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DIFFICULTIES IN THE GERMINATION OF CYPRIPEDIUM SEED

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THESIS

DIFFICULTIES IN THE GERMINATION OF CYPRIPEDIUM SEED

By

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Introduction

Cypripedium, a genus of the family Orchidaceae, includes terrestrial and epiphytic orchids belonging to the monocotyledonous group of plants. The plants of this genus have sympodial type of vegetative structure, terminal inflorescence and solitary, irregular and perfect flowers. According to Swingle (25) the Monocotyledons reach the climax of their development in the Orchidaceae and in floral complexity they surpass everything else in the plant kingdom.

In addition to being valuable from a taxonomic viewpoint, the flowers of the Orchidaceae are among the most cherished of any in the plant kingdom. Their culture in the greenhouse has become a fine art and their colors, form and profusion of blossoms are pleasant sights to all that observe them. The Cypripedium, although not as important commercially as other genera such as the Cattleya and Laelia, is an important commercial cut flower, pot and decorative plant. This genus contains many interspecific hybrids which offer a field of opportunity fascinating to the plant breeder.

Unfortunately the Orchidaceae, especially Cypripedium, are sensitive to abuse and difficult to propagate sexually. The seeds are extremely small and, according to Costantin (7), are without stored food material, inclosed in an integument, and the embryo is formed of a simple minute mass of undifferentiated cells. The difficulties encountered in germination of the seeds, the time required by the life cycle of the plant from pollination to the production of blossoms, and detailed cultural methods contribute to make the cost of production of flowers relatively high in this group of plants. Veitch (26) states that the time that elapses from the pollination of the stigma to the fertilization of the ovule of

Orchidaceae is about four months. He also noted that it required eleven months for the capsule of the Cypripedium to ripen completely. After the seeds are dispersed from a ripened capsule, their germination and the growth of the plant to maturity and blossom requires an additional four to eight years, depending upon the genus. Cypripedium requires four to five years to produce blossoms from the seedling stage.

Review of Literature

For years there has been a controversy regarding the most satisfactory method for the germination of orchid seeds. White (27) lists the three most important and common methods, namely: (1) On a substratum not artificially inoculated with a fungus, as practiced by G. E. Baldwin and Company; (2) In flasks containing peat mixtures inoculated by a fungus, as practiced by Charlesworth and Company; (3) In flasks containing a nutritive solution, as practiced by Armacost and Royston, Inc.

The older method of Orchid seed germination, on a substratum not artificially inoculated with a fungus, was used by Dominy and Dean Herbert. According to Veitch (26), the first hybrid orchid "raised by hand" was Calanthe X Dominii which flowered in October, 1856, a cross of Calanthe masuca and Calanthe fucata by Dominy; the first Cypripedium hybrid was made by Dominy in 1869, Cypripedium Harrisianum. One of the earliest and probably the first attempt to raise orchids from seed produced by the cross fertilization of different species was made by Dean Herbert (10) who stated that cross breeding among Orchidaceae plants would perhaps lead to very startling results, but unfortunately they are not easily raised from seed.

Williams (28), Moore (20), Rand (23), Burberry (4), and Boyle (3)

describe the earliest method used in germinating orchid seeds, and one which was used by Dominy and Herbert. The seeds are sown on a block of wood or in a pot where an orchid plant is growing. This method requires the use of peat and moss as a medium and is still employed. Osborne (21) reports fair success in the germination of Cypripedium seeds on pots of the parent plant. Lambeau (18) does not favor the use of fiber and compost for the medium as did the preceding authorities, due to the fact that seeds are easily washed down into the compost. He uses yellow loam, allowing the seeds to germinate around the parent plant. Hill (11), the orchid grower for Lionel De Rothschild, suggests the sowing of Cypripedium seeds on the surface of fibrous compost around a newly potted seedling of their own genus. Hill selects a free-rooting, young plant and repots it into a 3 1/2 inch pot early in the season, January, and then sows his Cypripedium seeds around this plant in March of the same year.

White (27) reports the use of new, sterilized 3 1/2 inch pots filled with broken crock, chopped osmundine and charcoal; then topped with live sphagnum moss. This is covered with a sterilized disk of toweling or coarse muslin, and the entire pot content is saturated with water. The seeds are scattered thinly over the surface of the toweling.

The use of flasks artificially inoculated with a fungus, the symbiotic method of germination, was first described by Bernard (1) and Burgeff (5). This method was soon adopted by Charlesworth and Company and used commercially. It calls for the use of a mixture of peat and sand, stiffened with agar, in a flask as a desirable medium for fungi and seeds. The flasks are sterilized in an autoclave, inoculated with the proper fungus, and the seeds are sown on the surface of the medium.

Orchid plants, growing naturally, have within the cells of the root cortex fungal hyphae with a very characteristic appearance (See plate 1).

This is the "special fungus" referred to later by Knudson (14) and Ramsbottom (22) and is the fungus used by Bernard (1) and Burgeff (5) described in the preceding paragraph. It was known from the studies of Wahrlich, 1886, (7) that the roots of orchids invariably contain fungi. Costantin (7) stated that it seemed to him that important consequences must result from this verification (fungi on roots) and that this invasion of the roots of these plants by fungi must have affected their evolution and been the cause of their peculiarities of structure and modes of life. The seeds were undifferentiated and so minute in size that Costantin (7) was led to affirm that the minuteness of orchid seeds was one of the remote results of the presence of mycorhiza and due in all probability to toxins, which, acting at a distance, prevented the development and production, as in nearly all seeds, of a radicle, a hypocotyl and cotyledons. Costantin (8) states also that aerial roots do not contain fungus, and that the fungus is never found at the tip of a root, but somewhat (one to two inches) back from the root tip. Costantin and Bernard (8) obtained desired fungus in one out of ten trials.

Ramsbottom (22) describes the method of attack by the fungus on seeds. The embryo is usually undifferentiated, or nearly so; the cells of the seed differ in size, those cells at the suspensor end being the larger. The fungus always enters the seed at the suspensor end (See plate 3). It does not pass through the cells of the seed, the most usual mode of growth of a parasitic fungus, but grows in a tangled manner, filling one cell before passing on to the next, and remains restricted to the cells at the suspensor end. The symbiotic view is that a special fungus which enters the seed supplies the necessary "stimulus" to germination. Knudson (14) observed that many fungi (including the special fungus) have a

stimulating action on seed germination, but he noted that this stimulus was due to the production of different forms of sugar from the substratum and not necessarily required the entrance of the fungus into the seed.

Costantin (7 and 8) describes three species of fungi beneficial in the germination of orchid seeds; One inhabits the roots of Cypripedium, Cattleya, and Laelia; another is associated with Phalaenopsis and Vanda; the third is restricted to Odontoglossum. The experiments of Bernard, according to Costantin (8), show that by changing the fungi of orchids one may create monstrosities.

Mercier (19) used a Rhizoctonia in germinating Cattleya and Laelia seeds, both in Brazil and in France. He makes the statement that it is possible to germinate orchid seeds without the presence of Rhizoctonia, but that it is then necessary to furnish them an appropriate food consisting of very concentrated salep (jelly of crushed orchid roots), an added solution of carbohydrates and nitrogenous matter. Mercier (19) in summation, however, states that the seeds of orchids, except for those rare ones which contain a sufficient reserve of food, are not able to germinate without contact with symbiotic Rhizoctonia.

Reychler (24) suggests a still different idea on the germination of orchid seeds. He states, "The germination of orchid seeds is in no real sense dependent upon the assistance of the fungus, but that on the contrary, the seeds contain within themselves all the nutrients they require. I am convinced that the reason is that the too-feeble germ is unable to pierce the outer covering."

According to Knudson (12-13), the difficulty of germinating orchid seeds is due in part to inherent causes, but undoubtedly is due also to environmental factors. The small size of the embryo renders it liable to

death if it becomes desiccated; also, fungi attacking the seeds quickly and easily result in the death of the embryo. Knudson (12-13) germinated seeds successfully without the aid of a fungus when sugars were supplied in flasks containing a nutritive solution, the asymbiotic method of germination. Fructose appeared more favorable than glucose. Knudson (12) gives other media for successful germination. He states that germination is also possible on certain plant extracts containing traces of sugars; also, Bacillus radicicola from alfalfa, and Penicillium have a favorable influence on the germination and the development of chlorophyll.

Knudson (14) isolated a fungus, Rhizoctonia repens, and used it along with starch and sugar media. He obtained some germination but observed that the starch was completely digested to sugars, and that the hydrogen ion concentration was changed from a value which was unfavorable for growth to one which was favorable--usually from a pH 7 to a pH 5. He also found that germination was obtained without the infection of the seeds by the fungus.

Costantin and Magrou (9) stated that seedlings produced by the non-symbiotic method would not flower; however, Knudson (15) sowed seeds of a Laelia-Cattleya in a tube culture which flowered seven years later on agar-agar medium. There was no fungus in the roots.

La Garde (17) used the non-symbiotic method of germination for Cattleya hybrids. He used slanted agar in flasks of 250 cc. capacity containing solution B of Knudson and one solution of his own derivation. La Garde also tried out several different kinds of sugars and varied the hydrogen ion concentrations. The sugars he used were levulose, glucose, sucrose, and maltose. He observed that the best results were obtained with maltose and a pH value of 5.2 to 5.4. Seedlings grown on a medium with a pH above

5.6 showed very slow growth and chlorosis. Clements (6) showed that maltose was not readily utilized by plants and that glucose, if in excess, produces discoloration of the protocorm with probable malformation. Clements believes that the hydrogen-ion concentration of the medium is important for successful germination. His most successful pH value was 4.5.

Materials and Methods

For the germination of the Cypripedium seeds the three commercial methods were used, as reported previously by White (27). Flasks and test tubes stoppered with cotton were used as containers for those seeds to be sown on an agar medium. The test tubes were six inches long and one inch in diameter, and the flasks were of one liter volume size; Knudson (13-16) states that the larger flasks should be used in order that a better exchange of gases can be obtained.

Asymbiotic Method

The nutrient solution suggested by Knudson (12), solution B, was used in an agar-agar base. This solution contained:

Calcium nitrate	1 gram
Potassium acid phosphate	0.25 gram
Magnesium sulfate	0.25 gram
Ferric phosphate	0.05 gram
Ammonium sulfate	0.50 gram
Distilled water	1000 cc.

To this nutrient solution was added 17 grams of agar-agar (pure stripped form) and 20 grams of sugars*, making a 2% sugar solution. This nutrient agar solution was heated just to boiling and kept at that temperature for two or three hours, until the agar was completely dissolved; this prepared solution was then strained through a cotton filter and poured into the flasks and tubes. These flasks and tubes were then sterilized in the autoclave for 30 minutes at 17 pounds pressure. Immediately after removing the hot solutions from the autoclave, the hydrogen-ion concentrations

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* In some cases the sugars were omitted.

were determined* and corrected to the desired values by the use of a 1% hydrochloric acid solution. The acid was added at this point instead of before sterilization because the pressure and heat together with the acid conditions caused the agar and sugars to break down. This break-down resulted in a watery material instead of a solid jell. This was especially true with sucrose and maltose.

The sugars used were fructose, maltose, sucrose, and glucose at pH values of 5.0, 5.5, and 6.0. The number of samples of each used is shown in Table 1.

Most of the flasks were slanted, but some were allowed to remain upright, the latter being the general commercial method. It was observed that with the slanted flasks, inoculation was easier and there was less cause for contamination. The slanted flasks allowed the neck of the flasks to remain in the flame during the inoculation of the agar with seeds, preventing fungi spores from entering. These flasks and tubes of prepared sterilized nutrient agar were allowed to stand for four or five days in order to be sure of sterile conditions within the container.

The orchid seeds were sterilized for 30 minutes in a calcium hypochlorite solution previous to sowing; seeds were then sown directly from this sterilizing agent with a looped platinum wire. Wilson (29) suggested the use of calcium hypochlorite, and Knudson (12) used ten grams of calcium hypochlorite to 140 cc. of water, filtering, and using the filtrate as the sterilizing agent. The seeds are sown thinly, about 100 per liter flask, over the surface of the agar. The flasks were then plugged with

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* The hydrogen-ion concentrations were determined by the addition of methyl red indicator to a sample of the nutrient agar solution and comparisions made colorimetrically. The pH range of methyl red is 4.4--6.0, the desired range for optimum seed germination according to Knudson (13) and La Garde (11).

cotton and capped with paper to cut down contamination through the cotton plugs.

Symbiotic Method

The preparation of the nutrient agar medium for the symbiotic method was the same as described previously for the asymbiotic method. The procedure for sterilizing and inoculating the seeds into the flasks was identical in both methods.

The fungus that was isolated and used in the symbiotic method was a Rhizoctonia which is found in the cortical cells of the roots of the Cypripedium. Two methods of inoculation were used. One was very similar to the "salep" method of Bernard (1); segments of the orchid root were placed in the flasks with the seeds. The other, a more difficult procedure, was the isolation of the Rhizoctonia and inoculation of this fungus into the flasks in the pure state. The latter method requires about two weeks isolation period before the fungus is ready to introduce into the flasks.

To obtain the desired fungus, free-rooting, healthy plants of Cypripedium were selected; the underground roots were cut about three to four inches from the tip. These root samples were then cut into strips about 1/2 inch long, washed carefully, and placed in a calcium hypochlorite solution, where they remained for 24 hours. After this cleaning period the roots are removed and passed slowly through an open flame; then they are carefully scraped and placed into the hypochlorite solution again. After remaining in the sterilizing solution for two days the root strips are removed and ready for use in flasks with seeds or in petri dishes filled with agar for the isolation period. This is the point where the two fungus methods differ. In the "salep" method, the cleaned roots were placed in the

flasks of agar and allowed to stand for a week before the seeds were sown around the root segments. This delay previous to seed inoculation was long enough for the Rhizoctonia to become visible on the agar surface. The other method, using a pure culture of fungus, requires much time and careful technique. After the root particles are cleaned, they are placed in agar-filled petri dishes and allowed to stand for a week; the fungus appears and then the Rhizoctonia is isolated into another petri dish. This procedure is continued until a pure culture of Rhizoctonia is obtained; then the flasks are inoculated with the fungus and are ready for seeding within four or five days.

Fiber and Peat Medium in Pots

This older method requires less equipment, time and skill. Four inch pots were used as containers, half filled with broken crockery. The pots and broken crockery were washed thoroughly before they were filled with Osmundine fern roots and peat, ratio of two to one. Over the surface of this fibrous mixture was placed a disk of clean toweling. The prepared pot was soaked for ten minutes in water and sterilized in the autoclave at 17 pounds pressure for 30 minutes. Upon removal from the autoclave, clean bell jars were placed over the steaming pots. The sterilized seeds were thinly sown over the surface of the toweling, and these seeds were watered every day with an atomizer. Table 1 shows the number of pots used and seeds sown.

Following the suggestion of Osborne (21), some seeds were sown on the surface of Osmundine fern roots and peat around the base of a Cypripedium plant.

TABLE 1. Media prepared for Cypripedium seeds; number of prepared flasks and pots at a given pH value.

Media	Number of Containers		
	pH 5.0	pH 5.5	pH 6.0
2% Glucose in agar-agar	6*	10*	6*
2% Fructose in agar-agar	5	5	4
2% Maltose in agar-agar	4	5	4
2% Sucrose in agar-agar	5	11	7
Fungus and nutrient solution in agar-agar	4	4	-
2% Sucrose and fungus (salep) in agar-agar	-	8	-
Nutrient solution alone in agar-agar	3	3	4
Sand and nutrient solution	-	0	4
Nutrient solution and Colchicine in agar-agar	1	4	1
Pots--fern root and peat	-	2	3

Media prepared for other orchid seeds; number of prepared containers at a given pH value.

Kinds of seeds	Number of Containers		
	Agar-agar 2% Sucrose	Solution 2% Glucose	Pots
	pH 5.5	pH 5.5	pH 5.5
Laelia X Epidendron	-	6	1
Bletia spp.	-	-	2
Cattleya spp.	2	1	2
Laelia autumnalis	8	-	-
Laelia atropurpurea X Cattleya Schroderii	8	-	4

* Approximately 100 seeds were sown in each flask or pot.

TABLE 2. Per cent germination of Cypripedium seeds to the green, protocorm stage in each medium prepared as given in Table 1.

Media	Per cent germination		
	pH 5.0	pH 5.5	pH 6.0
2% Glucose in agar-agar	1	1	0
2% Fructose in agar-agar	0	0	0
2% Maltose in agar-agar	0	0	0
2% Sucrose in agar-agar	1	3	1
2% Sucrose and fungus in agar-agar	-	0	-
Fungus and nutrient solution in agar-agar	0	0	-
Nutrient solution alone in agar-agar	0	0	0
Sand and nutrient solution	0	0	0
Colchicine and nutrient solution in agar-agar	0	0	0
Pots--fern root and peat	-	20	0

Per cent germination of seeds of other genera to the green, protocorm stage.

Kinds of seeds	Per cent germination		
	2% Sucrose	2% Glucose	Pots
Laelia X Epidendron	-	90	75
Bletia spp.	-	-	70
Cattleya spp.	16	6	10
Laelia autumnalis	0	-	-
Laelia atropurpurea X Cattleya Schroderii	90	-	60

Results

The primary interest in the germination of the orchid seeds was through the protocorm stage to the formation of the leaf. The protocorm stage is the globular, green stage produced after the testa is broken and the enbryo is enlarging. During the time of the breaking of the testa, a papilla* is formed.

The seeds of the Cypripedium in 90 per cent of the cases on sucrose, glucose, and fructose media broke their testae and formed papilla within a period of two weeks; then within 30 days an enlarged white spherical body was formed. This enlarged spherical body remained such for approximately 30 days, then turned brown and showed no further advancement. In 3 per cent of the seeds of Cypripedium on the sucrose medium with a hydrogen-ion concentration of 5.5, there was formed a green protocorm stage. Cypripedium seeds sown on a maltose medium were a complete failure, none developing beyond the papilla stage.

From the two observations mentioned below a question may still be asked: "Is fungus necessary for the germination of orchid seeds?" The seeds sown on a nutrient solution with fungus segments or "salep" were examined under a microscope. Those around the root sections contained the fungus, entering the suspensor end of the seed, but those seeds away from the root sections were not contaminated and showed no signs of germination. The seeds containing the fungus (See plate 3) did show papillae and broken testae; the seeds that were not contaminated with the fungus had not

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* Papilla is a hair-like protuberance formed by an epidermal cell, according to Costantin (8), and used by the seed as a primary feeding root.

broken their testae or formed papillae. This mode of germination is strictly symbiotic. Other observations were made under the microscope of seeds from the fungus-sucrose medium. The embryos of 80 per cent of the seeds from this medium were not contaminated with the fungus; however, the seeds had broken their testae and formed feeding hairs. Such an observation would lend confidence in the asymbiotic method of germination. Seeds sown on the pure fungus culture were contaminated and killed by the fungus which agrees with Knudson's statement (12-13) that the small embryo of the orchid seeds renders it liable to death by desiccation or attack of a fungus.

The Cattleya seeds that were sown on sucrose and glucose media have shown to date some germination to the advanced green protocorm stage.

Seeds obtained from a Laelia autumnalis-Epidendron ciliare cross made seven months previous to inoculation were sown on a glucose medium. Six flasks of these seeds were started to germinate in the botanical stock room. After 75 per cent of the seeds had reached a green protocorm stage, two of the flasks were moved to the germinating frame in the greenhouse. At the present, April, 1940, the spherical bodies present in these two flasks are brown and give no further signs of advancement, whereas the seeds in the four remaining flasks kept in the stockroom are forming leaves and show 90 per cent germination. The temperature variation in the greenhouse ranged from 21°C. to 32°C., whereas in the botany stockroom the temperature ranged from 23°C. to 29°C.

The seeds of Cypripedium, Cattleya and Vanda sown on the nutrient agar solution without sugar or fungus were a complete failure; in only about 20 per cent of the seeds did papillae form or testae break. The sand cultures were completely lost; no form or germination was observed.

The older methods of orchid seed germination, that is, on peat and osmundine around the base of the parent plant and on sterilized toweling in pots covered with bell jars, proved as successful as the two more scientific methods suggested by Bernard (1) and Knudson (12). The seeds around the parent plant were completely lost, but germination of seeds sown in pots and kept under a bell jar was more successful. The first three pots that were sown with Cypripedium seeds gave negative results, only brownish colored globular tissue was formed. Seeds of Cypripedium sown in two pots in August, 1939, were breaking their testae and forming white globular tissues; 90 per cent of these seeds have developed to this white spherical stage.

Cattleya seeds were sown in two pots and have remained under the bell jar since August, 1939. During this time about 90 per cent of the seeds have developed to the white spherical stage with green color showing in about 1 per cent of the seeds, but no further development has been noticed.

The pots containing seeds of Bletia spp. have produced up to the present time 60 per cent of germinated green globular bodies and in most instances these have produced leaves and have been potted in individual pots.

Seeds of the Laelia-Epidendron cross have shown 75 per cent germination in the pots, but seeds of Laelia autumnalis (selfed at Michigan State College) have shown no development. Careful examination under the microscope shows no papilla formation or broken testa, but some differentiation and enlarging of cells (see plates 7 and 8) has occurred; the examination also showed about 75 per cent of the seeds to be in perfect condition.

In practically every method used for germination of orchid seeds

the testa was broken and papilla formation was observed disproving Reychler's statement (24) that poor germination is due to the testa being too hard for penetration by the embryo.

Discussion

A statement mentioned previously in the review of literature by Knudson (12-13): "The difficulty of germinating orchid seeds is due in part to inherent causes, but undoubtedly is due also to environmental factors", divides the difficulties of germinating Cypripedium seeds into two distinct groups--inherent and environmental.

The difficulty in obtaining good viable seeds of the Cypripedium is based on inherent causes. Cypripedium seeds, being a mass of similar undifferentiated cells with no stored food matter, quickly becomes less viable after removal from the capsule. They are liable to death if they become desiccated; therefore, they should be sown very soon after ripening, usually within six months (30). Seeds purchased from two commercial sources were complete failures; some packages of seeds were contaminated with fern spores. The seeds of all genera that were successfully germinated to the protocorm stage were purchased from a single source, and those seeds that were produced as a result of our own pollination at Michigan State College.

One of the greatest difficulties encountered in the germination of Cypripedium seeds is the selection of a desirable medium for them, a long disputed question. This problem may be closely associated with inherent causes, but undoubtedly is due also to environmental factors. Do Cypripedium seeds require supplementary food in the form of sugar? Does the fungus supply a stimulus to the seed? Does the fungus break down the medium to a form more available for the seeds? Or do the seeds require either of these media for successful germination? The results showed no germination of seeds to the protocorm stage on a nutrient agar solution without sugars, but when the sugars were used the seeds germinated to the

protocorm stage; however, little advancement was made beyond this stage. The seeds infected with the fungus on a nutrient solution without sugars germinated to the protocorm stage and made no further advancement. Seeds on the fungus-sucrose medium were not infected with the fungus but developed equally as well as the seeds that were infected previously. These observations made of results obtained uphold both Bernard and Knudson's statements described in the review of literature. The germinating Cypripedium seeds in every instance made no further advancement from the protocorm stage, which may be due to other environmental difficulties such as temperature, light and moisture conditions. However, the last question asked, do the seeds require either of these media for germination, may be answered no by results obtained from seeds germinating on the sterile toweling in pots, but even on this medium the Cypripedium seeds did not germinate beyond the protocorm stage. The seeds of other genera such as Cattleya, Bletia, and Laelia X Epidendron germinated successfully on a sugar medium and sterile toweling, but no successful germination beyond the protocorm stage was observed on a fungus-infected medium. . The seeds of all genera showed no signs of desiccation on the agar media; therefore, it is questionable if moisture was a limiting factor in the germination of Cypripedium seeds in this study.

The hydrogen-ion concentration of the medium may be another environmental factor of importance in germinating Cypripedium seeds; however, no definite results were obtained through the use of media of different hydrogen-ion concentrations.

The browning of the protocorms may possibly be due to two environmental difficulties, temperature and light. This browning of the Bletia protocorms may have been due to the greater variation of temperature in

the greenhouses as compared to their original place in the stockroom, or it may have been due to the change in light that they were subjected to in each place. The temperature variation over a 14-hour day in the greenhouse was 11°C., whereas in the stockroom the temperature variation was 6°C. Maintenance of a constant temperature of 24°C.-28°C. is considered desirable. The seeds were subjected to approximately five hours direct sunlight through a newspaper covering every 24 hours in the stockroom, and approximately 8 hours of daylight through the same covering, whereas in the greenhouse they were subjected to 8-10 hours sunlight and daylight through two sets of shaded glass. The greenhouse glass and the glass on the frame both being covered unevenly with whitewash.

Along with these many outstanding environmental and inherent difficulties in the germination of the Cypripedium seeds come several minor difficulties encountered in technique. These include sterilization of the medium and sterilization of the seeds. All combine to suggest why an orchid flower is such an expensive, highly valued blossom.

Summary

I. Three methods of germination were used for Cypripedium seeds, namely, Symbiotic, Asymbiotic, and in pots under bell jars.

II. Sucrose, glucose, and fructose used as media for the seeds produced 90 per cent germination to the protocorm stage, but these seeds made no further advancement.

III. Seeds did not germinate on maltose medium.

IV. Cypripedium seeds supplemented with fungus root strips were contaminated and germinated to the protocorm stage, but ceased development at that point. Seeds on fungus-sucrose medium were not contaminated and germinated likewise to the protocorm stage and ceased development. The pure fungus culture killed the seeds.

V. Nutrient agar solution and sand cultures gave complete negative results.

VI. Seeds of Cattleya and Bletia on sucrose and glucose media showed some germination and production of leaves.

VII. Most of the seeds in pots under the bell jars germinated; however, only Bletia spp., Cattleya, and Laelia X Epidendron seeds had developed any green color at the close of this study.

VIII. Temperature, moisture, viability of the seeds, media, and hydrogen-ion concentration are the many inherent and environmental difficulties encountered in the germination of Cypripedium seeds.

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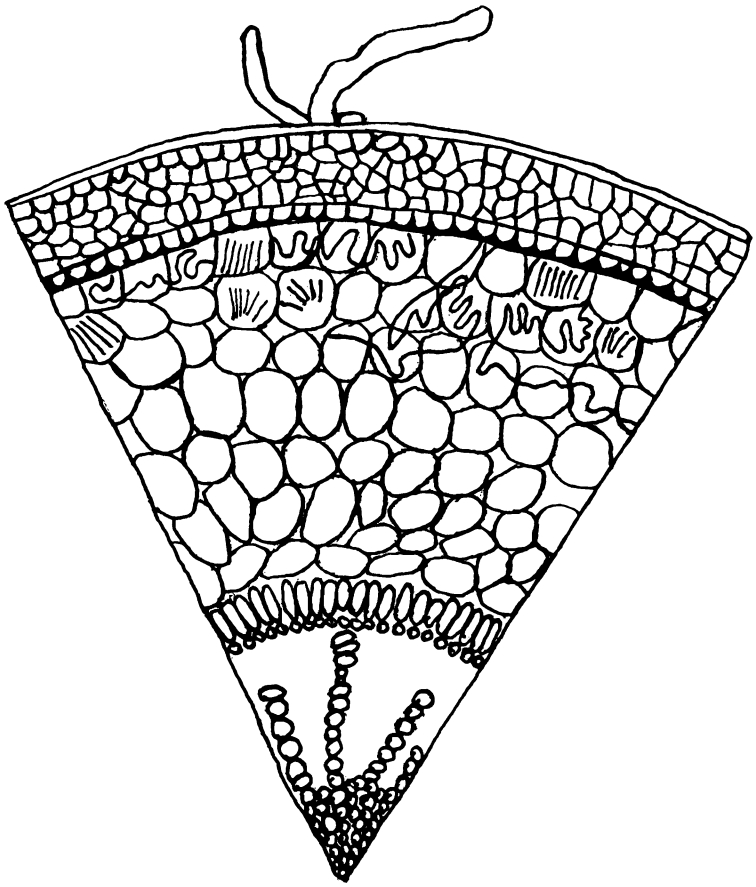

Plate 1

Cross section of Cypripedium root showing fungus infected cortical area.

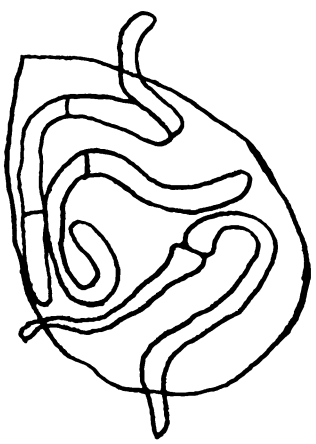

Plate 2

Enlarged cortical cell from Cypripedium root showing fungal mycelium.

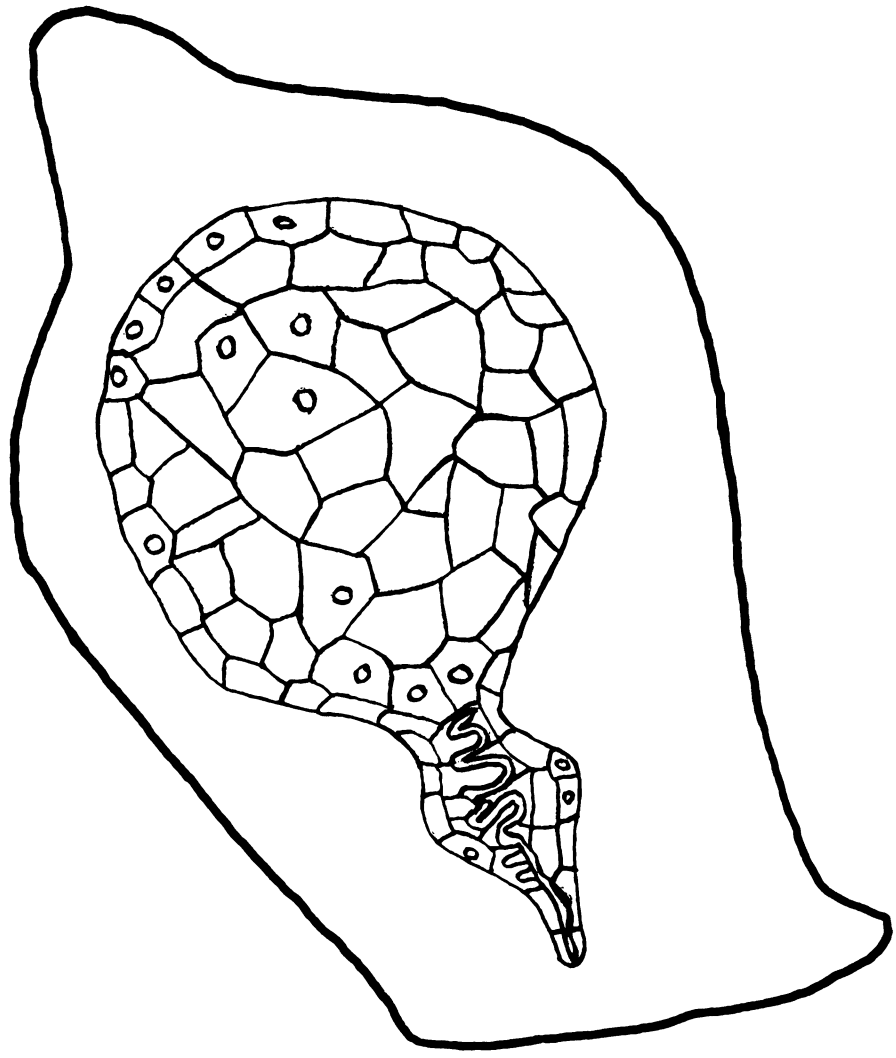

Plate 3

Laelia seed--Infected with fungus at suspensor end of seed.

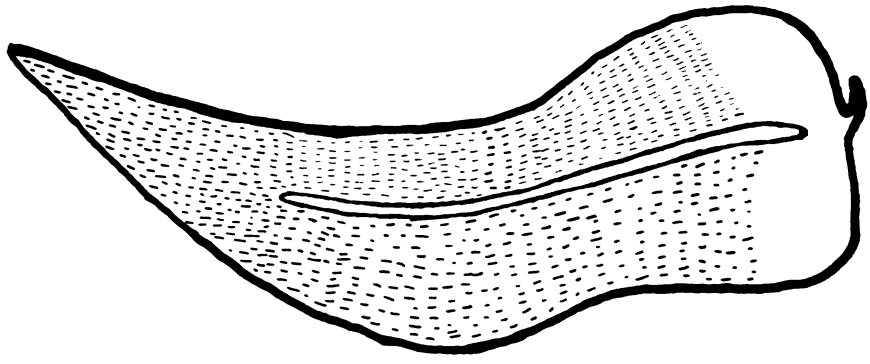

Plate 4

Protocorm of Laelia infected with fungus.
Center cylinder may be identified as the prostele.

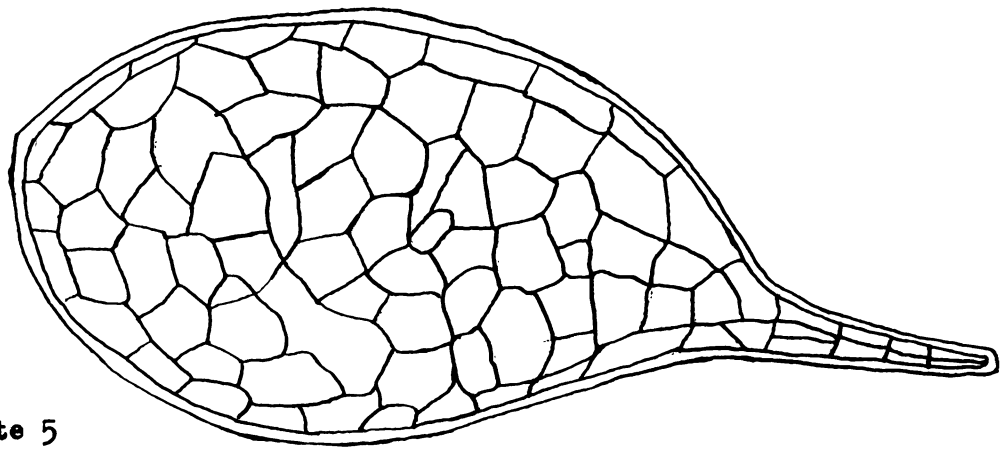

Plate 5

Cattleya--Undifferentiated cells of embryo sac.

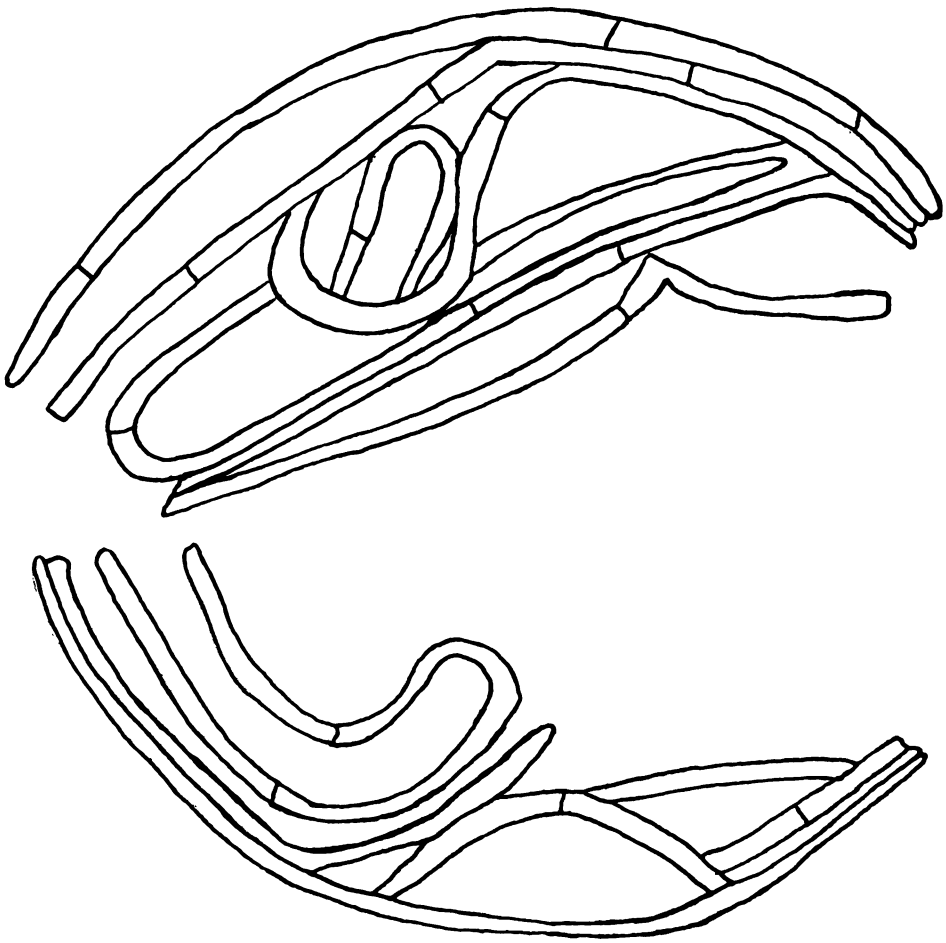

Plate 6

Cattleya--Distorted integument of embryo of Plate 5.

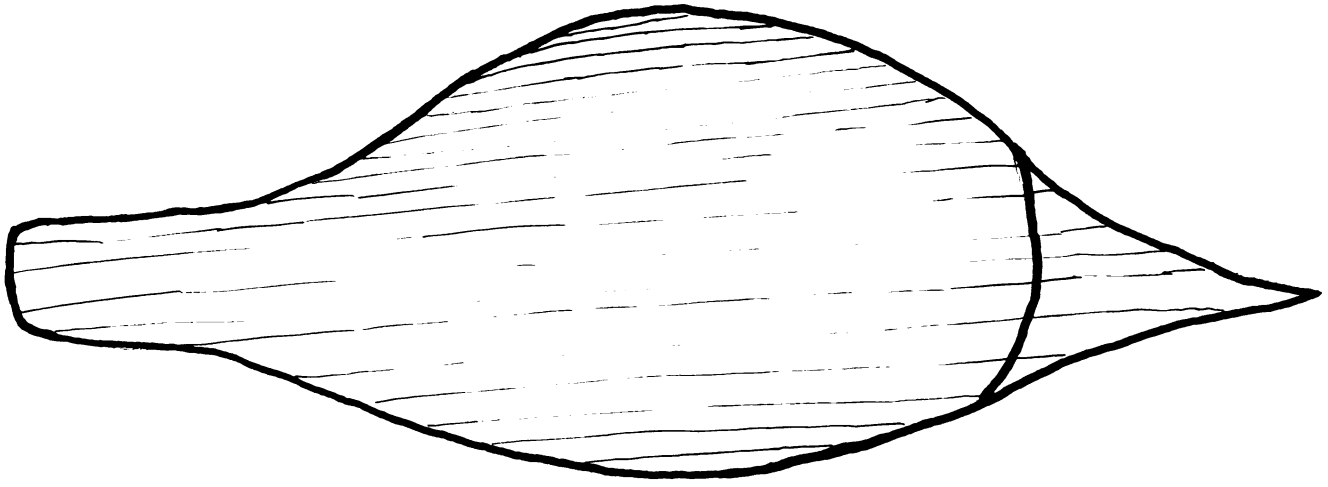

Plate 7

Laelia autumnalis--Seed with integument.

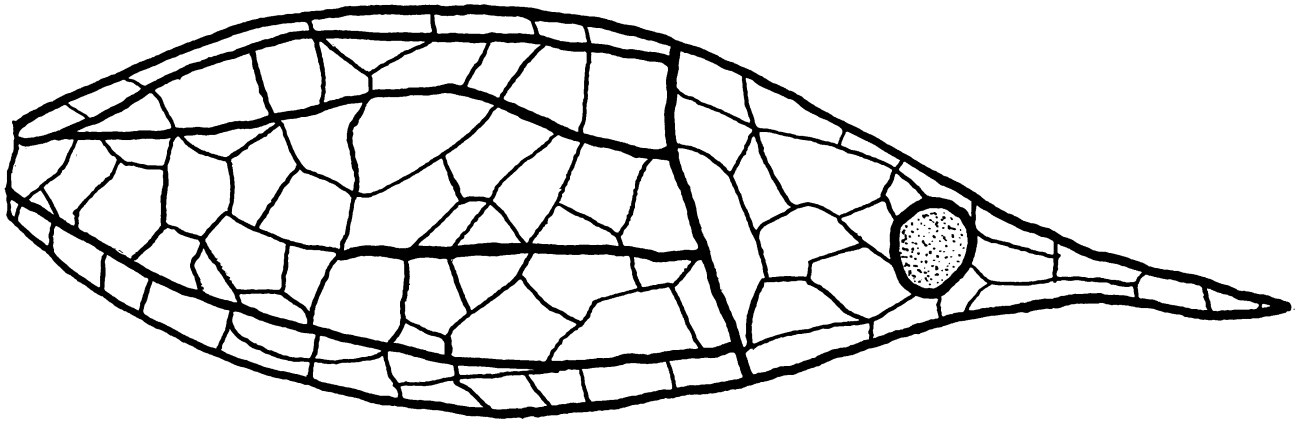

Plate 8

Laelia autumnalis--Differentiating cells of embryo sac.

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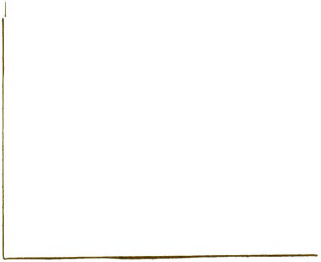

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