PAEONIA

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Suggested yearly contribution:
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I'll try to order any of these clones from Germany if you are interested.

- Chris

SEED PROPAGATION OF PEONIES

Don Hollingsworth

Seed propagation of peonies (genus Paeonia) is used for growing species and in breeding new cultivars. The cultivars, which are highly ornamental plants, are propagated asexually, but the annual rates of increase are low, being limited by a low annual increase of growth points. Accordingly, the production of peony plants for commerce has a poor economic outlook. Matters pertaining to the peony have not competed well for research and commercial capital. Research in tissue culture propagation offers the best encouragement for this outlook to change in the future.

Seedling production in peonies is complicated by "slow" root germination, followed by epicotyl dormancy. The process of seedling production may be completed in either the first or second year after seed maturation. These complications seem to be characteristic not only in the long used peony species ¡ª the Chinese peonies (Paeonia lactiflora Pallas), the tree peonies (P. suffruticosa Andrews) and the European peony (P. officinalis Linnaeus) ¡ª but are also characteristic of the other species which have been used in interspecific hybridization by breeders.

The overriding problem in producing new cultivars of peonies lies not only in their slow propagation, but in the relatively long life cycle from seed, maturation to first flowering of the seedlings. Only as an exception is the life cycle as short as three years. Five years is more typical. Evaluation and seedling selection require additional seasons. A reliable means of obtaining a high yield of first year germinations is of great interest to breeders.

Despite historical accounts of domestic peony culture dating more than 2000 years and despite modern advances in seed science which are of great magnitude, the germination process in peonies remains incompletely defined.

Paeonia lactiflora and P. suffruticosa have been subjects of herbal medicine and ornamental gardening interest in China and Japan for centuries. A Western translator (Hoffman, 1848) quotes an early Japanese writer concerning the propagation of tree peonies from seeds. It was cautioned that the seeds should be planted without being allowed to dry from the seedpod, for, if permitted to become dry, "scarcely one in a hundred can be expected to germinate." However, in the natural course of events, tree peony seeds typically become dry before reaching the ground.

The deciduous portion of the tree peony shoot, which is the infructescence (was the inflorescence), does not separate by an abscission layer. The dead portion of the stem is persistent until weakened by decay, then breaking apart progressively and the pieces falling at random times. As a consequence of this persistence, the typically up-facing, opened follicles tend to form vessels which retain the seeds exposed to the air until they are well dried and loosened, thereafter being free to fall as a result of physical disturbance. Thus, the seeds tend to reach the soil during mid-autumn or later, eventually becoming covered by organic debris, ultimately coming up in the second spring after maturation. However, some of the seeds may also fall earlier in autumn or late summer, and may be subject to either first year germination or failure. In some herbaceous species, seed ripening and senescence of the stems occurs in midsummer. In these instances, the seeds typically reach the soil by late summer. Other herbaceous species characteristically have more persistent stems and tend to hold the seeds much later in the season.

Morphological events in germinating peony seeds were described by A. P. Saunders (1928), agriculturist and chemist at Hamilton College, Clinton, New York. He is credited with having grown and attempted to hybridize all of the Paeonia species which he could acquire as plants or seeds and induce to grow and mature in his area, approximate latitude 43°. He wrote, "The normal course with fully ripened seed is for it to remain quiescent until the following summer, when, in July or August, a root is thrust out which may go down six inches into the soil, but no leaf growth is made until the following spring. . . . ". Of the proposition that seeds planted while still moist from the pod will give good germination the first year, Saunders observed, "This would probably depend on latitude, for in regions to the south the seed not only matures much earlier but has also a longer period of warm weather in autumn before winter closes in."

The roles of two temperature-dependent developmental phases in germinating seeds of tree peony were shown in studies at the Boyce Thompson Institute of Plant Research, Yonkers, New York. (Barton, 1933.) The production of a root by a germinating seed — "root-germination," considered in this study to be tree germination — was found to depend upon having a warm/moist environment for several weeks. Roots began appearing after 2 months and continued over a period of 4 months. "The effective germination environments were 20°C and daily alternating 15 -30° treatments. However, the yield from different seed sources varied greatly. As low as 4% and not more than 53% germination was reported.

When the germinated seedlings were kept at these high temperatures (20°C and daily alternating 15-30°), leafy shoots failed to develop and the roots eventually died. However, when small seedlings were planted and kept at low temperatures for 2 or 3 months, leafy shoot production was successful following their transfer to a cool greenhouse (about 13°C). Epicotyl dormancy was identified. Reduction of bud dormancy by exposure to low temperatures is now known to be a widespread phenomenon among temperate zone species and the low temperature is commonly known as chilling.

Successful production of leafy shoots after 2 or 3 months of chilling was found to be dependent upon the cool greenhouse. When similarly chilled seedlings were transferred to a warm greenhouse (21°C), the epicotyls all died, even if the plumule was starting to break through the soil when transferred. In light of the Vegis hypothesis (1963), success in the cool greenhouse compared with failure in the warm greenhouse can be viewed as evidence that dormancy reduction was only partially complete after the chilling durations used. The Vegis hypothesis holds, in part, that the external limits (conditions) under which growth can occur become wider during the course of dormancy reduction.

The most favorable chilling results were obtained at 5°C, 10°, and refrigerator (fluctuating 3 -15°C, average about 8°) for two to three months. When transferred to the greenhouse after two months of chilling, leafy shoot production ranged 27-71% and after three months ranged 55-100%. These less-than-complete results indicate variability among individual seeds in their time/temperature requirement for chilling and are also consistent with the previously stated view that reduction of dormancy was not complete.

Subsequent studies of epicotyl dormancy in tree peonies were conducted during many years at the Boyce Thompson Institute including studies of the physiological and morphological effects of gibberellic acid (GA) applied to dormant epicotyls (Barton and Chandler, 1957). The application of 1, 10 or 100 micrograms of GA directly to the hypocotyl of root-germinated seeds before planting caused the emergence of green shoots above ground in three weeks or less. The most rapid growth resulted from 100 micrograms, but more normal growth resulted from the lower dosages. Other chemicals which had been tried unsuccessfully include indoleacetic acid, kinetin, thiourea, ethylene chloro-hydrin, and ethylene-diaminetetraacetic acid (EDTA). Other studies were conducted on the biochemical changes associated with the reduction of epicotyl dormancy. (Fine and Barton, 1958, Barton, 1961, Barton and Bray, 1967.)

In the later studies, the daily alternating temperature of 15 -30°C was used for the production of root-germinated seeds. Wolfe (1955), a grower in Southern Illinois, working principally with tree peonies, reported very high rates of root-germination were obtained while using sub-surface soil temperatures to maintain the rooting environment. Freshly harvested seeds, showing the coloration of matured seeds, but still moist from the seed pod, were layered with friable soil in an ordinary clay pot. The pot was then sunk in moist soil to a depth such that the rim of the pot was covered 18 inches. The surface was well mulched. When the pot was taken up in about six weeks, he expected to have every viable seed germinated. Percentage yields were not given.

While Wolfe did not report temperature levels obtained in the buried pot, it seems likely that diurnal fluctuation was minimized and that the average temperature would have been a few degrees lower than the 20°C constant temperature environment that was of limited effectiveness in the early Boyce Thompson Institute studies.

Pehrson (1968), a grower in Minnesota (his approximate latitude 44°30'), carried out extensive peony breeding activities using a range of interspecific hybrids interbred and crossed back to cultivars of *Paeonia lactiflora*. In the context of promoting more widespread participation in peony breeding, he offered "Early Germination Method", recommended to obtain a significant yield of first year seedling production, thereby shortening the long life cycle by one year. Fully matured seeds, harvested without allowing them to become dry, were enclosed with moist horticultural grade vermiculite in thin polyethylene plastic bags (e.g., Baggies brand sandwich bags) and closed tightly against drying. The bags were to be kept at room temperature and inspections for germinated seeds should begin in about 5 weeks. Clearly, Pehrson felt he was getting favorable yields of root germination at whatever temperature range was represented by his "room" temperature.

However, almost simultaneously with publication of "Early Germination Method" Pehrson (1969) encountered a series of hybrid crosses the seeds of which did not show significant incidence of root-germination after 18 weeks at "room" temperature. Upon being moved to a cooler location, about 55°F (13°C), a high rate of root-germination was found when inspected after 2 weeks. The seeds were of crosses in which *Paeonia lactiflora* were the pod parents and the pollen parents were a plant of *P. peregrina* Miller and a hybrid of *P. officinalis* Linnaeus x *P. peregrina*. Obviously, the "room" temperature treatment at which other seeds had been germinating in satisfactory proportions was not suitable for this group of hybrids. It should be noted that both *P. officinalis* and *P. peregrina* are tetraploid and would have contributed about twice the chromosomal information to the progeny as the amount contributed by the diploid *P. lactiflora* parent. The implication is that the requirement for a lower than expected root-germination temperature is a characteristic of *P. peregrina* or both it and the *P. officinalis* ancestor.

Although the room temperature treatment was not suitable for promoting root-germination in the Pehrson hybrids, it apparently promoted readiness to germinate, for when a suitable temperature was given there was no further delay in the germination response.

From the foregoing, four tentative conclusions were drawn:

1. Paeonia embryos may require a period of post-maturation conditioning (or the reduction of a block) after seed maturation and before germination can take place.

2. Post-maturation conditioning can take place in a temperature range above which germination remains blocked. Germination-ready seeds remain quiescent until a favorable temperature obtains.

3. Post-maturation conditioning is completed in different lengths of time by different seeds under a given set of conditions, thus leading to the accumulation of germination-ready seeds over time.

4. Germination (root-germination) is supported at different temperature levels in different populations and probably in different seeds of the same population, leading to the expectation that the control is genetically variable.

The view represented in items 1, 2 and 3 separates the Boyce Thompson root-germination phase into two parts: warm/moist environment for post-maturation conditioning (the development of germination readiness) and a root-germination environment. Although the effective temperature ranges of these two phenomena may overlap, the latter range is somewhat cooler.

All four tentative conclusions were supported in the performance of seed lots which were followed by the author in 1969-70 (Hollingsworth, 1970). Seeds used included a sample of the previously mentioned Pehrson hybrids from one-year storage; homebred seeds of the current season, involving Paeonia lactiflora cultivars backcrossed to hybrids; current crop seeds of (*P. lutea* Pelavav ex Franchet x *P. delavayi* Franchet) and *P. lutea* var. *Ludlowii* Stern and Taylor; and, current and old crop seeds of *P. suffruticosa*. When the seeds were held for durations ranging from 5 to 13 weeks at a temperature range of 24°c. and above, 2 germinations were found in the (*P. lutea* x *P. delavayi*) lots. No other root germinations were found in more than 700 seeds.

Upon transfer to a cooler location, which ranged 10-16°C for the majority of the time, germinations were found in all lots at inspection two weeks later. Eleven of the 20 lots had reached 50% or higher root germination. At the third-week inspection, most of the lots which had been allowed 8 weeks or more of warm/moist conditioning had reached or were near their peak level of germination for the season.

In another set of observations (Hollingsworth, 1974), a large sample of seeds (708) from one pod parent, *Paeonia lactiflora* '**Opha**', of open pollination, was divided and carried for two relatively long durations at 24°C and above. Group A, 517 seeds, after 96 days showed no root germinations at the time of transfer to cooler temperature. In 17 days root germination was 44.1% and afterward peaked at 54.0%. The second lot, Group B, 197 seeds, was carried just 25 days longer and had 1 long root when inspected before transfer to a cooler location. After 18 days 95.5% root germinations were found, a difference of more than 40% for a seemingly brief additional period of warm/moist conditioning. These results suggest that when not all viable seeds germinate the failure is due to insufficient time for the development of germination readiness in all of the seeds.

Meyer (1976), at the University of Illinois, used in vitro embryo culture techniques to produce seedlings of several Paeonia lactiflora cultivars. He used lighted racks in an 80°F (26.5°C) room for the initial development. On a modified Linsmaier and Skoog medium, excised embryos enlarged rapidly and the hypocotyl-root axis extended as much as 3 or 4 inches in two months. (Results were negative on Knop's and Knudson's mediums.) When the enlarged embryos were transferred to a cold chamber at 35°F (2°C) for 28 or more days and returned to the light racks, true leaves commenced to develop immediately. It appears that the root germination block was either not present or was overriden and that the chilling requirement was substantially less than was found necessary for the tree peony seedlings in the Barton studies, yet the degree of dormancy reduction was able to proceed normally at a much higher temperature, 26.5°C, whereas tree peony seedlings from intact seeds failed at 21°C after a much longer period of chilling. However, in the absence of data which would define basic differences in the adaptations of the two species, no firm conjecture is very defensible. In this connection, it can be said that the superficial evidence from this observer's experience is that tree peony seedlings are less tolerant of high temperatures during leafy shoot production — in the range of 30°C and above, for example.

Yoneda and others (1978) at Osaka University, Japan, root-germinated excised embryos of *Paeonia lactiflora* on a culture medium which included gibberellin A3 (GA3)at concentrations of 0.01, 0.1 and 1.0 ppm and using temperatures of 11°C and 20°C. At both temperatures a graded response was obtained, the percentage yield increasing as the concentration of GA3 was increased, reaching 100% at 1 ppm.

In another part of the Yoneda (and others) study, intact seeds were root germinated, followed by leafy shoot production, without chilling, while held at 20°C when they had been previously soaked in an aqueous solution of GA3 at 1000 ppm for 48 hours. Germination commenced in 19 days and was complete in 34 days at 100%, values which are comparable to those obtained by this writer only after an extensive period of warm/moist conditioning, as is referenced above. Untreated controls started germinating in 24 days and reached 95% after 94 days. No leafy shoots were obtained in the control lot.

Germination percentage and germination rate are two of the commonly used dimensions upon which seed quality is measured for commercial use (Hartmann and Kester, 1975). These dimensions are equally relevant in the economy of seedling production by breeders. Growers want to germinate a high percentage of the viable seeds and to know how to obtain the seedlings at a season when adequate conditions can be given for their survival. Germination percentage is the total germination over the entire period of measurement. Germination rate considers how promptly the germinations are obtained. For example, in cases of slow germinating species, a statement of germination percentage may include a time element, indicating the number of seedlings produced in a given length of time.

In the Boyce Thompson Institute 1933 report, the yield of seedlings was not very high under the best performing conditions which were identified. Root germinations were reported at 4-53%, followed by leafy shoot production of 27-100% of the rooted seeds. Applying the leafy shoot rates to seeds started, the two percentage ranges can be multiplied, which gives the range of 1-53%. These values are not appealing when one is working with rare seeds as is often the case in a breeding program. It should be noted, however, that the report did not account for the failed seeds between those which may have remained quiescent and those which rotted. This low yield is therefore a matter of uncertain significance.

The principal concern in attempting first year seedling production in peonies continues to be the unreliability of results. Yields are often good, particularly when the seeds can be put into germination procedures as soon as they have reached maturity. This allows a maximum of calendar time before the onset of early spring growing temperatures, the normal time of seedling emergence out of doors.

When the results arc not good, they are often especially disappointing, owing to the irretrievable loss of partially germinated seeds. When the grower has followed the indications of the Boyce Thompson studies, such loss comes principally as incomplete reduction of epicotyl dormancy, the last stage of development before seedling emergence. When the seedlings are planted in a warm spring environment, where the temperatures are no longer low enough to adequately support dormancy reduction, those which are not already free to grow do not produce a leafy shoot and eventually perish.

When a grower has access to suitable facilities, the technology-intensive studies cited above offer very promising prospects. Meyer (1976) produced seedlings ready to go to the greenhouse in four months by making use of in vitro techniques with excised embryos. This production schedule permits a starting time well into autumn while still having the seedlings into a cool greenhouse for the early part of their growing period. The Yoneda and others procedures (1978) using GA3 apparently produced seedlings in an even shorter time. However, the techniques of both procedures entail an unusual amount of skill and knowledge, as do the procedures necessary for carrying the plants on their own afterwards.

The present study is concerned with the question of what may be done to improve the control of a more natural process of germination. The basic objectives are twofold — additional definition of the germination process in Paeonia, and enlargement of the knowledge base available to growers for projecting the probability that a particular seed lot may be a favorable candidate for success with first year germination procedures.

In particular, the experiments will seek to:

1. Verify the indicated reduction of a post-maturation barrier to germination, a process which leads to the accumulation of germination-ready seeds over time in warm/moist environment.

2. Verify the indicated temperature-limited germination of germination-ready seeds.

3. Examine factors which may influence the length of time required for reduction of the post-maturation germination barrier, specifically: 31°C compared with 25°C; dry seed weight; parent source cross; duration of after-harvest dry storage.

4. Verify time/temperature-dependent reduction of epicotyl dormancy; attempt to obtain complete reduction of the dormancy, such that normal leafy shoot growth will take place in a warm greenhouse (21°C and above); and, look for easily apparent morphological indicators that dormancy is completely reduced.

5. Evaluate seed covers permeability to water as a factor in the delay of germination.

- Don Hollingsworth

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SEED DISTRIBUTION:

lactiflora - from dark red clones
- from mixed colors
- from Minnie Shaylor
tetraploid - from dark red clones of Dad F_2 and #114 and #113. (#114 is Crimson Classis)

- tetraploid - advanced generation clones - mixed colors
tetraploid - from Roy's Best Yellow (pale yellow F_2)
And a few Nosegay F_4's which are mlokosewitschi x tenuifolia, nice 24" plants with pale yellow flowers
suffruticosa-tree peony seed from named varieties

109 Benedict Road
Pittsford, N.Y. 14534
February 21, 1993

Mr . Chris Laning,
553 W. F . Ave.
Kalamazoo, Michigan 49007

Dear Chris,

Don Hollingsworth has forwarded the species Peony seed, mlokosewitschii and obovata to me. It is safely planted in vermiculite and plastic bags in a warm spot. Results are being anxiously awaited . My sincere thanks to you for running the seed exchange and making it possible for me to have mloko again . The seeds turned a beautiful turqoise after being soaked. That is a hopeful sign.

After a lapse of twenty years,. I really plan on getting back into peonies again. My name is on the original Paeonia list as Mrs. Robert Stokes. I was a member until March 74 and still have all the back copies, which I was poring over this week.

In the 1990 book, The American Hybrid Peony, it mentions that you have been the editor of Paeonia for twenty years. Does that by any chance mean that Paeonia is still in existence? If it is, I would be very interested in receiving it again. Please let me know what the dues would be.

I was truly saddened to see that Roy Pehrson passed away in 1982. He had such a gift for explaining complex hybridizing points.

Is there any archive anywhere where the back copies of Paeonia are available for study? I feel a little behind in my knowledge of the field and would like to catch up. Back then all the excitement was in the new Ito crosses and the chance for a better yellow. That seems to have been achieved now with your '**Sunny Boy**' and Don Hollingsworth 's '**Garden Treasure**'. Is any nursery propagating Roy's '**Roy Pehrson 's Best Yellow**' which you registered for him?

At present I am trying to figure out what breeding stock I should purchase and what my goals as a hybridizer should be. I know that I want to breed for fragrance. Just ordered Early and Late Windflowers from Caprice to capture the Emodi bloodlines and fragrance. Now if I can just get Reath's to send me a '**White Innocence**', I will be all set. I ordered one from them last year, but they substituted 'Archangel' instead. Not at all satisfactory from my point of view, but I understand it is a good one for breeding stock although not fragrant.

Anyway, thank you again for obtaining the mloko and the wonderful surprise of the obovata rosea seeds . They will have every care humanly possible and a lot of hopes for the future.

Very sincerely yours,

Donna J. Linsley

553 West F Ave.
Kalamazoo MI 49004
March 5, 1993

Donna J. Linsley
109 Benedict Rd.
Pittsford, NY 14534

Dear Donna:

The Emodi hybrids are a difficult group for hybridizing since so rarely is a viable seed obtained.

'**White Innocence**' is available from me for $25.00 — I have a whole row of them and am always hoping to get a seed which will germinate.

Saunders' '**Early Windflower**' and '**Late Windflower**' only rarely give a seed. I have a row of them too and offer a division for $25.00. Dr. Saunders succeeded in getting four plants from them so the four are F_2's. These clones have red flowers on big bushes — not at all charming like his F. Windflowers.

Roy Pehrson succeeded in getting a seedling from '**Laura Dessert**' x emodi mlokosewitschii. This plant is fertile, has red flowers on a fair sized bush and is definitely a hybrid. I'll offer a division of it for $25.00.

Nothing, as yet, has been developed that is as pretty as Saunders' Windflowers.

Why don't you try something that is less difficult?

'**Roy Pehrson's Best Yellow**' has not been giving any seedlings with a good yellow color, most of them are of a cream color. (I'll send you some seeds from it.) But the new yellow singles, semi-double and double yellows are producing good offspring, some of which I'll name next fall.

Under separate cover, past issues of "Paeonia" are being sent to you.
Sincerely,
Chris Laning

ED. NOTE:
Don Hollingsworth-says: "Super D merits registration and not just because it has a uniquely beautiful flower for its season. Registration should also be viewed as a measure to reduce the risk of the genetics being lost (as when we devotees are gone). A name enables increased interest for commercial propagation and distribution. Distribution of any kind reduces risk of loss and grown in gardens for itself gets into more use than it will ever attract for breeding alone."