STUDIES ON THE GERMINATION, EARLY SEEDLING GROWTH AND NUTRITION OF CINCHONA

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INTRODUCTION

The early history of the use of the quinine bark as an antimalarial febrifuge will probably remain an unsolved mystery. The first reliable record of its use was in the year 1630, when, according to Suppan (24), the Spanish Corregidor of Loxa was cured of an intermittent fever by the bark. The most popular and persistent legend as to its discovery and introduction involves the Countess of Chinchon, but this legend has recently been thoroughly discredited by Haggis (12), who believes that the drug was introduced into Europe soon after 1633, through Belgium rather than Spain, either through ordinary commercial channels, or through the efforts of the Jesuits.

Regardless of the point or mode of its introduction into Europe, the next two hundred years saw an increasing recognition of its curative powers, given particular impetus when Pelletier and Caventou isolated the quinine alkaloid in 1820, resulting in an extravagant exploitation of the only natural source, a rather limited area in South America. Holland and England, because each had highly malarious colonies, finally became fearful of exhausting the natural supply, and took steps to inaugurate the cultivation of the plant.

Their early attempts at cultivation were discouraging. The few plants they were successful in establishing in Java and India were difficult to grow and returned low yields of alkaloids. Private enterprise could not be interested in the attempt until about 1873, when the very high yield from the plants from seeds collected by Ledger in Bolivia were reported from Java. There then began a production race between Ceylon, where the plantation owners had turned to cinchona when

their coffee acreages were being destroyed by disease, and Java. The result was overproduction and a consequent drastic reduction in returns. The climate of Ceylon not being as suitable for growing Cinchona ledgeriana, another species, C. succirubra, had been planted, but because of its lower quinine content, the Ceylon growers were forced to turn to another crop (tea) leaving the Dutch East Indies with a virtual monopoly of the cinchona industry.1/

This monopoly has been effectively protected since 1913, when an agreement was signed between growers and manufacturers which has controlled prices and prevented over-supply. Though it has been stated that the Java plantations could produce all of the bark that could be sold on the world market at a price that would sustain the growers, there never has been sufficient quinine available to adequately treat the estimated eight hundred million cases or prevent the current three million deaths annually from malaria. Other than in Java, only in India is a significant amount of cinchona being grown, and that largely as a governmental enterprise and in quantities far below what is needed for its own use.

Though the possibility was foreseen and large stockpiles of quinine accumulated by several governments, the occupation of the Dutch East Indies by the Japanese military forces in the current war has created an emergency situation for the United Nations. Plans are now being carried

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1/ A recent note by Fosberg (9) on the taxonomy of the genus Cinchona recognizes only twelve valid species. The names of the two principal forms of commercial importance, long referred to as C. ledgeriana Moens and C. succirubra Pav. ex Klotzsch., are currently considered invalid synonyms of C. officinalis L. and C. pubescens Vahl, though the author feels this classification requires further substantiation. The former names, however, are retained in this thesis to conform to conventional usage. The C. hybrid seeds used in some of the experiments resulted from a cross between selected individuals of C. ledgeriana and C. succirubra.

out or are under consideration to cultivate cinchona in the Americas and elsewhere, to provide an emergency supply of the drug in the event of a long war and to safeguard against a future interruption of the supply of this indispensable material. Although atabrine, the synthetic compound of German origin and now in large-scale production in America, plasmochin, another synthetic with less desirable properties and effectiveness, and totaquine, a total-alkaloid product that can be produced in a relatively short time from young cinchona plants, are all partially effective against the four main forms of malaria, quinine is still the preferred specific antimalarial febrifuge.

Thus, though emergency requirements could probably be met with drugs produced more quickly, until improved chemical substitutes are discovered, any conclusive campaign against malaria must still be based on quinine from the cultivation of cinchona. Furthermore, until higher-yielding strains of other species can be developed or discovered, past experiences indicate that the proven Ledger strain should be heavily relied upon.

From the numerous recorded accounts of previous attempts to cultivate the Ledger strain in many parts of the world, and from a knowledge of the conditions in Java which were obviously favorable for its growth, a knowledge of the general climatic limitations within which successful growth may be expected can be deduced with reasonable accuracy. A rich, deep, light loamy soil, at an elevation between 4,000 and 5,500 feet, with uniform temperatures averaging about 22°C. and always between 8° and 30°C. and a uniformly distributed annual rainfall of between 100 and 200 inches are known to be required for the best growth of this form. It will not tolerate frost or prolonged drought,

and excessive heat or poorly-drained soil seriously reduce growth and increase losses from diseases and insects. Within these general limits the plant is very reactive to environmental changes. Cultural practices as followed in Java, including propagation, nursery and plantation practices, cultivation and harvesting methods, have been recorded.

Most of the cultural details are recorded as observations before 1900; later information and experimental data are largely lacking. As the culture of this form has been almost exclusively confined to the Dutch East Indies since its discovery, and since it succeeded there so well, there has been little incentive for its subjection to modern experimental techniques in other localities. It has been repeatedly observed that the first few months are the most critical period in the life of the plant, and that its success is considered assured if it survives this period. Yet information as to the effects of conditions other than those encountered in Java, as will be the case in many of the localities in the Americas where the attempts are being made, is very meager, and such data as presumably have been collected in Java, largely by private plantation owners and their staffs, have not been recorded in the literature.

The present investigation was undertaken to provide at least preliminary data on this critical growth stage of the fastidious C. ledgeriana, of immediate value and importance to the current emergency program, as the rather limited supply of available seed must be utilized with the greatest efficiency. This investigation furnishes data on (1) the effect and importance of humidity and temperature and their inter-relationships on the maintenance of viability of the stored seeds, (2) the effect of various temperatures, light and media factors

on germination, (3) an evaluation of some of the factors affecting early seedling growth, and (4) nutritional requirements and nutrient deficiency symptoms during the earlier growth stages. These studies were conducted at the U. S. Plant Introduction Garden of the Department of Agriculture, and at the Horticulture Department of the University of Maryland.

LITERATURE REVIEW

As stated in the introduction, recorded experimentation on the horticultural aspects of cinchona cultivation is almost non-existent, and particularly as regards the early growth stages. Such references as are pertinent to the scope of these investigations will be cited under the various section headings to which they directly apply. For a recent review of the voluminous popular literature on the general subject of cinchona, the reader is referred to Morrison (18), and to Posniak (19).

PROCEDURES AND RESULTS

Germination Studies

Part I. Effect of Seed Storage Environment

It is often necessary that harvested seeds be held in an artificial environment for an indefinite period, and where such seeds are to be used for propagation purposes it is highly desirable that their maximum inherent vitality be retained. Though many seeds tolerate a wide range of conditions with little loss of viability, others rapidly lose their germinative power unless their storage environment is carefully controlled.

Few generalizations can safely be made as to optimum storage environments for seeds, as these vary widely with species, natural habitats and local conditions. In temperate species the effect of storage conditions on processes such as after-ripening, dormancies and stratification requirements may be involved, whereas in tropical species and those lacking these special adaptations an environment resulting in the suspension or reduction of germinative processes is sought.

Probably the most important readily controllable factor in the keeping quality of seeds lacking such special adaptations is the humidity of the storage atmosphere, but also important is its interaction with the temperature effect. As high temperatures and humidities favor germination it is logical to assume that a reduction in either

will have an inhibitory effect, and that optimum storage conditions should result from the reduction of both factors as low as is possible without inflicting permanent injury on the seeds. Akamine (1) found the control of either temperature or humidity of approximately equal value in prolonging the life of stored seeds in Hawaii, and in other investigations both factors have been found to be mutually dependent.

The extent to which seeds can safely be dried varies widely. Seeds of some aquatic plants are normally stored under water (2); some types have a fairly high critical moisture content below which there is a serious loss of viability, as oaks and hickories (8)(16), some maples (6), strawberry (13) and others; and still other types require a low moisture content for storage, as with mulberry (25) and elm (21)(3). The reaction of seeds to lowered temperatures during storage is usually less critical and temperatures somewhat above the freezing point are frequently employed with safety, though some seeds may be severely injured by below-freezing temperatures.

Cinchona seeds have long been known to lose their viability rapidly when held under the conditions of high humidity and temperature in which the plant succeeds, and though there are many observations recorded in the popular literature which indicate that high humidity was recognized as a chief cause of the decline in viability, not until 1920 were there experimental data to support the belief. Kerbosch (14) reports the details of an experiment in which a range of nine vapor tensions, from 7 to 92 percent, were maintained for three years by the use of various chemicals in well-closed bottles. All C. ledgeriana seeds stored at a relative humidity of 30 to 60 percent gave germinations of over 90 percent after three years, but those stored at higher or lower humidities

suffered a decline in viability. Kerbosch concluded that some drying was necessary for successful storage, but that a humidity of below 30 percent resulted in too strong a desiccation. Though a supplementary experiment in the same work indicated that viability was maintained in white glass bottles somewhat better than in yellow glass bottles, nevertheless Kerbosch recommended that cinchona seeds be stored over calcium chloride in bottles placed in the dark for best results.

Kerbosch did not study the effect of temperature on the maintenance of viability, but stored the samples at room temperature (stated to average 18°C.). Only one preliminary experiment on the effect of temperature has been recorded. A one-year test by Kevorkian (15) with C. ledgeriana seeds stored over calcium chloride at room temperature and in a refrigerator led to the conclusion that viability of the seeds so stored was prolonged regardless of the storage temperature. Though significantly superior to the checks, the stored seeds germinated only 30 and 33½ percent at the end of the test.

The rather limited supply of fresh seeds of C. ledgeriana received by the U. S. Department of Agriculture soon after the occupation of the Dutch East Indies was immediately stored over calcium chloride, in accordance with the recommendations of Kerbosch. But as the program for re-establishment of the species in the Americas made it probable that the stored seeds would be exposed to various temperatures, information as to the effect of this temperature factor was desired. This phase of these studies accordingly considers (1) the effect of four humidities at each of five storage temperatures on maintaining the viability of seeds of C. ledgeriana, (2) the effect of four humidities and three temperatures on maintaining the viability of C. succirubra and C. hybrid seeds,

(3) a comparison of C. ledgeriana seeds stored at five humidities in daylight with those stored in the dark, and (4) the effect of storing well-dried C. ledgeriana seeds in sealed tin containers placed at two temperatures.

Materials and Methods

In the investigation of the effect of both temperature and humidity on prolonging the viability of cinchona seeds, two-quart screw top glass jars were used as containers for the samples. The jars were fitted with metal caps which had waxed cardboard inserts, so that reliably airtight closures were easily made. A waxed screenwire rack was placed in each jar, upon which the small cheesecloth bags containing the seed samples were placed.

To control the humidity within the jars, the data and chart furnished by Wilson (27) of vapor pressures of sulfuric acid solutions were employed. Humidities of 0, 33, 66 and 100 percent were established in the jars. Pure concentrated sulfuric acid was used in the minimum, and water alone was used in the maximum humidity jars. The intermediate humidities were established by means of Wilson's data and frequently checked by properly calibrated hydrometers. Storage temperatures of 2°, 7°, 16° and 32°C. were accurately maintained. The fifth temperature used was that of the laboratory at the U. S. Plant Introduction Garden, which varied somewhat, but averaged about 24°C., and will be hereinafter designated as ±24°C. The specific gravities of the solutions at 25°C. which produced humidities of 33 and 66 percent in the jars at the desired temperatures are given in table 1.

One and one-half gram samples of C. ledgeriana seeds were weighed,

put in small cheesecloth bags and one bag placed in each of the twenty jars. One-gram samples of C. succirubra and C. hybrid seeds were placed in twelve of the jars, none being stored at 7° and ±24°C. Figure 1 is a natural size photograph of these three seed lots. The arrangement of the samples and solutions in the jars is shown in figure 2.

Table 1. Specific gravities of sulfuric acid-water solutions at 25°C. which when placed at the temperatures indicated produced relative humidities of 33 and 66 percent in the jars.

Storage temperature °C.	Specific gravity of solutions to produce relative humidities of 33 percent	66 percent
2	1.407	1.273
7	1.405	1.271
16	1.402	1.268
±24	1.400	1.265
32	1.397	1.262

The C. ledgeriana seeds had been harvested only a few months previous, and had been kept quite dry during this holding period. These seeds were in very good condition, for the tests at the start of the experiment resulted in 100 percent germinations. The previous history and care of the C. succirubra and C. hybrid seeds were not known, but test germinations of 86 percent for the C. succirubra and 90 percent for the C. hybrid seeds, under the controlled germination conditions used in this experiment, indicated that these seed lots may have already begun to decline in viability.

An arbitrary set of uniform germination conditions was selected to eliminate the effect of season on the results. Four replicates of fifty seeds were counted from each of the storage samples on each test date. The seeds were sown on seed flats of sphagnum moss, the location of a replicate on a flat being chosen at random. After sowing the seeds on

the previously moistened moss, the flats were again watered and covered with a pane of glass. They were then placed in a dark room at about 25°C. where a uniform exposure of 6 hours of light approximating 300 foot-candles at seed level could be automatically provided. The position of the flats in the room was changed several times during the germination period to minimize place effect.

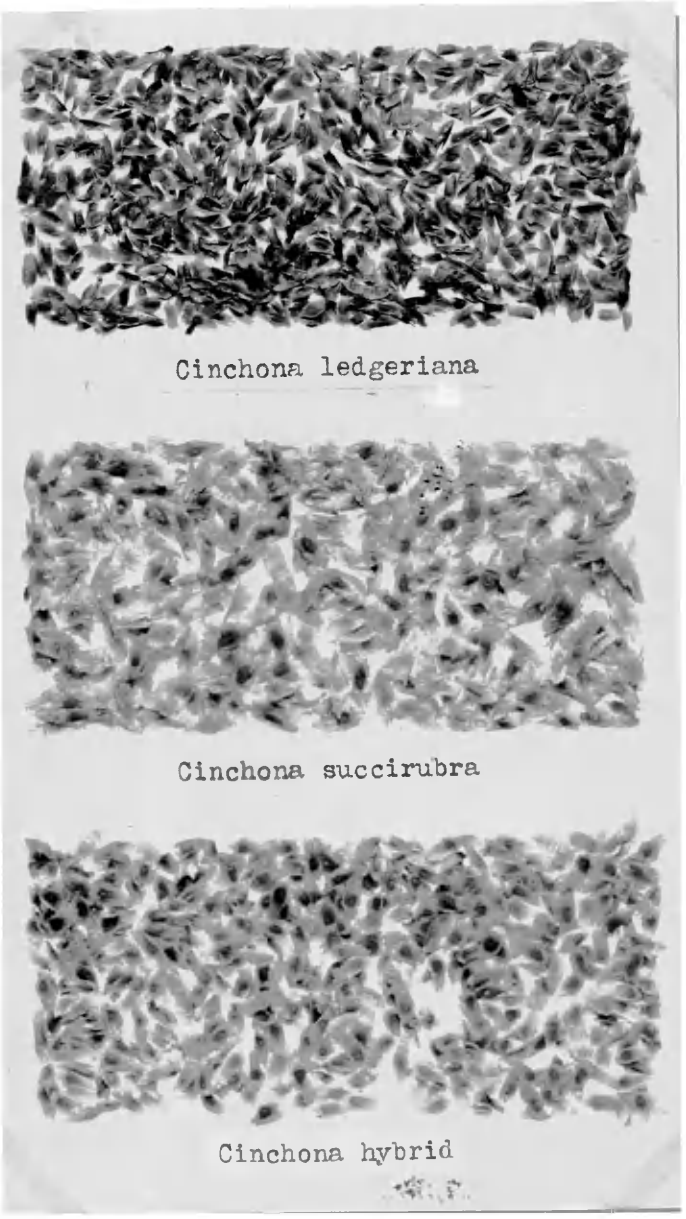

Figure 1. Natural size photograph of seeds of C. ledgeriana, C. succirubra and C. hybrid used in the storage experiments.

Figure 2. Arrangement of seed samples and solutions controlling humidity in treatment jars.

The experiment was started on July 10, 1942, and samples were germinated in the manner described at six progressive nine-weeks sampling dates. Figure 3 shows a flat of seedlings so germinated.

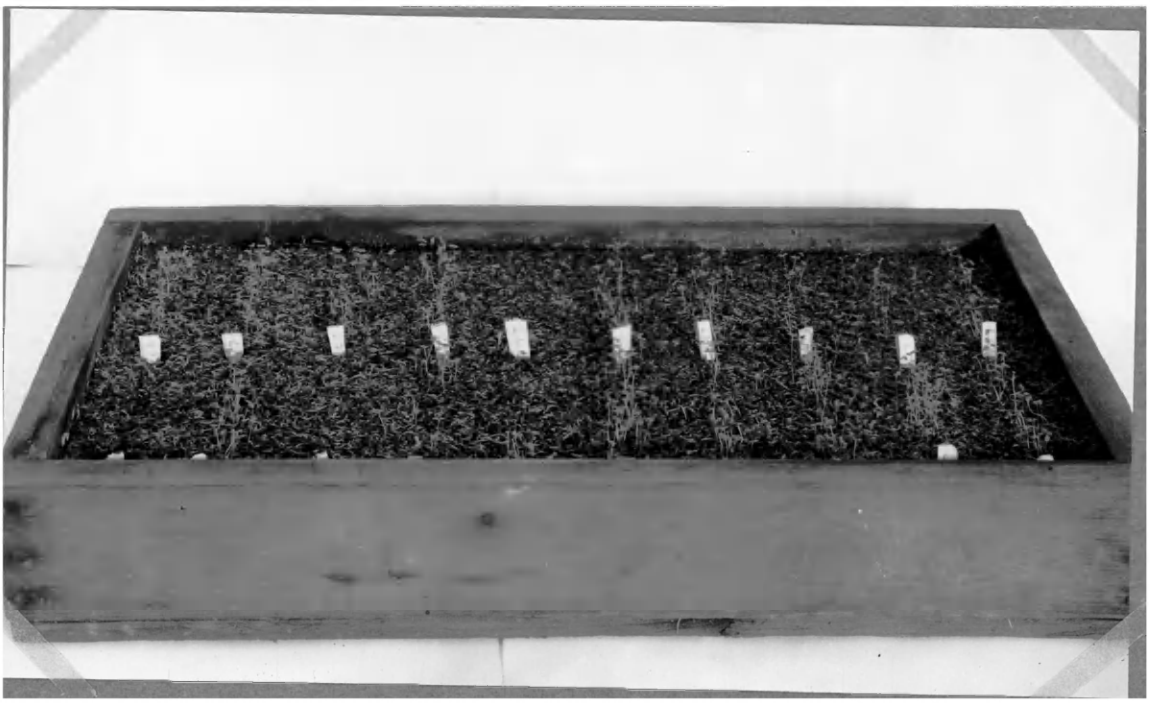

Figure 3. Photograph of seedlings of C. ledgeriana from seeds stored under various combinations of temperature and humidity, illustrating the arrangement of the fifty-seed samples on the sphagnum germination medium.

For the comparison of storing seeds in the light as against storage in the dark, as recommended by Kerbosch (14), fresh seeds of C. ledgeriana only were used. Again using the sulfuric acid-water solution technique to control humidities, seed samples were stored at room temperature and humidities of 0, 25, 50, 75 and 100 percent. Narrow-mouth, eight ounce glass bottles with rubber stoppers were used as containers. A piece of 7 mm. glass tubing was held in the center of 15 x 75 mm. vials by pouring melted paraffin into the vial until the paraffin level in the vial was slightly higher than the level of the humidity-controlling solution in the bottle. The seed samples were then

placed in the vials around the glass tubing so that the seed mass was no more than 4 mm. thick at any place, assuring that a very large percentage of the seeds would be exposed to the light. The vials were then placed in the bottles and samples were withdrawn, counted and germinated at three successive 18-week intervals under the standard conditions previously described.

For the samples to be stored at two temperatures in tin cans, again only the fresh C. ledgeriana seeds were used. Two No. 2 cans were well filled with previously dried seeds on a warm, dry day, and the cans were sealed by the canning laboratory machinery at the University of Maryland. It was thought that this procedure might profitably be considered either for storage for relatively long periods or for shipping seeds for considerable distances. A sample of the seed was oven dried at the start of the experiment, and was found to contain only 3.35 percent moisture. The cans were stored at two temperatures, 16° and 32°C., and after one year 400-seed samples were germinated under the standardized conditions previously described.

Results

Effect of storage temperature-humidity inter-relationships upon germination of C. ledgeriana seeds. In recording the germination of samples stored under the different temperature and humidity conditions, two counts were made, at 21 and 30 days from sowing. A large percentage of fresh seed in good condition germinates in 21 days from sowing. A deviation from the optimum, either in condition of seeds or of germination procedure, results in slower germination. Though germination of poor seeds continues for a longer period under certain

conditions, the 30-day period was arbitrarily selected as the maximum, beyond which germinations would have little practical value.

The average germination of the four replicates of fifty seeds each at each of the six progressive sampling dates following storage at various combinations of temperature and humidity is given in table 2.

A study of the data in table 2 shows that both the temperature and humidity of the storage atmosphere are extremely important factors in determining the viability of C. ledgeriana seeds.

Though check samples germinated 100 percent at the beginning of the experiment, those stored for only nine weeks at the various temperatures and 100 percent humidity varied widely in their germination. Storage at either 2° or 32°C. resulted in less than 5 percent germination, while those at 7°, 16° or ±24°C. germinated between 65 and 90 percent. The average germination for all temperatures at 100 percent humidity after 9 weeks was only 46 percent. For the same period, the samples stored at 66 percent humidity averaged 96 percent germination, those at 33 percent humidity 97.4 percent germination and at 0 percent humidity 88.9 percent germinated. However, storage at 0 percent humidity and 32°C. resulted in much reduction of germination after nine weeks and a complete loss of germinability by 27 weeks. Thus it is clear that storage at either extreme of the humidity range for even a short period seriously reduced germination. The extent of the reduction depended on the temperature of the storage.

Storage at 32°C. resulted in a rapid decline and finally a complete loss of germinability in 18 weeks at 100 percent humidity, in 27 weeks at 0 percent humidity and in 36 weeks at 66 percent humidity, while those stored at 33 percent humidity germinated nearly 80 percent even

Table 2. Average percent germination of C. ledgeriana at six progressive nine-weeks sampling dates after storage under twenty combinations of temperature and humidity (four fifty-seed replicates for each test).

Storage environment		Average percent germination in 21 and 30 days after storage under twenty temperature and humidity combinations for --											
temp. °C.	humidity (%)	9 weeks		18 weeks		27 weeks		36 weeks		45 weeks		54 weeks	
		21 da.	30 da.	21 da.	30 da.	21 da.	30 da.	21 da.	30 da.	21 da.	30 da.	21 da.	30 da.
2	0	89.0	89.0	72.5	94.0	74.6	82.5	90.0	91.0	----	----*		
7	0	96.5	100.0	----	----*								
16	0	88.0	90.0	63.5	85.5	78.6	96.0	80.5	82.5	89.5	95.5	69.5	71.0
±24	0	96.5	97.0	70.0	95.5	72.0	87.0	83.5	84.0	73.5	85.0	77.0	82.5
32	0	60.0	69.5	19.5	43.0	0.0	0.0						
2	33	100.0	100.0	71.0	87.5	78.0	87.5	94.0	95.0	----	----*		
7	33	93.7	99.0	81.5	91.5	77.6	87.0	91.5	94.5	84.5	92.5	89.0	91.5
16	33	100.0	100.0	67.5	96.0	82.5	90.5	89.5	90.5	92.0	97.0	86.5	88.0
±24	33	96.0	97.5	73.0	94.0	84.0	94.5	83.0	85.0	88.5	93.5	85.0	85.5
32	33	89.5	91.0	71.0	82.5	76.5	87.0	74.0	81.0	65.0	72.0	77.5	78.5
2	66	91.0	95.0	75.0	89.0	96.5	99.0	90.0	99.0	89.0	97.0	72.5	74.0
7	66	98.5	100.0	88.0	99.5	85.5	96.0	96.0	96.0	89.5	93.5	92.0	95.0
16	66	91.5	99.0	75.0	95.5	87.0	90.5	89.5	89.5	75.0	88.0	85.0	85.5
±24	66	97.5	98.0	73.5	93.0	65.5	84.5	77.0	77.0	73.0	73.0	81.0	82.0
32	66	85.0	89.5	48.0	84.5	0.0	1.5	0.0	0.0				
2	100	2.0	3.0	0.0	0.0								
7	100	69.0	69.5	47.5	55.5	53.5	69.0	----	----*				
16	100	64.0	69.0	37.5	50.5	43.5	45.5	40.0	40.0	31.0	36.5	28.5	30.0
±24	100	89.5	89.5	47.0	72.0	68.5	88.5	76.5	79.0	67.5	73.5	62.5	64.0
32	100	2.0	2.0	0.0	0.0								

* Samples destroyed by contact with humidity-controlling solutions.

after 54 weeks. Storage at 2°C. and 100 percent humidity resulted in only 3 percent germination after 9 weeks, and a complete loss of germinability after 18 weeks.

The effect of the interaction of temperature and humidity in determining the viability of stored C. ledgeriana seeds is perhaps shown more clearly in figure 4. This figure graphically illustrates the data for the germination after 30 days for the samples stored under the various treatment conditions for the 54-week period.

Effect of storage temperature-humidity inter-relationships upon germination of C. succirubra and C. hybrid seeds. Though a lack of definite knowledge regarding the history of the seeds of C. succirubra and C. hybrid possibly renders data on their reaction to the various temperature and humidity storage conditions less subject to accurate interpretation, the results do illustrate two points of importance and are therefore presented in tables 3 and 4.

These data serve as further proof that both temperature and humidity controls are necessary to prolong the viability of stored cinchona seeds. The combinations that proved deleterious to the C. ledgeriana seeds also resulted in drastic reductions in the viability of C. succirubra and C. hybrid seeds. High humidity is again revealed as the critical factor, particularly at either extreme of temperature. Thus, as was the case with C. ledgeriana seeds, storage of C. succirubra and C. hybrid seeds at 2°C. and 100 percent humidity resulted in a complete loss of germinability in 27 weeks, and at the 32°C. and 100 percent humidity combination loss of viability was complete after only nine weeks.

The effect of high temperature on the viability of C. succirubra

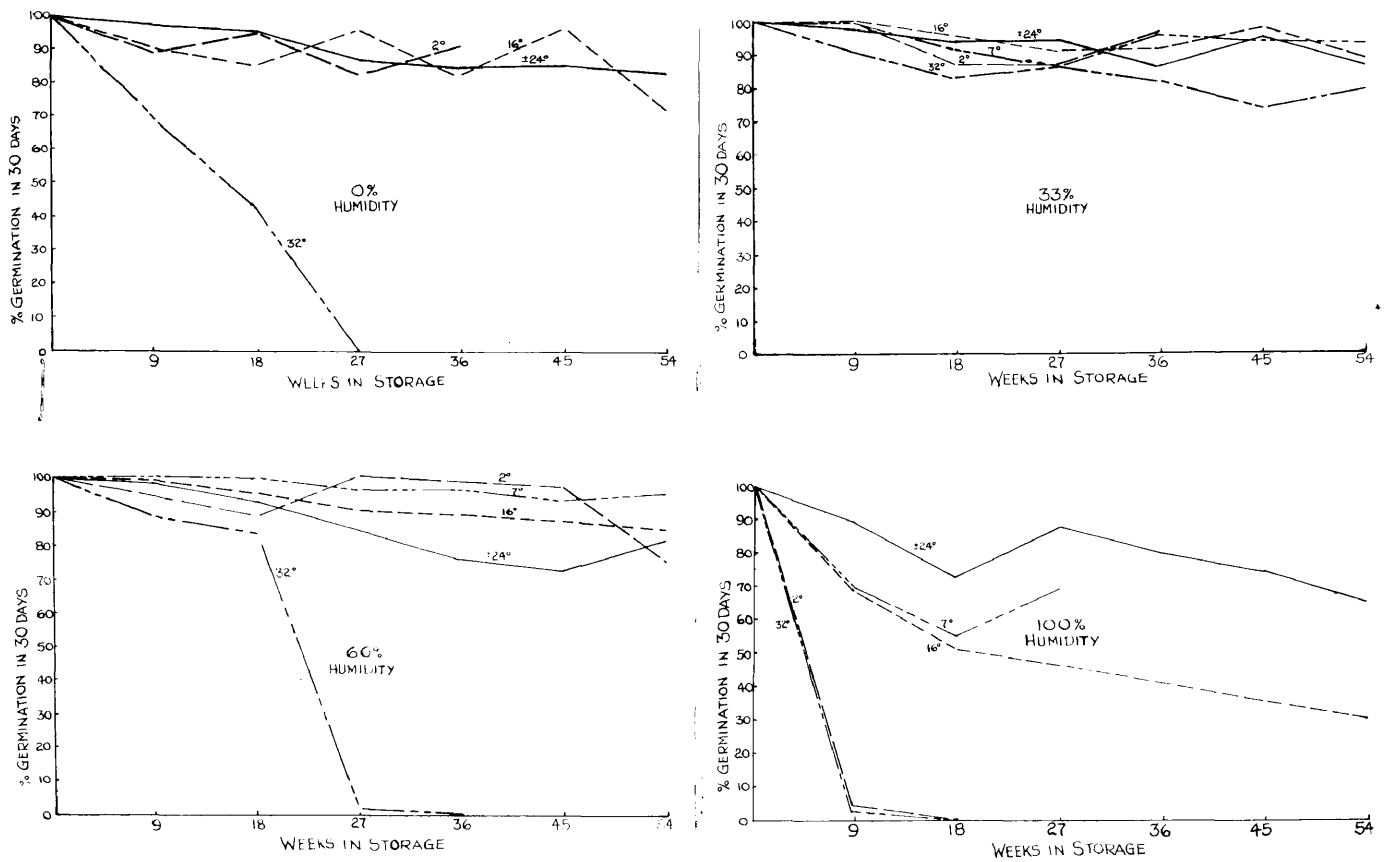

Figure 4. Graphs of the effect of temperature (°C.) and humidity conditions on prolonging the viability of stored C. ledgeriana seeds.

and C. hybrid seeds also was similar to the effect on C. ledgeriana seeds. Seeds stored at 32°C. and 0 percent humidity rapidly lost viability, failing to germinate after 27 weeks, and those at the same temperature and 66 percent humidity suffered a similar loss in viability.

Table 3. Average percent germination of C. succirubra seeds at six progressive nine-weeks sampling dates after storage under various combinations of temperature and humidity (four fifty-seed replicates for each test).

Storage environment		Average percent germination in 21 and 30 days after storage under conditions indicated for --											
		9 wks.		18 wks.		27 wks.		36 wks.		45 wks.		54 wks.	
temp. °C.	humidity (%)	21da.	30da.	21da.	30da.	21da.	30da.	21da.	30da.	21da.	30da.	21da.	30da.
2	0	71.5	71.5	61.5	66.0	52.0	55.0	59.5	60.0	----	----*		
16	0	76.0	76.5	44.0	65.0	50.0	55.0	56.5	57.0	35.0	56.5	47.5	47.5
32	0	68.0	70.0	2.0	20.0	0.0	0.0						
2	33	77.5	78.0	55.5	65.0	64.5	67.5	71.0	72.0	----	----*		
16	33	84.5	85.0	54.0	69.5	65.5	75.0	57.0	60.0	47.0	65.5	58.0	59.0
32	33	75.0	76.0	44.5	68.5	48.0	58.5	49.0	51.5	21.0	36.5	39.0	39.0
2	66	80.0	80.5	50.0	72.0	69.5	78.0	69.5	70.5	34.5	52.0	64.5	65.0
16	66	76.5	77.0	59.0	77.5	65.0	65.0	71.0	71.0	51.5	68.5	41.5	41.5
32	66	70.0	72.0	15.0	54.5	0.0	0.0						
2	100	49.5	51.5	19.0	31.5	16.0	19.5	----	----*				
16	100	58.5	61.5	6.0	26.5	13.5	21.5	14.0	14.0	2.5	5.0	8.5	8.5
32	100	0.0	0.0										

* Samples destroyed by contact with humidity-controlling solutions.

It is of considerable interest to note that the older seeds have reacted to the various combinations of temperature and humidity in a manner quite similar to the fresh seeds, as this lends support to any general conclusion which may be drawn as to the optimum storage conditions for cinchona seeds.

An examination of the data for all three lots of cinchona seeds reveals that in general, under the conditions of this experiment, there was little relationship between the germination recorded at 21 days and that at 30 days, and that the wide variations in this ratio were not

related to treatment variations. However, a wide variation in germination between the two periods did exist following storage under conditions proving decidedly unfavorable and during which the seed had apparently approached a very low stage in vitality. This is seen most clearly in the case of the 32°C. and 66 percent humidity combination at 18 weeks with all three seed lots, and with the 32°C. and 0 percent humidity combination at 18 weeks with C. ledgeriana and C. succirubra seeds. This fact indicates that there is probably some point in the process of deterioration at which germination is considerably slowed. Consequently such germination behavior may hold practical importance, indicating that such seed has reached a critical condition and should be used promptly.

Table 4. Average percent germination of C. hybrid seeds at six progressive nine-weeks sampling dates after storage under various combinations of temperature and humidity (four fifty-seed replicates for each test).

Storage environment		Average percent germination in 21 and 30 days after storage under conditions indicated for --											
temp. °C.	humid-ity (%)	9 wks.		18 wks.		27 wks.		36 wks.		45 wks.		54 wks.	
		21da.	30da.	21da.	30da.	21da.	30da.	21da.	30da.	21da.	30da.	21da.	30da.
2	0	57.0	62.0	39.0	64.0	35.5	52.5	46.5	51.5	----	----*		
16	0	50.0	59.0	29.0	51.5	28.5	46.5	40.0	44.5	38.5	44.0	22.5	23.0
32	0	15.0	25.0	0.5	4.0	0.0	0.0						
2	33	85.0	87.0	64.5	83.5	58.5	76.5	69.0	73.0	----	----*		
16	33	75.0	77.0	66.5	80.0	71.5	87.5	64.5	70.0	63.5	74.0	68.5	69.0
32	33	74.0	77.0	41.0	64.0	34.5	42.0	28.0	30.0	29.0	32.5	25.5	26.0
2	66	85.0	89.0	60.0	75.5	65.0	82.0	79.0	83.5	73.5	80.0	71.0	71.0
16	66	83.5	88.0	60.0	77.5	52.5	65.5	71.5	73.5	69.0	75.5	67.0	68.0
32	66	67.0	71.0	16.0	54.0	0.0	0.0						
2	100	20.0	22.0	8.0	11.5	2.5	3.0	----	----*				
16	100	26.0	30.0	13.5	14.5	9.5	12.5	6.5	9.5	4.5	5.0	5.0	5.0
32	100	0.0	0.0										

* Samples destroyed by contact with humidity-controlling solutions.

Effect of storage in light vs. darkness. The data for the germination of samples of C. ledgeriana seeds stored at room temperature under five humidities and in the presence of daylight are presented in table 5. For purposes of comparison, the data for the same seed stored in the dark at the same temperature and under four humidities are included from table 2.

Table 5. Average percent germination of samples of C. ledgeriana seeds stored in the presence of light at room temperature under five humidities, compared with storage at the same temperature under four humidities in the dark (four fifty-seed replicates for each test).

Storage environment		Average percent germination after storage for --											
		18 weeks in				36 weeks in				54 weeks in			
		DARK		LIGHT		DARK		LIGHT		DARK		LIGHT	
temp. °C.	humidity (%)	21da.	30da.	21da.	30da.	21da.	30da.	21da.	30da.	21da.	30da.	21da.	30da.
±24	0	70.0	95.5	71.5	89.5	83.5	84.0	51.5	77.5	77.0	82.5	65.0	75.5
±24	25			76.0	85.5			58.5	84.5			68.5	82.5
±24	33	73.0	94.0			83.0	85.0			85.0	85.5		
±24	50			80.5	87.0			58.5	85.5			70.5	76.5
±24	66	73.5	93.0			77.0	77.0			81.0	82.0		
±24	75			61.5	70.5			24.0	59.0			9.0	31.5
±24	100	47.0	72.0	0.5	0.5	76.5	79.0	0.0	0.0	62.5	64.0	0.0	0.0

These data show that storage of C. ledgeriana seeds in daylight at room temperature and high humidity significantly hastened deterioration over those stored in the dark. This might have been anticipated, as these conditions approach those favorable for germination, though no germination was observed in the storage bottles. The seeds stored in the light at 0 percent humidity germinated consistently less than comparable seeds stored in the dark, though the differences were not very large. No direct comparisons are available at the intermediate humidities, but germinations of seeds stored in the light were below what might be expected on the basis of the results from those stored in the dark.

Effect of storage in sealed tin containers. As stated previously, only one germination test was made of the seeds stored in tin cans at two temperatures for one year. A 400-seed sample of the seeds stored at 16°C. averaged 81.5 percent germination in 30 days, and a like sample of the seeds stored at 32°C. averaged only 52.5 percent germination in the same period. This again demonstrated the deleterious effect of high storage temperature on seed viability, and though at the lower temperature germination was nearly 20 percent below that of the check at the beginning of the experiment, it compared favorably with the better germinations recorded in table 2, resulting after storage for a similar period at various temperature and humidity combinations.

On the basis of the results of this preliminary test, the use of tin cans as storage or shipping containers would seem to warrant further consideration.

Part II. Effect of Various Media

The popular literature on the propagation of cinchona contains several detailed accounts of the preparation of the germination beds and of the exacting care they require. All are agreed that success or failure in the enterprise depends primarily on this phase of the work, and repeatedly urge that no expense or care be spared.

van Gorkom (10) states that when the famous Dutch propagator Junghuhn first collected good seeds in Java, he tried many methods of germination.

> From 200 well developed seeds, sown broadcast upon well prepared beds, there was hardly one healthy plant obtained. Junghuhn ascribed this misfortune to the fineness and lightness of the seed, which were there exposed to all the caprices of wind and weather, and further pointed out the dangers without number, which existed also from the first sprouting of the seed.

Junghuhn then tried sowing the seeds in bamboo pots filled with purified, finely sifted wood earth, or else with a mixture of this and a fourth part of black volcanic sand.

> In the first experiments, each bamboo pot served for the reception of a single seed, afterwards two or three were put in; this was done in a small superficial pit, made by the pressure of the finger in the loose soil in the center of the pot, which was then sprinkled with the black sand just mentioned, to a thickness of ½ or at most 1 millimeter to hinder the seed being blown away, and to prevent it being dried too much by the air. From this time the soil in the pots was kept constantly moist by means of a very fine watering pot.

So handled, the majority are stated to have germinated in six weeks, in a few cases after 20 or 22 days, never after 100 days.

When van Gorkom took over the direction of the cinchona culture, he found a hundred thousand seeds so sown that had failed completely to germinate. He then used baked earthen pots, filled two-thirds of

their depth with sand, on which was spread out a layer of loamy soil, properly moistened, of a few centimeters in thickness. On this prepared soil the seeds were scattered as thickly as possible and the pots then put in a saucer of water. He states that in this way

> . . . a sufficient quantity of moisture is drawn up by capillary attraction, whilst in the sprinkling of the seeds the limits of the desired moisture may be easily overstepped, moreover the seeds themselves may be damaged, and sometimes dislodged or removed. It is quite clear that favorable conditions for the germination of the seeds and the development of the plants cannot be sufficiently ensured in the open ground, and thus it is necessary to have well-devised propagating houses.

As further experience was gained, however, a system of outdoor propagation was developed which yielded satisfactory results. Sands (20) reported that the system extensively used in Java was to construct special germination sheds of bamboo poles, covering the roof with dried grass and providing removable grass or bamboo shades for the sides. A trench 2 feet deep was dug and filled with fresh jungle mould, and the seed sown thickly on the top of the mould. The seeds germinated in about three weeks and

> . . . from then onwards extreme care and watchfulness have to be exercised in the watering, lighting and sheltering of the young plants. If the soil is too wet or too dry, or if there is insufficient diffused light for the seedlings, damping-off diseases often destroy them; if kept too dry or exposed to wind, red spider or orange mite may cause damage to the tender leaves and shoots.

The germination methods employed in Java were undoubtedly successful, and have been imitated in many other localities. Experimental data on germination media other than jungle mould were not available until 1939, when Stoffels (23) reported an experiment to determine the effect of media and altitude on germination. Six media, including (1) arable soil dug to a depth of 25 cm., (2) arable soil disinfected with fire, (3) arable soil and fine gravel, (4) fine gravel, (5) subsoil, and

(6) a layer of forest litter, were tested at altitudes of 1650 and 1880 meters. Somewhat better germination was recorded with the subsoil and gravel media than with other media, with an indication of superiority for the higher altitude. The germination was recorded as the number of plants per square decimeter, and the sowings were apparently duplicated, but there is no statistical evidence to indicate that the germination differences were significant. The superiority of the subsoil and gravel media was attributed to the lack of losses due to fungi.

Nevorkian (15) reported a recent experiment in which sphagnum moss, peat moss, forest mulch and sand were tested as germination media. Though the earliest germination was recorded on peat moss, a highly significant difference in the number of seedlings surviving by the end of the eleventh week was reported in favor of the sphagnum moss medium.

Materials and Methods

To obtain data on the influence of the medium on germination of C. ledgeriana seeds, an experiment was conducted in which sphagnum moss, peat, filter paper, blotters, sand, sterilized composted soil and distilled water were all compared as germination media. Four hundred seeds were sown on each of the media and placed in a small room in which identical light and temperature conditions were supplied all treatments. The sowings were inspected daily for 30 days and, with the first appearance of the radicle as the criterion of germination, the rapidity of germination as well as the maximum was recorded. Five weeks from the date of sowing the experiment was discontinued, and the number of plants remaining at that time recorded.

Results

Maximum germination was obtained with all media in 18 days, with no significant difference in rapidity of germination. However, there was a significant difference in the survival of the seedlings, with losses ranging from 0 percent in the case of sphagnum moss, to 25 percent in the case of the soil medium. These data are presented in table 6.

Table 6. Influence of medium on the germination of C. ledgeriana seeds and survival of the young seedlings (400 seeds in each test).

Medium	Days to 1st germination	Maximum germination (no.pits.)	Days to maximum germination	No. sdlgs. surviving after 5 weeks	Percent loss after germination
Sphagnum moss	7	400	15	400	0.0
Sand	10	385	18	369	3.5
Soil	8	387	16	290	25.0
Peat	7	393	14	310	20.9
Filter paper	6	390	16	354	9.2
Blotters	9	387	18	365	5.7
Distilled water	8	391	18	380	2.8

Nearly all of the losses occurred shortly after germination, probably due almost entirely to damping-off fungi. Mandarang (17) reported 20 to 30 percent losses of cinchona seedlings due to a Rhizoctonia damping-off in soil in the Philippines.

From this data, it appears that the medium exerts little influence on actual germination, and that complete germination will result on any substrate that provides a proper moisture condition and is not actually toxic to the seeds. However, as the seedlings are extremely susceptible to damping-off fungi, and the high moisture and temperature conditions

favorable for germination are also conducive to fungal growth, there is a close correlation between the medium used and the survival of the young seedlings.

Part III. Effect of Light

The rôle of light in seed germination has been the subject of numerous investigations, from which it may be concluded that some seeds require light for the initiation of germinative processes, the germination of others is favored by exposure to light, exposure to light interferes with the germination of a third group, and a final group is indifferent to the availability of light during germination. These investigations have been recently reviewed by Burkholder (4), and Crocker (7).

Kerbosch (14), in the only published studies on the requirement of light for cinchona seeds, concluded that (1) other environmental factors being favorable for germination, germination will not take place until the seeds are exposed to some light, (2) germination is the more rapid and complete with increasing exposure to light, maximum germinations resulted from conditions of continuous illumination, and (3) in an experiment in which light intensities from 55 cm. from a light source (50 candle Philips' metal-filament light) through four intermediate intensities to 330 cm. from the light source, germination was the more rapid and complete as the light intensity was increased.

The experiments to be reported were designed to verify the light requirement of cinchona seeds and to determine quantitatively in universally recognized foot-candle and time units the intensity and duration of exposure to light necessary for complete germination.

Materials

These experiments were conducted in a small underground dark room equipped with automatic temperature and light controls. The temperature was maintained with a range of 22° to 24°C. Light conditions were varied in accordance with each particular study and the specific details are presented with each test. A large quantity of sphagnum moss was kept moist in the room, which effectively maintained a humid atmosphere.

Procedures and Results

Verification of light requirement. To determine that some light is necessary for germination, four replicates of fifty seeds each of Cinchona ledgeriana were sown on sphagnum, thoroughly moistened, covered with a pane of glass, and held in total darkness for 21 days. When then inspected it was found that the seeds were apparently in good condition and had imbibed water, but there was no trace of germination. A duplicate sowing was prepared and held in the dark for 50 days. Though there was ample moisture, there was again no indication of germination. In the latter case, however, some of the seeds had deteriorated through long exposure to moist conditions. When sowings thus held in the dark were subsequently brought into the light, the seeds undamaged by rot germinated normally. Those held dark for 21 days reached maximum germination in an additional 21 days, or 42 days from sowing, and those held dark for 50 days reached maximum germination in 71 days from sowing.

The experiment was again repeated, using C. succirubra and C. hybrid seeds as well as C. ledgeriana, and using sand, soil, peat, filter paper and sphagnum as germination media. In all cases the result

was the same, none germinated in the dark, but when brought into the light the remaining good seed germinated normally. These results are in complete agreement with those of Kerbosch, and conclusively prove that some light is required for the initiation of the germinative process in cinchona.

Light exposure required for germination. For experiments on the exposure required for germination of cinchona seeds, the dark room was subdivided into a series of booths, each equipped with a light which was controlled independently of the others. New 100-watt frosted Mazda bulbs in reflectors were suspended 15 inches above the sowing medium, which resulted in a light intensity of about 200 foot-candles at the seed level, as measured by the Weston illumination meter.

Metal bread pans, 5 by 10½ by 3 inches were used as seed flats. These accommodated four replicates of 100 seeds each. A preliminary experiment was conducted in which sand, soil, peat and sphagnum were compared as germination media. Considerable difficulty was encountered in maintaining adequate moisture conditions in the sand, and damping-off occurred in the peat and soil trials, consequently sphagnum was selected as the standard medium. The lower portion of the flats was filled with soil, over which a layer of at least one-half inch of finely shredded sphagnum was placed. This was thoroughly watered, the seeds were sown on the surface, and the pan covered with a pane of glass. No further watering was usually required during the germination period.

Daily exposures to the 200 foot-candles of light of 24 hours (continuous), 12 hours, 6 hours, 1 hour, 10 minutes, 5 minutes, 1 minute and 10 seconds were supplied to four replicates of 100 seeds each of C. ledgeriana. As the results of the first experiment failed to agree with Kerbosch's conclusion that maximum germination resulted from

continuous illumination, the entire experiment was repeated three times. The data from the four experiments are presented in table 7.

The data in table 7 show that daily exposures to 200 foot-candles of light for as short a period as 10 seconds were sufficient to result in nearly complete germination of C. ledgeriana seeds in 21 days. For the purpose of this experiment, a seed was considered germinated only after the hypocotyl had begun to elongate.

However, as the light quantity supplied was decreased, though germination was nearly complete, the resulting seedlings had longer hypocotyls, were progressively more etiolated, and their roots failed to penetrate the medium normally. This response is illustrated in figures 5 through 7. When these weak seedlings were gradually exposed to larger daily light exposures, however, they rapidly developed more chlorophyll and were soon normal in appearance.

Table 7. Germination of *C. ledgeriana* seeds after 21 daily exposures to 200 foot-candles of light for the lengths of time indicated.

Length of daily light exposure	Repl.	Germination of 100-seed replicates after 21 days			
		Exper.I	Exper.II	Exper.III	Exper.IV
24 hours (continuous)	A	100	97	100	99
	B	98	99	96	95
	C	99	95	95	97
	D	99	100	98	99
	Ave.	99	98	97	97.5
12 hours	A	98	96	98	98
	B	99	94	100	95
	C	98	99	99	97
	D	97	100	95	99
	Ave.	98	97	98	97
6 hours	A	98	94	96	98
	B	93	90	89	92
	C	92	97	97	90
	D	99	95	95	95
	Ave.	95.5	94	94	94
1 hour	A	96	96	98	100
	B	98	92	92	95
	C	94	90	99	96
	D	100	99	99	88
	Ave.	97	94	97	95
10 minutes	A	94	94	91	88
	B	100	90	96	92
	C	96	95	98	97
	D	94	91	94	93
	Ave.	96	92.5	95	92.5
5 minutes	A	94	100	94	98
	B	92	91	91	96
	C	96	93	87	91
	D	100	90	93	95
	Ave.	95.5	93.5	91	95
1 minute	A	88	97	96	88
	B	96	90	91	85
	C	98	86	90	93
	D	100	89	93	93
	Ave.	95.5	90.5	92.5	90
10 seconds	A	80	88	91	96
	B	92	97	87	93
	C	90	93	91	98
	D	96	93	94	91
	Ave.	89.5	94	91	94.5

Figure 5. Cinchona seedlings germinated with short exposures to 200 foot-candles of light. Left, 1 hour; center, 10 minutes; right, 5 minutes daily for 21 days. Note increasing length of hypocotyls with decrease in length of exposure.

Figure 6. As in figure 5, with (left) 1 minute and (center) 10 seconds daily exposure to 200 foot-candles of light. Pan on right was held in the dark.

Figure 7. Enlargement of a section of a flat exposed to 10 seconds of light daily (figure 6, center). Note long, etiolated hypocotyls and roots which failed to penetrate the medium.

Figure 8. Enlargement of part of a flat held in the dark for 21 days. Though temperature and moisture conditions were favorable, the absence of light completely inhibited germination.

Light intensity required for germination. To determine the effect of light intensity on germination, an experiment was conducted in which several light intensities were provided by varying the distance from the sown seed to a single light source. A Photoflood No. 2 bulb was used as the light source, and positions for the seed flats were so selected that 2000, 700, 300, 150, 75 and 25 foot-candles of light would be supplied at seed level. As an exposure of 10 seconds daily had previously proved to be sufficient, it was used in this experiment. This experiment was repeated twice, using four replicates of fifty seeds each of C. ledgeriana at each of the treatment conditions in each of the experiments. The results are summarized in table 8.

Table 8. Average percent germination of C. ledgeriana seeds given a daily exposure of 10 seconds for 21 days to light of several intensities (four fifty-seed replicates for each test).

Light intensity (foot-candles)	Average percent germination in 21 days		
	Experiment I	Experiment II	Experiment III
2000	99.0	98.0	95.5
700	95.5	93.0	96.0
300	89.0	84.5	90.0
150	43.0	47.5	46.5
75	15.5	12.0	10.0
25	7.5	10.5	13.5

Figures 9 and 10 are photographs of representative flats given daily 10-second exposures to light of the several intensities.

Figure 9. Germination of C. ledgeriana seeds supplied 21 daily 10-second exposures to light of different intensities. Left, 2000 foot-candles; center, 700 foot-candles; right, 300 foot-candles.

Figure 10. As in figure 9, but supplied (left) 150 foot-candles, (center) 75 foot-candles, and (right) 25 foot-candles of light daily.

It is apparent from these data that there was a rapid decline in germination as the light intensity supplied was reduced below 300 foot-candles. This corroborates the results of the previous experiments which indicated that there is a minimum point in the light requirement of cinchona seeds, below which reduced germination results, but above which germination is not greatly increased. This minimum point was determined to be a combination of 10 seconds per day exposure to light of between 150 and 300 foot-candles intensity, under which conditions nearly complete germination was recorded in 21 days.

It is of interest to note in figures 9 and 10 that the increased intensities of light supplied were not effective in preventing the etiolation or elongated hypocotyls previously recorded as resulting from short daily light exposures during the germination period.

The results of the effect of light intensities here reported may not be in disagreement with the work of Kerbosch (14) from which it was concluded that maximum germinations resulted at the greater light intensities. No means are provided for determining the range of light supplied in the previous work in universal units, but if the light intensities were below 700 foot-candles such a conclusion would have been justified.

Stage of germination period at which light is required. The experiments on the light required for germination of cinchona seeds have recorded the effect of daily exposures to light of a known quantity. To determine whether exposures less frequent in occurrence are effective, two experiments were conducted.

In the first experiment, the total light quantity supplied by 21 daily 10-second exposures to light of 300 foot-candles intensity, which

is near the minimum amount that results in complete germination, was supplied at less frequent intervals by lengthening the individual exposures. The results obtained with four 100-seed replicates of C. ledgeriana are given in table 9.

Table 9. Germination of C. ledgeriana seeds supplied a total of 63,000 foot-candle-seconds of light at several stages during the 21-day germination period.

Length and frequency of exposures to light of 300 foot-candles intensity	Germination after 21 days				
Replicate	A	B	C	D	ave.
10 seconds daily	98	96	100	97	97.5
20 sec. every 2nd day	87	85	85	90	87
30 sec. every 3rd day	55	63	61	64	61
70 sec. on 1st, 7th and 14th days	11	9	8	4	8
105 sec. on 1st and 10th days	6	9	9	7	8
210 sec. on 1st day only	0	0	1	1	0.5
210 sec. on 10th day only	1	0	2	1	1
unlighted control	0	0	0	0	0

From the data in table 9 it is apparent that the 63,000 foot-candle-seconds light quantity was sufficient to supply the minimum requirements for the germination of cinchona seeds only when some portion of it was supplied at least every second day. The failure of less frequent exposures to result in reasonable germination suggested that light must be required throughout the germination process, rather than being required only as a possible catalytic stimulus for the initiation of germination.

Following this, a second experiment was conducted in which a much greater light quantity was supplied. Four 100-seed replicate sowings of C. ledgeriana were supplied 2100 foot-candles of light for (1) 7 minutes on the first day, (2) 7 minutes on the 10th day, and (3) 140 seconds on each of the 1st, 7th and 14th days. The germinations recorded are presented in table 10. This shows that even with a light quantity

far greater than the 63,000 foot-candle-seconds previously shown to be near the minimum required for germination of C. ledgeriana seeds, germination failed to occur when the light was supplied at infrequent intervals.

Table 10. Germination of C. ledgeriana seeds supplied a total of 882,000 foot-candle-seconds of light at several stages during the 21-day germination period.

Length and frequency of exposures to light of 2100 foot-candles intensity	Germination after 21 days				
Replicate	A	B	C	D	Ave.
7 minutes on 1st day	2	0	1	4	2
7 minutes on 10th day	1	1	3	3	2
140 sec. on 1st, 7th and 14th days	15	18	24	19	19

Part IV. Effect of Temperature

There is known to be a direct relationship between the temperature of the environment and seed germination, due in large part to the effect of temperature on the rate of chemical reactions. The temperature requirements for germination of different species is quite varied, but fairly definite minimum, optimum and maximum temperatures can be determined for a given species.

There are no published data on the temperature requirements for the germination of cinchona seeds. Herbosch (14) recognized that differences might be expected from variations in germination temperatures, but since at the uniform temperatures of 18° to 22°C. at Java excellent germinations were being obtained, no study was reported for this factor. However, since it is not feasible to maintain these temperatures in outdoor beds in many of the places in the Americas where the attempt to reestablish this plant is being made, a critical study of the effect of this factor

assumes considerable importance.

Minimum, optimum and maximum temperatures for the germination of C. ledgeriana seeds have been determined in this investigation, and the effect of relatively short exposures to above-maximal temperatures, such as might occur in full sunlight under glass or other translucent cover, on the viability of the sown seed has been investigated.

Materials

The study of the effect of the temperature of the environment on the germination of cinchona seeds was made with the aid of an improvised constant-temperature chamber. A metal cabinet was partitioned into four small chambers, in each of which was placed a light bulb to furnish both heat and light. The bulbs were operated individually by thermostats. The cabinet was placed in a cold storage room that was held at 7°C. By carefully adjusting the thermostats, the individual chambers could be maintained within 1 degree of any desired temperature, from 7° to 40°C. Figures 11 and 12 respectively illustrate the exterior and interior arrangement of the germination chambers.

Thermographic recordings were made during each of the experiments to guard against the possibility of temporary current failure or bulb failure which might have altered the continuity of the desired temperature. C. ledgeriana seeds were used in all of these experiments. They were sown on sphagnum moss in metal pans in the manner previously described, and covered with a pane of clear glass.

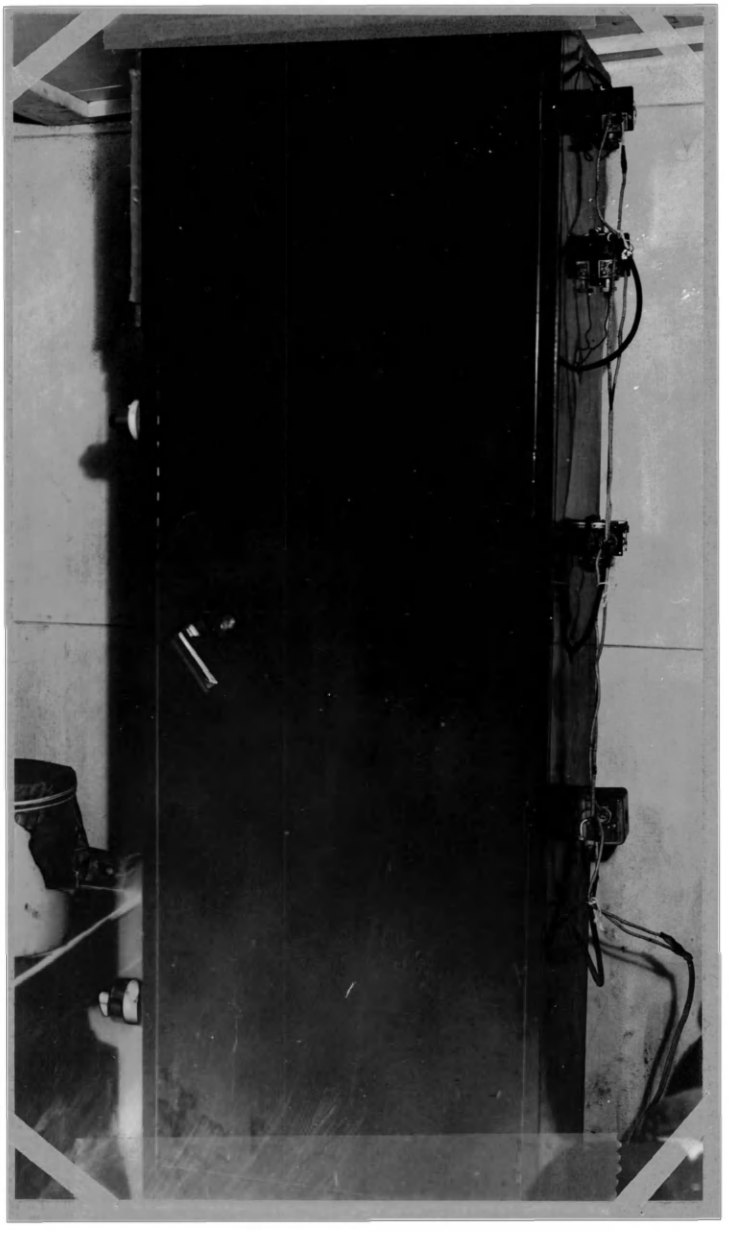

Figure 11. Exterior view of the improvised germination chamber used to study the effect of controlled temperatures on the germination of cinchona seeds. Note location of thermostats.

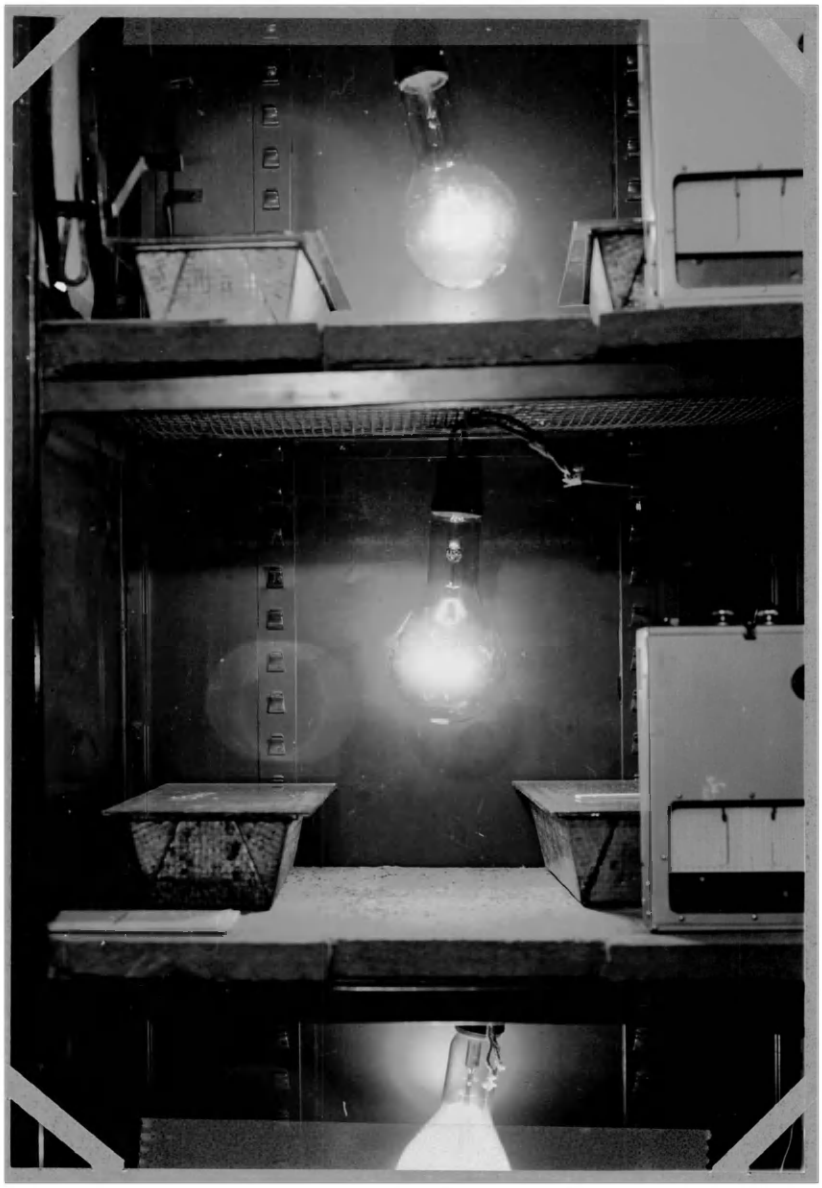

Figure 12. Interior view of improvised germination chamber, showing insulated individual compartments and arrangement of bulbs, seed flats and hygrothermographs.

Procedures and Results

Determination of minimum, optimum and maximum temperatures for germination. A preliminary experiment was conducted to determine the extremes of temperature at which germination would take place. Sowings were made at 7° and 40°C. and resulted in complete failure. The seeds sown at 7°C., though they retained their vitality, failed to germinate even after 75 days, whereas those sown at 40°C. deteriorated rapidly and had all become mouldy in only 5 days. Therefore 7° was below the minimum and 40°C. was above the maximum temperature for germination.

A series was then sown in the controlled-temperature chambers, adjusted to maintain temperatures of 12.5°, 18.5°, 24° and 29.5°C. Four replicates of fifty seeds each were placed at each of the temperatures and the resulting germinations recorded after 20, 30 and 50 days to determine the effect of temperature on the rapidity of germination as well as on total germination. At the conclusion of the experiment a new set of samples were sown and the experiment repeated. The results of the two experiments are given in table 11.

The complete failure of the seeds at 12.5°C., and the increasingly rapid and complete germination at the three higher temperatures indicated that though 12.5° was below the minimum, 29.5° was also below the maximum for germination.

Table 11. Average percent germination of C. ledgeriana seeds at 12.5°, 18.5°, 24° and 29.5°C. (four-fifty seed replicates for each test).

Temperature °C.	Experiment I Ave. percent germ. after 20 days	30 days	50 days	Experiment II Ave. percent germ. after 20 days	30 days	50 days
12.5	0	0	0	0	0	0
18.5	68	95	98	60	93	95
24.0	94	100	100	95	100	100
29.5	98	100	100	91	100	100

The thermostats were then adjusted to maintain temperatures of 14°, 16.5°, 32° and 35°C. in the compartments and two separate experiments were again conducted in the manner described, using four replicates of 50 seeds each for each of the temperatures indicated. In table 12 are found the average germination percentages for this series of experiments.

Table 12. Average percent germination of C. ledgeriana seeds at 14°, 16.5°, 32° and 35°C. (four fifty seed replicates for each test).

Temperature °C.	Experiment I Ave. percent germ. after 20 days	30 days	50 days	Experiment II Ave. percent germ. after 20 days	30 days	50 days
14	0	0.5	1.0	0	0	1.0
16.5	3.5	31.5	51.0	4.0	28.5	58.0
32	2.0	4.0	11.5	0	2.5	12.0
35	0	2.0	7.5	0	2.5	8.0

The slow and incomplete germination at these temperatures indicated that the maximum and minimum temperatures were closely approximated. The data in tables 11 and 12 were combined in the preparation of figure 13, which graphically presents the effect of temperatures on the germination of C. ledgeriana seeds.

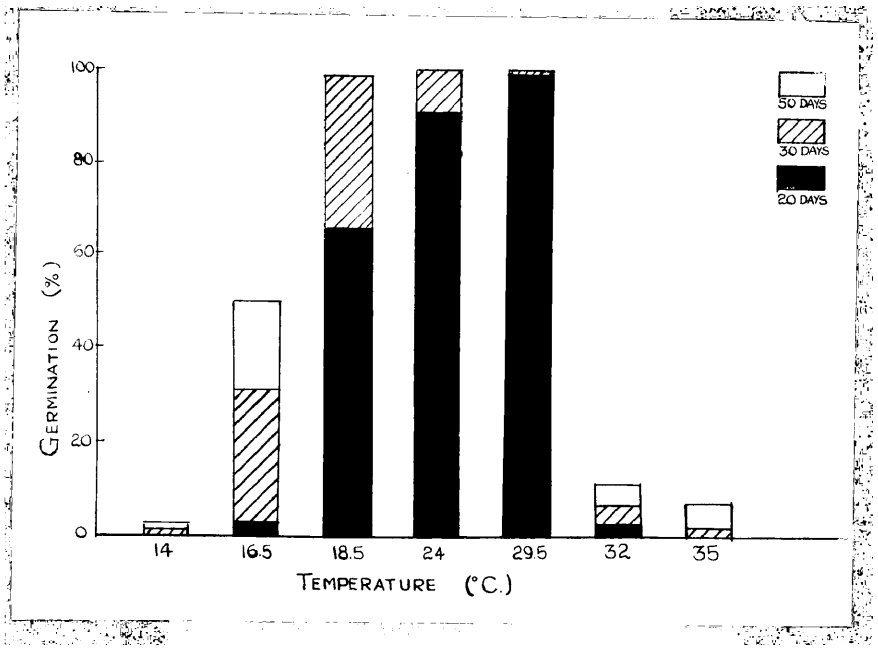

Figure 13. Effect of constant temperatures on the germination of C. ledgeriana seeds. From data in tables 11 and 12.

It is apparent from figure 13 that there was a definite reaction between the temperature of the environment and the resultant germination of C. ledgeriana seeds. There exists a critical minimum near 14°C. below which complete failure results, and a critical maximum near 35°C. above which germination will not take place. Between these two extremes the germination obtained increases in both rapidity and amount with an increase in temperature, attaining a maximum between 24° and 29.5°C., with an extremely rapid loss in germinability at temperatures above 29.5°C.

It should be emphasized, however, that the temperatures used in this series of experiments were maintained constantly throughout the entire germination period. In germinations under natural conditions, prompt and complete germinations have been obtained at temperatures

averaging about 20°C. with diurnal fluctuations of several degrees. Little practical significance can be attached to the fluctuations between 18.5° and 29.5°C., and possibly to short exposures to temperatures below this range.

Effect of short exposures to above-maximal temperatures on germination. As previously stated, seeds sown at temperatures below the minimum for germination retain their vitality for some time. One sowing that was held at 7°C. for 75 days germinated 87 percent after an additional 30-day exposure to 24°C. Seeds sown at temperatures above the maximum, however, rapidly lose their vitality. To obtain data on the effect of short exposures to high temperatures, 200-seed samples of C. ledgeriana were sown on sphagnum and allowed to imbibe moisture overnight, and then exposed to 40° or 50°C. for time periods of 2, 6, 12, 24, 48 and 92 hours, after which they were placed at 24°C. for the remainder of a 30-day germination period. These data are presented in table 13.

Table 13. Average percent germination of C. ledgeriana seeds exposed to 40° or 50°C. for the time period indicated, thereafter held at 24°C. for the remainder of a 30-day germination period (four fifty seed replicates for each test).

Temperature °C.	Germ. period days	Average germ. percentage after initial exposure to temperature indicated for 2 hrs.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	92 hrs.
40	21	93.0	95.0	98.0	90.0	51.5	0.0
	30	95.0	95.0	98.0	91.0	53.0	0.0
50	21	70.5	28.5	11.0	0.5	0.0	0.0
	30	72.5	31.0	12.0	0.5	0.0	0.0

The data in table 13 show that an exposure of 48 hours to a temperature of 40°C. resulted in a germination of only 51.5 percent, and that a 92-hour exposure to the same temperature resulted in a complete

failure of germination. Short exposures to 50°C. reduced germination even more drastically, only 72.5 percent germinating in 30 days following an exposure of two hours, and only 0.5 percent after an exposure of 24 hours.

Studies Concerning Early Seedling Growth

Assuming that the current program for the cultivation of cinchona in the Americas will be carried out in localities where the above-ground environmental conditions conform to the general requirements which have been held necessary for adequate growth, a knowledge of the below-ground environmental limitations is nevertheless essential to the successful prosecution of the project.

A few general statements on the soil requirements of the plants in plantation culture exist in the literature. Thus, we have the statements of Sands (20) that in Java "the best soil is a deep, light, friable loam, rich in organic matter, well-drained and with a gravelly or other porous subsoil. A sloping yet sheltered situation is necessary." Further, that "cinchona, particularly the high-yielding and valuable 'Ledgeriana' type, is most liable to the ill effects of unfavorable soil conditions"; and, finally, that

> . . . chemical analyses of some of the best cinchona soils in Java indicate that they are well supplied with nitrogen and phosphorous, but the writer was surprised to find in an extensive series of analyses of soils of a large group of estates no mention of potassium, and it may be that the lack of available potash is the limiting factor on the poorer or previously cultivated lands, judging by the physiological effects produced.

The general practice in Java was to use freshly-cleared jungle land and grow one crop only. Re-use of the land resulted in poorer growth and yield, though details as to the cause, or of attempts to remedy the trouble, are not recorded.

The statements of Wilson and Mirchandani (26) agree with those of Sands, and add that a nitrogen level of about 0.2 percent in the top

foot and not less than 0.1 percent in the third foot is necessary for successful growth. They further state that the cinchona tree requires a deep, rich, well-drained soil, acidic in reaction (pH 5.2 to 5.6), with less than 35 percent clay. Concerning fertilizer practices, they state:

> This aspect of cinchona cultivation has not received as much attention in India as it deserves. There have been a few isolated trials both in Bengal and Madras, but no systematic experiments with adequate controls have yet been attempted. Though the details of manuring cinchona in Java are not available in the literature, it is, however, mentioned that at the Government plantations in Java stable manure increased the quinine content of the bark and ammonium sulphate reduced it slightly. No information is available regarding the effect on yield of bark per acre.

They urge that experiments should be undertaken to determine the effect of nitrogen alone and in combination with phosphoric acid on the growth and the yield of the bark, and of potash on the alkaloid content of the bark, and that the general effect of liming be studied.

These statements are representative of the recorded observations on the requirements of the older plants, and illustrate the lack of specific data on this phase of cinchona cultivation. However, though it is frequently stated that young seedlings in the seed bed and nursery are more exacting in their requirements, there is a complete lack of even general information as to the effects of the soil environment on this phase of their growth. Probably the chief cause of losses in the early growth stages is the incidence of damping-off fungi, though malnutrition and incompatible media are doubtless important contributing factors to this loss. Yet there are no available data on the effectiveness of various cultural or nutritional practices on the reduction of these losses.

There are few statements in the literature as to the usual rate of growth of young seedlings of cinchona. Sands (20) reports that in Java when the young plants have developed two or three pairs of leaves and are 3 to 4 inches high they are transplanted into specially prepared nursery beds. As to rate of growth, he states

> . . . the seedlings often grow unevenly with the result that some may be removed at 5 months from the time of sowing the seed, while others in the same bed may not be ready for 12 months . . . . the seedlings of ledgeriana are ready for planting out in their permanent position in 2 to 3 years from the time of sowing the seed. They should then be about 3 feet high.

According to Stoffels (23), in the Belgian Congo the seedlings are planted into the nursery when they are about 6 cm. high. At about 1650 m. of altitude they attain this size in about 7 months, and at 1860 m. after 11 months from seeding. Final placing in the plantation is done after they have grown 8 to 10 months in the nursery, when the plants measure about 25 cm. The period from seed to plantation thus varies from 15 to 20 months.

A photograph (figure 1) published by Mandarang (17) shows three-months-old seedlings in soil in the Philippines that apparently are scarcely an inch tall, though no data are given.

These statements indicate that the early growth of cinchona seedlings proceeds rather slowly. As the tender young plants are doubtless most susceptible to damping-off losses until such time as the lower portion of the stem is sufficiently woody to offer mechanical resistance to hyphal penetration, this slow early growth may well be a limiting factor in the successful culture of the plant, and further, may also have been responsible for the lack of success with C. ledgeriana in localities other than Java.

When the limited amount of C. ledgeriana seed was received by the U. S. Department of Agriculture, urgency dictated that existing facilities in the United States be utilized to start production of as many seedlings as possible, while sites in areas in the Americas thought to be suitable for their later growth could be prepared to receive them. The investigations to be here reported were inaugurated to furnish data to assist in the most efficient production of vigorous, disease-free seedlings in the greenhouse, and to provide controlled experimental data on the media and nutrient requirements that will result in shortening the time interval between seed sowing and final placement in the plantation. This data would be of great value to cooperators in the program to be charged with the care of the seedlings in the field.

Part I. Effect of Various Media

Early in the course of the greenhouse production of seedlings for the program just referred to, it appeared that wide differences in their early growth resulted from the use of various growing media. For example, growth resulting when seedlings newly germinated on sphagnum were carefully planted in a soil mixture consisting of three parts compost to one part each of sand and peat was slow, and there was an appreciable loss of plants following the transplanting. The seedlings that did survive grew unevenly, averaging 6 to 8 cm. in height in about four months, with some individual plants attaining 10 to 12 cm. in height. This rate of growth approximates that reported by Sands (20) and Steffels(23). However, when the same seedings were transplanted into sphagnum moss as a growing medium, transplanting losses were negligible and subsequent growth, when

sufficient nutrients were supplied, was many times as rapid as in the case of the soil medium. This response is illustrated in figure 14.

Figure 14. Growth of C. ledgeriana seedlings in a greenhouse soil mixture (left) and in sphagnum moss (right).

Preliminary tests showed that there was apparently an adequate supply of nutrients in the soil medium. The pH of the soil was 5.7, while that of the sphagnum medium was 4.4, and there is a wide difference in the physical structure of the two media. The effect of these factors on the growth of young seedlings is considered in the following series of experiments.

Procedures and Results

Effect of pH adjustment on growth of seedlings in soil. Young, active C. ledgeriana seedlings, from seeds sown June 1, were transplanted into flats of a soil medium on August 11, 1942. The greenhouse soil, as prepared, gave a pH reading of 5.7. By the use of sulfuric acid one series was prepared at a pH of 4.4, a second series was maintained at pH 5.7, and a third series was prepared at a pH of 7.4 by the addition of calcium carbonate. The flats were all fertilized with 8 g. per flat of 6-10-6 fertilizer applied in solution form.

Two flats of 60 plants each were used for each treatment, and similar flats of sphagnum were grown for purposes of comparison. The experiment was continued for three months, or until November 10. The growth of the seedlings in these treatments is illustrated in figure 15.

Total dry weight of 36-plant samples of each of the three soil treatments was 1.85, 3.62 and 3.35 g. for the soil at pH 4.4, 5.7 and 7.4, respectively. The similar plants in the sphagnum medium gave a dry weight yield of 19.30 g. Thus growth in the soil medium was again negligible as compared with growth in the organic medium, and the artificial adjustment of the pH was not effective in increasing growth.

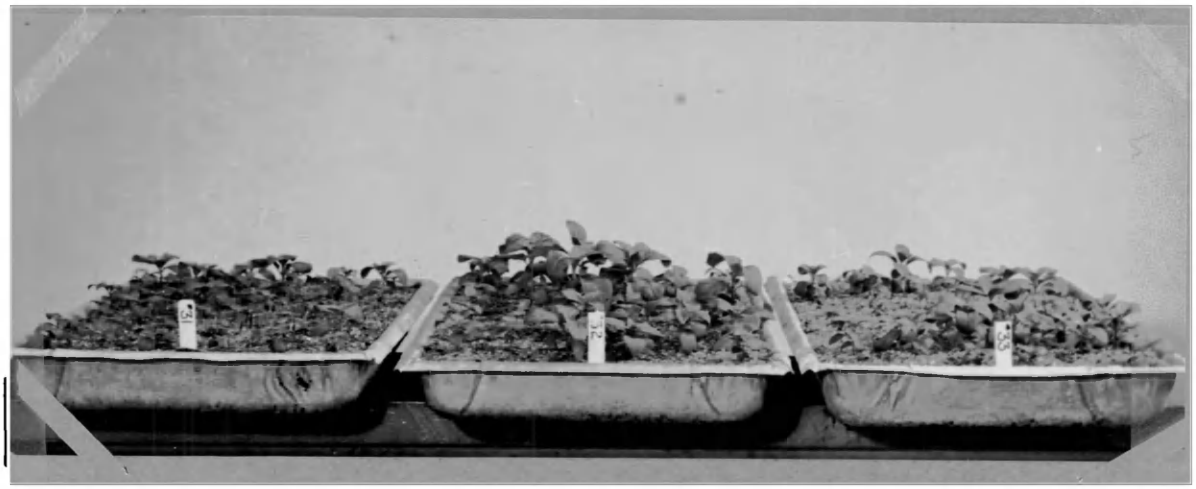

Figure 15. C. ledgeriana seedings in soil mixture, fertilized with 8 grams of 6-10-6 fertilizer. Flat on left adjusted with sulfuric acid to a pH of 4.4, center flat unadjusted, with a pH of 5.7, and flat on right adjusted with calcium carbonate to a pH of 7.4.

Effect of aeration on growth of seedlings in various media. Seeds of C. ledgeriana were sown in August, 1942, and the resulting seedlings transplanted into flats of sphagnum in October of the same year. Because of the sub-optimal growing conditions during the winter at this latitude, these seedlings had attained a height of only 20 mm. by March, 1943. These short, well-hardened seedlings were transplanted in March to 7-inch clay florist's pots in which copper tubing, coiled within 1 inch of the pot walls and with holes drilled with phonograph needles at one-inch intervals along the length, was fitted so that air could be pumped into the media, as described in detail by Crane (5). The seven media used were (1) a mixture of three parts of sphagnum and one part of peat, (2) 100 percent leaf mould, (3) 75 percent leaf mould and 25 percent potting soil, (4) equal parts of leaf mould and potting soil, (5) 25 percent leaf mould and 75 percent potting soil, (6) 100 percent

potting soil, consisting of three parts composted sterilized soil, one part each of bank sand and Michigan peat by volume, and (7) the composted, sterilized soil, without sand or peat. Weighed quantities of each medium were put in five pots, and the moisture-holding capacity determined by both the glass funnel and the Buchner porcelain filter methods. The amount of the media in each pot and the average of the moisture-holding capacity determinations are given in table 14.

Table 14. Amount of oven-dry material in each pot, and the average moisture-holding capacity of the media.

Medium	Oven-dry material per pot (grams)	Average moisture-holding capacity (percent)
1. Sphagnum and peat	164.5	1,108
2. 100% leaf mould	608	427
3. 75% leaf mould, 25% soil	1,808	122
4. 50% leaf mould, 50% soil	2,320	71
5. 25% leaf mould, 75% soil	3,335	42
6. 100% potting soil	4,020	35
7. Compost	3,535	33

The original experimental design called for the maintenance of a moisture level of between 50 and 66 percent of the moisture-holding capacity of the medium used in each of the pots. As the weight of the individual pots and all contents was known, this was accomplished by calculating a weight range for the two moisture percentages, which was maintained by frequent weighings and water additions. Three of the pots of each medium were supplied 15 minutes of air each day, by pumping air through the tubing. The other two pots, though they also contained the tubing, had no additional air pumped through them. There were ten seedlings in each pot, making a total of fifty plants in each medium, thirty of which received additional air by pumping. The pots were placed on racks on a greenhouse bench, the position occupied by a

treatment chosen at random and the pots systematically rotated to minimize place effect. Figure 16 is a photograph of the experiment taken March 10, six days after the transplanting.

Figure 16. Experiment to determine the effect of aeration and various media on the early seedling growth of C. ledgeriana. Air was pumped through three pots of each of the seven media 15 minutes daily. Note uniform size of seedlings.

Differences in growth rate attributable to the additional aeration failed to appear. Whether this was due to the method of aeration, to the amount, or to a lack of a critical requirement was not determined in this experiment. However, early differences in growth rate due to the different media were soon apparent. The seedlings in the sphagnum and peat mixture soon began to grow rapidly, as was expected on the

basis of previous experiences, but growth in some of the other media, notably the potting soil and some of the potting soil and leaf mould combinations, was not significantly less rapid than in the case of the sphagnum and peat mixture. Figure 17 is a photograph of the experiment taken June 6, or after the experiment had continued for three months.

Figure 17. As in figure 16, but after the experiment had continued for three months. Note differences in growth between media (rows), but lack of growth differences due to aeration (within rows). The media are (left to right) (1) 25% leaf mould, 75% soil, (2) 100% soil, (3) compost, (4) 100% leaf mould, (5) sphagnum and peat, (6) 75% leaf mould, 25% soil, and (7) equal parts of leaf mould and soil.

When it was apparent that supplying additional air was not affecting growth rate, the pumping was discontinued, and water was added as needed rather than maintaining a particular moisture content. The experiment was continued to determine the effect of media.

The early differences in growth rate in the different media began to disappear, however, and very rapid growth was observed to occur in

all media, during the warm months of June to August. By September 15, somewhat shorter days and lower temperatures resulted in a reduction of growth in all media, and the plants were measured and photographed and the experiment discontinued. Figure 18 is a photograph of all of the pots taken September 15, from which it is apparent that differences between the various media are slight, and that total growth in all media was excellent. A representative pot of each medium is shown in figure 19.

Figure 18. Photograph of all media treatments, September 15. Note uniformity and excellence of growth.

Figure 19. Representative pot from each media treatment, September 15. Differences in growth between treatments are slight.

A summary of the height measurements of the plants in the seven media is presented in table 15.

Table 15. Summary of height measurements of C. ledgeriana seedlings grown for six months in the greenhouse in pots of seven media. Plants were 2 cm. tall at the beginning of the experiment.

Medium	Average height (cm.) of plants in pot A	B	C	D	E	Ave.
1. Sphagnum and peat	34	39	38	36	44	38
2. 100% leaf mould	29	36	36	40	30	34
3. 75% leaf mould, 25% soil	33	36	35	41	33	36
4. 50% leaf mould, 50% soil	33	34	35	34	32	34
5. 25% leaf mould, 75% soil	36	36	31	29	31	33
6. 100% potting soil	44	39	34	34	41	38
7. Compost	32	34	42	40	34	36

Statistical treatment of the data in table 15 by the variance analysis method revealed that none of the height differences was significant. A minimum of 7 cm. difference in the average heights would be required for significance at the 1 percent point. Therefore,

well-hardened seedlings that were 2 cm. tall in March, made an average additional growth of 86 cm. in six months in the greenhouse, regardless of the medium in which they were growing.

Effect of age of seedlings at time of transplanting on growth in soil. In another experiment the age of the seedling at the time of transplanting has been considered. One lot of seedlings from seeds sown in May of 1942 on sphagnum and subsequently held without nutrients, a second lot from seeds sown in January of 1943 and hardened by withholding nutrients, and a third lot from seeds sown in May of 1943 and similarly hardened, were transplanted into two media, namely soil and sphagnum and peat, on July 10, 1943. Ten 7-inch pots of soil were planted with each of the three lots of seedlings, ten seedlings in each pot, or a total of 100 plants of each lot. Five pots of sphagnum and peat were similarly planted with 10 seedlings of the same lots. Figure 20 illustrates the survival and early post-transplanting growth in the two media.

It is apparent from figure 20 that in the case of the younger seedlings (May and January 1943) there is a slight difference in early post-transplanting growth in favor of the sphagnum and peat medium. In the case of the older seedlings, however, the transplants in the soil are fully equal in height to those in the sphagnum and peat medium, and are notably more sturdy.

The results of these experiments are in direct contrast with earlier experiences to the effect that growth of the young seedlings is slow in a soil medium as compared with growth in an organic medium. The fact that as good growth was recorded in soil as in the sphagnum and peat in these experiments is believed to be due to the fact that

the seedlings were well hardened before being transplanted to the media. The conclusion drawn from these experiments is that when newly germinated, actively growing seedlings are transplanted into soil they fail to make as rapid growth as similar seedlings transplanted into the sphagnum and peat medium in which they were germinated. However, when the seedlings were hardened before transplanting, the difference in subsequent growth was negligible. This peculiarity has also been observed in the case of seedlings to be transplanted into sand for sand culture studies. Considerable difficulty has been experienced in attempting to establish active seedlings in the new medium, whereas a prior period of hardening has resulted in excellent transplanting survival and subsequent growth.

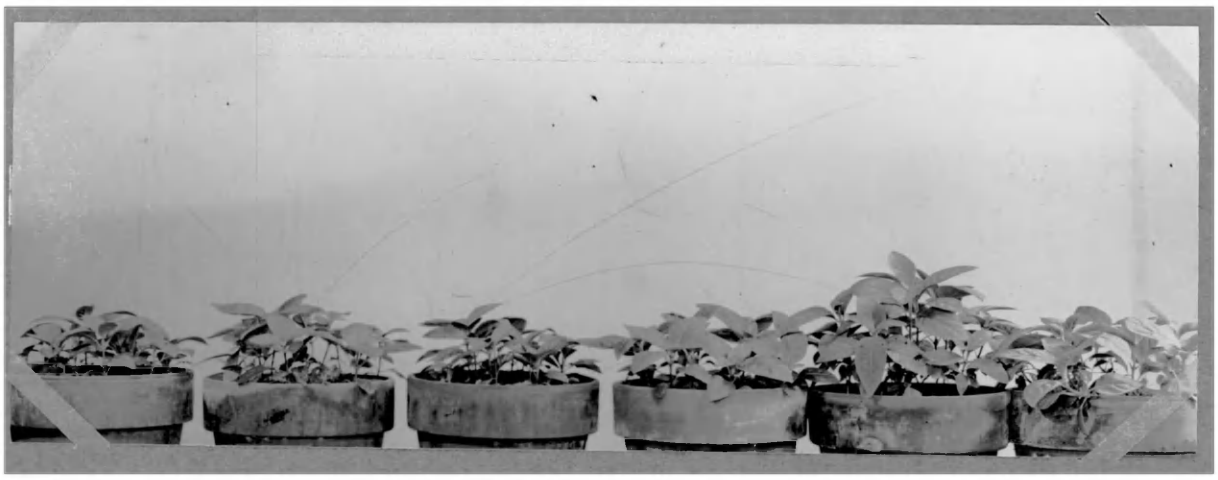

Figure 20. C. ledgeriana seedlings of three ages transplanted into pots of soil compared with similar seedlings in pots of sphagnum and peat. Left to right (1) May, 1943 seedlings in soil, (2) same in sphagnum and peat, (3) January, 1943 seedlings in soil, (4) same in sphagnum and peat, and (5) May, 1942 seedlings in soil and (6) in sphagnum and peat. Transplanted to the media July 10, photographed September 15, 1943.

Part II. Effect of Fertilizer Applications and Lime on Growth in a Sphagnum and Peat Medium

Though it is of considerable practical interest to know that seedlings of C. lederiana germinated on sphagnum can be grown rapidly in a soil medium when the precaution of a previous hardening is taken, for the purpose of growing large numbers of vigorous, disease-free seedlings for subsequent shipment over considerable distances, the use of an organic medium offers several advantages. In the first place, the remarkable freedom from damping-off losses in the seed bed resulting from the use of sphagnum moss as the germination medium has also been found to be true in the case of the transplanted seedling; of the hundreds of thousands of seedlings transplanted into sphagnum the transplanting loss has been considerably less than 1 percent, and these due largely to mechanical causes and not to damping-off. Furthermore, seedlings grown to a height of 6 to 15 inches in three to six months in the sphagnum medium in the greenhouses have been found to ship remarkably well over long distances. Of great practical significance in the present program also is the fact that seedlings so grown can be packaged with an economy of weight of over 1500 percent as compared with similar plants grown in soil, making feasible the use of air express as the method of transport.

However, as sphagnum contains only negligible amounts of nutrients, a fertilization program was necessary to obtain satisfactory growth in this medium. To furnish experimental evidence on the effect of fertilizer, lime and micro-element applications to the sphagnum medium on the growth

of young C. ledgeriana seedlings, the following series of experiments was conducted.

Materials and Methods

For these experiments, seeds of C. ledgeriana were sown on sphagnum June 1, and the seedlings transplanted without a previous hardening period during the week of August 3, 1942. Sixty uniform plants were transplanted in each metal flat, and two such flats, or 120 plants were used for each treatment. A mixture of three parts sphagnum and one part peat, shown by preliminary experiments to be slightly superior to all-sphagnum as a growing medium, was used in the flats.

All treatments were placed on a single greenhouse bench, divided lengthwise into two plots, within which the treatments were located at random. Twice weekly for the duration of the experiment the flats were systematically shifted as to position and exposure, to minimize place effect.

The fertilizers were all applied in solution form, and except where mentioned, the same supplementary solution containing calcium, magnesium and the micro-elements were applied to all flats. At no time during the experiment was enough liquid added to the flats to cause leaching. Thus, the total amounts of nutrients supplied are assumed to have been available to the seedlings.

The experiment was conducted for three months, or until the second week in November. A 48-plant sample from each treatment, using only the innermost 24 plants in each flat, was then measured as to height, width and length of largest leaf. Three central rows of each flat, or 36 plants for each treatment, were cut at the media line, and fresh and dry weights determined.

The experimental layout, photographed in November, is shown in figure 21.

Figure 21. General view of the experiments on the nutrition of young C. ledgeriana seedlings grown in sphagnum and peat. Experiment begun in August, photographed in November.

In the absence of any information in the literature as to a suitable fertilizer formula, a complete nutrient formula containing 6 percent nitrogen, 10 percent phosphoric acid and 6 percent potassium was arbitrarily selected. The following treatments were provided: (1) Applications of the 6-10-6 fertilizer at the rate of 2 g., 4 g., and 8 g. per flat, as compared with an unfertilized check. (The 2 g. per flat application is roughly equivalent to 260 lbs. per acre on an acre

six inches basis.) (2) The preceding rates applied in one application, as compared to the same rate in 3 monthly and in 6 bi-weekly applications. (3) Applications at the 2 and 4 g. rates in which each of the nutrient components in the 6-10-6 fertilizer formula was doubled. (4) The addition of lime in rates of 10 and 30 g. per flat to the sphagnum and peat medium. (5) The customary application of micro-elements (3 ppm. boron, ½ ppm. copper, 1 ppm. manganese and zinc, and 10 ppm. iron) as compared to no supplementary application of micro-elements.

Results

Effect of rate of application. Growth resulting when 6-10-6 fertilizer was applied to the sphagnum and peat plots in six equal portions at bi-weekly intervals in rates of 2 g. and 4 g. per flat, as compared to an unfertilized check plot in the same medium is illustrated in figure 22. Measurements of these seedlings taken at the conclusion of the experiment are summarized in table 16.

Figure 22. C. ledgeriana seedlings grown in sphagnum and peat medium. Flat on left is unfertilized check, center flat received 2 grams 6-10-6 fertilizer and flat on right received 4 grams 6-10-6 fertilizer.

Table 16. Summary of measurements of C. ledgeriana seedlings grown in sphagnum and peat fertilized with 2 and 4 grams of 6-10-6 fertilizer, compared with unfertilized check.

Treatment	Ave. height 48 plts. (cm.)	Ave. width largest leaf (cm.)	Ave. length largest leaf (cm.)	Total fresh wt. 36 plts. (g.)	Total dry wt. 36 plts. (g.)
Unfertilized	5.67	2.71	5.00	53.5	11.58
2 g. 6-10-6	8.58	3.39	6.78	81.9	16.98
4 g. 6-10-6	9.00	3.33	6.90	93.8	18.55

The effect of further increasing the rate of application is illustrated in figure 23, which is a photograph of the flats receiving 4 and 8 g. of 6-10-6 fertilizer compared with the unfertilized check. The measurement data from these treatments are given in table 17.

Figure 23. C. ledgeriana seedlings in sphagnum and peat medium. Flat on left is unfertilized check, center flat received 4 grams 6-10-6 fertilizer, and flat on right received 8 grams 6-10-6 fertilizer.

From figures 22 and 23, and the data in tables 16 and 17, it is apparent that the use of fertilizers has resulted in significant increases in growth over the unfertilized plots. The dry weight data,

however, indicate that there was little difference in the response between the 2 and 4 g. applications, while the use of 8 g. resulted in a further increase in growth. The 8 g. application rate yielded plants that were 101 percent greater in dry weight than the unfertilized check plants. Of particular interest also is the fact that even with the 8 g. rate of application, which is roughly the equivalent of an application of 1040 lbs. per acre six inches, there was no evidence of leaf burning or other symptom of overfeeding.

Table 17. Summary of measurements of C. ledgeriana seedlings grown in sphagnum and peat medium unfertilized, compared with fertilized with 4 and 8 grams of 6-10-6 fertilizer.

Treatment	Ave. height 48 plts. (cm.)	Ave. width largest leaf (cm.)	Ave. length largest leaf (cm.)	Total fresh weight 36 plts. (g.)	Total dry weight 36 plts. (g.)
Unfertilized	5.67	2.71	5.00	53.5	11.58
4 g. 6-10-6	8.20	3.18	6.23	84.2	16.95
8 g. 6-10-6	10.21	3.64	7.37	122.4	23.26

Effect of time of application. Growth resulting when 4 g. of 6-10-6 fertilizer was applied to the sphagnum and peat medium in six equal portions at bi-weekly intervals, as compared with the same amount applied in three equal portions at monthly intervals and all in one application at the beginning of the experiment, is illustrated in figure 24, and the final measurements are summarized in table 18.

Figure 24. C. ledgeriana seedlings grown in a sphagnum and peat medium to which 4 grams of 6-10-6 fertilizer was applied (left) in six equal portions bi-weekly, (center) in three equal portions monthly, and (right) all in one application at the beginning of the experiment.

Table 18. Summary of measurements of C. ledgeriana seedlings illustrated in figure 24.

Treatment	Ave. height 48 plts. (cm.)	Ave. width largest leaf (cm.)	Ave. length largest leaf (cm.)	Total fresh weight 36 plts. (g.)	Total dry weight 36 plts. (g.)
6 applications	9.00	3.33	6.90	93.8	18.55
3 applications	8.12	3.28	6.78	85.7	17.75
1 application	7.13	3.13	6.02	75.7	15.32

Similarly, growth resulting when 8 g. of the 6-10-6 fertilizer was added to the sphagnum and peat medium in six, three and one applications is illustrated in figure 25, and the measurements summarized in table 19.

Figure 25. C. ledgeriana seedlings in a sphagnum and peat medium to which 8 grams of 6-10-6 fertilizer was supplied (left) in six equal portions bi-weekly, (center) in three equal portions monthly, and (right) all in one application at the beginning of the experiment.

Table 19. Summary of measurements of C. ledgeriana seedlings illustrated in figure 25.

Treatment	Ave. height 48 plts. (cm.)	Ave. width largest leaf (cm.)	Ave. length largest leaf (cm.)	Total fresh weight 36 plts. (g.)	Total dry weight 36 plts. (g.)
6 applications	10.21	3.64	7.37	122.4	23.26
3 applications	8.53	3.28	6.76	96.1	19.30
1 application	7.21	3.20	6.50	79.5	16.32

As shown by the data in tables 18 and 19, there was an increase in growth resulting from split applications. Dry weight data for the 4 g. rate shows a 21 and 28 percent increase for the three and six portion applications, respectively, over the weight for the single application. Similarly, with the 8 g. rate, there was a significant increase in

growth when the fertilizer was applied in split applications. In the latter case, the differences are more marked, and it is again of interest to note that even when the 8 g. were applied in one application, no injury to the young seedlings resulted. Data from preliminary experiments have shown that this rate, if applied to young seedlings in soil, will result in serious injury to the foliage, in most cases resulting in the death of the plants. One possible explanation of this difference is that there is a very great difference in the moisture-holding capacity of the two media, and thus a large difference in the quantity of solution present. Hence, though the amount for a given area or volume may be the same, there is nevertheless considerable difference in actual concentration. It is thus possible to apply seemingly large quantities of fertilizer to plants in the sphagnum and peat medium without danger of injury to the seedlings.

Effect of doubling a nutrient component. To obtain preliminary information on the effect of different ratios of N, P and K in the fertilizer formula, an experiment was conducted in which each of the three components was individually doubled. Figure 26 illustrates the growth response of C. ledgeriana seedlings in the sphagnum and peat medium fertilized with 2 g. per flat of 12-10-6, 6-20-6 and 6-10-12 fertilizers. Final measurements are summarized in table 20.

Figure 26. C. ledgeriana seedlings in sphagnum and peat medium. Flat on left received 2 grams of 12-10-6, center flat 2 grams of 6-20-6, and flat on right 2 grams of 6-10-12 fertilizer.

Table 20. Summary of measurements of C. ledgeriana seedlings illustrated in figure 26.

Treatment	Ave. height 48 plts. (cm.)	Ave. width largest leaf (cm.)	Ave. length largest leaf (cm.)	Total fresh weight 36 plts. (g.)	Total dry weight 36 plts. (g.)
2 g. 12-10-6	8.28	3.25	6.70	77.8	15.03
2 g. 6-20-6	8.21	3.20	6.54	86.8	17.83
2 g. 6-10-12	9.87	3.58	7.23	103.1	21.76

Similar plants receiving 2 g. of 6-10-6 fertilizer yielded 16.98 g. dry matter. Thus, only in the case of the 6-10-12 fertilizer, or where the potassium was doubled, was there an increase in yield that was significant.

Growth resulting when 4 g. of fertilizer was supplied is illustrated in figure 27, and the measurement data summarized in table 21.

Figure 27. C. ledgeriana seedlings in sphagnum and peat medium. Flat on left received 4 grams of 12-10-6, center flat 4 grams of 6-20-6, and flat on right 4 grams of 6-10-12 fertilizer.

Table 21. Summary of measurements of C. ledgeriana seedlings illustrated in figure 27.

Treatment	Ave. height 48 plts. (cm.)	Ave. width largest leaf (cm.)	Ave. length largest leaf (cm.)	Total fresh weight 36 plts. (g.)	Total dry weight 36 plts. (g.)
4 g. 12-10-6	9.53	3.53	6.76	91.4	18.20
4 g. 6-20-6	8.75	3.44	6.59	89.0	17.69
4 g. 6-10-12	8.88	3.51	6.57	89.6	17.80

Similar plots receiving 4 g. 6-10-6 fertilizer yielded 16.95 g. dry matter. Thus doubling each of the nutrient components was not effective in significantly increasing growth of the seedlings.

Effect of liming. The pH of the sphagnum and peat mixture used as the growing medium in these experiments was between 4.2 and 4.4. As this was below the 5.2 to 5.6 range stated by Wilson and Mirchandani (26) to be the optimum for growth of this form in India, an experiment in which the pH was controlled by the addition of calcium carbonate was conducted. Ten grams of calcium carbonate thoroughly mixed with the medium in each flat before the plants were set resulted in a pH of 5.8. The growth that resulted at the higher pH in an unfertilized check, as compared with similar plots to which 2 and 4 g. of 6-10-6 fertilizer was applied in six bi-weekly portions is illustrated in figure 28, and a summary of the measurements taken at the conclusion of the experiment presented in table 22.

Figure 28. C. ledgeriana seedlings in sphagnum and peat medium limed to a pH of 5.8. Flat on left is unfertilized, center flat received 2 grams of 6-10-6, and flat on right 4 grams of 6-10-6 fertilizer in six bi-weekly portions.

Table 22. Summary of measurements of C. ledgeriana seedlings grown in sphagnum and peat limed to pH 5.8, and supplied 0, 2 and 4 grams of 6-10-6 fertilizer.

Treatment	Ave. height 48 plts. (cm.)	Ave. width largest leaf (cm.)	Ave. length largest leaf (cm.)	Total fresh weight 36 plts. (g.)	Total dry weight 36 plts. (g.)
Unfertilized	3.16	1.90	3.38	24.3	5.28
2 g. 6-10-6	6.04	2.77	5.49	56.1	12.20
4 g. 6-10-6	7.76	3.16	6.28	73.1	13.80

It can be seen from figure 28 and the data in table 22 that there was a response to the application of fertilizer by the plants in the limed medium which was similar to the response in the unlimed medium (see table 16), though growth in the limed medium was significantly less than in the unlimed medium, the dry weight yield of the former being 61, 42 and 41 percent below the comparable plots in the latter.

A similar response is seen in figure 29 and table 23, in which seedlings grown in plots limed to a pH of 5.8 and supplied 4 and 8 g. of 6-10-6 fertilizer in six bi-weekly portions are illustrated and the growth measurements summarized.

Here again, though there was a significant increase in growth resulting from the application of fertilizers to the limed medium, growth remained significantly less than in the case of the unlimed medium (see figure 23 and table 17). Thus, regardless of the application of fertilizer, growth in the limed medium was less than in the unlimed medium.

Figure 29. C. ledgeriana seedlings in sphagnum and peat limed to a pH of 5.8. Flat on left is unfertilized, center flat received 4 grams and flat on right 8 grams of 6-10-6 fertilizer in six bi-weekly portions.

Table 23. Summary of measurements of C. ledgeriana seedlings grown in sphagnum and peat limed to pH 5.8 and supplied 0, 4 and 8 grams of 6-10-6 fertilizer.

Treatment	Ave. height 48 plts. (cm.)	Ave. width largest leaf (cm.)	Ave. length largest leaf (cm.)	Total fresh weight 36 plts. (g.)	Total dry weight 36 plts. (g.)
Unfertilized	3.16	1.90	3.38	24.3	5.28
4 g. 6-10-6	7.70	3.26	6.62	87.3	18.08
8 g. 6-10-6	8.33	3.32	7.00	97.9	18.82

An application of thirty grams of calcium carbonate per flat to the sphagnum and peat medium before the seedlings were planted resulted in a pH reading of 7.2. Figure 30 illustrates the growth of seedlings in the medium which had received 2 g. per flat of 6-10-6 fertilizer, and similar plots to which had been added 10 and 30 g. of calcium carbonate, resulting in pH readings of 5.8 and 7.2.

Figure 30. C. ledgeriana seedlings in sphagnum and peat medium fertilized with 2 grams per flat of 6-10-6 fertilizer. Flat on left was not limed (pH 4.4), center flat received 10 grams of calcium carbonate (pH 5.8), and flat on right 30 grams of calcium carbonate(pH 7.2).

Table 24. Summary of growth measurements of C. ledgeriana seedlings illustrated in figure 30.

Treatment	Ave. height 48 plts. (cm.)	Ave. width largest leaf (cm.)	Ave. length largest leaf (cm.)	Total fresh weight 36 plts. (g.)	Total dry weight 36 plts. (g.)
pH 4.4	8.58	3.39	6.78	81.9	16.98
pH 5.8	6.04	2.77	5.49	56.1	12.20
pH 7.2	1.25	0.86	1.40	5.8	0.89

It is apparent that the addition of lime to the medium has resulted in a reduction of growth. Growth in the medium limed to pH 7.2, though 2 g. of fertilizer was applied, was the least of any of the various treatments in this series of experiments. The dry weight yield, shown in table 24, was less than 10 percent of that of the unlimed, unfertilized check plot (table 16, p. 64).

A similar comparison is seen in figure 31, in which the same liming treatments were applied to flats which received a double rate of the 6-10-6 fertilizer.

Figure 31. C. ledgeriana seedlings in sphagnum and peat medium fertilized with 4 grams per flat of 6-10-6 fertilizer. Flat on left was not limed (pH 4.4), center flat received 10 grams of calcium carbonate (pH 5.8), and flat on right 30 grams of calcium carbonate (pH 7.2).

Table 25. Summary of growth measurements of C. ledgeriana seedlings illustrated in figure 31.

Treatment	Ave. height 48 plts. (cm.)	Ave. width largest leaf (cm.)	Ave. length largest leaf (cm.)	Total fresh weight 36 plts. (g.)	Total dry weight 36 plts. (g.)
pH 4.4	8.20	3.18	6.23	84.2	16.95
pH 5.8	7.70	3.26	6.62	87.3	18.08
pH 7.2	3.25	1.93	3.82	28.8	5.55

From the data in table 25 it is apparent that the deleterious effect of the addition of lime on the growth of the young seedlings in the sphagnum and peat medium is overcome to a certain extent by increasing the rate of fertilizer application. Whereas there was a consistent

reduction in growth from the addition of lime when 2 g. of 6-10-6 fertilizer were applied per flat, in the case of the 4 g. application such reduction in growth did not materialize when the medium was limed to pH 5.8, and was to a significantly less degree when the medium was limed to pH 7.2. In neither case, however, did liming materially increase growth.

Effect of withholding micro-elements. Preliminary trials to establish young C. ledgeriana seedlings in water culture, and the question as to whether micro-element fertilization would be required when plants were grown in the sphagnum and peat medium, prompted the inclusion in this series of experiments of a comparison to determine the effect of the presence or absence of additional micro-elements in the fertilizer mixture employed. Figure 32 illustrates the growth of the seedlings in the unfertilized medium, compared with similar seedlings fertilized with 2 g. of 6-10-6 per flat supplemented by a solution containing 3 ppm. boron, ½ ppm. copper, 1 ppm. manganese and zinc, and 10 ppm. iron. A third plot received the 2 g. of fertilizer without the micro-element supplement. Growth measurements are summarized in table 26.

Table 26. Summary of growth measurements of C. ledgeriana seedlings in sphagnum and peat medium unfertilized, compared with fertilized at the rate of 2 grams of 6-10-6, with and without micro-element supplement.

Treatment	Ave. height 48 plts. (cm.)	Ave. width largest leaf (cm.)	Ave. length largest leaf (cm.)	Total fresh weight 36 plts. (g.)	Total dry weight 36 plts. (g.)
Unfertilized	5.67	2.71	5.00	57.5	11.58
2 g. 6-10-6 + micro-elem.	8.58	3.39	6.78	81.9	16.98
2 g. 6-10-6, no micro-elem.	8.83	3.38	6.80	94.5	18.98

Figure 32. C. ledgeriana seedlings in sphagnum and peat medium. Flat on left is unfertilized check, center flat received 2 grams 6-10-6 fertilizer, supplemented by the addition of a micro-element solution, and flat on right received fertilizer but without micro-elements.

To determine the effect of level of nutrition on this response, 4 g. per flat of the 6-10-6 fertilizer was applied with and without the same micro-element supplement. The response to these treatments is illustrated in figure 33, and the summarized growth measurements are presented in table 27.

Table 27. Summary of growth measurements of C. ledgeriana seedlings in sphagnum and peat medium unfertilized, compared with fertilized at the rate of 4 grams of 6-10-6, with and without micro-element supplement.

Treatment	Ave. height 48 plts. (cm.)	Ave.width largest leaf (cm.)	Ave.length largest leaf (cm.)	Total fresh weight 36 plts. (g.)	Total dry weight 36 plts. (g.)
Unfertilized	5.67	2.71	5.00	53.5	11.38
4 g. 6-10-6 + micro-elements	8.20	3.18	6.23	84.2	16.95
4 g. 6-10-6, no micro-elements	8.41	3.38	6.68	94.0	17.90

Figure 32. C. ledgeriana seedlings in sphagnum and peat medium. Flat on left is unfertilized check, center flat received 4 grams of 6-10-6 fertilizer supplemented by the addition of a micro-element solution, and flat on right received fertilizer but without micro-elements.

It is apparent from these data that supplying additional micro-elements did not result in increased growth. In fact, there was a consistent, though non-significant difference in growth in favor of the treatments where no additional micro-elements were supplied. Also, failure to supply additional micro-elements to the seedlings growing in the sphagnum and peat medium, regardless of the other nutrients applied, did not result in visible symptoms of malnutrition in the plants.

Nutritional Studies in Sand Cultures

Though a final determination of the most feasible fertilizer practice for any crop must be made in the locality and particular site on which it is grown, controlled experimentation in the greenhouse often provides the basic knowledge upon which an efficient field experiment can be based. No recorded accounts of such controlled experimentation with cinchona are available, however, though correspondence by Stoddard (22) with the author mentions an unsuccessful attempt to grow the plants in sand cultures in Puerto Rico.

Preliminary experiments with sand cultures and water cultures indicated that the plants can be successfully grown in the former. Therefore, a series of experiments were conducted to obtain information on (1) the general nutrient level required and the effect of the pH of the nutrient solution on seedling growth, (2) the effect on the seedling of all combinations of three levels of nitrogen, phosphorous and potassium in the nutrient solution, (3) the effect of various calcium, magnesium and potassium levels, and (4) the mineral deficiency symptoms of some of the important nutrient elements as evidenced by young seedlings.

Part I. The General Nutrient Level Required by Cinchona Seedlings, and the Effect of the pH of the Nutrient Solution on their Growth

Materials and Methods

Crocks and sand. One-gallon earthenware crocks, with the drainage hole covered by a small piece of glass wool, were used as containers for the media. As it had been frequently stated in the popular literature that cinchona plants require a well-aerated medium, the fairly coarse 18-mesh white quartz sand was employed as the growing medium.

Solutions. C. P. chemicals were used exclusively in the preparation of the stock solutions, and were dissolved and diluted with distilled water throughout. A composite solution was prepared to contain 140 ppm. nitrogen, 124 ppm. phosphorous, 156 ppm. potassium, 200 ppm. calcium, 99 ppm. magnesium and 64 ppm. sulfur. This solution is hereinafter designated as Solution I. To obtain information on the effect of the general nutrient level on growth of the seedlings, a second solution was prepared in which the concentration of the above nutrient elements was doubled. This second solution will be designated as Solution II. Both solutions were prepared using calcium nitrate, magnesium sulfate, and monopotassium phosphate, and are thus a modification of the usual three-salt solution in wide use in artificial culture work.

A micro-element solution was prepared which when diluted to feeding strength delivered $\frac{1}{2}$ ppm. copper, 1 ppm. of zinc and manganese, and 2 ppm. boron. Iron, from ferric tartrate, was supplied separately in the amount of 10 ppm. The micro-elements were supplied to all treatments equally.

Plants. Seedlings of C. ledgeriana from seeds sown on sphagnum in May, 1942, were hardened by withholding nutrients before transplanting. Ten seedlings were used in each of the crocks, and all of the plants were taken from the same seed flat and were apparently uniform as to growth and appearance. They were about 3 cm. tall and had formed their second pair of true leaves when they were set in the crocks on August 17, and watered with distilled water only for a few days before the solutions were applied.

Methods. Two crocks, or twenty plants, were used for each treatment. The pH of the unadjusted composite solutions as prepared for application was 5.2 for Solution I and 5.6 for Solution II. Five pH treatments, from 4.0 to 8.0 at 1.0 point intervals, were established by adding the necessary amount of 1 percent HCl or NaOH to the solutions. Indicator dyes were used as standards. Each of the composite solutions was applied at each of the five pH levels, making a total of ten treatments.

The crocks were placed on a table in the greenhouse, and the position occupied by any treatment or its replicate chosen at random. Twice each week all of the crocks were systematically rotated as to position, and turned half around to equalize exposure.

The pH of the solutions was adjusted immediately before applications, and 250 ml. of the prepared solutions was applied to each crock twice each week. This amount was found to thoroughly moisten the medium and furnish about 150 ml. of leachate. At two-weeks intervals during the course of the experiment the crocks were flushed through with a liter of distilled water, to leach out any accumulated chlorides and sulfates.

The experiment was continued for six weeks, or until the first week in October, when it was apparent that autumn conditions were reducing the growth rate. Final records consisted of height measurements, width and length of the largest leaf produced, and fresh and dry weight determinations of the aerial portion of the plants. Photographs were taken of both tops and representative root systems.

Results

Main experiment. The leaves of the seedlings supplied the composite solutions at the low end of the pH range were observed to change from the reddish color indicative of the hardened condition to a dark green color after only 10 days. The terminal buds increased greatly in size and the new pair of leaves produced compared favorably in size and color with plants grown in sphagnum.

The plants receiving the composite solutions adjusted to pH 7 and 8, on the other hand, either resumed growth much more slowly or failed to survive.

Growth measurements made at the end of the six-weeks period are summarized in table 28. This data shows that there was no significant increase in growth resulting from increasing the nutrient concentration. This suggests that some of the nutrient components were in sufficient concentration in Solution I and possibly may have been in excess of optimum in Solution II. Data presented elsewhere in this thesis show that the phosphorous concentration, 124 ppm. in Solution I and twice that amount in Solution II, was considerably above the level at which maximum growth was recorded in a factorial experiment.

The data in table 28 also show that a highly significant reduction in growth resulted from the application of nutrient solutions adjusted to a pH higher than 6.0. This was true in the case of both nutrient solutions. With Solution II, in which the nutrient elements were in twice the concentration as in Solution I, there seemed also to be a relationship between the pH of the solution and the number of plants surviving.

Table 28. Growth measurements of C. ledgeriana supplied composite nutrient solutions at two concentrations and five pH levels.

Treatment	Plants surviving (no.)	Ave. height (mm.)	Ave.width largest leaf (mm.)	Ave.length largest leaf (mm.)	Ave. fresh weight per crock (g.)	Ave.dry weight per crock (g.)
Solution I						
pH 4	20	45	20	37	10.74	1.408
pH 5	20	45	19	32	7.93	1.134
pH 6	20	55	21	37	10.11	1.389
pH 7	20	42	15	25	3.62	.538
pH 8	20	44	15	24	2.76	.458
Solution II						
pH 4	20	51	18	33	7.17	1.081
pH 5	19	44	20	35	7.49	1.221
pH 6	17	41	17	31	5.62	.876
pH 7	11	45	13	23	0.90	.357
pH 8	16	42	14	25	1.35	.481

Figure 34 is a photograph showing the comparative response of the plants supplied Solution I adjusted to pH 4, 5 and 6. These are seen to be quite similar in appearance. This substantiates the results obtained in sphagnum cultures, in which maximum growth was seen to result from a pH of 4.4, and also the statement of Wilson and Mirchandani (26) that in India a pH range of 5.2 to 5.6 was optimum for growth.

Figures 35 and 36 provide a comparison of the response of the plants which received solutions adjusted to pH 5, 6, 7 and 8, in which it is apparent that with both Solutions I and II the solutions at pH 7 and 8 resulted in a marked depression of growth, though the greatest reduction in growth and highest mortality resulted from a combination of Solution II at a pH of 7 or 8.

Figure 34. C. ledgerianæ seedlings supplied Solution I adjusted to pH 4, 5 and 6 (left to right).

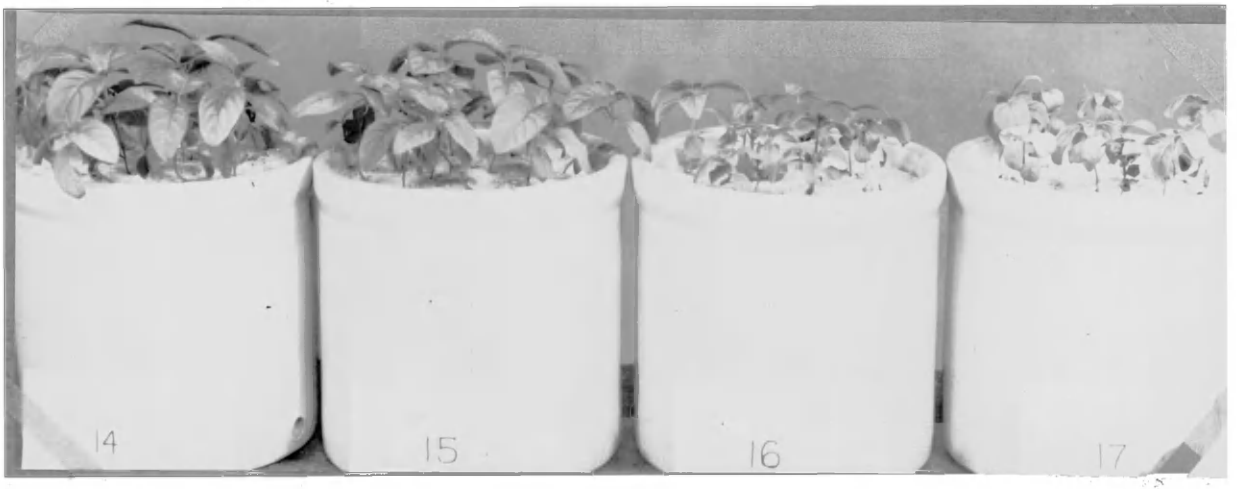

Figure 35. C. ledgerianæ seedlings supplied solution I adjusted to pH 5, 6, 7 and 8 before application (left to right).

Figure 38. C. ledgeriana seedlings supplied Solution II adjusted to pH 5, 6, 7 and 8 before application (left to right).

Though it was not possible to obtain reliable physical data on the roots of the plants in these treatments, because of their fragility and small quantity, photographs were taken of representative root systems of the plants in the various cultures. Figure 37 illustrates the root systems of the plants supplied Solution I at pH 5, 6, 7 and 8. The root systems of the seedlings supplied Solution II adjusted to pH 5, 6, 7 and 8 are illustrated in figure 38.

The differences in the tops produced, both as a result of the variation of the nutrient concentration and the various pH levels, are seen to be reflected also in the gross configuration of the root systems.

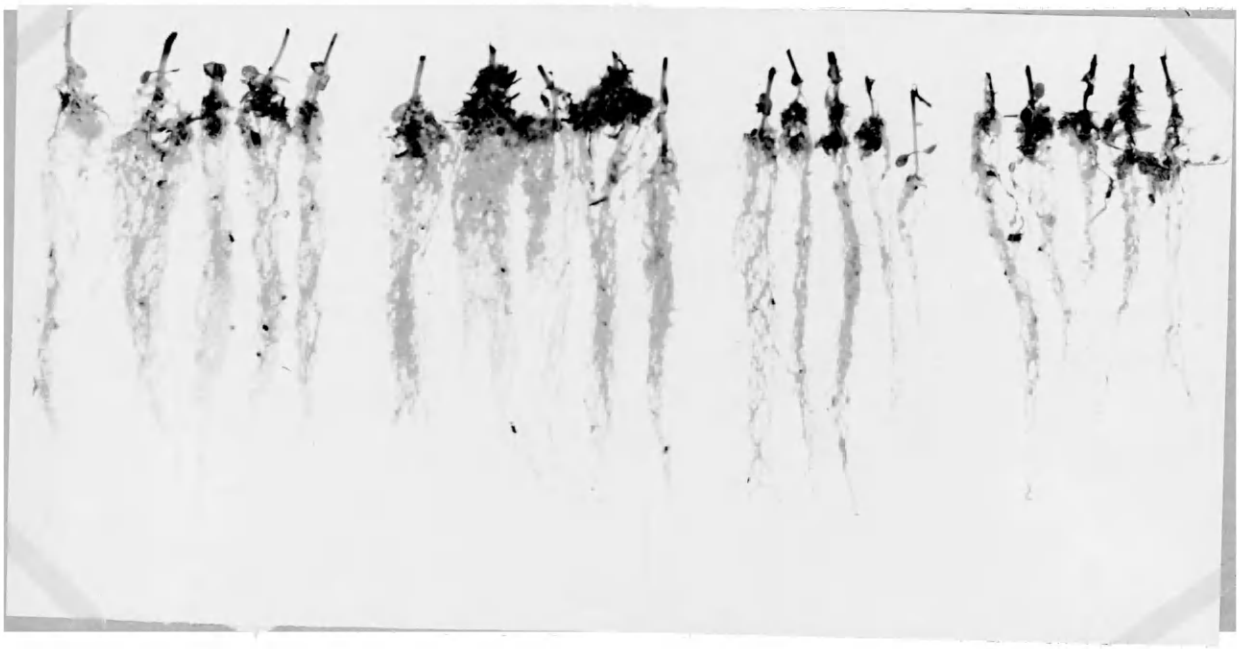

Figure 37. Representative root systems of C. ledgeriana seedlings supplied Solution I adjusted to pH 5, 6, 7 and 8 (left to right).

Figure 38. Root systems of C. ledgeriana seedlings supplied Solution II adjusted to pH 5, 6, 7 and 8 (left to right).

Supplementary pH experiment. To obtain information on the effect of the pH of the nutrient solution on growth of larger seedlings in sand cultures, sphagnum-grown C. ledgeriana seedlings, averaging 12 cm. in height, were hardened by withholding nutrients, most of the sphagnum removed, and planted three to each one-gallon crock of 18-mesh quartz sand. The nutrient solution was the same as Solution I in the preceding experiment, with the exception that the phosphorous quantity was reduced to 62 ppm.

Six pH treatments, namely 2.5, 3.0, 4.0, 5.0, 6.0 and 7.0, were established by daily adjusting the pH of 10 liters of solution with 1 percent HCl or NaOH and thoroughly saturating the medium. The solution was allowed to drain back into the respective bottles and re-used for three weeks, when the solution was renewed. The pH determinations were made on a Leeds and Northrup calomel eletrode potentiometer.

The experiment was started on November 10, and continued until June 3. Since it was desired to then use a part of these plants in another experiment, final records were limited to photographs.

The plants supplied the nutrient solution adjusted to pH 2.5 were severely injured by the treatment, and died within three weeks. The plants in all other treatments are illustrated in figure 39.

It is evident that there was considerable variation in the growth rates of the individual plants in the crocks, preventing any precise determination of an optimum pH for growth of these plants in sand cultures. As illustrated in figure 40, however, there was a definite trend in the type and amount of growth made in response to the pH of the nutrient solutions. Plants supplied the solutions adjusted to pH 4, 5 and 6, with a few exceptions, were very similar in appearance, making

a luxuriant growth with large leaves and long internodes. Plants supplied the neutral solution, though all remained alive, made but little growth in height, and the leaf color was notably more pale than in the other treatments. Thus it appears that larger plants may be tolerant of a somewhat wider range of pH values, nevertheless these results support the results obtained with younger seedlings in that optimum growth occurred when the nutrient solution was adjusted to between pH 4 and 6.

Figure 39. General view of older C. ledgeriana seedlings in supplementary pH experiment.

Figure 40. Representative pots of the supplemental pH experiment. Growth of older C. ledgeriana seedlings supplied a nutrient solution adjusted to pH 3.0, 4.0, 5.0, 6.0 and 7.0 (left to right). All plants were 12 cm. high at the start of the experiment on November 10. Photographed May 10.

Part II. Factorial Experiment on the Nitrogen, Phosphorous and Potassium Nutrition of Young C. ledgeriana Seedlings

The first successful attempt to grow C. ledgeriana seedlings in sand culture indicated that they responded favorably to the arbitrarily chosen nutrient concentrations when applied at the relatively acid pH of 4.0 to 5.0. It was deemed highly desirable to continue the studies by experimentally varying the concentration of the nutrient components to determine the combination most favorable for growth. This is best accomplished by means of factorial experiments, in which the effect of all combinations of chosen concentrations of more than one nutrient component can be simultaneously studied.

As nitrogen, phosphorous and potassium have been the most widely studied elements in plant nutrition work, an experiment of the factorial type was conducted in which the effect of three levels of each of these elements was determined. The nitrogen levels selected were 20, 80 and 320 ppm., to be combined with 5, 20 and 80 ppm. of phosphorous and 25, 100 and 400 ppm. of potassium. The nitrogen and potassium levels selected were so chosen as to extend the range above and below that used in the previous experiment, but as there was some indication from the preliminary work that the 124 and 248 ppm. concentrations of phosphorous may have been above the optimum for this element, a much lower range of concentrations was employed in the present experiment.

Materials and Methods

Crocks and sand. A factorial experiment with three levels of three elements requires 27 pots per replicate to include all possible combinations of the variates. In this experiment, each treatment was triplicated, making a total of 81 pots. Two-gallon glazed coffee-liners were used as containers, and the drainage hole in the bottom was covered with glass wool. The 18-mesh white quartz sand previously used was again employed as the medium.

Solutions. Stock solutions were prepared with reagent chemicals and tap water. The composition of the stock solutions, the dilutions used, and the resulting concentration of the elements in the feeding solution are given in table 29.

The solutions were prepared just prior to their application, at which time they were adjusted to a pH of 4.5 to 5.0 by means of 1 percent HCl.

Plants. Twelve seedlings of C. ledgeriana were planted in each of the 81 pots on February 18, 1943. The plants were from seeds sown the previous December 7, and had just begun to develop their first pair of true leaves. All plants required for the experiment were taken from the same seed flat, and were very uniform in growth and appearance.

Methods. The crocks were placed on a single bench in one of the Horticulture greenhouses of the University of Maryland. The position of a treatment in each of the replicates was chosen at random, leaving all of the interaction degrees of freedom unconfounded. As the plant is known to be susceptible to rather slight environmental differences,

several precautions were taken in this experiment to reduce place effect or other factors contributing to replicate variance. The greenhouse was shaded as uniformly as possible, a double layer of aster cloth was suspended over the bench, and in the first three weeks after transplanting cheesecloth shades were placed directly over the pots. In addition to these precautions, the position of the pots on the bench was systematically rotated, without disturbing the randomized distribution of the treatments within the replicates.

Table 29. Composition of stock solutions, dilution rate and final concentration of the nutrient elements in the feeding solution.

Element	Source	Stock concentration (g./l.)	Dilution rate (ml. stock in 3 l.)	Concentration of element (ppm.)
Nitrogen	NH_4NO_3	8.58	5	5
			20	20
			80	80
	$NaNO_3$	53.70	5	15
			20	60
			80	240
Phosphorous	$NaH_2PO_4 \cdot H_2O$	13.76	5	5
			20	20
			80	80
Potassium	KCl	28.65	5	25
			20	100
			80	400
Calcium	CaCl	110.99	10	133.4
Magnesium and Sulfur	$MgSO_4 \cdot 7H_2O$	246.49	6	48.6 and 64.1
Boron	H_3BO_3	2.290	7.5	1
Copper	$CuSO_4 \cdot 5H_2O$	0.786	7.5	0.5
Manganese	$MnCl_2 \cdot 4H_2O$	1.438	7.5	1
Zinc	$ZnSO_4 \cdot 7H_2O$	1.760	7.5	1
Iron	$Fe_2(C_4H_6O_6)_3 \cdot H_2O$	10.0	15	10

The arrangement of the pots on the bench and the shading employed are illustrated in figures 41 and 42.

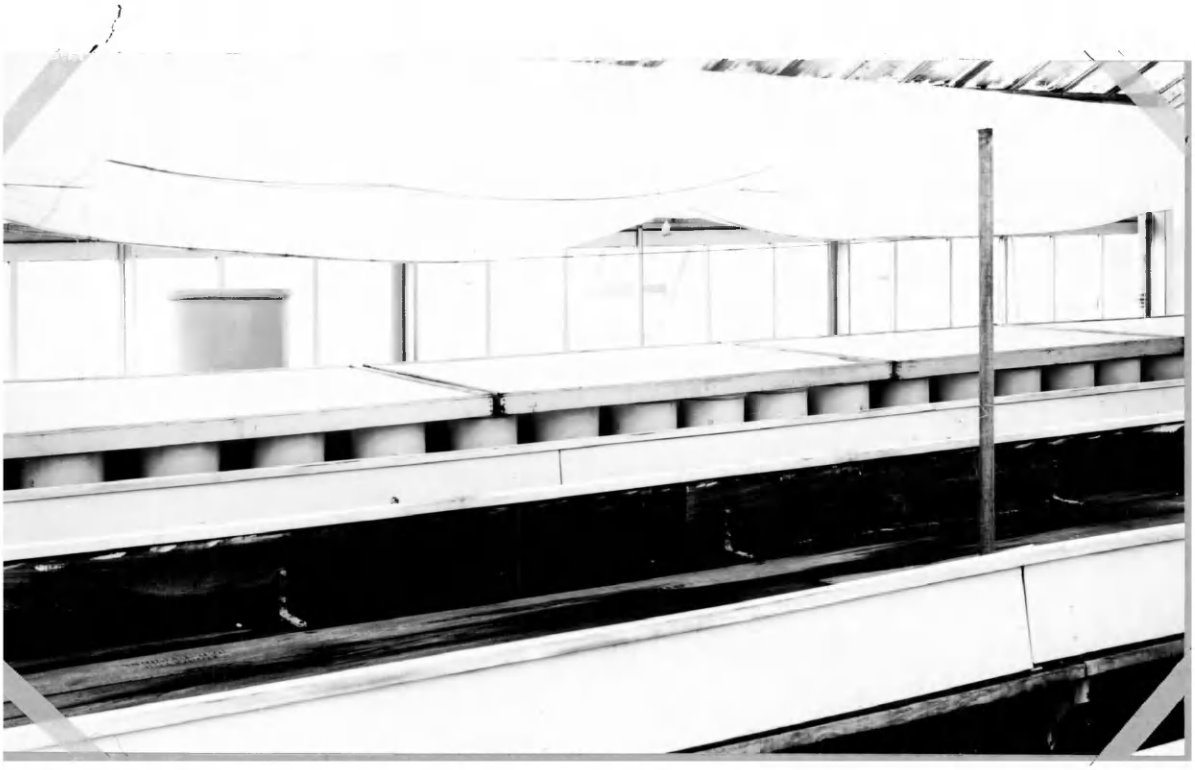

Figure 41. Crocks of the 3 x 3 x 3 factorial nutrition experiment, showing amount and arrangement of shading materials.

Figure 42. Arrangement of crocks on bench. Photographed May 25, 1943.

Considerable difficulty was encountered in maintaining the humidity of the greenhouse atmosphere as high as desired. Though the floor was kept wet and the cloth shading was frequently syringed, it was often necessary to add water to the crocks between nutrient applications to maintain sufficient moisture in the sand. The additional water was applied uniformly to all treatments, however, and though somewhat less total growth may have resulted than if this factor could have been more favorably controlled, it is not believed that this fact militates against the validity of the comparison of the effects of the treatments used, which was the principal objective of the experiment.

The solutions were applied to the pots for the first time on February 20, and the experiment was continued for five months from that date, at which time photographs and final measurements were taken. For the first three months, one liter of solution containing the desired concentration of nitrogen, phosphorous, potassium, calcium, magnesium and sulfur was applied to each of the pots at weekly intervals, and the iron and micro-elements solutions applied bi-weekly. During the final two months, when the plants were larger and the greenhouse temperatures higher, the interval between applications was reduced one-half.

Results

The first differences due to treatments, following the initial check in growth due to transplanting, were seen after three weeks, at which time it was apparent that the high-nitrogen plants were resuming growth more quickly than those of the other treatments. The medium-nitrogen plants became visibly active about a week later, but those receiving low nitrogen failed to become fully active, as evidenced by

leaf color, for the duration of the experiment.

Photographs (figures 43 through 45) taken on May 25, or about three months after the differential treatments were first applied, show the pronounced effect of the nitrogen level on the growth of the plants.

Figure 43. C. ledgeriana seedlings supplied LOW NITROGEN solutions.

Figure 44. C. ledgeriana seedlings supplied MEDIUM NITROGEN solutions.

Figure 45. C. ledmeriana seedlings supplied HIGH NITROGEN solutions. Photographed May 25, 1947.

An examination of figure 44, which shows the seedlings supplied the medium nitrogen levels, indicates that growth as a result of medium phosphorous applications (center row) was apparently greater than with either the high or low phosphorous levels (left and right rows, respectively). Finally, a marked reduction in growth is seen to have resulted at the high nitrogen applications when low phosphorous was supplied (figure 45, right row), and the reduction appears to be correlated with an increase in the potassium concentration (back to front). The quantitative data later obtained substantiates this observation. Compared with any other treatment, the survival in the high nitrogen, low potassium combination was by far the lowest.

At the discontinuation of the experiment on July 20, the plots were again photographed (figures 46 through 48), and the treatment differences noted in the previous photographs are seen to have become further intensified.

Figure 46. C. ledgeriana seedlings supplied LOW NITROGEN solutions. Photographed at the conclusion of the experiment, July 20, 1943.

Figure 47. C. ledgeriana seedlings supplied MEDIUM NITROGEN solutions.

Figure 48. C. ledgeriana seedlings supplied HIGH NITROGEN solutions

Individual heights of all the seedlings were taken to the nearest millimeter, the plants cut at the media line, dried at 70°C. and individually weighed. These measurements are summarized in table 30.

The variance analysis of the dry weight averages, according to the outline in Goulden (11), is given in table 31. Somewhat larger differences would have resulted from the use of pot totals instead of pot averages, as the former figure would also have given weight to differences in survival, but on the assumption that survival differences may have been due in part to factors other than those under consideration, the use of pot averages in the analysis is believed to reflect more accurately the true effects of the treatment variations.

Table 30. Summary of heights and dry weights of the three replicates of 12 plants each of C. ledgeriana in a 3 x 3 x 3 factorial experiment on the nitrogen, phosphorous and potassium nutrition of the young seedlings in sand cultures.

Nutrient combination			Average heights in mm. Replicate				Average weights in mg. Replicate			
N	P	K	A	B	C	Ave.	A	B	C	Ave.
L	L	L	17	24	20	20	37	52	41	43
L	L	M	15	19	16	17	28	41	39	36
L	L	H	23	18	19	20	63	43	40	49
L	M	L	22	17	28	22	63	49	65	59
L	M	M	21	16	20	19	43	52	51	49
L	M	H	20	17	21	20	55	48	65	56
L	H	L	20	20	19	20	56	69	54	60
L	H	M	22	17	20	20	59	48	58	55
L	H	H	26	19	28	24	69	57	97	75
M	L	L	21	28	24	24	58	102	81	79
M	L	M	20	35	28	28	48	109	95	84
M	L	H	22	25	21	23	59	70	52	61
M	M	L	41	38	39	39	169	149	198	172
M	M	M	49	47	51	49	217	224	222	221
M	M	H	47	66	42	52	191	236	181	217
M	H	L	41	31	40	37	171	133	200	167
M	H	M	39	21	44	38	158	122	229	166
M	H	H	38	37	29	35	157	172	118	149
H	L	L	30	28	28	29	102	92	91	95
H	L	M	16	27	23	22	34	75	45	50
H	L	H	17	17	21	18	39	23	54	39
H	M	L	42	38	47	42	159	101	164	152
H	M	M	42	58	41	47	157	225	163	193
H	M	H	49	53	57	53	191	238	265	232
H	H	L	43	47	39	43	187	166	155	170
H	H	M	55	35	45	45	254	108	189	181
H	H	H	65	53	59	59	274	238	291	267

Nitrogen (N): Low = 20 ppm., Medium = 80 ppm., High = 320 ppm.
Phosphorous (P): Low = 5 ppm., Medium = 20 ppm., High = 80 ppm.
Potassium (K): Low = 25 ppm., Medium = 100 ppm., High = 400 ppm.

Table 31. Summary of variance analysis of average dry weights per pot of plants supplied all combinations of three levels of nitrogen, phosphorous and potassium in a sand culture factorial experiment on the nutrition of C. ledgeriana seedlings.

Source	DF	SS	DF	SS	MS	F	1%	5%
Replicates	2	1,414	2	1,414	707	1.88		3.18
N_r	1	128,676	2	161,958	80,979	215.94	5.06	
N_d	1	33,282						
P_r	1	95,929	2	130,777	65,388	174.37	5.06	
P_d	1	34,848						
K_r	1	3,266	2	3,428	1,714	4.57	5.06	3.18
K_d	1	162						
$N_r \times P_r$	1	35,094						
$N_r \times P_d$	1	7,073	4	53,893	13,473	35.93	3.72	
$N_d \times P_r$	1	62						
$N_d \times P_d$	1	11,664						
$N_r \times K_r$	1	3,268						
$N_r \times K_d$	1	18,934	4	29,027	7,257	19.35	3.72	
$N_d \times K_r$	1	2,269						
$N_d \times K_d$	1	4,556						
$P_r \times K_r$	1	6,806						
$P_r \times K_d$	1	189	4	11,575	2,894	7.72	3.72	
$P_d \times K_r$	1	3,710						
$P_d \times K_d$	1	870						
N x P x K	1	15,357	8	15,357	1,919	5.12	2.88	
Error	52	19,861	52	19,861	375			
Total	80	427,290						

A study of the statistical analysis of the average dry weights per pot reveals several points of interest. The high significance of the variance from nearly all sources proves beyond doubt that the seedlings in this experiment have shown a marked response to variations in the nutrition supplied, and from the very small variance due to replicates it is concluded that the various precautions taken to eliminate error due to place effect were effective.

More than three-fourths of the total variance is seen to have been due to the nitrogen and phosphorous treatments and their interactions. This demonstrates that careful consideration should be given these two elements in the nutrition of the young seedlings. A comparison of their variance shows that the nitrogen levels have contributed somewhat more than have the phosphorous levels. The subdivision of the effect of nitrogen yields the information that $N_r : N_d = 80 : 20$, from which it is concluded that though a major portion of the variance was due to the tendency of the plants to increase in weight in proportion to the amount of nitrogen supplied (N_r), a considerable amount of the variance was also due to a deviation (N_d) from this proportionate response. Either the high nitrogen level or the low nitrogen level was therefore a limiting factor in the growth of the seedlings as compared with the growth resulting at the medium level. A study of the data in table 30 and the photographs (figures 43 through 48) shows that the low nitrogen level was definitely limiting, even to the point of rendering non-significant the effect of wide variations in the amounts of phosphorous and potassium supplied.

It is similarly evident from the subdivision of the variance due to phosphorous levels that a considerable portion is due to the deviation effect ($P_d : P = .27$), which again is interpreted as indicating

that the growth resulting from the medium level is disproportionate with that at the high and low levels. The data for the phosphorous levels, particularly in combination with a medium level of nitrogen, strongly suggest that the high phosphorous level (80 ppm.) resulted in a decided reduction in growth. Coincidentally, at the high nitrogen level, low phosphorous was again a definite limiting factor, the smallest plants in the entire experiment resulting from the low phosphorous, high nitrogen and high potassium combination. These data provide two conclusions as regards the phosphorous nutrition of C. ledgeriana seedlings, (1) this element is required in relatively small amounts by young seedlings, and may limit growth under certain conditions if available in concentrations as high as 80 ppm., and (2) though the relative requirement is low, it must be available in the proper proportion to nitrogen and potassium, since 5 ppm. of phosphorous in combination with high nitrogen and high potassium resulted in significantly less growth than the same phosphorous level in other treatments.

Though the total variance due to potassium levels exhibits only questionable significance (between the 1 and 5 percent points), it is of interest to note that 95 percent of this variance is due to the regression effect (K_r). Therefore, under the conditions of this experiment, though the young C. ledgeriana seedlings failed to respond with high significance to applications of potassium, the partitioning of the variance yields the information that the levels used produced proportionate effects, and that neither the high nor the low level limited growth when compared with the medium level. In contrast to the effects produced by nitrogen and phosphorous applications, the data for potassium indicate a relatively low requirement and a relatively high tolerance.

A similar variance analysis was calculated on the basis of the average heights per pot, and the summary is presented in table 32.

Table 32. Summary of variance analysis of average heights per pot of C. ledgeriana seedlings supplied all combinations of three levels of nitrogen, phosphorous and potassium in a factorial sand culture experiment.

Source	DF	SS	DF	SS	MS	F	1%	5%
Replicates	2	1	2	1	0.5			
N_r	1	5,222	2	5,890	2,945	101.55	5.06	
N_d	1	668						
P_r	1	2,400	2	3,906	1,953	67.34	5.06	
P_d	1	1,506						
K_r	1	110	2	118	59	2.03	5.06	3.18
K_d	1	8						
$N_r \times P_r$	1	1,272						
$N_r \times P_d$	1	385	4	2,083	521	17.96	3.72	
$N_d \times P_r$	1	16						
$N_d \times P_d$	1	410						
$N_r \times K_r$	1	57						
$N_r \times K_d$	1	*	4	194	49	1.68		2.56
$N_d \times K_r$	1	*						
$N_d \times K_d$	1	137						
$P_r \times K_r$	1	230						
$P_r \times K_d$	1	10	4	347	87	3.00	3.72	2.56
$P_d \times K_r$	1	98						
$P_d \times K_d$	1	9						
N x P x K	8	531	8	531	66	2.27	2.88	2.13
Error	52	1,497	52	1,497	29			
Total	80	14,567						

* Less than one.

In the analysis of the plant heights, the error variance constituted a larger portion of the total variance than with the height data, and the F values were correspondingly reduced. There was, however, a close correlation between the relative effects of the treatments on heights and weights. The nitrogen, phosphorous, and the nitrogen x phosphorous interaction variances are seen to be highly significant, while the value for potassium and for the nitrogen x potassium interaction are not significant.

Partitioning the variance into regression and deviation effects yielded the information that the deviation effect of nitrogen levels on height (N_d : N = .11) was not as great, while the deviation effect of the phosphorous levels (P_d : P = .39) was considerably larger than was true with the weight data. The proportion of the two components of the small potassium variance was the same in both cases. These calculations further substantiate the correlation between the effect of the treatments on both weight and height, though with phosphorous it appears that the high level (80 ppm.) was somewhat more effective in reducing height than it was in reducing weight, in comparison with the medium and low levels.

Part III. Factorial Experiment on the Calcium, Magnesium and Potassium Nutrition of young C. ledgeriana Seedlings

The effect on the growth of C. ledgeriana seedlings of all combinations of three concentrations of calcium, three of magnesium and two of potassium was also determined by the use of a factorial experiment.

Materials and Methods.

Crocks and sand. Two-gallon glazed earthenware coffee-liners were used as containers for the growing medium. The 18-mesh white quartz sand previously employed in sand culture studies was again used in this experiment. There were a total of 18 treatments with each treatment triplicated, making a total of 54 pots.

Solutions. Stock solutions of calcium chloride, magnesium sulfate and potassium sulfate were prepared, which when diluted to feeding strength delivered 50, 200 and 400 ppm. calcium, 15, 60 and 240 ppm. magnesium, and 100 and 400 ppm. potassium. A fourth stock solution was prepared containing ammonium nitrate, sodium nitrate, acid sodium phosphate and the micro-elements. When diluted to feeding strength this solution delivered 140 ppm. nitrogen, 20 ppm. phosphorous, 1 ppm. zinc and manganese, ½ ppm. copper and 2 ppm. boron. These elements were supplied equally to all treatments. A final stock solution was prepared with iron tartrate, which was diluted to supply 10 ppm. iron to all crocks.

Plants. C. ledgeriana seedlings were germinated on sphagnum in May. They were hardened well before transplanting by withholding nutrients, and 12 uniform seedlings were planted in each of the 54 crocks on

July 10. The crocks were shaded and watered with tap water for 10 days to allow them to begin the initiation of new roots before the differential treatments were applied.

Methods. The crocks were placed on a ground bed in the greenhouse on individual stands which held the crocks above the soil. The location of the treatments was selected at random within the three replicates. One liter of the nutrient solutions diluted with tap water was applied to each of the crocks twice weekly. Any additional watering required to maintain the sand in a moist condition was supplied as tap water.

The treatments were first applied on July 20 and the experiment was continued until September 20, when the treatments were photographed. Final measurements taken were height measurements and individual dry weights of the aerial portion of the seedlings.

Results

It soon became apparent that the plants in the high calcium crocks were being injured by the treatment. Many of the plants died, and the few surviving made very little growth, regardless of the other nutrients supplied. Plants in all of the other treatments grew rapidly and uniformly. The final photographs of all treatments are presented in figures 49 through 51.

The data on the height measurements of the plants in the various treatments are summarized in table 33. As in the case of the N, P and K factorial experiment, the average height of the plants in a pot is given. It is felt that this statistic affords a better comparison between treatments than does the use of total heights, which would give weight to differences in survival regardless of cause.

Figure 49. C. ledgeriana seedlings supplied LOW CALCIUM. Back row was supplied low potassium, front row high potassium. Pots on right received low magnesium, with medium and high magnesium treatments in center and on left, respectively. Note excellence and uniformity of growth.

Figure 50. C. ledgeriana seedlings supplied MEDIUM CALCIUM. Back row was supplied low potassium, front row high potassium. Pots on right received low magnesium, with medium and high magnesium treatments in center and on left, respectively.

Figure 51. C. ledgeriana seedlings supplied HIGH CALCIUM. Back row was supplied low potassium, front row high potassium. Pots on right received low magnesium. with medium and high magnesium treatments in center and on left, respectively. Note uniformly poor appearance of all plants.

Table 33. Average heights of plants per pot of C. ledgeriana seedlings supplied three levels of calcium, three of magnesium and two of potassium. (12 plants in each pot)

Treatment			Average height in millimeters of plants in replicate				Plants surviving
Ca	Mg	K	A	B	C	Ave.	
L	L	L	57	43	74	58	34
L	L	H	41	55	60	52	35
L	M	L	70	68	80	73	36
L	M	H	63	52	77	64	35
L	H	L	54	80	57	64	36
L	H	H	58	55	44	52	35
M	L	L	56	52	50	53	36
M	L	H	55	44	64	54	36
M	M	L	74	62	55	64	36
M	M	H	74	55	51	60	35
M	H	L	82	53	52	62	35
M	H	H	49	49	54	51	36
H	L	L	41	50	42	44	19
H	L	H	33	37	58	43	8
H	M	L	41	42	35	39	10
H	M	H	55	49	37	47	9
H	H	L	54	46	43	48	16
H	H	H	50	49	43	47	14

The summarized variance analysis of the height measurements of the C. ledgeriana seedlings is presented in table 34. As the plants that received the high calcium treatment were obviously not comparable with those in the medium and low calcium treatments, they were not included in the analysis.

Table 34. Analysis of variance summary of average heights per pot in millimeters of C. ledgeriana seedlings supplied two levels of calcium, three levels of magnesium and two levels of potassium.

Source	DF	SS	MS	F	5%
Replicates	2	193	97		
Calcium	1	90	90		
Magnesium	2	751	375	3.32	3.40
Potassium	1	393	393	3.48	4.26
Ca x Mg	2	50	25		
Ca x K	1	38	38		
Mg x K	2	132	66		
Error	24	2,721	113		
Total	35	4,368			

A study of the data in table 34 reveals that statistical significance was not shown by any of the treatments. The values for magnesium and potassium most nearly approach significance, but even these are below the values required for odds of 19 to 1.

The relatively low variance due to replicates indicates a lack of error due to place effect, while the relatively high error variance demonstrates the failure of the plants in the individual pots of each treatment to respond similarly. This is apparent from a study of the data in table 33.

The plants were cut at the media line for dry weight determinations, dried at 70°C., and individually weighed. A summary of these data is presented in table 35.

Table 35. Average dry weight per pot of C. ledgeriana seedlings supplied three levels of calcium, three of magnesium and two of potassium in sand cultures.

Treatment Ca	Mg	K	Average weight (milligrams) of plants in replicate A	B	C	Ave.	Plants surviving
L	L	L	228	141	171	180	34
L	L	H	210	198	112	173	35
L	M	L	182	174	184	180	36
L	M	H	161	211	187	186	35
L	H	L	197	223	233	218	36
L	H	H	217	200	215	211	35
M	L	L	100	131	94	108	36
M	L	H	118	110	182	127	36
M	M	L	147	151	189	162	36
M	M	H	156	174	174	168	35
M	H	L	243	158	205	202	35
M	H	H	202	112	129	148	36
H	L	L	27	42	57	42	19
H	L	H	27	12	40	26	8
H	M	L	41	75	27	48	10
H	M	H	66	34	27	42	9
H	H	L	40	45	42	42	16
H	H	H	36	48	31	38	14

The data in table 35 again demonstrates the significantly inferior survival and growth of all treatments with the high calcium level, regardless of the levels of the other two nutrients.

The data for the high calcium treatments were not included in the variance analysis of the dry weights, as the plants in these combinations were not homogeneous with those in the medium and low calcium treatments.

A summary of the variance analysis of the dry weight data is presented in table 36.

Table 36. Summary of variance analysis of dry weights per pot in milligrams of aerial portions of C. ledgeriana seedlings supplied two levels of calcium, three of magnesium and two of potassium in sand cultures.

Source	DF	SS	MS	F	1%	5%
Replicates	2	1,320	660			
Calcium	1	12,432	12,432	11.29	7.82	
Magnesium	2	12,141	6,071	5.51	5.61	3.40
Potassium	1	192	192			
Ca x Mg	2	1,984	992			
Ca x K	1	42	42			
Mg x K	2	3,090	1,545	1.40		3.40
Error	24	26,420	1,101			
Total	35	57,621				

The data in table 36 show that there was a highly significant variance due to the two levels of calcium. From the data in table 35 it is seen that the medium calcium level (200 ppm.) produced plants of less weight than those receiving low calcium (50 ppm.).

The variance due to magnesium levels was significant between the 1 and 5 percent points. Again referring to the data in table 35, it is seen that there was a consistent increase in yield as the concentration of magnesium in the nutrient solution was increased from 15 to 240 ppm.

No significance can be ascribed to the yield differences due to potassium levels. This corroborates the data from the factorial experiment on the nitrogen, phosphorous and potassium nutrition in which it was found that the young seedlings are unresponsive to a rather wide range of concentrations of potassium when grown in sand cultures.

Part IV. Nutrient Deficiency Symptoms of young C. ledgeriana Seedlings

An important contributing factor to the belief that C. ledgeriana is difficult to grow arises from the fact that considerable losses are incurred at the time of the first transplanting of the seedlings from the germination medium to the prepared nursery beds. Except when unusually severe insect or disease infestations occur somewhat later, or damping-off losses are particularly heavy in the seedling bed, this first transplanting loss is normally the point in the culture of the species at which the most severe losses are encountered.

One method of reducing this heavy loss of seedlings has been reported in the section on the effect of various media on seedling growth. Another, which under certain conditions may be the controlling factor, is the possibility of an incompatible nutritional condition in the transplant bed.

The effect of various levels of some of the more important nutrient elements on early seedling growth, as determined by sand culture studies, has been considered previously in this investigation. The present studies were inaugurated to determine the effect of a deficiency of some of the nutrient elements on the survival and the subsequent appearance of young transplanted seedlings. Also, in a supplemental experiment, the leaf symptoms exhibited by older seedlings well established in sand cultures and supplied nutrient-deficient solutions are briefly described.

Materials and Methods

Crocks and sand. One-gallon crocks of 18-mesh white quartz sand were used as the growing medium. The drainage hole in the bottom of the crock was covered with a piece of glass wool.

Solutions. The nutrient solutions were prepared with C. P. chemicals and distilled water. The basic composite solution was prepared to contain 140 ppm. nitrogen, 124 ppm. phosphorous, 156 ppm. potassium, 200 ppm. calcium, 98 ppm. magnesium and 64 ppm. sulfur, using calcium nitrate, magnesium sulfate and monopotassium phosphate. Chemicals omitted and substituted in the preparation of the nutrient-deficient solutions are shown in table 37.

A micro-element solution was prepared, which when diluted to feeding strength would deliver $\frac{1}{2}$ ppm. copper, 2 ppm. boron, and 1 ppm. of zinc and manganese. This was supplied equally to all treatments. Likewise, a stock solution of ferric tartrate was prepared, and 10 ppm. of iron was supplied to all treatments. The pH of the solutions was not adjusted, since it was determined to lie between 4.8 and 5.2 as prepared.

Plants. Seedlings of *C. ledgeriana* from seeds sown on sphagnum in May, 1942, were hardened by withholding nutrients before transplanting. Ten seedlings were used in each of the crocks, and all of the plants were taken from the same seed flat and were apparently uniform as to growth and appearance. They were about 3 cm. tall and had formed their second pair of true leaves when they were set in the crocks on August 17 and watered with distilled water for a few days.

Table 37. Chemicals omitted and substituted in the preparation of nutrient-deficient solutions for the determination of deficiency symptoms in young C. ledgeriana seedlings.

Deficiency desired	Chemicals omitted	Chemicals substituted
Nitrogen	Calcium nitrate	Calcium chloride
Phosphorous	Monopotassium phosphate	Potassium chloride
Potassium	Monopotassium phosphate	Acid sodium phosphate
Nitrogen and phosphorous	Calcium nitrate, monopotassium phosphate	Calcium chloride, potassium chloride
Nitrogen and potassium	Calcium nitrate, monopotassium phosphate	Calcium chloride, acid sodium phosphate
Phosphorous and potassium	Monopotassium phosphate	none
Calcium	Calcium nitrate	Sodium nitrate
Magnesium	Magnesium sulfate	Sodium sulfate
Calcium and magnesium	Calcium nitrate, magnesium sulfate	Sodium nitrate, sodium sulfate
Calcium and potassium	Calcium nitrate, monopotassium phosphate	Sodium nitrate, acid sodium phosphate
Magnesium and potassium	Magnesium sulfate, monopotassium phosphate	Sodium sulfate, acid sodium phosphate
Nitrogen, phosphorous and potassium	Calcium nitrate, monopotassium phosphate	Calcium chloride, acid sodium phosphate

Methods. Two crocks, or twenty plants, were used for each treatment. The crocks were placed on a table in the greenhouse, and the position occupied by any treatment or its replicate chosen at random. Twice each week all of the crocks were systematically rotated as to position, and turned half around to equalize exposure.

The prepared solutions were applied to the crocks twice each week, 250 ml. to each crock at each application. At two-weeks intervals during the course of the experiment the crocks were flushed through with a liter of distilled water, to leach out any accumulated chlorides and sulfates.

The solutions were first applied to the crocks on August 20, and the experiment continued for seven weeks, by which time the symptoms of most of the deficiencies were well defined, and complete collapse of some of the plants had occurred.

Results

Nitrogen. Within 10 days from the time the differential treatments were begun the effect of the -N solutions could be easily recognized. As previously stated, the plants had been hardened before transplanting, but the plants in the -N, -NP, -NK and -NPK treatments all appeared similar in that the leaves became deeply reddened, in comparison with the lighter coloration resulting from the hardening treatment. Particularly notable was the deeply reddened condition produced on the stems of the young transplants, starting at the media line and progressing upward until the entire stem was deeply colored. The leaf coloration appeared more intense on the upper leaves, particularly near the margins, but at the discontinuation of the experiment the entire leaf area was affected to an approximately equal extent.

All of the plants in the -N treatment survived the transplanting, and new growth was produced during the experiment, though the new leaves were significantly smaller and the internodes shorter than in the case of treatments where nitrogen was supplied. As the new leaves

unfolded, they were only slightly colored, but as they matured the red coloration became evenly distributed over the leaf. The -N plants are illustrated in figure 52.

Figure 52. Color photograph of C. ledgeriana transplants in sand culture supplied nutrient solution deficient in nitrogen.

Phosphorous. No symptom of phosphorous deficiency appeared in the plants receiving a nutrient solution deficient in that element during the period of the experiment. The plants grew equally as well as those receiving the composite solution, and showed no discoloration or other symptom indicating a lack of this element. This failure of a -P symptom to develop was noted also in the -PK plot. In the latter case the -K symptoms which developed were no more severe than in the treatment where potassium alone was deficient.

Potassium. The lack of potassium produced a pronounced effect by September 10, or after the treatments had been applied for three weeks. The leaves were pale in color and small in size, but no chlorotic or necrotic areas developed. The most striking symptoms were that the leaf tips curled downward and then toward the stem within 10 days after

the treatment was started. Within an additional 10 days the leaf margins, at first near the petioles and later along most of the length of the blade, also rolled downward. Concurrently with the margin rolling, the veins in the leaf became conspicuously sunken into the intervenal tissues. This effect was first evident on the leaves already formed on the plant, but as the new leaves were formed, the same sequence of events was observed. These plants are illustrated in color in figure 54.

Figure 53. Color photograph of C. ledgeriana transplants in sand culture supplied a nutrient solution deficient in phosphorous.

Figure 54. Color photograph of C. ledgeriana transplants in sand culture supplied a nutrient solution deficient in potassium.

A comparison of the effect of the deficiency of nitrogen, phosphorous, and potassium is afforded by figure 55. The appearance of the plants in the -P treatment is particularly striking.

Figure 55. C. ledgeriana transplants supplied nutrient solutions deficient in (left) nitrogen, (center) phosphorous and (right) potassium. Note excellence of growth in -P treatment.

Nitrogen and Phosphorous. The lack of both nitrogen and phosphorous in the nutrient solution resulted in plants that were indistinguishable from those supplied a solution deficient in nitrogen only. The only difference noted in the two treatments was that four of the 20 seedlings failed to survive in the -NP treatment, whereas all survived the -N treatment, but the experiment was not sufficiently extensive to ascribe significance to this survival difference.

Nitrogen and Potassium. Plants supplied a solution deficient in both nitrogen and potassium were also strikingly similar to the -N plants. In this case all of the plants survived, but growth apparently was inhibited to such an extent that the -K symptoms were not evident.

Phosphorous and Potassium. Where both phosphorous and potassium were omitted from the nutrient solution, the resulting plants were superior in growth and appearance to those in the -K plots, but inferior to that

in the -P plots. Here the -K symptoms of leaf curling and sunken veins were strikingly exhibited on the developing leaves, but the leaf color was equally as good as in the plants receiving a composite solution. That the -PK plants made significantly greater growth than the -K plants strongly suggests that the level of phosphorous in the composite solution may have been above the optimum for early seedling growth. These plants are illustrated in figure 56.

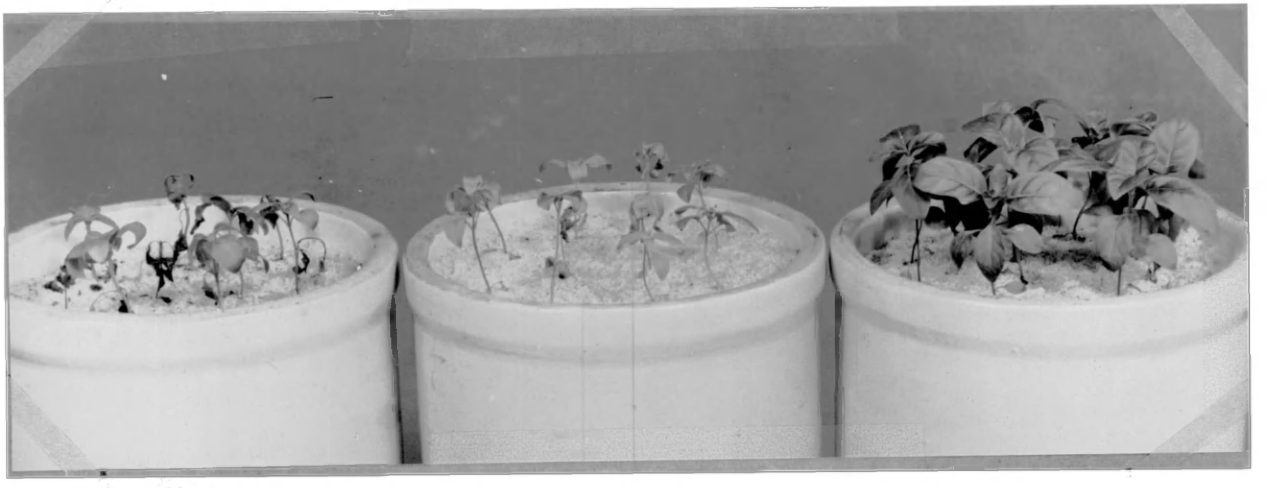

Figure 56. C. ledgeriana transplants supplied nutrient solutions deficient in (left) nitrogen and phosphorous, (center) nitrogen and potassium, and (right) phosphorous and potassium.

Calcium. C. ledgeriana transplants in a sand culture supplied a nutrient solution deficient in calcium failed to exhibit any striking symptom of the deficiency for the period of the experiment. The leaf color was notably paler than similar plants receiving a composite solution, particularly in the intervenal tissues, but the leaves compared favorably in size with those on the full-nutrient plants, and no chlorosis or necrotic areas developed. These plants are illustrated in color in figure 57.

Figure 57. Color photograph of C. ledgeriana transplants in sand culture supplied a nutrient solution deficient in calcium.

Magnesium. When magnesium was omitted from the nutrient solution supplied, the appearance of the growth of the plants was strikingly similar to plants in the -Ca treatment. Leaf color and size was so similar in both instances, in fact, that the plants could not be distinguished on the basis of appearance. Figure 58 is a color photograph of the -Mg plants.

Figure 58. Color photograph of C. ledgeriana transplants in sand culture supplied a nutrient solution deficient in magnesium.

Calcium and Magnesium. When calcium and magnesium were simultaneously omitted from the nutrient solution supplied, a pronounced effect was observed after only 10 days. Several of the plants failed to survive the transplanting. The leaf tips turned brown and died, then the entire leaves and finally the stems. The plants that survived the transplanting produced leaves that were chlorotic except on the veins. The older leaves collapsed and died, from the tip toward the base. Figure 59 is a color photograph of the -CaMg plants, and figure 60 affords a comparison of the plants supplied the -Ca, -Mg and -CaMg solutions.

Figure 59. Color photograph of C. ledgeriana transplants in sand culture supplied a solution deficient in both calcium and magnesium.

Figure 60. C. ledgeriana transplants supplied nutrient solutions deficient in (left) calcium, (center) magnesium and (right) both calcium and magnesium.

Calcium and Potassium. Transplants in sand supplied a solution deficient in both calcium and potassium exhibited symptoms similar to those described for the -CaMg plots. Several of the seedlings failed to survive the transplanting, those that survived differed from the -CaMg plants mainly in the appearance of the -K symptoms which were absent in the -CaMg plants. Lack of both calcium and potassium in the solution intensified the -K symptoms, as is illustrated in the color photograph (figure 61).

Figure 61. Color photograph of C. ledgeriana transplants in sand culture supplied a nutrient solution deficient in both calcium and potassium.

Magnesium and Potassium. When both magnesium and potassium were withheld from the solution the result on the seedlings was very similar to that produced by the -CaK treatment. The effect when both magnesium and potassium were omitted was similar in the intensification of the -K symptoms, but the effect on transplant survival was much less severe. The surviving plants produced leaves that were larger, with pale interveinal tissues as in the plants from which calcium and magnesium were individually withheld. These plants are illustrated in figure 62.

Figure 62. Color photograph of C. ledgeriana transplants in sand culture supplied a nutrient solution deficient in both magnesium and potassium.

Nitrogen, Phosphorous and Potassium. Plants in the -NPK pots exhibited the -N symptoms of reddening and the -K symptoms of leaf curling and sunken veins, but none of the plants showed any signs of collapse for the rather short duration of the experiment, and the depression of growth was not significantly greater than in the case of those plants supplied a solution deficient only in nitrogen.

A comparison of the plants produced in the -CaK, the -MgK, and the -NPK plots is afforded by figure 63.

Figure 63. C. ledgerima transplants supplied nutrient solutions deficient in (left) calcium and potassium, (center) magnesium and potassium, and (right) nitrogen, phosphorous and potassium.

Leaf symptoms of larger seedlings. C. ledgeriana seedlings, germinated in May on sphagnum and grown rapidly in sphagnum during the summer, were hardened and transplanted to sand in November. They were supplied a composite nutrient solution until the following June, when they were supplied only distilled water for three weeks to leach out as much of the nutrients as possible. The active, vigorously growing seedlings, averaging 35 cm. in height, were then supplied nutrient-deficient solutions to determine the expression of a lack of a given element on larger plants.

Solutions deficient in nitrogen, phosphorous, potassium, calcium, magnesium, and calcium and magnesium were prepared as described in the previous experiment. Solutions deficient in iron and in manganese were also prepared.

Only one pot of three plants was available for each of the deficiency treatments, so that the results are not considered conclusive. Most of the lower leaves were lost from these plants when the composite nutrient solution was replaced for three weeks by distilled water. The nutrient-deficient treatments were continued for three months, and the symptoms on the leaves produced during that period were described and photographed.

Plants growing in the nitrogen-deficient culture produced leaves that were narrower, shorter and thinner than those receiving the composite solution. The leaves were notably paler in color, but not until the shorter days and cooler temperatures of autumn did they assume the red coloration so characteristic in the small seedlings.

Phosphorous deficiency symptoms again failed to appear, though the leaves were somewhat darker green than those receiving the full nutrient, particularly in the area near the midrib. The difference in color quality was apparently more evident to the photographic film, however, as may be seen in figure 64. This photograph, taken on panchromatic film through a light green filter, resulted in a much lighter print tone with the -P leaf than in the case of the composite-solution leaf.

Symptoms of potassium deficiency were easily recognizable in the new leaves of the plants supplied the -K solution. In addition to the leaf curling and sunken veins as noted with the smaller seedlings, there appeared a mottled reddish coloration along the edges of the leaf blade, which was soon followed by a breakdown of the tissues, or marginal necrosis.

The application of -Ca and of -Mg solutions resulted in pale green leaves and occasional intervenal necrotic areas. No distinction could be made between the plants under the two treatments. When both elements were simultaneously withheld, a marked increase in the amount of necrotic tissue occurred.

Marked intervenal chlorosis soon appeared in the -Fe plants, and the contrasting green veins can be seen in figure 64. The experiment was discontinued before there was a significant effect on the growth rate.

The symptoms of a lack of manganese were not very clearly defined. The leaves were pale and thin, though of normal size, and the plants grew slowly, but no further symptoms were evident when the experiment was discontinued.

Figure 64. Leaf symptoms of larger seedlings. Representative leaves from C. ledgeriana seedlings in sand culture supplied a composite nutrient solution (1), and solutions deficient in (2) nitrogen, (3) phosphorous, (4) potassium, (5) calcium, (6) magnesium, (7) calcium and magnesium, (8) iron and (9) manganese.

DISCUSSION

The investigations reported herein furnish data which, though they must be interpreted and verified for local conditions in the field, establish certain fundamentals concerning the effect of various factors on germination, early seedling growth and nutrition of *C. ledgeriana*, and suggest methods of approach in obtaining further necessary knowledge of these and other growth stages.

Germination Studies

The results of the studies on the effectiveness of temperature and humidity controls in prolonging the viability of stored *C. ledgeriana* seeds largely corroborate those of Verbosch (14) which resulted in the recommendation of maintaining a humidity of about 20 percent (by the use of $CaCl_2 \cdot 6H_2O$) as optimum for this species under the natural temperatures prevailing. However, the results here reported show that the data presented by Verbosch are capable of only a strict interpretation because of the fortunately favorable temperatures existing under his conditions. The present data demonstrate that temperatures other than the one he used exert a pronounced influence on the viability of cinchona seeds. Of particular importance to the current emergency program is the discovery of the drastic effect of a storage temperature of 32°C. for even a relatively short period on the viability of the seeds, particularly at humidities other than those around 20 percent. This condition may be approached in many places in the Americas and because of the importance of the available seed supply should receive the utmost consideration. Of

similar importance would be the danger of reducing the storage temperature too low without some concurrent reduction in humidity, as is evidenced by the very low germination of 3 percent obtained in these studies after only 9 weeks when seeds were stored at 2°C. under high humidity.

The data presented show that when extremes of both temperature and humidity are avoided, C. ledgeriana seeds can be stored for at least 54 weeks without material reduction in germinability. That this is probably true also of C. succirubra and C. hybrid seeds is suggested by the fact that the trend of responses to storage conditions of fresh C. ledgeriana seeds was followed closely by seeds of the other species whose previous treatment and age were not known.

The studies pertaining to the light requirements of cinchona seeds herein reported not only corroborate the findings of Herbosch (14) that germination will not occur in total darkness, but also contribute much new information concerning the minimum quantity and intensity of light required for successful germination. Unawareness of this requirement for light may have been the cause of some of the failures in the early attempts to cultivate the plant, for it is obvious that covering the sown seeds with even a shallow layer of black volcanic sand, as was the case in some of the early attempts, would have effectively prevented sufficient light from penetrating to the seeds, particularly when the entire procedure was carried on under the shade of forest cover.

These studies establish the fact that the total light quantity required for germination is small, considerably smaller than that required for the production of stocky seedlings. This, too, may have

been a factor leading to the widespread belief that the plant is difficult to manage in its early stages. In situations where only borderline light intensities were supplied, the resulting weak seedlings would have been highly subject to early losses from damping-off fungi, as well as losses resulting from mechanical causes.

The rather elaborate germination beds developed in Java and since widely adopted as standard for cinchona germinations were so constructed that a fair quantity of diffused daylight could reach the seed sown on the surface of the germination medium. Data gathered in these studies indicate that so far as the light requirement of the seeds is concerned, germinations could be obtained in the presence of much greater light intensities. Bearing in mind the effect of increased light on the temperature and humidity of the environment and assuming that these factors could be satisfactorily controlled by ventilation, the possibility that a modification in the design of the germinator which would permit more light to reach the seedlings would result in less susceptibility to damping-off losses is considered worthy of future investigation.

The temperature requirements for the germination of C. ledgeriana seeds are apparently recorded for the first time as a result of the experiments reported in this investigation. It has been determined that there is a critical minimum temperature between 14° and 16.5°C. below which germination is completely inhibited, and a critical maximum lying between 29.5° and 32°C. above which a rapid deterioration of the seed is initiated. The optimum sustained temperature for germination was near 29.5°C., though as complete but not as prompt germinations were recorded at temperatures between 18.5° and 29.5°C.

It should perhaps be reiterated that the experiments conducted on the effect of temperature have not considered the effect of fluctuating temperatures. Apparently excellent germinations have been reported under Javanese conditions, where temperature is stated to vary, usually between 18° and 22°C.

Of particular importance to the present emergency program is the deleterious effect shown to result from short exposures to above-maximal temperatures. Serious reduction in germination resulted when the sown seeds were exposed to a temperature of 50°C. for only 2 hours, and exposures of over 24 hours to temperatures of 40°C. likewise reduced germination. Seed sowings out-of-doors must usually be covered to maintain adequate moisture conditions, and in some cases translucent materials such as cheesecloth or Cello-glass are being used to admit sufficient light. Unless the effect of relatively short exposures to high temperatures is considered and provided against, valuable seed may unnecessarily be lost.

Studies Concerning Early Seedling Growth

It seems clear that the usual rather slow rate of growth of the seedlings during the first few months after their germination can be considerably hastened under a combination of more favorable media, nutritional and environmental conditions. A greatly increased rate of early growth, besides shortening the time interval during which the plants are most susceptible to losses, would be desirable also from the standpoint of hastening the production of quinine.

It has been found that the use of sphagnum as a growing medium for actively growing seedlings germinated on sphagnum has resulted in several times the rate of early seedling growth obtained with similar

seedlings transplanted to a soil medium. When the seedlings were hardened by withholding nutrients prior to transplanting, however, early growth of sphagnum-germinated seedlings was equally as rapid in several soil and leaf mould media as in the sphagnum medium, regardless of the age of the seedlings at the time of transplanting. The possibility that early growth of seedlings transplanted from a jungle-mould germination medium to a soil nursery medium may be benefited in a tropical environment by a prior hardening of the seedlings by increasing light quantities is considered worthy of investigation. Of special value to the current program also is the marked reduction in transplanting losses when the seedlings are previously hardened.

In the production of large numbers of C. ledgeriana seedlings at the U. S. Plant Introduction Garden for later shipment to cooperators in the emergency program in the Americas, the use of a sphagnum and peat medium for early seedling growth has proven ideal, both from the standpoint of producing vigorous, disease-free seedlings, and from the enormous economy of the packaged weight of plants so grown, over soil-grown plants. Experiments herein recorded establish a fertilization program for optimum growth in this medium. Most rapid growth was obtained from the application in six bi-weekly portions of a 6-10-6 fertilizer in an amount equivalent to 1040 pounds per acre six inches. Data from sand culture studies reported strongly suggest that the proportion of phosphorous and potassium to nitrogen can profitably be considerably reduced over the ratio used in this series of experiments.

No study has yet been made of the relative effectiveness of

various nitrogen carriers. In all of the work herein reported, nitrogen has been supplied in the form of calcium nitrate, sodium nitrate or ammonium nitrate. However, in none of the formulae has more than one-fourth of the total nitrogen been supplied in the form of the ammonium salt.

The pH of the sphagnum and peat medium is 4.4, which sand culture experimentation has shown to be very favorable for active growth. Additions of lime to the medium exerted a pronounced inhibitory effect on growth of the seedlings. Whether this effect was due to a change in the pH of the solution of the medium, to the presence of above-optimal quantities of calcium, or to the effect of the lime application on the physical structure of the sphagnum has not been determined.

Failure of the plants in sphagnum to significantly respond to the application of a micro-element supplement suggests either that there is a relatively low requirement for these elements or that they naturally occur in sufficient quantities in this medium.

Nutritional Studies in Sand Cultures

The results of sand culture experiments reported in this investigation indicate that nitrogen is the especially important element in the nutrition of the young seedlings. Growth of seedlings in sand cultures supplied a solution containing 20 ppm. nitrogen was significantly inferior to growth when 80 ppm. or 320 ppm. nitrogen was made available. Though preliminary experiments indicate that the seedlings tolerate concentrations as high as 600 ppm. in sand culture solutions, most satisfactory growth resulted from the use of 140 to 200 ppm. concentration of nitrogen.

Results of greenhouse experiments on the phosphorous nutrition of young P. ledgeriana seedlings indicate that the requirement for this element is very small, though evidence of an inter-relationship between nitrogen and phosphorous requirements was obtained. With solutions containing 80 ppm. nitrogen, seedling growth was good with as low as 5 ppm. and as much as 80 ppm. phosphorous. With solutions containing 320 ppm. nitrogen, however, seedling growth was significantly inferior at 5 ppm. phosphorous, though there was no benefit from increasing the concentration above 20 ppm. It is inferred from these experiments that with a nitrogen concentration of 150 to 200 ppm., which is probably as great as can be practically maintained, phosphorous need not be supplied in excess of about 20 ppm. The possibility that growing media containing significantly greater quantities of available phosphorous are not suited for maximum growth of young seedlings should be subjected to field experimentation at an early date.

That the requirement for potassium was also relatively low was shown by the growth of seedlings in sand cultures supplied potassium in 25 ppm. concentration. Little benefit resulted from increasing the potassium supplied to 400 ppm., though at the same time no decrease in growth resulted from the higher concentrations. Thus the seedlings tolerate high concentrations, though a low concentration is apparently adequate for early growth.

Growth during the early seedling stage is reduced only slightly when either calcium or magnesium are omitted from the nutrient solution. The simultaneous deletion of both elements from the nutrient solution, however, results in an almost complete inhibition of growth. The failure to produce severe symptoms when only one of the elements

is lacking suggests that calcium and magnesium may substitute for each other in the metabolism of the young seedlings. Evidence from sand culture experiments further indicates that a concentration of magnesium between 100 and 240 ppm. in the nutrient solution results in very satisfactory growth, while concentrations of calcium in excess of 200 ppm. are to be avoided.

Symptoms of the deficiency of nutritional elements as evidenced by young seedlings grown in sand cultures are illustrated and described in this investigation. As the most serious losses are usually encountered as a result of the first transplanting from the seedling bed, this information should be of great value in determining whether such losses may be attributed to a deficiency in the nutrition supplied.

In general, the studies on the early growth stages of C. ledgeriana reported in this investigation show that the species is very responsive to environmental conditions. It is felt, however, that the plant is considered 'difficult to grow' only because knowledge of its requirements is so incomplete, and that horticultural practices can conceivably be developed by experimentation which will result in the successful culture of the form under a diversity of conditions now generally considered impossible. Such experimentation, in conjunction with the application of modern breeding techniques, may ultimately produce a form with a reasonably high alkaloid content in the young plant that can be subjected to large-scale cultivation at a great economy of space over the current plantation system. Certainly the successful completion of such a project in the Americas would result in complete independence of the Far East for the supply of a vital necessity, and go far in providing medicine with the available means of waging an effective campaign against malaria.

SUMMARY AND CONCLUSIONS

Cinchona ledgeriana seeds were stored under twenty combinations of temperature and humidity and the effect on germination determined at nine-weeks intervals for 54 weeks. It was found that both temperature and humidity were factors in determining seed viability. Storage at either extreme of the temperature or humidity range used resulted in reduced germination, the most rapid loss in viability occurring with a combination of high temperature and humidity. Conversely, excellent germinations were obtained even after 54 weeks of storage, with seeds stored at 33 and 66 percent humidity and temperatures of 16° and ±24°C. Storage in the dark proved slightly superior to storage in the light.

No pronounced effect of media on actual germination was found, but an important indirect effect on the survival of the seedlings resulted. Of the several media tested, a significantly higher seedling survival resulted from the use of sphagnum moss as a germination medium, principally because of the complete freedom from damping-off losses of the young seedlings.

Cinchona seeds require light for germination. It was found, however, that the minimum light requirement is relatively low. Exposures to 300 foot-candles of light for 10 seconds daily resulted in nearly complete germinations in 21 days, though the resultant seedlings had long hypocotyls, were etiolated, and the roots failed to penetrate the medium normally. Light was required throughout the germination period, exposures less frequently than every second day resulting in incomplete germination.

Critical minimum and maximum temperatures for germination of C. ledgeriana seeds were determined. Germination did not take place at sustained temperatures below 14°C. or at temperatures above 35°C. At below-minimum temperatures seed vitality was retained for weeks, whereas at above-maximal temperatures a rapid deterioration of the sown seeds resulted. Optimum sustained germination temperatures were determined to be between 24° and 29.5°C.

Early seedling growth of sphagnum-germinated seedlings was uniformly excellent in seven different media providing the seedlings were hardened before transplanting. When sphagnum-germinated seedlings were transplanted in an actively growing condition to soil, however, subsequent growth was far less than when similar seedlings were grown in the sphagnum medium.

The use of sphagnum as a growing medium required the development of a fertilization program. Of the several variables tested, it was found that most rapid growth resulted from the application in six bi-weekly portions of a total of 6-10-6 fertilizer equivalent to 1040 pounds per acre six inches. Applications of lime to the sphagnum medium markedly reduced growth. It was found unnecessary to supplement the fertilizer with a micro-element solution.

It was found that C. ledgeriana seedlings could be successfully grown in coarse quartz sand for nutritional studies by adjusting the solution to pH 4 to 6 and using a modification of a three salt nutrient with micro-element supplement.

Results of a 3 x 3 x 3 factorial experiment on the nitrogen, phosphorous and potassium nutrition of the young seedlings showed that they required a greater concentration than 15 ppm. nitrogen in the solution for active growth, and more growth was made at the highest

level used, 240 ppm., than at the medium level of 60 ppm. Five ppm. of phosphorous was too low only when in combination with high nitrogen and high potassium, and 80 ppm. phosphorous actually limited growth in combination with a medium nitrogen level. A low level of potassium, 25 ppm., was apparently sufficient for the young seedlings, no increase in growth resulting from concentrations up to 400 ppm. in the nutrient solution applied.

A second factorial experiment, on the calcium, magnesium and potassium nutrition of the young seedlings showed that 400 ppm. calcium from $CaCl_2$ resulted in the death of most of the seedlings, regardless of the levels of the other two elements, with 200 ppm. calcium resulting in more growth than 50 ppm. calcium. There was an increase in dry weight as the magnesium concentration was increased from 15 to 240 ppm. No significant response resulted from varying the level of potassium, corroborating the results of the NPK factorial experiment, and no marked interaction between levels of the nutrients was observed.

Deficiency symptoms of young seedlings supplied nutrient solutions deficient in nitrogen, phosphorous, potassium, nitrogen and phosphorous, nitrogen and potassium, phosphorous and potassium, magnesium and potassium, and nitrogen, phosphorous and potassium are illustrated and described. Leaf symptoms of plants several months old grown with solutions deficient in nitrogen, phosphorous, potassium, calcium, magnesium, calcium and magnesium, iron, and manganese are also illustrated.

It is believed that the application of the results of these studies will prove valuable in the current emergency program for reestablishing in the Americas a dependable source of natural quinine from the bark of the high-yielding ledgeriana species, by reducing to a minimum the loss of the valuable seed, and by furnishing experimental data on the environmental and nutritional requirements of the young seedlings. Further investigational work in the localities where the plants are to be grown is urgently needed to assure the success of the program.

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