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BOTANY

POLLINATION STUDIES WITH NATIVE MINNESOTA *PYROLA* AND *MONESES* SPECIES¹

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INTRODUCTION. The closely related ericaceous genera, *Pyrola* L. and *Monese* Salisb. ex. Gray, are common throughout the world in coniferous woods of north temperate areas. Characteristically they are small herbaceous plants with basal evergreen leaves and decorative inflorescences of green, white or pink flowers appearing in the early summer. In Minnesota there are five species of *Pyrola*—*P. asarifolia* Michx., *P. elliptica* Nutt., *P. rotundifolia* L., *P. secunda* L., and *P. virens* Schweigger. There is also the one species of *Moneses*, *M. uniflora* (L.) Gray. All these occur together in abundant quantities and bloom at approximately the same time. Consequently they are suitable as experimental material for a field problem. The aspects chosen for investigation were those of interspecific hybridization and pollination mechanisms.

Although Hagerup (1954) and Vogelaar (1958) believe *Pyrola* species to be reliant upon self-pollination, their conclusions are not based upon thorough pollination studies. Neither is it known if *Pyrola* species are capable of crossing with each other in nature or if they and *Moneses uniflora* are capable of cross-pollination. The object of the present study was to answer these questions in so far as possible, utilizing the available native species in their natural habitats. A program of hand pollination was carried out at the University of Minnesota Biological Station in Itasca Park during the summer of 1960. Capsules and seeds were collected and then examined and sown during the following winter and spring at the Department of Botany, University of Minnesota, Minneapolis.

ECOLOGY. *Pyrola asarifolia* var. *asarifolia* was the least shade tolerant of the plants studied, preferring open coniferous or coniferous-deciduous forest and even occasionally competing with grasses on the edges of fields. *P. asarifolia* var. *purpurea* (Bunge) Fern., was found in swamps bordering Lake Itasca. It was also observed growing in both the tamarack and the sedge zones of floating bogs. In the tamarack zone, the stems of plants with buried leaves were often traced

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six to fourteen inches straight down to their rhizomes. It appears that the plants grew upward as the sphagnum moss accumulated at the surface. In contrast to this the rhizome, of both upland plants and those in the sedge zone of bogs, was seldom more than a few inches below the surface.

A preliminary investigation designed to determine whether or not the two varieties of *P. asarifolia* represent distinct ecotypes was initiated. Several non-flowering plants of *P. asarifolia* var. *purpurea* were transplanted to an upland area. Likewise, several plants of *P. asarifolia* var. *asarifolia* were transplanted to a sphagnum bed in the bog. Both sets of plants were checked 15 months later and were found to be in good condition but with no evidence of having flowered or changed in appearance.

Pyrola secunda var. *secunda* was most common in dense stands of balsam fir, *Abies balsamea* (L.) Mill., where it had little competition from other herbaceous plants, due apparently to the extreme shade. *P. secunda* var. *obtusata* Yurcz. was found growing in the tamarack zone of floating bogs.

Pyrola virens favored the same habitat as *P. secunda* but was also frequently found in open stands of red pine, *Pinus resinosa* Ait., where there was a sparse ground cover.

Pyrola elliptica was found in moist areas of deciduous-coniferous woods where there usually was present a fairly dense shrub and herb cover.

Pyrola rotundifolia had the narrowest range of habitats of any of the species. It was found only in relatively dry, open, jack pine, *Pinus banksiana* Lamb., forests. Such areas were usually characterized by a moss covered floor, and an abundance of other ericaceous plants.

Moneses uniflora was not widespread; it occurred in moist locations of coniferous woods, ranging from open red pine stands to densely shaded stands of balsam fir.

FLORAL MORPHOLOGY.

Androecium: In most of the *Pyrola* and *Moneses* species, prior to the start of anthesis, the anthers are in an upright position with the filament attached just above the base on the inside. The anthers are green, have pores at their base, and are arranged symmetrically around the pistil. As Copeland (1947) and others have observed, the anthers become inverted during anthesis, with the point of attachment of the filament to the anther serving as a fulcrum. The filaments on the lower side of the slightly pendulous flowers in many species bend upwards during anthesis forming a cluster above the stigma. However, such a distortion of the filaments was not observed in *Pyrola secunda* and in *Moneses uniflora*. With the exception of *P. virens* which retains a slightly chlorophyllous condition, the anthers lose their green appearance and turn a pale cream-yellow at maturity. The period of time involved from the start of anthesis to dehiscence varies from two to five days, being shorter in warm weather.

The pollen of all species, except *Pyrola secunda*, occurs in tetrads.

P. secunda has pollen in monads. This supplements Erdtman's observations (1952) on *P. rotundifolia*, *P. secunda* and *P. virens*.

Gynoecium: At the advent of anthesis the flowers of all species are actinomorphic and have a straight, short style, and no stigma lobes. Within the next day or two, several changes occur. In all species but *Pyrola secunda* and *Moneses uniflora*, the style grows to a length of 1 to 3 mm, usually downward and outward giving it an S-shaped configuration as was observed by Copeland (1947). The actinomorphic configuration of the corolla of these species gradually changes as the style develops, resulting in a slightly zygomorphic condition at maturity. Zygomorphy seems to result from the downward growth or movement of the style, forcing the lower petals to extend at a ninety degree angle from the receptacle. This is probably related to the fact that the pendant flowers in these species are at an angle of approximately 45° from the horizontal. In *Moneses uniflora* and *Pyrola secunda*, the style continues to lengthen during anthesis, growing straight out and not affecting the posture of the petals. Hence, the corolla remains actinomorphic. This condition in turn is correlated with the fact that in *Moneses* and in *Pyrola secunda*, the flower is usually hanging straight down at maturity. It is interesting to note that after maturity, the flower of *Moneses* gradually turns upward until it is finally completely upright.

As Copeland (1947) has observed, all *Pyrola* species, with the exception of *P. secunda*, have five lobes of stigmatal tissue prominent in the mature flowers. However, prior to anthesis these lobes are scarcely distinguishable from the tissue of the style proper. During anthesis they increase in length, so that at maturity of the flower, they often are raised 0.5 to 1 mm. above the collar at which time they are covered with a viscid exudate. *Pyrola secunda* characteristically has the end of the style flaring out to form a circular, peltate stigma. The stigma was observed to swell slightly prior to maturity and to develop five broadly rounded and raised areas, separated by furrows radiating toward the center.

The style of *Moneses uniflora* is characterized by very pronounced stigma lobes. The plants at Itasca have five elongate, acute lobes, arising from the edge of the broad tip of the style. Their length is approximately 1 to 1.5 mm. and the center of the tip of the style is flat except for a slight depression. At the start of anthesis, the stigma lobes are folded together over this central area, and their unfolding progresses as the flower reaches maturity.

EXPERIMENTAL METHODS. Nine crosses were made for each species—two controls, one self-pollination, one intraspecific cross and five interspecific crosses.

Control No. 1: This involved bagging racemes before anthesis and leaving them untouched for the rest of the season. The purpose was to determine if self-pollination occurs.

Control No. 2: This involved emasculating the flowers on several racemes for each species and then bagging them. This was done

to determine if there had been any penetration of foreign pollen into the bag or if apomixis had taken place.

Self-Pollinations: In each of these crosses several racemes were pollinated with their own pollen to determine if they were self-compatible.

Intraspecific Crosses: Stigmas of several emasculated flowers were dusted with pollen from plants of the same species growing several miles distant, to avoid the possibility of both parents coming from the same rhizome.

Interspecific Crosses: Each species was reciprocally cross-pollinated with the other five. The custom of mentioning the recipient first when discussing crosses will be followed in this paper.

In the five species of *Pyrola*, each of the nine crosses involved an average of six racemes, each raceme possessing about three flowers. This resulted in a total of 810 flowers treated. In *Moneses* where there is only one flower per inflorescence, only five flowers could be used in each of the crosses due to scarcity of flowering material. Therefore, a total of only 45 flowers of this species was involved in the crosses.

Racemes of plants to be used as pollen donors were inclosed in rice paper bags and fastened at the bottom with paper clips with utmost care being taken to leave no space for insects to enter. This bagging was done before anthesis to avoid contamination by wind or animals. Fine jeweler's forceps sterilized with 70% ethyl alcohol were used in collecting pollen. Entire anthers were placed in gelatin capsules for transport to the recipient flower. Shaking the capsule was sufficient to release the pollen from the anthers.

Racemes used as recipients were left unbagged until the flowers showed signs of anthesis. At this time, the petals were opened and the stamens removed by pinching off the filaments with sterilized forceps. This process often involved damaging or removing the petals due to the small size of the flower, but in the majority of cases this did not affect the subsequent development of the flower. After removal of the stamens, the raceme was bagged in the same manner as the donor. As the flower continued to mature, close observation was required to determine when the stigma was receptive. In all species the receptiveness of the stigma was judged largely by the presence of a liquid exudate. Pollen was removed from the capsule on the tip of sterilized forceps and transferred to the recipient stigma. The stigma received a thorough dusting visible to the naked eye. After applying the pollen, the raceme was bagged and labeled with the information necessary for identifying the cross for seed collection.

Voucher specimens were made for all species and were deposited in the herbarium of the University of Minnesota. These plants were taken from the immediate vicinity of the experimental plants.

In the second week of September the capsules were collected. They were then sorted and examined and put into cork-stoppered glass vials. The vials were transferred to a root cellar with a constant temperature of 2° C and a relative humidity of about 80-90%. Care

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was taken to fit the corks into the vials loosely to allow the moist air to come in contact with the seeds, and prevent their drying out.

Three months after collection, seeds from all crosses were sown on forest loam taken from localities where several *Pyrola* species grew. Seven months after collection, a limited number of seeds were sown on sterile media modified from Lihnell's methods (1942). This involved using two different media, one being a combination malt-yeast agar, the other a standard preparation for germinating orchid seeds (Difco). As space was limited, not all the seeds were sown on sterile media. Instead, six different batches of seeds were chosen, with each being sown on the malt-yeast and the orchid agar. The crosses from which the seed was chosen were *Pyrola rotundifolia* × *P. virens*, *P. asarifolia* × *P. elliptica*, *P. virens* × *P. elliptica*, and *P. elliptica* × *P. rotundifolia*. Seeds from untouched plants of *P. asarifolia* and *P. elliptica* were also used:

RESULTS.

Morphology of Capsules and Seeds: The majority of flowers pollinated produced mature capsules (see Table No. 1) which were two to three times the size of the ovary present at anthesis. Those flowers that did not yield mature capsules had shriveled up small ovaries.

Most of the mature capsules bore seemingly mature seeds. According to Copeland (1933, 1947) and Soueges (1939) the seed is characterized by a single integument, and a single layer of endodermis covering a centrally located, circular undifferentiated embryo. In this study the presence of an embryo was presumed to coincide with the incidence of a translucent yellow, circular mass located in the center of the tubular, transparent epidermal layer of the seed coat. Such yellow structures were observed in the seeds of mature capsules of plants not used in experiments. Seeds of most mature capsules from controlled pollinations had a central yellow body whereas those from immature capsules did not.

The mature seeds of all *Pyrola* species (both those plants used in experiments and those not touched) had a length of 510u to 765u, and a diameter at the middle of 75u to 128u. The length of the testa of *Moneses uniflora* was greater, ranging from 638u to 1,020u but the diameter was the same as in the *Pyrola* species. This difference in length is consistent with Beijerinck's measurements (1947). It is interesting to note here that not only does *Moneses uniflora* differ from *Pyrola* by having a greater seed length, it differs also in the character of the integument. Whereas the integument in the *Pyrola* species examined was very wrinkled and shriveled at the ends of the cigar-shaped seed, it was much smoother in *Moneses uniflora*, and retained a cylindrical shape.

In those crosses which yielded mature capsules but embryoless seeds, the length of the testa was considerably shorter. The cross *Pyrola asarifolia* × *P. virens* produced seeds that were 204u to 255u in length. In the crosses *P. secunda* × *P. asarifolia*, *P. secunda* ×

TABLE 1. Capsule and Seed Data.

Recipients:

Donors:

	Control 1	Control 9 ¹	Self-pollinations	Intra-specific	Asarifolia	Elliptica	Rotundifolia	Secunda	Virens	Moneses
capsule	—	—	Missing ¹	+	=====	+	+	+	+	—
<i>Asarifolia</i>	0/33	0/24	Missing ¹	24/37=65%	=====	21/24=88%	7/9=78%	4/24=17%	3/19=15%	0/25
seed	—	—	Missing	+	=====	+	+	+	—	—
capsule	—	—	+	+	+	=====	+	+	+	—
<i>Elliptica</i>	0/30	0/4	9/10=90%	17/29=59%	17/17=100%	=====	12/14=86%	10/18=55%	20/23=87%	0/20
seed	—	—	+	+	+	=====	+	+	+	—
capsule	—	—	Missing	+	+	Missing	=====	—	+	—
<i>Rotundifolia</i>	0/24	0/42	Missing	32/34=94%	11/11=100%	Missing	=====	0/12	10/22=45%	0/12
seed	—	—	Missing	+	+	Missing	=====	—	+	—
capsule	—	—	—	+	+	+	—	=====	+	+
<i>Secunda</i>	0/24	0/10	0/3	21/31=63%	10/17=57%	13/28=47%	0/7	=====	9/21=43%	7/14=50%
seed	—	—	—	+	—	—	—	=====	—	—
capsule	+	—	+	+	+	+	+	+	=====	+
<i>Virens</i>	10/30=33%	0/42	15/15=100%	10/13=76%	5/6=83%	18/18=100%	10/13=77%	17/17=100%	=====	9/16=56%
seed	+	—	+	+	+	+	+	+	=====	+
capsule	—	—	+	+	+	+	+	+	+	=====
<i>Moneses</i>	0/24	0/18	1/3=33%	2/3=67%	2/3=67%	1/1=100%	2/2=100%	1/4=25%	5/7=7%	=====
seed	—	—	+	+	+	+	+	+	+	=====

+—capsules mature
 ——capsules immature
 numerator—No. of capsules matured
 denominator—total No. of capsules

¹ Capsules recorded as missing were found destroyed at the end of the summer, presumably due to animals or tourists.

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P. elliptica, *P. secunda* × *P. virens*, and *P. secunda* × *Moneses uniflora*, the length of the seed was 255u to 306u.

In those crosses which did not result in mature capsules, the testa was very short, being in the range of 77u to 179u. No embryo was observed in these seeds.

Germination: Seeds of all crosses as well as seeds from non-experimental plants sown on forest soil did not germinate during a seven month period of observation. Seeds sown on sterile media have also failed to germinate in eight months. Forty percent of the flasks were contaminated with molds at five months.

DISCUSSION. The failure of the seeds to germinate makes the production of F₁ and F₂ generations impossible. The interpretation of results is therefore dependent upon the formation of fruit and seeds.

The results from control 1 and the self-pollinations indicate that the *Pyrola* and *Moneses* species studied do not necessarily rely on self-pollination for the production of seeds. This is contrary to Hagerup's belief (1954) which, however, is based upon two species of *Pyrola* not involved in this study, viz., *P. minor* L. and *P. media* Hayne. Hagerup believed that the release of pollen is dependent upon the flower's being shaken by the wind. It is possible that the presence of the rice-paper bag prevented a release of pollen by sheltering the flower from any breezes. This would account for the lack of seed formation by *Moneses uniflora* and *P. elliptica* in control 1, and its subsequent formation when the pollination was done by hand. However, this could not account for the absence of seed in *P. secunda* even when it was pollinated by hand.

The results from control 2 indicate that there were no contaminations due to entrance of foreign pollen and that apomixis did not occur in unpollinated plants.

The results from the intraspecific crosses show that all species, as would be expected, were capable of yielding fruit when crossed with other plants of the same species. However, these crosses yielded smaller percentages of capsules than did many of the inter-specific ones. The reasons for this are explained with difficulty.

Whereas in most instances all of the species successfully crossed with each other, some showed better yields of capsules when employed as donors while others showed better yields as recipients. *Pyrola asarifolia* and *P. secunda* exemplify the former condition, *P. virens* and *Moneses uniflora* the latter. This could be due to difficulties in the compatibility between pollen and stigmas or to differences in pollen tube growth, style length or other factors.

The negative germination results may be due in part to the very small size of the embryo with the attendant scarcity of stored food; and in part to the fact that *Pyrola* and *Moneses* are dependent on mycorrhizal fungi for proper growth. According to Christoph (1921), these genera depend upon mycorrhizal fungi which form a dense hyphal mat around the rhizomes and roots with haustoria penetrating the epidermal cells. According to Lück (1940) these fungi are ba-

sidiomycetes. Lihnell (1942) investigated the importance of the mycorrhizal fungi for germination of *Pyrola* seeds. The results from his work indicate that while a fungus is not necessary for germination it does tend to increase the percentage of seeds that germinate. However, not any fungus in the soil would aid in germination of seeds; only a few isolated strains were of any value. In most cases, Lihnell reported a fungus was necessary for the survival of the seedling after germination, yet in no instance was he able to bring a seedling to maturity.

It is significant that in this study *Pyrola secunda* was found to differ greatly from the other *Pyrola* species. Differences occurred in the ontogeny and morphology of the style, the symmetry of the flower, the morphology of the pollen, and the ability to cross-hybridize. It is pertinent to this discussion to mention that *P. secunda* has a chromosome number of 19 compared with 23 in the other species (Darlington, 1955; Tischler 1950). In the past *P. secunda* has been considered sufficiently distinct from the other *Pyrola* species to justify segregation into a separate genus. The genus *Actinocyclus* was established by Klotzch (1851) and the genus *Ramischia* by Opiz (1852) for the express purpose of separating this species from *Pyrola*. *Ramischia* was recognized by Rydberg (1914) and Copeland (1947), but two of the current field manuals for the northeastern United States (Fernald 1950; Gleason 1952) recognize only *Pyrola* and *Moneses*. The evidence presented in this paper tends to confirm the separation of *P. secunda* from *Pyrola*.

It has been noted that *Moneses uniflora* revealed distinct characteristics in the ontogeny and morphology of the style and the symmetry of the flower. These characteristics, combined with its restricted ability to hybridize with *Pyrola* species, indicate that its position as a genus distinct from *Pyrola* is justified.

Copeland (1947) considered *Ramischia* (*P. secunda*), with its disk and actinomorphic flowers, as representing the primitive stock of *Pyrola* and *Moneses*. *Pyrola* (*sensu stricto*) has in common with *Ramischia* a racemose inflorescence, an extensive underground system of rhizomes, and a style traversed by five dorsal carpel bundles. However, it illustrates a trend away from *Ramischia* toward a reduction of the glandular disk and a zygomorphic configuration of the flower. *Moneses*, according to Copeland, exhibits a trend toward a solitary flower, an underground system primarily of roots, and a style traversed by ten vascular bundles.

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