true for many other species.

Studies have been conducted on the development of a number of species of Ericales [Palser (14), Copeland (4,5), Kavaljian (8), Palsitrot (16) and Chou (3)] but none of these workers have observed developmental stages of the embryo and endosperm of Rhododendron. Embryo development is very difficult to follow and only the early divisions have been determined for

a few species. In R. japonicum, the first division is transverse which eliminates the Piperad Type [Johansen (7)] but no further details were observed. Palser (15) reported that the first division in Cassiope hypnoides D. Don was transverse and the second probably the same thus indicating the Solanad Type. The only clear case of embryo development is Pyrola rotundifolia L. which Johansen classified as the Myriophyllum Variation, Caryophyllad Type. More recently, Chou (3) studied three species of Gaultheria but conclusions were similar to those of Palser.

In this study, the development of the endosperm of R. japonicum was observed in detail, in R. mucronatum to a lesser extent. In both species, the first two divisions were clearly nuclear, resulting in a box-like arrangement of four nuclei. These subsequently migrated to form a linear arrangement, transverse walls were formed and all subsequent divisions were cellular. Maheshwari (10) has stated that the cellular type of endosperm is a general characteristic for Ericales and most workers have agreed with this [Palser (14), Chou (3) and Copeland (5)]. Some consideration must be given, however, to the fact that two authors [Pelsitrot (16) and Brough (2)] did not observe cellular endosperm development in the initial stages.

It has been observed by Palser (15) that the megagametophyte development in Cassiope mertensiana (Bong.) G. Don does not agree with the Polygonum Type that Maheshwari has considered to be standard for Ericales but rather was a modified Allium Type.

The progressive development of embryo and endosperm for both species studied here agrees with Palser (14) in that first division of the zygote does not occur until the endosperm has completed the first two divisions. In the present study, this occurred subsequent to 240 hours following

pollination. Other papers have not reported a time sequence since collections were not made following timed, mass pollinations but depended on variations occurring in materials gathered in the wild.

b. Development following intersectional pollinations

Deviations in pollen tube growth following intersectional crosses were discussed under barriers but morphological abnormalities found in the ovules when pollen tubes did not penetrate the ovary or did so to a very limited extent are discussed here. Even up to 576 hours after pollination, unfertilized ovules remained intact but the polar nuclei had not fused. In some species of Ericales, polar nuclei may fuse prior to fertilization [Palser (14)] but here the process was not autonomous, requiring the impact of fertilization to initiate their merging. Changes in the ovule associated with embryo and endosperm growth were not observed where pollen tubes failed to enter the ovary, thus there was no intensifying of stains due to increased cell contents or enlargement of the ovule and the assumption of the "winged" appearance denoting mature seeds. This indicates that the mere presence of the pollen tubes in the style was not sufficient stimulus to initiate parthenocarpic development; furthermore, failure to find enlarged capsules after certain of the intersectional crosses is in accordance with this evidence. As compared to the above results, the one instance of ovary penetration following the cross R. mucronatum x R. japonicum resulted in ovules with a normal rate of development as well as those which remained in the same undeveloped stage as when there had been no pollen tube penetration.

SUMMARY

A wide range of intrasectional and intersectional crosses between thirteen species of Rhododendron L. from sections Tsutsutsi, Pentanthera and Sciadorhodion was attempted. Results showed that in the first two sections interspecific crosses may be made with ease but in a more difficult manner in section Sciadorhodion. Intersectional crosses generally failed and several barriers that isolated the sections were noted. Chiefly, gamete isolation due to failure of pollen tubes to reach the ovules and lethal chlorophyll deficiencies when seedlings did result were responsible. Such crosses were not impossible and R. canescens x R. mucronatum, a new combination, gave a limited number of seedlings that have survived the chlorophyll defect. One such seedling flowered and it was established, on the basis of flower type, to be a sexual hybrid. Other seedlings from different intersectional crosses were normal green and showed no influence of the pollen parent when they flowered. These were concluded to be apomictic.

Embryological studies showed that the embryo sac of R. japonicum and R. mucronatum is normal for Ericales and that zygote development as far as discernible is in accordance with other species. The endosperm development deviated somewhat in that the first two divisions were nuclear followed by cellular division while other species of Ericales are reported to be cellular throughout. Further study of endosperm development of Rhododendron is suggested to determine if this deviation has any generic significance.

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APPENDIX

PLATE I

- Figure 1. Seedling of the cross R. canescens x R. mucronatum showing extent of chlorophyll deficiency after three years. Note both terminal shoot and basal growth is chlorotic. (Approximately 2x)
- Figure 2. Seedling of the cross R. canescens x R. mucronatum that produced only green foliage during second year and initiated white shoots at the base of the plant during the third year. (Natural size)
- Figure 3. Capsules of azaleas collected on July 28, 1952. Upper left, R. mucronatum (selfed); upper right, R. mucronatum x R. japonicum, note capsules are beginning to dehisce and one is distorted; lower left, R. japonicum (selfed); lower right, R. japonicum x R. mucronatum. (Natural size)
- Figure 4. Stigma of R. japonicum, showing pollen tetrads and pollen tubes penetrating clefts between stylar lobes. (48 hours) (200x)



FIG. 1



FIG. 2

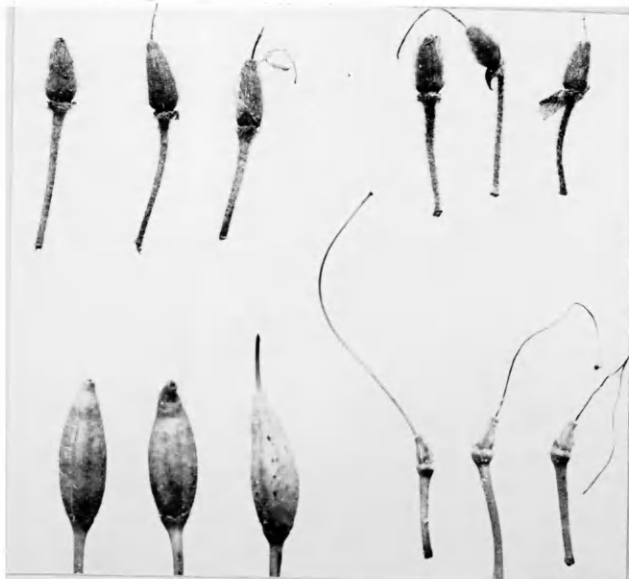


FIG. 3

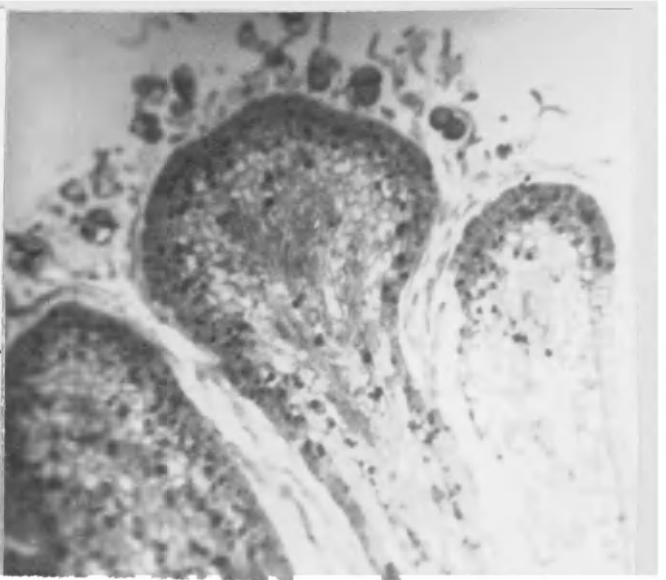
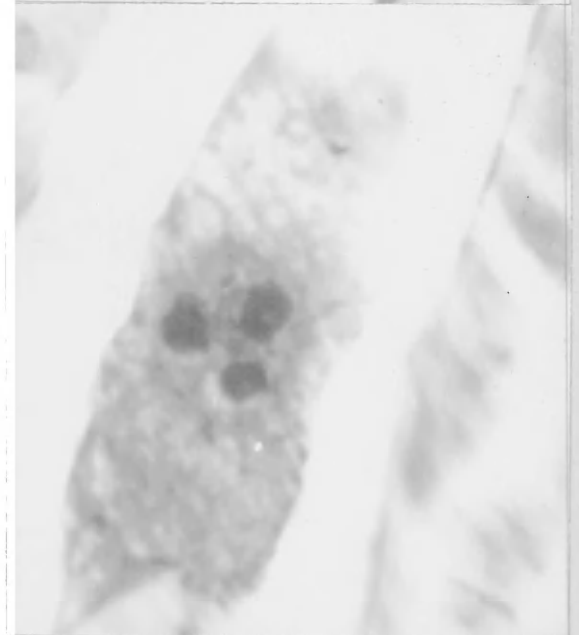
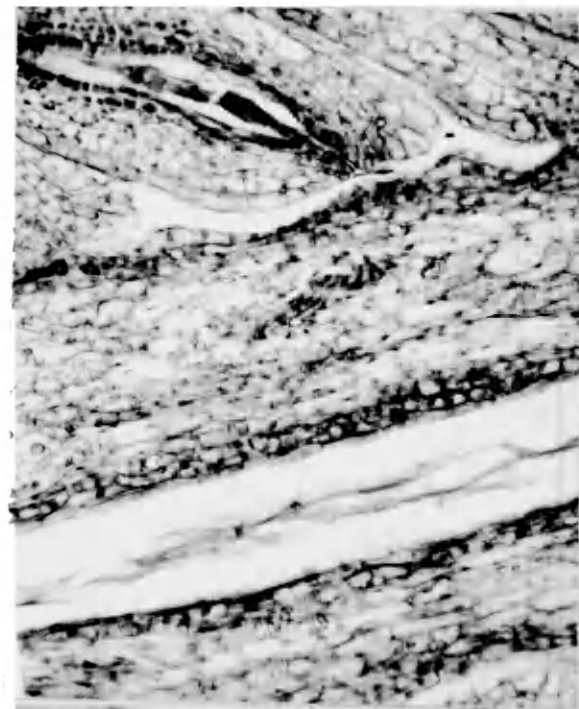
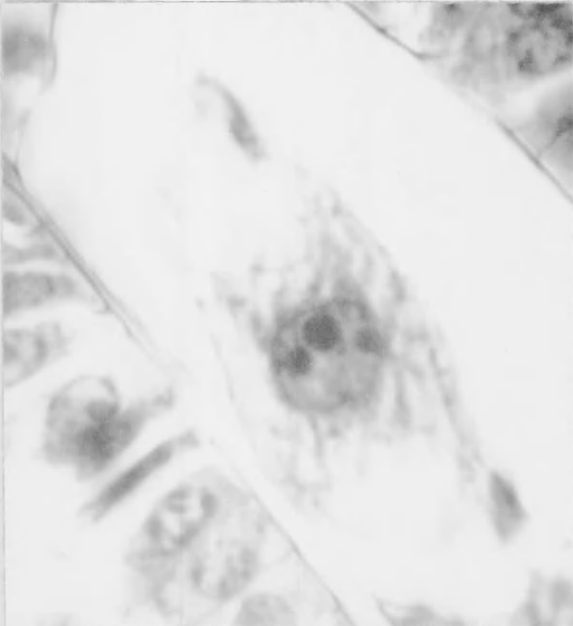
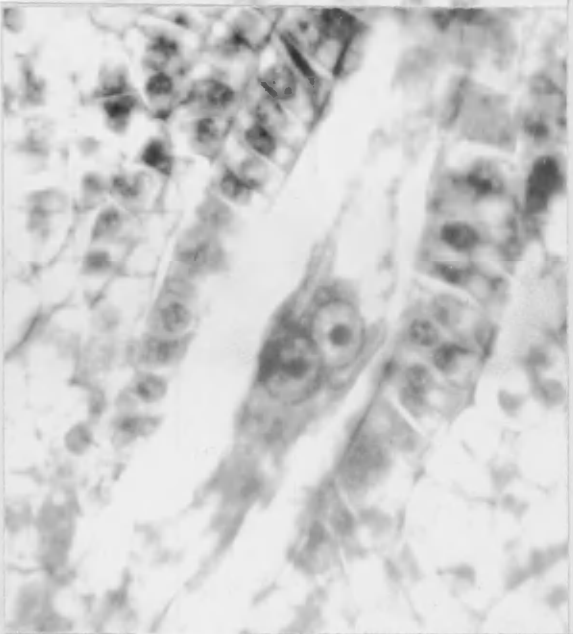
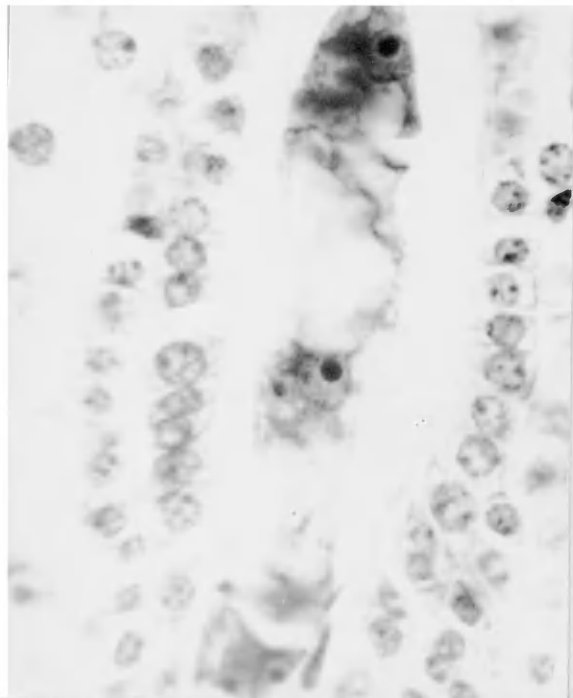


FIG. 4

PLATE 2

- Figure 5. Embryo sac of R. japonicum at anthesis. Egg apparatus at bottom, polar nuclei at center of cavity and antipodals at top. (1200x)
- Figure 6. Portion of ovary of R. japonicum with pollen tubes in the lumen and anatropous ovule which has been fertilized. Note micropylar opening and darkly stained area of egg apparatus. (144 hours) (200x)
- Figure 7. Embryo sac of R. japonicum in which the endothelium can be clearly identified by uniform cells oriented at right angles to the embryo sac. Polar nuclei remain unfused at center. (48 hours) (1200x)
- Figure 8. Developmental stage following intraspecific pollination of R. japonicum. Polar nuclei have fused but nucleoli remain distinct and vermiform sperm is visible. (96 hours) (2200x)
- Figure 9. Developmental stage following intraspecific pollination of R. japonicum. Secondary fusion has occurred but nucleoli contributed by polar nuclei and sperm have not merged. (144 hours) (2200x)
- Figure 10. Similar to Figure 9 but from a later section. Three distinct nucleoli can be observed. (192 hours) (2200x)



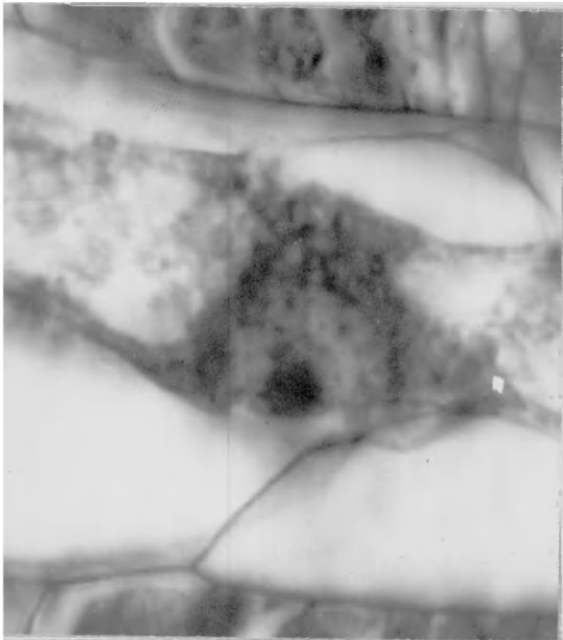
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15.
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FIGS.
9-10

PLATE 3

- Figure 11. Developmental stage following intraspecific pollination of R. japonicum. Primary endosperm nucleus in which the single large nucleolus is present. Secondary fusion has been completed. (120 hours) (2200x)
- Figure 12. Endosperm development following intraspecific pollination of R. japonicum. Linear arrangement of nuclei following second division. (144 hours) (2200x)
- Figure 13. Endosperm development following intraspecific pollination of R. japonicum. Largest nucleus is toward chalazal end of the embryo sac and presumably the result of the first division. (240 hours) (2200x)
- Figure 14. Endosperm nuclear arrangement after first series of divisions have been completed. (R. japonicum x R. japonicum) (120 hours) (2200x)
- Figure 15. Endosperm nuclei subsequent to the second paired divisions, showing the typical diamond-shaped arrangement. (R. japonicum x R. japonicum) (144 hours) (2200x)
- Figure 16. Embryo sac showing migration of endosperm nuclei. Those moving toward micropylar end have already separated. (R. japonicum x R. japonicum) (240 hours) (2200x)



FIGS.
11-12



FIGS.
13-14



FIGS.
15-16

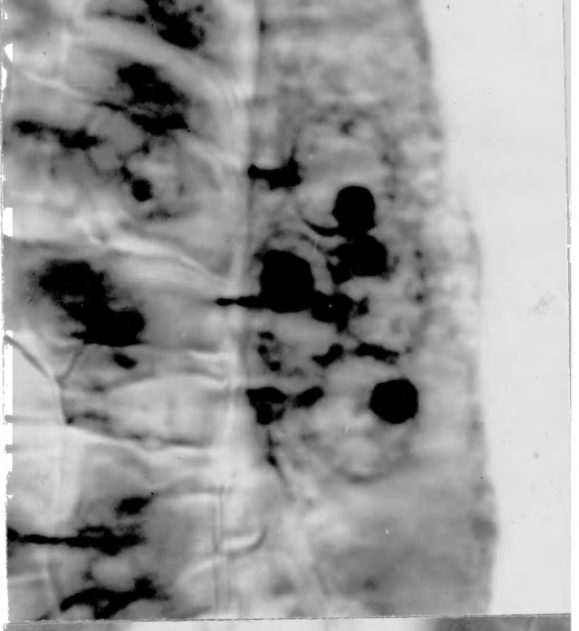
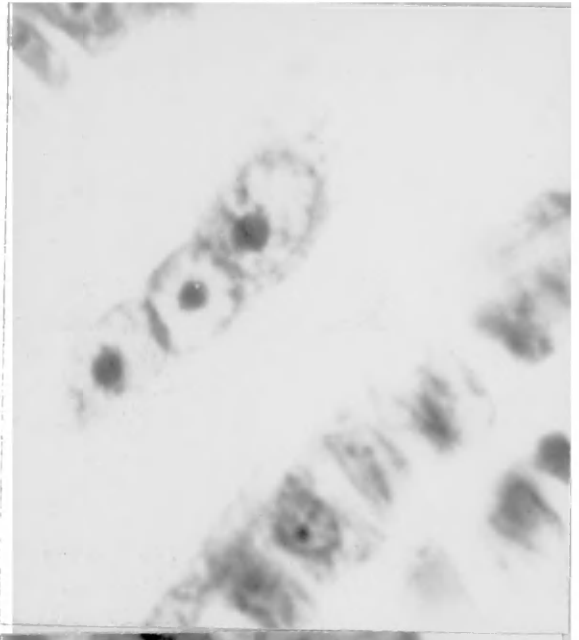
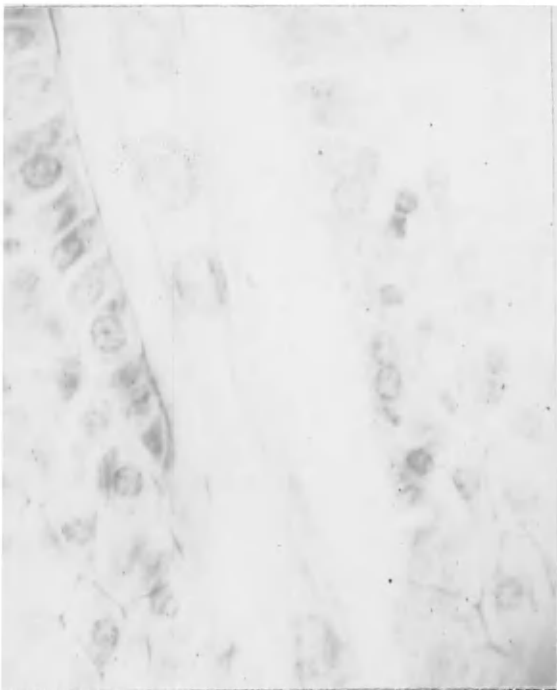
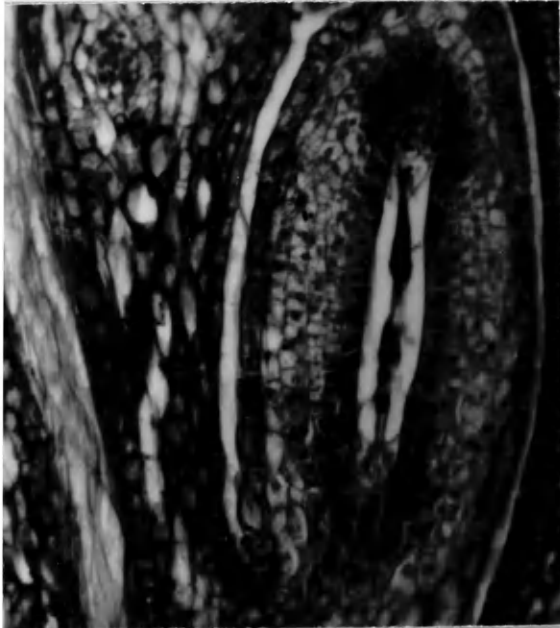
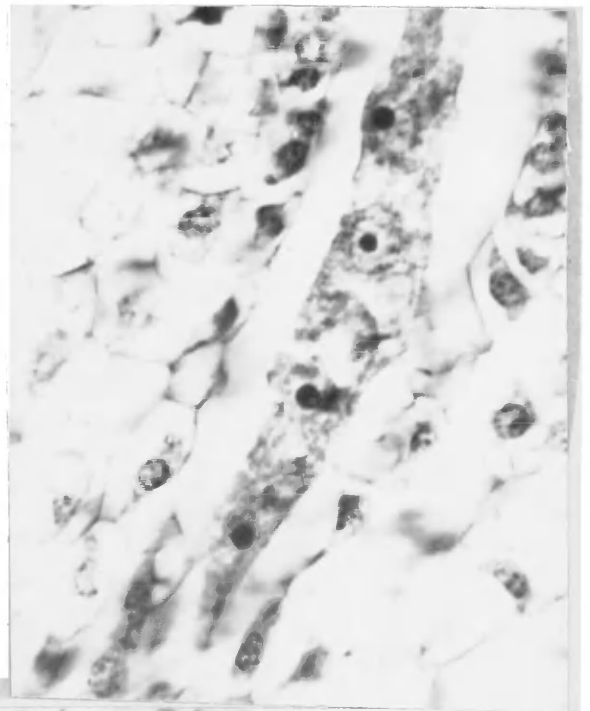


PLATE 4

- Figure 17. Embryo sac showing migration of endosperm nuclei into a linear arrangement. Nucleus at bottom is toward the micropylar end. Note tapering of the endothelium as it reaches the micropylar end of the embryo sac. (R. japonicum x R. japonicum) (240 hours) (1200x)
- Figure 18. Endosperm nuclei oriented equally throughout embryo sac, clearly distinguished by large nucleoli. (R. japonicum x R. japonicum) (240 hours) (1200x)
- Figure 19. Ovary showing developing endosperm with walls between nuclei partially visible. Note numerous pollen tubes in lumen at left of Figure. Heavily stained cells of chalazal haustorium can be observed at top of ovule. (R. japonicum x R. japonicum) (240 hours) (400x)
- Figure 20. Zygote development showing elongation out of micropylar end of embryo sac into main cavity. First division has resulted in a small terminal cell and an elongate basal cell. (R. japonicum x R. japonicum) (240 hours) (2200x)
- Figure 21. Developmental characteristics in ovule of R. japonicum. Note endosperm transverse walls and the partially visible longitudinal wall. Chalazal haustorium is visible at the top of plate. Ovary walls at right have become filled with deeply staining material. Compare size of ovular cavity with that in Figure 19. (R. japonicum x R. japonicum) (576 hours) (400x)
- Figure 22. Early endosperm divisions of R. mucronatum x R. mucronatum showing tetrad of nuclei similar to those shown in Figures 14-15 for R. japonicum. (144 hours) (2200x)



**FIGS.
17-18**



**FIGS.
19-20**



**FIGS.
21-22**

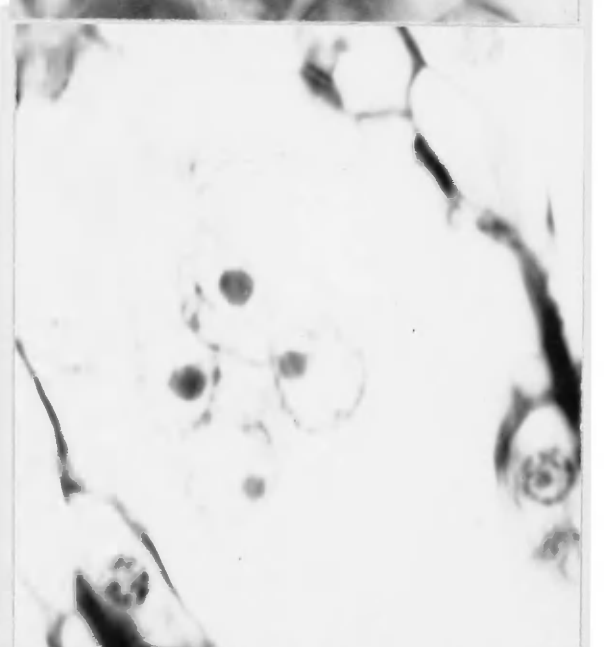
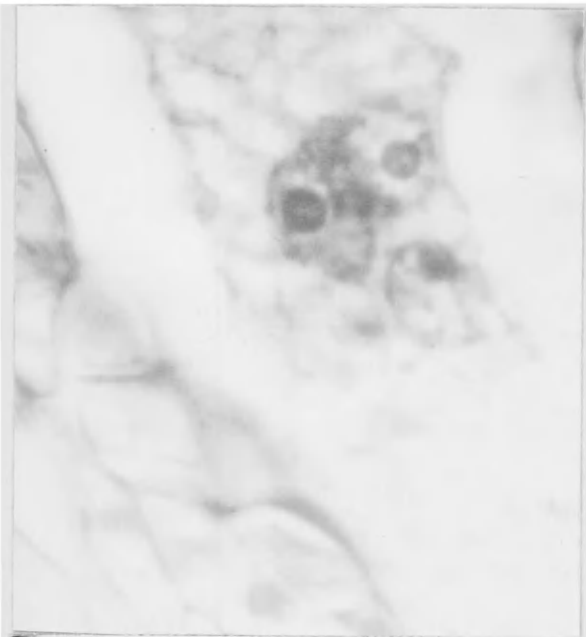


PLATE 5

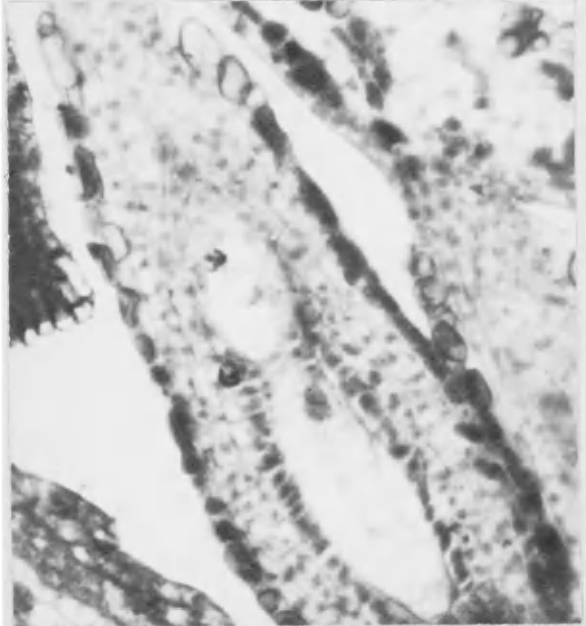
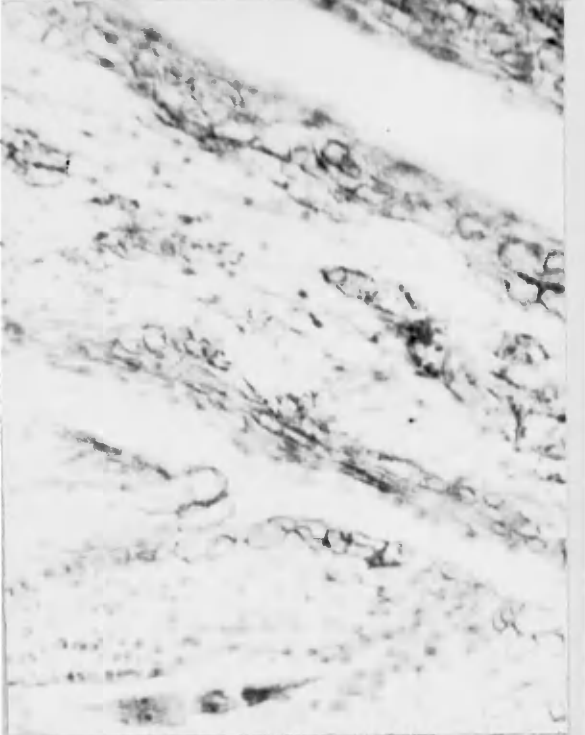
- Figure 23. Endosperm developmental stage of R. mucronatum x R. mucronatum also showing the typical diamond or box-like formation that the nuclei assume. (144 hours) (2200x)
- Figure 24. Advanced ovule of R. mucronatum x R. mucronatum showing typical longitudinal wall formation. Micropylar end of embryo sac at lower end of Figure. (576 hours) (400x)
- Figure 25. Ovary following pollination of R. japonicum x R. mucronatum. Lumen at top of Figure is void of pollen tubes; ovule at center shows no indication of fertilization; polar nuclei remain unfused. (120 hours) (200x)
- Figure 26. Ovary following pollination of R. japonicum x R. mucronatum similar to Figure 25. Note that no pollen tubes are present in lumen and ovule at bottom of Figure shows none of the development characteristic following fertilization. (336 hours) (200x)
- Figure 27. Ovule following cross of R. mucronatum x R. japonicum showing similar condition as in Figures 25 and 26. Fertilization had not been effected and polar bodies were unfused. (576 hours) (200x)
- Figure 28. Fertilized ovule of R. mucronatum x R. japonicum. Compare development with unfertilized ovule from same cross in Figure 27. Development will also be noted as well advanced as in Figure 24. All three figures are from ovaries of the same age. (576 hours) (400x)



**FIGS.
23-24**



**FIGS.
25-26**



**FIGS.
27-28**



VITA

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