CONTROLLED ENVIRONMENT PROPAGATION AND CULTURE OF *CASTILLEJA COCCINEA* (L.) SPRENG., *ANEMONE PATENS* VAR. *WOLFGANGIANA* (BESS.) KOCH, AND *CYPRIPEDIUM CALCEOLUS* VAR. *PARVIFLORUM* (SALISB.) FERN.

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By

Judith Anne Zastre

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ABSTRACT

Zastre, Judith Anne. M.Sc., The University of Manitoba, April, 1985. Controlled Environment Propagation and Culture of Castilleja coccinea (L.) Spreng., Anemone patens var. wolfgangiana (Bess.) Koch, and Cypripedium calceolus var. parviflorum (Salisbury.) Fern.

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Three native plants were propagated and cultured indoors for possible future commercial use as cutflowers or pot plants.

Seed germination experiments were conducted on Castilleja coccinea and Anemone patens var. wolfgangiana while Cypripedium calceolus var. parviflorum was propagated by plant division. The results on Castilleja seed indicated that the germination percent increased with increased light intensity. Germination was inhibited by red filtered light. Seed germinated readily at temperatures between 3.8 and 20°C but was inhibited at 2±2°C. The results indicated that light was not required for Anemone seed germination. The germination percent increased as temperature increased from 2.2 to 21°C. Extensive damping-off in vitro was controlled only by dry benomyl. Moist after-ripening of Anemone seed decreased the percent germination. Transplanting and dividing

Cypripedium stimulated approximately an 80 percent increase in plant numbers.

Culture studies were undertaken to determine the effects of various factors on the growth of the plants. Results indicated that the root parasitic Castilleja coccinea and C. coccinea forma lutescens can survive without host contact but different hosts appear to enhance plant growth. The results indicated that storage at temperatures at/or below 0°C for six to 16 weeks is essential for the termination of plant dormancy of Anemone. Cypripedium required storage at temperatures above 0°C for a minimum of 10 weeks for vernalization and termination of plant dormancy. Devernalization appeared to occur after 17 weeks of storage for Cypripedium.

Media type and amounts of N, P and K were important for Anemone plant survival and appearance. Combinations of several media components result in improved plant survival. Increased amounts of the nutrients N, P and K: N increased leaf numbers and dry shoot weights, P decreased dry shoot and root weights and K decreased leaf numbers and dry shoot weights.

On the basis of the research conducted, it is concluded that the three native plants can be propagated and cultured under controlled environmental conditions.

FOREWARD

This thesis has been written in paper style with three separate papers and one general literature review. The papers will be submitted to the Canadian Journal of Botany.

INTRODUCTION

Wildflower seed has been commercially available in the United States since the 1930's when the Atlantic Richfield Oil Company gave away packages of wildflower seed as an incentive to buy their gasoline and encouraged customers to scatter the seeds along highways to make travels more scenic (Tessene, 1980). However, according to Tessene (1980), only two species out of a total of approximately 150,000 species of flowering plants in North America are used extensively today in the seed and florist trade. These are the Tagetes species, the marigolds, and Zinnia elegans, the common zinnia which are both native to Mexico. No plants native to the United States or Canada have successfully gained a large share of the market.

Researchers (Salac et al., 1973, and Tessene, 1980) have set up criteria for selecting and evaluating wildflowers and bedding plants. Included in the list is that the plants must not be a noxious weed or poisonous, should display a showy vegetative or reproductive structure, be adaptable to varying conditions, and be easily recognized by the public.

For this study, three native Manitoba wildflowers: Catilleja coccinea (L.) Spreng., Cypripedium calceolus L. var. parviflorum (Salisb.) Fern, and Anemone patens L. var. wolfgangiana (Bess.) Koch, were selected from a group of plant species as being possible future assets to the florist

trade. The flowering plants C. coccinea, C. calceolus var. parviflorum, and A. patens var. wolfgangiana also known as Indian paintbrush, Small yellow lady's slipper, and Prairie crocus respectively, were once common sights on the prairie provinces. Today, due to cities and farms encroaching on native habitats, these plants are becoming rare. In addition, the ever present needs of florists to increase variety and sales of houseplants and cutflowers has contributed to the interest of conserving our native plants and possibly turning them into a commercial asset. The conditions for propagation and culture of these plants are discussed in this thesis.

LITERATURE REVIEW

Successful commercial production of cutflowers and pot plants involves the appropriate methods of propagation and culture for each plant species. The plants can be propagated sexually by seed germination or asexually by vegetative propagation. The growth of the plant and subsequent flowering is affected by several environmental and cultural factors.

The native plants C. coccinea, A. patens var. wolfgangiana and C. calceolus var. parviflorum have distinctly different flower sizes, shapes and numbers. The plant species also have different growth habits, life cycles and habitats.

Seed Propagation

Germination

Devlin (1969) defines germination "as the sequence of steps beginning with the uptake of water and leading to the rupture of the seed coat by the radicle or the shoot". In practice, germination is usually considered to have occurred upon observation of the radicle or shoot having protruded through the seed covering.

As outlined by Hartmann and Kester (1975), there are three stages of germination. The first stage involves the imbibition of water and activation of various RNA and DNA

molecules. Digestion of storage reserves and translocation of water and materials occurs in the second stage. In the third and final stage of germination, cell division occurs in the meristems of the embryonic axis. In order for the seed to undergo these changes, the appropriate conditions of water, temperature, gas mixture, and sometimes light must be supplied. If a viable seed fails to germinate under these conditions, it is said to be dormant (Devlin, 1969, and Hartmann and Kester, 1975).

Dormancy

The recognition of seed dormancy was first discussed fully by Crocker (1916). He classified dormancy into five different types. The first type of dormancy is the result of rudimentary embryos. The family Ranunculaceae, of which *Anemone* is a member, has small, few celled embryos (Atwater, 1978) requiring a post-harvest maturation period or after-ripening of dry seed or a low temperature moist chilling treatment prior to germination (Horovitz *et al.*, 1975, and Janick, 1972). Applications of the plant hormone gibberillic acid are unsuccessful in shortening the germination lag period of *Anemone* (Horovitz *et al.*, 1975).

The second dormancy type mentioned by Crocker (1916), was complete inhibition of water absorption due to a hard seed covering. A hard seed covering can be found in several plant families including the legumes (Devlin, 1969). The

water repellent hairy coats of the Anemone may also play a role in the long lag period for germination following exposure to water (Bullowa et al., 1975). Artificial or natural scarification may be required to terminate the dormancy.

Mechanical resistance to the expansion of the embryo by the seed coats is the third type of dormancy (Crocker, 1916). Small Xanthium pennsylvanicum Wally embryos are thought to be unable to produce enough pressure to rupture the testa surrounding the seed thus inhibiting germination (Esashi and Leopold, 1968).

The fourth dormancy mechanism listed by Crocker (1916) was the seed coats interfering with gas exchange and CO_2 elimination. The dormant embryos of Xanthium have shown rapid germination when placed under high oxygen conditions (Crocker, 1906). The seed coats of Xanthium permit free imbibition of water but inhibit or restrict uptake of oxygen (Crocker, 1906, and Devlin, 1969).

Dormant embryos are the fifth form of dormancy noted by Crocker (1916). Light, seed scarification and moist chilling or warm periods are used to overcome this type of dormancy (Hartmann and Kester, 1975).

Light Requirement

Light requirement for seed is a common complex cause of lack of germination (Devlin, 1969). One of the earliest in-depth studies on the effect of light on germination was con-

ducted by Flint and McAllister in 1935. They classified seed into the following: "1. seeds germinating equally well in light or darkness, 2. seeds whose germination is hindered by light, and 3. seeds whose germination is favoured by light." Those seed that respond to white light by germinating are known as positively photoblastic. If white light inhibits germination the seed is negatively photoblastic (Smith, 1975).

In 1934, Flint discovered that certain colors which make up white light are responsible for affecting germination. He discovered that red, orange, and yellow light promote germination while violet, blue, and green light inhibit germination. Flint and McAllister (1935) narrowed down the inhibition maximums to 4400 and 4800 A in the blue region and 7600 A in the near infra-red (IR) region. The promotion maximum was found to be near 6700 A in the red region (Flint and McAllister, 1937).

Borthwick et al. (1952) discovered that a reversible photoreaction existed between red and far-red (FR) light. They determined that the last treatment decided whether or not germination occurred. If near IR light was projected on the seed last, germination was low. If the seed was treated with red light last, the germination was near 100 percent. These results were confirmed by numerous researchers (Black and Wareing, 1954, Jones and Bailey, 1956, and Mohr and Appuhr, 1963).

The role of blue light in germination became an important issue after Borthwick et al. (1952) reported that blue light with peak activity at 4600 A was an effective promoter of germination. In 1954, Borthwick et al. stated that blue light can inhibit or promote germination depending on imbibition time and incident energy. Evenari et al. (1957) also found that blue light promoted and inhibited germination, similar to red or far red light. Toole et al. (1955b) found no response by Lepidium seed in the 4000 to 5260 A region. Wareing and Black (1958) found a blue/red reversible photoreaction present similar to the red/far red photoreaction although the energy required by the former reaction was thirty times greater to achieve 50 percent germination.

High Energy Reaction

Another type of light reaction was discovered in 1959 when Hendricks et al. noted that only under high energy irradiation between 6500 and 7000 A of greater than 0.1 joules, germination of Lactuca sativa 'Great Lakes' seed was inhibited. With low energy irradiances in the same region the germination was not inhibited. This became known as the high energy reaction (HER) or high irradiance response (HIR). The energy required for the HER is much greater than for the typical low energy response (Borthwick et al., 1969).

Photopigments

The pigment or pigments involved in the high and low energy photoreactions posed problems for researchers. The actual pigment responsible for the low energy germination response was unknown until the late 1950's although many of the properties had already been suggested. Butler et al. (1964) purified the spectrophotometerically active pigment phytochrome and obtained the two forms known as Pfr and Pr. Pr changes to Pfr upon exposure to red light while Pfr changes to Pr upon exposure to far-red light or in the dark (Butler et al., 1964a, and Mumford and Jenner, 1971). Butler et al., (1964b) discovered that blue and near ultra violet light will also transform phytochrome.

It is questionable today whether or not the HER is controlled by phytochrome. Contradictory reports on the correlation of phytochrome and the HER were made as soon as the reaction was noted. Mohr and Appuhr (1963) stated that phytochrome could not account for the high energy effects. Schneider and Stimson (1971 and 1972) reported that photosynthesis was involved by demonstrating that inhibitors of cyclic photophosphororylation also inhibited the HER response. These reports were discredited by later researchers who found no definite correlation between chlorophyll and the HER response (Mancinelli et al., 1974, Drumm et al., 1975, and Duke et al., 1976). Further research revealed that in fact the HIR processes were in competition for substrates

with the chloroplast development (Duke et al., 1976) and that an inverse linear relationship existed between an HIR of anthocyanin synthesis and decrease of chlorophyll content (Mancinelli et al., 1976). At present it is believed that the HER is mediated only through phytochrome (Drumm et al., 1975, and Mancinelli and Robino, 1975).

Low Energy Reaction Mechanisms

As mentioned above, the low energy phytochrome reaction is reversible. Borthwick et al. (1954), postulated that a monomolecular isomerization of the pigment P_{660nm} to P_{735nm} and vice versa, explained the changes during exposure to light. Kendrick and Spruit (1973) using very low temperatures proposed a scheme of phytochrome intermediates Pr, P_{650}, P_{690}, P_{698}, P_{710}, and Pfr due to conformational changes of the protein in the chromophore. Pfr is much more susceptible to breakdown than Pr (Butler et al., 1964). Continuous FR inhibits germination by moving the reaction of phytochrome Pfr ⟶ Pr (Kendrick et al., 1969).

The amount and type of phytochrome present in the dry seed is responsible for the promotion of germination (Mancinelli et al., 1967). Pfr was found to be the active part of phytochrome responsible for germination (Kendrick et al., 1969). Researchers found Pfr varies from 25 to 75 percent of the total phytochrome in the seed (Kendrick et al., 1969, Mancinelli et al., 1967, and Spruit and Mancinelli,

1969). After imbibition the total phytochrome increases but the increase is due to an increase in Pr only, the amount of Pfr remains the same (Spruit and Mancinelli, 1969).

The low energy reaction is also sensitive to temperature (Eisenstadt and Mancinelli, 1974, Gutterman and Evenarei, 1972, Kendrick et al., 1969, Mancinelli et al., 1967, Suzuki et al., 1980, and Toole et al., 1955). Toole et al. (1955a) reported that high constant temperature decreased germination but an alternating or single change in temperature allowed germination. Low temperatures slow down the first phase of germination but allow hydration or rehydration of phytochrome to occur. However, the low temperatures do not allow the synthesis of phytochrome necessary for the second phase of growth (Kendrick et al., 1969).

Jones and Bailey (1956) state that henbit seed is sensitive to the time of exposure to light after imbibition begins. Shorter or longer periods than the ideal result in reduced sensitivity to light. Other types of seed show different time course responses to light with increasing or decreasing photosensitivity as imbibition continues (Smith, 1975). Some seed, such as that of Lamium are sensitive to light intensities as low as one footcandle (Jones and Bailey, 1956).

High Energy Reaction Mechanisms

Hartmann (1966) stated that HER inhibited the second

phase of germination by destroying the Pfr present in the seed. Increasing the intensity of the light increased Pfr destruction and increased the HIR. The intensity and duration dependence of the HIR first explained by dependence on photosynthesis (Schneider and Stimson, 1972) was disproved by Mancinelli et al. (1974). Mancinelli and Rabino (1975) explain the irradiance dependence of the HIR as a requirement of a minimum level of the membrane bound Pfr-X'. The Pfr binds to the receptor sites, X, very quickly thus decreasing the concentrations of available X and soluble P. They also state that the membrane bound Pfr has a much higher rate of irreversible decay than does the unbound soluble Pfr. The duration dependence can also be explained by the requirement for a minimum Pfr level (Schneider and Stimson, 1972).

Castilleja Seed Germination

The seed of the Castilleja species are very small with each seed capsule containing hundreds of the tiny seed (Malcolm, 1962a). Heckard (1962) found that most seed of the Castilleja species maintained in cold storage for 3 1/2 to 4 1/2 years germinated rapidly upon sowing, although a few species from high altitudes failed to do so. The germination of Castilleja requires the presence of light. According to Malcolm (1962b) the seed will not germinate in the dark but the light requirement can be satisfied with very low

intensities of light. Malcolm (1962b) also notes that the seed can germinate at temperatures as low as 4°C. Although the germination rate of untreated seed is already high at approximately 70 percent, a cold treatment of four weeks at 4°C increases the rate and level of germination.

In contrast to many parasitic plants, the seed of Castilleja will germinate without stimulation by a host plant (Kuijt, 1969, and Malcolm, 1962a). Kuijt suggests that a chemical exudate from the roots of the host plants is required for germination in many parasitic plant seed. Melampyrum lineare, a common parasite, possesses a complex set of requirements for germination. The seed normally requires a moist cold treatment of some length following an initial warm storage in order to overcome separate radical and epicotyl dormancies. The requirement for an initial warm activation period can be overcome with exogenous applications of gibberellic acid, a growth stimulant or activator (Curtis and Cantlon, 1963).

Anemone Seed Germination

The germination of Anemone seed is not difficult to achieve. Little scientific research has been conducted on A. patens var. wolfgangiana, but other Anemone species have been tested. Olver (1981) in her article on Anemone patens suggests collecting a few ripe seed and drying them in a paper envelope prior to storage for the winter in the

refrigerator. Taylor and Hamblin (1976) suggest removing the 'plumey tails' or styles prior to sowing in the spring. A sterile, porous, slightly acid, fairly sandy soil mixture is recommended for seed germination. The seed and seedlings should receive good light and careful watering with good drainage allowed (Olver, 1981).

As mentioned previously, the Anemone has immature embryos resulting in delayed germination (Atwater, 1978, and Bullowa et al., 1975). Even under optimal conditions a critical number of days must elapse, six for A. coronaria, before embryo protrusion occurs (Bullowa et al., 1975). Removing the stylar end of the seed coat reduced the lag time by one or two days (Bullowa et al., 1975). Nichols (1934) found that refrigerating three species of Anemone seed tended to decrease the number of days required for germination and slightly increase the number of seed germinating. Bullowa et al. (1975) found that light inhibited germination of A. coronaria, slowing down the rate of germination and lowering the final percent germination. However, Mitchell (1926) found that A. virginiana required light for germination as no seed germinated in the dark.

Cypripedium Seed Germination

The germination of orchid seed requires special care. The North American terrestrial orchid seed, including Cypripedium, is among the most difficult to germinate. The ger-

mination percent can range from as low as 1 percent up to as high as 80 percent if no infestations occur (Arditti et al., 1982). In addition to poor germination of seed, the seedlings grow very slowly. Plants in the wild will not flower until they are 14 to 16 years old (Curtis, 1943).

The seed of the orchid contains almost no storage reserves. An external food source is required for germination. In nature this food source is supplied by the mycelium of a soil fungus, usually of the genus Rhizoctonia (Case, 1974). The tissue formed of both the fungus and orchid is known as mycorrhiza. As cited by Curtis (1943) Burges states that the soil fungi "digest insoluble carbohydrates and proteinaceous components of the outer cells of the substrate into soluble form". The Rhizoctonia digest the outer cells of the seed allowing the seed to absorb the water and nutrients directly from the mycelium (Case, 1974, and Muick, 1978). Chemical secretions from the seed prevent the fungus from penetrating farther than the first few cell layers (Vandersar, 1978). The Rhizoctonia supplies the orchids with the pyrimidine moiety of thiamine and niacin. The orchids produce para-amino benzoic acid which is required by the fungus (Hijner and Arditti, 1973).

Seed germination in the laboratory requires completely sterile conditions (Arditti et al., 1982, and Harvais, 1972). Following a short sterilization treatment the slightly immature seed is placed on to a Norstog, Curtis, or modified

Curtis media. Harvais (1972) developed his own medium and sterilization procedures in working with Cypripedium reginae. The apparent darkness requiring dormancy of C. reginae can in part be overcome by using a slightly acidic medium (Reyburn, 1978). Prior to development of roots and leaves, at about five or six months, a protocorm develops (Harvais, 1972). The orchid seed germination undergoes similar growth rates in the laboratory (Harvais, 1972) as noted in the field (Curtis, 1943), although unfavourable weather in the field can greatly retard growth.

Seed and Seedling Damping-off

Seed and seedling damping-off is a very common problem. The disease is caused by several types of fungi including Pythium, Rhizoctonia, Botrytis, and Phytophthora (Hartmann and Kester, 1975 and Janick, 1972). The disease can occur at various stages of plant growth. Pre-emergence damping-off occurs before the seed has emerged from the soil. Seedlings may suffer post-emergence damping-off and develop a stem rot near the soil surface. The third type of damping-off occurs when the seedlings are established. The wire-stem disease girdles the stems of the plants resulting in plant death. The fourth type of damping-off can strike plants at any stage. Root rot attacks the rootlets of the plants causing stunting and if not treated, eventual death (Hartmann and Kester, 1975).

Damping-off is controlled by eliminating the organisms during propagation and protecting the plants by regulating the environmental conditions (Hartmann and Kester, 1975, and Janick, 1972). Treating the soil and seed and using good sanitation practices reduces damping-off. The three basic methods of pre-planting soil treatments are: 1. heat treatment or pasteurization where the soil is steamed at temperatures from 60 to 80°C for about 30 minutes, 2. soil fumigation with chemicals such as Vapam (Sodium N-methyl dithiocarbamate dihydrate), formaldehyde, chloropicrin, or methyl bromide, and 3. fungicidal soil drenches with dexon (p-dimethylaminobenzene diazo sodium sulfonate), terraclor (pentachloronitrobenzene) or benomyl. The first two treatments may kill weed seed and nematodes as well as reducing damping-off. The chemical fumigation requires a waiting period of two to fourteen days before the medium can be used. When soil treatments are not used, it is desirable to use seed treatments. Disinfestants, of which calcium hypochlorite is an example, eliminate surface organisms on the seed. Disinfectants are used when the disease organisms are within the seed itself. Hot water, formaldehyde and aerated steam are the common methods used. The third type of seed treatment is to protect the seed by using a fungicidal soil drench or by coating the seed with an appropriate fungicide such as captan or benomyl (Hartmann and Kester, 1975). The fungicide can be applied to the seed dry or in a slurry (Strider, 1980).

Vegetative Propagation

Asexual or vegetative propagation involves the non-sexual reproduction of plants (Janick, 1972). This method is used when seed propagation is not practical or possible. True seed propagation is not possible when plants such as banana produce no or few viable seed (Janick, 1972). Seed is also not used when the plants are highly heterozygous and would result in unacceptable variable progeny (Hartmann and Kester, 1975). Plants such as many orchids (Hawkes, 1961) or English ivy have a long juvenile stage making seed propagation impractical (Hartmann and Kester, 1975). The minute size and susceptibility to infection of orchid seed makes seed germination almost impossible for all but the professional grower (Hawkes, 1961). The above reasons, along with others, have led to developing numerous methods of propagating the plants vegetatively.

Janick (1972) and Hartmann and Kester (1975) have grouped the types of vegetative propagation into five categories. The first and most common involves the induction of adventitious roots or shoots by cutting or layering the plants (Janick, 1972). Cutting involves the regeneration of a vegetative part after the piece has been removed from the parent plant. Layering, on the other hand, allows the regeneration to occur while the piece is still attached to the parent plant. A second common, but more complicated

method of propagation utilizes the joining of separate plant parts by tissue regeneration (Janick, 1972). This method involves the budding and grafting of plants onto other plants when the desired plant cannot be rooted satisfactorily, is not hardy for the area or is incompatible with a certain plant (Hartmann and Kester, 1975). A third method of propagation utilizes the production of apomictic seed (Janick, 1972). The seed of citrus and Kentucky blue grass is usually not produced by sexual means and is therefore a duplicate of the parent plant. Tissue culture is the fourth way of vegetatively propagating plants. A very small piece of a plant is removed and induced under sterile conditions on a special medium to regenerate an entire new plant (Janick, 1972).

The final method of vegetative propagation utilizes specialized vegetative structures. Examples of specialized structures are the runners of strawberries, bulbs of tulips, corms of gladious, tuberous roots of sweet potato, stem tubers of potato, the rhizomes of irises, lily-of-the-valley, and orchids and offshoots of day-lilies and orchids (Janick, 1972). The latter two types are of concern here. Hartmann and Kester (1972) define a rhizome as a "specialized stem structure in which the main axis of the plant grows horizontally at or just below the ground surface". Most plants having rhizomes are monocots (Hartmann and Kester, 1975). The two main types of rhizomes are listed by Hartmann and Kester as the pachymorph rhizome and the leptomorph rhizome.

The former, typified by the iris and ginger have thick, fleshy short stems with determinate growth. The latter type, the leptomorph rhizome, is found in Convallaria (Hartmann and Kester, 1975). This slender rhizome grows continuously from the terminal axis and branches during the spring and summer. During fall the rhizome forms a 'pip' or crown which survives underground in the dormant state until more favourable conditions occur. The rhizome grows vegetatively early in the growth period and flowers later in the same period (Hartmann and Kester, 1975).

Rhizomatous plants can usually be easily propagated by dividing the rhizome (Janick, 1972). Reynolds (1979) and Holman (1975) found that division of Cypripedium stimulates the growth of new stems. Division of the rhizome by cutting them into sections with at least one bud per section can be done at the beginning or near the end of the yearly growth period (Hartmann and Kester, 1975). Taylor and Hamblin, (1976) and Muick (1978) recommended that Cypripedium be divided in spring while Holman (1975) stated that they can be divided at any time in the season. Muick (1978) recommended that only two or three offshoots of Cypripedium be taken off yearly making sure that all divisions have plenty of roots.

Vegetative offshoots formed by orchids are known as keiki (Batchelor, 1982, Hartmann and Kester, 1975, and Hawkes, 1961). They develop from buds on the flower spikes (Batchelor, 1982), stems (Burke, 1982), or roots (Northern,

1982) of mature plants. Formation of keiki on the roots of Paphiopedilum, a close relative of the Cypripedium, is questionable. Most likely the Paphiopedilum is weakly rhizomatous (Whitson, 1980), producing the offshoots on short rhizomes rather than true roots. According to Batchelor (1982), keiki are usually formed when the plants are under stress although some plants such as Phalaenopsis luedmanniana tend to form numerous keiki under most conditions. A chemical mixture of a cytokinin in a lanolin paste known as Keiki Grow is produced at McMaster University. Applying the mixture to the flower nodes is reported to induce keiki instead of flowers (Brasch, 1982). Keiki often produce flowers within two to three years of being removed from the mother plant (Hawkes, 1961).

Vegetative and Reproductive Growth

Growing Media

The important characteristics that a good medium must have, as outlined by Hartmann and Kester (1975), are as follows: 1. firm, dense enough to hold cuttings in place, 2. volume fairly constant wet or dry, 3. good moisture holding capacity, 4. porous to provide good aeration, 5. free from weed seeds, nematodes, and other undesirable organisms, 6. low salinity, 7. able to withstand steam sterilization, and 8. adequate nutrients for seed germination. In the

greenhouse various combinations of soil, sand, peat, sphagnum moss, vermiculite, perlite, and compost are used to create a good growing media (Hartmann and Kester, 1975).

Soil is the most commonly used material in plant growing media. The soil is a mixture of inorganic and organic materials and gases (Hartmann and Kester, 1975). Organic soils contain a high percent of organic matter (Janick, 1972). The texture of the soil is determined by the proportion of each of the different sized particles of clay, silt and sand. The main texture classes range from sand and loamy sand to clay loam and clay (Hartmann and Kester, 1975). Each of the different classes have advantages and disadvantages for growing plants (Janick, 1972). The structure of the soil is determined by the arrangement of the particles into variously sized aggregates. For plant growth a granular or crumb-like structure is preferred (Hartmann and Kester, 1975).

Sand is composed of small grains of rock ranging in diameter from 0.05 to 2.0 mm (Hartmann and Kester, 1975). Sand as a soil textural class may also contain 5 to 20 percent silt and clay (Edmund et al., 1975). No mineral nutrients, no buffering capacity, and low fertility are the characteristics of sand (Edmund et al., 1975, and Hartmann and Kester, 1975).

Peat is made up of the remains of aquatic and bog vegetation. The various kinds of peat are divided into three

general types: 1. moss peat or peat moss, 2. reed sedge peat, and 3. peat humus. All three types vary greatly in moisture holding capacity and pH although most peats are very acid. Sphagnum moss is the dehydrated, not decomposed, residues of acid-bog plants and has a very high moisture holding capacity (Hartmann and Kester, 1975).

Vermiculite and perlite are often added to a soil mixture to improve the porosity and aeration. The former is a micaceous mineral with an excellent water holding capacity. Perlite is a silacaceous material that also has an excellent water holding capacity. Both retain water well and do not shrink when dry. Both are, or can be, sterilized readily (Hartmann and Kester, 1975).

Organic matter can be added to the soil by adding compost. Straw, sawdust, leaves, grass clippings and numerous other non-diseased materials are used to form a compost (Bienz, 1980). Sufficiently decomposed compost can be mixed in with the soil.

Other materials which can be used in developing a good growing medium, if readily available, are shredded bark, sawdust, and wood shavings. Their function is similar to peat moss in increasing the water holding capacity of the soil, although decomposition is slower (Hartmann and Kester, 1975). Osmunda fiber and bark chips are used together or separately as the main ingredients of the potting mix for orchids (Kramer, 1972).

Hartmann and Kester (1975) recommend two general types of soil mixtures for growing all plants. They recommend a 1 or 2 part sand: 1 part peat moss: 1 part loam for rooted cuttings and young seedlings. For general plant stock a mixture of 1 part sand: 1 part soil: 1 part peat moss is used. The University of California (U.C.) soil mixes contain no soil, only fine sand and peat moss plus lime and fertilizer. The John Innes soil mixes use 2 parts loam: 1 part peat moss: 1 part sand by volume for seeds. For normal potting, a mix of 7 parts loam: 3 parts peat moss: 2 parts sand plus fertilizer is used (Hartmann and Kester, 1975).

Plant Nutrition

The health and growth of plants depends among other things, on adequate nutrition. The fact that plants require certain elements in order to survive was established back in the early 1800's (Devlin, 1969). Today the study of plant nutrition is concerned with the types and amounts of essential elements and their role in the plant's life (Epstein, 1972).

The essential nutrients are artificially classified into macro and micro nutrients (Epstein, 1972). The known essential macro nutrients, required in relatively large quantities, are carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, sulphur, calcium, and magnesium. The known essential micro nutrients are manganese, iron, zinc, copper,

boron, molybdenum, and chlorine (Devlin, 1969, Epstein, 1972, and Roberts and Boothroyd, 1975). In addition some plants may require aluminum, sodium, selenium, gallium, cobalt, or silicon (Devlin, 1969, and Epstein, 1972).

The role of the macro elements and the deficiency symptoms if the element is unavailable in sufficient quantity, are very diverse (Epstein, 1972, and Roberts and Boothroyd, 1975). Without carbon, oxygen, or hydrogen, the building blocks of plants, no plant can survive. Nitrogen, an important constituent of chlorophyll, hormones, proteins, enzymes, vitamins, and amino acids. It causes very drastic symptoms in plants when deficient. Nitrogen deficient plants are usually light green or yellow in color. The plants may also display retarded etiolated growth. Phosphorous is important in the energy metabolism and nucleoproteins of plants. Deficiency symptoms range from blue-green to red or purple foliage with reduced growth. Potassium acts as a catalyst in photosynthesis and cambial activity. Yellowing of leaf margins with necrotic spotting are signals that the potassium level is low. Sulphur, important in the structure of amino acids, proteins and enzymes, causes the plant to become yellowed if deficient. The middle lamella of the cell walls require calcium. Without minimum levels of calcium, dieback of the meristematic regions, and curled and stunted foliage occurs. Magnesium, the final macro nutrient, is important in the structure of chlorophyll and also acts as a

catalyst in respiration. When magnesium is deficient the mature leaves show marginal or blotchy chlorosis.

A deficiency of one of the micro nutrients manganese, iron, zinc, copper, boron, molybdenum or chlorine usually results in localized chlorosis or necrosis. Zinc deficient plants, due to the role of zinc in several enzymes, show the failure of leaf and internode expansion. Decreased flower and seed production may be due to a boron deficiency as boron is involved in cell division, protein synthesis, and carbohydrate metabolism. The remaining micro nutrients are constituents or catalysts of enzymes or proteins (Epstein, 1972, and Roberts and Boothroyd, 1975).

Nutrient deficiencies can be prevented or resolved by the addition of appropriate fertilizers. General complete fertilizers usually contain nitrogen, phosphorus, potassium and sometimes sulphur. The concentration of the nutrients is expressed numerically such as 20-20-20 or 10-52-10 depending on the amounts of nitrogen, phosphorus, potassium present respectively. Specialized fertilizers are required to supply the plants with the other elements if found that they are deficient (Janick, 1972).

Nutrient excesses and unsuitable soil pH can also cause problems for the plant. Occasionally trace elements, or even macro nutrients, can accumulate in toxic amounts leading to severe chlorosis or necrosis of the plants. Excesses of one nutrient or incorrect pH can upset the mineral balance

causing the unavailability of one or more nutrients. Lime-induced iron chlorosis is caused by the unavailability of iron in calcareous soils. Alkaline soils can contain high amounts of soluble salts of magnesium, calcium, or sodium leading to severe problems in plant growth (Roberts and Boothroyd, 1975).

Vernalization

The term vernalization comes from the Russian word ioravization or javorization (Chouard, 1960, and McKinney, 1940) meaning 'god of the spring'. Lysenko, a Russian researcher, was the first person to name the phenomenon although many others realized it existed (Chouard, 1960). Chouard (1960) defined vernalization as "the acquisition or acceleration of the ability to flower by a chilling treatment". McKinney (1940) broadened the meaning of the term to "embrace practically all of the environmental factors and all the methods applied at any time in the plant's development and which are capable of accelerating sexual reproduction in any species of plant". Vernalization is not the overcoming of dormancy; this only removes an inhibition of growth and does not result in the formation of reproductive parts. Inducing dormant flower buds to flower is only overcoming the dormancy, not a case of vernalization (Chouard, 1960).

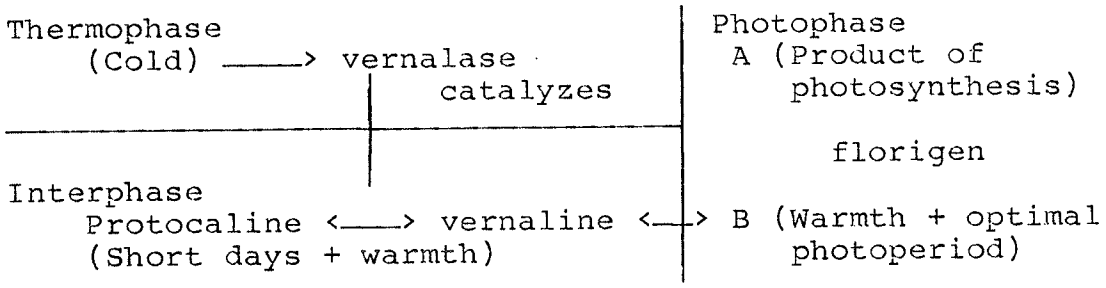
Vernalization is interrelated with photoperiodism (Lang, 1952). Whereas photoperiodism involves "florigen", "verna-

lin" is associated with vernalization. The former has distinct points of perception and response whereas in vernalization the sites need not be distinct. The vernalization changes must be completed before the florigen can be formed. Lang (1952) speculates that vernalin may be a precursor of florigen. Although there is no direct proof that either florigen or vernalin exist, the stimuli can be transmitted through grafts from a treated plant to a nontreated plant causing the nontreated plant to react as if treated (Lang, 1953, and Wellensiek, 1964).

The effectiveness of the vernalization treatments, usually chilling, depends on several factors. According to Devlin (1969) the plants ability to respond to vernalization is genetically controlled. The "ripeness-to-flower" depicts when the plant is sensitive to photoperiod. In plants that require vernalization, this is fulfilled when the necessary cold treatment is complete and the plant has formed a certain minimum number of leaves or nodes (Devlin, 1969). The presence of oxygen and the appropriate length and temperature of chilling are required before vernalization can be completed. Chouard (1960) suggested temperatures of +1 to +7°C are effective while temperatures below -6°C and above +14°C are ineffective. Devernalization can occur at temperatures above +17°C.

Several researchers have proposed theories to explain vernalization. Lang (1952) speculates that a precursor sub-

stance is supplied by the endosperm or synthesized by the embryo when the seed is subjected to cold temperatures. This precursor is transformed into a limiting substance by an oxidative process. The substance may then either go through another oxidative process in the presence of low temperature and become involved in floral initiation or can be lost during high temperatures. Purvis (1961) developed a scheme similar to the one above but also partially adopted a plan by Bakhuyzen. As cited by Purvis (1961), the three phase scheme is as follows:



(After Purvis, 1961)

The site of action of vernalization has been named by several researchers. Schwabe (1954) found that in Chrysanthemum the apical meristem must be subjected to cold in order that flowering can occur. In 1964 Wellensiek found that the common factor in vernalizable germinating seeds, apical meristems, and leaf and root cuttings was that all contained dividing cells. Thus any plant part which is undergoing active cell division may be vernalized.

Parasitic Plants

The fact that certain plants can parasitize other plants has been known for some time. The parasitic plants can be chlorophyllous or achlorophyllous and plants in the same genus can be of either type (Malcolm, 1962b). In order to be considered a parasite the following statement must hold:

> When a parasitic interaction is going on between two organisms or two populations, growth of the host decreases while growth of the parasite increases, and when the interaction is not going on, the host is unaffected while growth of the parasite decreases (Malcolm, 1964).

The production and location of the parasitic attachment is used for additional classification. Most attachments to the host plants are made through the roots (Malcolm, 1962b), although the agronomically destructive parasite Cuscata, as well as others, attach to the stems and leaves of hosts (Kuijt, 1969).

The mode of attachment of a parasite to the host is by means of a structure called an haustorium (Kuijt, 1969). Nutrients pass from one plant to the other through this specialized structure (Malcolm, 1962b). The two types of haustoria formed are the primary haustoria which are outgrowths of the apical meristem and secondary haustoria which arise as lateral organs (Kuijt, 1969). Malcolm (1962a) reports that although water, mineral nutrients, food materials, and phytohormones may pass through the haustoria from the host to the parasite, only the transport of water

and minerals has been proven.

In the family Scrophulariaceae several genera exhibit parasitism (Heckard, 1962). Included in this family are Melampyrum, Pedicularis, Striga, and Castilleja (Heckard, 1962, and Malcolm, 1962b). All are root parasites (Kiujt, 1969). Of these, Narrow leaved cow-wheat, Melampyrum lineare Desr. is an annual (Curtis and Cantlon, 1968), Common wood betony or lousewort, Pedicularis canadensis L., Swamp louse-coat, P. lanceolata L. (Piehl, 1963 and 1965), and Striga lutea Lour. (Stephens, 1912) are perennial while Indian paintbrush, Castilleja coccinea (L.) Spreng is biennial or annual in habit (Malcolm, 1962b).

Haustoria formed by plants of the family Scrophulariaceae are generally globular, varying from circular to broadly elliptic in shape (Piehl, 1963 and 1965). The haustoria consist of an outer portion of parenchyma with an inner area transversed either by a xylem vessel or tracheid (Piehl, 1963, and Stephens, 1912). Haustoria develop exogenously, unlike roots which originate endogenously (Stephens, 1912). In P. canadensis fine root hairs formed near the area of host contact may aid in attaching the parasitic root to the host (Piehl, 1963). In Castilleja the haustoria tend to surround the inner tissues of the host and push out the outer tissues (Kuijt, 1969). Stephens (1912) and Piehl (1963) suggested that chemical substances may be involved in the adhesion to the host and in the dissolving of

cell walls thus allowing access to the host vascular system. A plant can form haustoria not only on live host tissue but also on dead wood, pebbles and on to itself (Piehl, 1963). Also, P. canadensis can form haustoria when not attached to anything (Piehl, 1963). These haustoria often have suberized surfaces and may be due to former host contact or may act as water and food storage.

Root parasitic plants in the family Scrophulariaceae do not appear to be host specific. Piehl (1963) found that P. canadensis parasitized 80 species in Michigan. The plants attacked covered 35 families and three classes. M. lineare parasitized 12 species in Michigan (Piehl, 1962). Castilleja coccinea parasitized 17 hosts under greenhouse conditions, many of which are not native to the natural habitat of the paintbrush (Malcolm, 1962b).

The exact parasitic requirements of Castilleja are not known. It has been variously described as semiparasitic (Everett, 1981), "partially root parasitic, sometimes parasitic or more or less parasitic" (Heckard, 1962). Malcolm (1962, and 1964) states that C. coccinea cannot reach maturity unless successful host contact is made. On the other hand, Heckard (1962) found that the eleven Castilleja species tested completed their life cycle without a host.

The damage done to the hosts by the parasites is unclear. Heckard (1962) speculated that the effect of Castilleja on the host is negligible if water is abundant.

However, he noted that when the soil dries, the host plants wilted considerably while Castilleja showed no signs of wilting. Parasitized Lactuca canadensis had a much higher rate of die-off than did those L. canadenis free of parasites (Malcolm, 1962b).

Plant Description and Habitat

Castilleja coccinea

Castilleja coccinea is a member of tribe Euphasieae Benth in the family Scrophulariaceae (Kuijt, 1969, and Pennell, 1935). The family Orobanchaceae, the broomrapes, which contains several root parasites, are closely related to the Scrophulariaceae and are often difficult to distinguish (Kuijt, 1969). Thirty genera of the tribe Euphasieae occur in the cooler zone of the world, eight of which are in eastern temperate North America. All have zygomorphic flowers with the lower lip of the corolla shorter and smaller than the galea, the upper portion of the lip. The corolla is usually yellow or green (Pennell, 1935).

The members of the genus Castilleja Mutis are annual, biennial or perennial (Everett, 1981), although the perennials dominate in numbers (Kuijt, 1969). Approximately two hundred species make up the genus Castilleja (Bailey and Bailey, 1976, and Pennell, 1935). All the paintbrushes have a tubular corolla with the upper lip keeled and the calyx two

lobed (Bailey and Bailey, 1976 and Robinson and Fernald, 1908). The hairy slender stems grow to a height of 30 to 50 cm (Budd, 1979). The leaves of the rosette are usually entire with the alternate leaves on the stem deeply divided or lobed (Budd, 1979).

Pennell (1935) has divided the Castilleja into Anthrochroma, Callichroma, Perichroma, and Euchroma. The first two are perennial, have sepals that do not expand distally and have the thin and petalloid lower lip of the corolla 3 to 7 mm in length. The leaf blades are pinnately lobed and the stems are covered with long white hairs. Anthrochroma differs from Callichroma mainly in that the former has a much longer corolla which is yellow or purplish in color while the latter has a short green corolla. The third section, Perichroma is also a perennial but the lower lip of the corolla is thick and the leaf blades are entire. The fourth section Euchroma is an annual with the bracts and calyces usually red. Anthochroma contains four species which all produce colored corollas and are believed to be the most primitive existing species of Castilleja. They can be found growing in gravelly or sandy soil on the prairies. At least six species make up Callichroma. The bract color varies from yellow or green to pink, purple or red while the corolla is generally green. They can also be found on gravelly or rocky calcareous soil. The third section Perichroma is divided into three subsections: Lanatae, Septentrionales, and Litho-

spermoides which differ in terms of the structure of the leaf blades and the shape of the sepals. The habitats vary from wet cliffs to slopes and plains. The final section Euchroma, contains several species including C. coccinea which Pennell determined to be an annual. The bracts of the species in Euchroma are usually brightly colored in yellow or red. The plants grow in sandy bogs and glades and calcareous prairies.

The color of C. coccinea consists of the yellow, white or scarlet bracts and the yellowish green corolla (Pennell, 1935, and Scoggan, 1957). The yellow form is listed by Scoggan (1957) as C. coccinea forma lutescens Farw. Pennell (1935) prefers C. coccinea forma alba for describing both the yellow and white bracted forms rather than C. coccinea forma pallens Farw. for the white form and C. coccinea forma lutescens for the yellow form.

The habitat of C. coccinea varies from "moist sandy glades and bogs, subacid to slightly alkaline soil" (Pennell, 1935), low sandy ground (Robinson and Fernald, 1908), to the "thickets, meadows and prairie of south Manitoba" (Scoggan, 1957). The range of the species is from New Hampshire to southern Manitoba down to Florida and Oklahoma (Scoggan, 1957).

Anemone patens var. wolfgangiana

Anemone patens var. wolfgangiana is the floral emblem of Manitoba. This well known flower is a member of the Ranun-

culaceae or Buttercup family (Budd, 1979). Of the 120 to 140 species of Anemone, six are found in Manitoba (Dudley, 1930, and Everett, 1981). There are several common, as well as scientific names for this plant. The common names range from Prairie smoke (Everett, 1981), Prairie windflower (Dudley, 1930), Pasque flower, Crocus anemone to Prairie crocus (Wildeman and Steeves, 1982). The scientific names are even more diverse with controversy over the genus, species and varietal name. The earliest known name was Pulsatilla patens Mill. in 1768. For some unknown reason, in 1814 this plant was recognized as Clematis hirsutissima Pursh.. The name then changed through the following: A. nuttaliana D.C. 1818, P. nuttaliana Spreng. 1825, A. patens Hook 1830, P. patens Gray 1848, A. patens var. nuttaliana Gray 1867, A. patens var. hirsutissima Hitch 1891, P. hirsutissima Britton 1891, and finally to the accepted form today of A. patens var. wolfgangiana (Bess.) Koch (Dudley, 1930).

A. patens var. wolfgangiana is an herbaceous perennial as are all Anemone (Dudley, 1930). The large solitary flowers appearing in early spring measure approximately five cm across (Taylor and Hamblin, 1976). The flowers consist of petalloid sepals instead of petals (Dudley, 1930). When the buds first emerge they are enclosed in a grayish green pilose involucre (Dudley, 1930). The crocus name originates from the fact that at first when the flowers open they resemble the members of the genus Crocus. Later the flower stalk

elongates, changing the resemblance to the Crocus (Dudley, 1930, and Wildeman and Steeves, 1982). By anthesis the flower stalk averages a height of 25 cm above the ground (Wildeman and Steeves, 1982). Following pollination the sepals wither and drop off while the hairy styles elongate up to four cm in length (Dudley, 1930).

Unlike most prairie plants, the leaves of A. patens do not appear until after the flower has opened. The leaves initiated in the previous year emerge either at the bases of the flower stalks or from non-reproductive buds at the crown of the plant (Wildeman and Steeves, 1982). The mature leaves are ternately divided and dark green (Dudley, 1930).

Several researchers report hazardous qualities of A. patens. Dudley (1930) reported that the sap of the plant is poisonous due to a crystalline substance called anemonine. Anemonine vapors can temporarily inflame and close the eyes. Also, the extreme hairiness of the plant can cause problems if ingested. The digestive system may be disrupted by large balls of hair and can result in the death of sheep and possibly other animals (Budd, 1979, and Dudley, 1930).

The taprooted perennial is commonly found on the North American prairies in open fields, hillsides and beside railway tracks (Budd, 1979, Dudley, 1930, and Taylor and Hamlin, 1976). According to Budd (1979) they are very common on overgrazed pastures. Wildeman and Steeves (1982) note that the plants do very well on frequently mowed, grazed or burned

areas. Burning of the area in late summer or fall does not kill the plants as the dormant buds are close to or below the ground surface.

Cypripedium calceolus

C. calceolus is a member of the family Orchidaceae, subfamily Cypripedioideae. Other genera in the subfamily are Paphiopedilum, Phragmipedilum, and Selenipedium (Luer, 1975, and Teuscher, 1977). Selenipedium consists of only four species from the American tropics. Phragmipedium, also from tropical America, has eleven species. The 70 species of Paphiopedilum are all native to South East Asia and the East Indies. Cypripedium, the type genus, consists of 30 to 50 species from Europe, Asia, North America and several other countries (Bailey and Bailey, 1976, and Luer, 1975).

Orchid fanciers often grow plants known as Cypripedium, which are in fact Paphiopedilum or Phragmipedium (Bailey and Bailey, 1976, and Everett, 1981). The name Cypripedium comes from the Greek word 'kryris' meaning the goddess of love Aphrodite, and 'pedilon' a shoe (Luer, 1975). Paphiopedilum comes from the word 'Paphia' which is another name for Aphrodite. Therefore the names actually mean the same thing (Teuscher, 1977). Authors as recent as 1972 still refer to the Paphiopedilum as greenhouse Cypripedium (Kramer, 1972), even though the genera are quite distinct. Paphs, as they are often called, have a leafless scape, a unilocular ovary,

a perianth which is shed after pollination, and a basal rosette of leaves which are folded in the bud (Luer, 1975, and Teuscher, 1977). The true Cypripedium produces leafy stems (with one exception) carrying the inflorescence, leaves which are rolled up in the bud and a perianth which remains attached after withering (Luer, 1975, and Teuscher, 1977).

In North America, approximately 11 species of Cypripedium can be found growing wild (Everett, 1981, Luer, 1975, and Teuscher, 1977). Varieties and hybrids also exist (Luer, 1975, and Marshall et al., 1966). They range from C. montanum found only in the mountainous regions of North America, to the rare Ram's Head, C. arietinum, found only in moist coniferous forests (Budd, 1979). The species of interest here is C. calceolus L.. In Europe there is only C. calceolus (Teuscher, 1977) but in North America there are three varieties: C. calceolus var. pubescens, the Large yellow lady's slipper, C. calceolus var. parviflorum, the Small yellow lady's slipper and C. calceolus var. planipetalum the Flat-petalled lady's slipper (Luer, 1975). The plants form highly variable populations and all combinations of characteristics of the three varieties can be found in intergrading forms (Luer, 1975, and Niering and Olmstead, 1979).

The three varieties of C. calceolus have several similar characteristics. All are of terrestrial habit with the stems originating annually from short fibrous rhizomes. The small roots are covered with fine hairs (Luer, 1975). Also similar

is the colorful inflated yellow lip pouch striped or spotted with purple or red on the inside (Niering and Olmstead, 1979). An erect leaf-like bract is at the back of the base of the lip (Budd, 1979). The partially sheathing three to five leaves on the stem are ovate to elliptic-lanceolate.

According to Luer (1975) C. calceolus var. pubescens is the most common variety in North America. The pale yellow lip of 35 to 60 mm in length is the largest of the yellow lady's slippers. The sepals and petals are greenish brown or yellow in color and slightly twisted. The sepals range in length from 2.5 to 8 cm (Budd, 1979, and Luer, 1975). The vegetative plant parts are densely pubescent and can cause a rash in sensitive people (Luer, 1975). The plant height can vary from 45 to 80 cm (Luer, 1975, Taylor and Hamblin, 1976). The Large yellow lady's slipper is "less influenced by temperature, sunlight or water than the less common varieties" (Luer, 1975) and can therefore be found growing in habitats varying from forests to plains and even mountainous areas. The plants, however, are most commonly found near bogs and moist deciduous woods (Bailey and Bailey, 1976, Budd, 1979, and Luer, 1975).

The Small yellow lady's slipper, C. calceolus var. parviflorum which Luer (1975) states "is strikingly different from its common polymorphic relative, the Large yellow lady's slipper" has a deep yellow 20 to 50 mm long lip (Budd, 1979, Marshall et al., 1966, and Scoggan, 1975). In contrast with

the Large yellow, the Small yellow lady's slipper has reddish-brown to purple sepals and petals (Fuller, 1932 and Luer, 1975). The sepals are ovate lanceolate 20 to 70 mm long (Luer, 1975 and Marshall et al., 1966) and the lanceolate petals are 35 to 90 mm long (Fuller, 1932, and Marshall et al., 1966). Both are usually strongly spirally twisted several times (Bailey and Bailey, 1976, and Niering and Olmstead, 1979). Also, unlike C. calceolus var. pubescens, the C. calceolus var. parviflorum flower often has a strong fragrance (Bailey and Bailey, 1976, and Luer, 1975). The plant height varies from 17.2 to 70 cm (Marshall et al., 1966) and is less pubescent than the Large yellow lady's slipper (Luer, 1975). The natural habitat is moist woods, boreal forests, swamps, and bogs (Bailey and Bailey, 1976, Budd, 1976, and Luer, 1975).

The rare Flat-petalled lady's slipper, C. calceolus var. planipetalum is confined almost exclusively to southern Quebec and Newfoundland (Luer, 1975). The Flat-petalled lady's slipper grows in the open on treeless, limestone areas. Partially due to the harsh conditions, the plant stature is short and the flowers are small. The flat, relatively short petals and sepals are greenish yellow in color. The sparsely pubescent plant can reach a height of up to 20 cm. The yellow inflated lip is two to three cm in length, approximately the size of the small yellow lady's slipper.

MATERIALS AND METHODS

Preliminary Studies

This study was conducted to test the feasibility and methods of growing native plants indoors as pot plants or cut flowers. The plants used in the preliminary studies were: Anemone patens var. wolfgangiana (Bess) Koch, Echinacea purpurea (L.) Moench, Gentiana acaulis L., Gentiana andrewsii Griseb, Liatris spicata (L.) Wild., Petalostemon purpureum (Venten.) Rydb, Castilleja coccinea (L.) Spreng, Cypripedium reginae Walt, Cypripedium calceolus var. parviflorum (Salisb.) Fern, Campunula rotundifolia L., Viola adunca Sm., Viola cucullata Ait., Epilobium angustifolium L., Aquilegia canadensis L., Sisyrinchium angustifolium L., Lithospermum canescens (Michx.) Lehm, and Aster laevis L. The last nine species were collected as plants while the former plants were collected or received as seed, with the exception of Anemone, where both seed and plants were available. Species were eliminated on the basis of one or more of the following:

1. research already conducted on the plants,
2. unavailability of sufficient plants and/or seed, or
3. unsuitable plant or flower characteristics.

Anemone patens var. wolfgangiana, Castilleja coccinea, and Cypripedium calceolus var. parviflorum were selected on the basis of flower color and size, availability, and overall appearance.

General Methods

The plant and germination studies were conducted in Econaire Model GR-192(2) 192 square feet growth rooms illuminated by Gro-Lux WS and Cool White VHO (very high output) fluorescent lamps except as noted in individual experiments. The preliminary germination trials were conducted in several Econaire Model GC-15 15 square feet growth cabinets. The growth rooms were vented with electric ceiling fans running continuously. The fans were used to help regulate the temperature and air flow. The temperature was monitored and controlled continuously. The temperature in the growth room was 22°C for the 16 hour day and 16°C for the night. Plants were watered daily and fertilized alternately with 10-52-10 or 20-20-20 monthly. Pesticide applications of Insecticidal soap, Malathion and Diazinon, at the recommended rates, were used on the mature plants to reduce the aphid, mealy bug, and spider mite populations when numbers were sufficiently large as to cause possible damage to the plants. The systemic Diazinon was only used when the first two insecticides did not control the insects in the compact rosettes of Castilleja.

In the analysis of the results, transformations of the data were used to reduce the experimental variability. Square root transformations were used on data counts, arc sin transformations on data percentages and log transformations on the data measurements.

CHAPTER II

CONTROLLED ENVIRONMENT PROPAGATION AND CULTURE OF *CASTILLEJA COCCINEA* (L.) SPRENG

Introduction

The species *Castilleja coccinea* (L.) Spreng, the Indian paintbrush, is a member of the section Euchroma (Pennell, 1935) of the tribe Euphrasieae in the family Schrophulariaceae (Kuijt, 1969 and Pennell, 1935). Of the approximately 200 species of *Castilleja*, 10 are found in Canada (Budd, 1957). All are root parasitic (Kuijt, 1969). *C. coccinea*, the red or scarlet-bracted Indian paintbrush and *C. coccinea* forma *lutescens*, the yellow-bracted Indian paintbrush (Scoggan, 1957) were studied.

Germination of seed of parasitic plants often requires the stimulation of a host plant (Kuijt, 1969). Malcolm (1962a) reports *Castilleja* seed germinates without a host present and germinates at temperatures as low as 4°C. Malcolm (1962b) also reports that the light requirement for germination can be satisfied with very low intensities. Low light requirements controlled by phytochrome usually demonstrate germination inhibition in the blue (440 to 480 nm) and far-red (760 nm) regions and promotion in the red (670 nm) region (Flint and McAllister, 1935 and 1937). A reversible

photoreaction exists between the red and the far-red light (Borthwick et al., 1952). High energy light reactions (HER) demonstrate a dependence on light intensity (Hartmann, 1966). Increasing the intensity increases the destruction of far-red phytochrome and increases the HER. Duke et al. (1976) report an inverse linear relationship between the HER of anthocyanin synthesis and decrease of chlorophyll content which they report is due to competition for substrates between the HER and chloroplast development.

Castilleja parasitizes host plants through the roots. Malcolm (1962a) reports water, mineral nutrients, food materials and phytohormones may pass through the haustoria from the host to the parasite. Castilleja does not appear to be host specific but some hosts support better growth of the parasite (Malcolm 1962b).

Studies were initiated to explore the germination, light and host requirements of C. coccinea in a regulated indoor environment.

MATERIALS AND METHODS

Seed Collection and Storage

Ripe seed of C. coccinea was collected from plants growing in a roadside ditch at site 1 (Fig. 1) on August 25, 1981, and July 26, 1982. Seed was also collected from a field at site 2 on August 17, 1981, July 26, and August 11, 1982. The pods were dried for 4 to 5 days in a paper bag at 22°C ± 2°C. The pods were then opened and the seed removed with the aid of a dissecting needle and placed into Globe brand brown manila envelopes. The seed was stored dry at room temperatures of 25°C ± 5°C.

Seed Germination

Germination trials of C. coccinea were conducted at temperatures of 22° ± 2°C, 17/11°, 20/15°, 22/15°, and 22/18°C to determine the effect of temperature on the rate and percent germination. The latter four temperature regimes alternated between the first number for the 16 hour day and the second number for the 8 hour night. The seed was placed in Pyrex Petri dishes on three pieces of Whatman #1, 9 cm filter paper with 5 ml of distilled water for all experiments. The dishes were placed individually in plastic bags to reduce moisture loss. Half of the Petri dishes were covered with aluminum foil to exclude light and determine whether or not light was required. Each treatment consisted of six samples

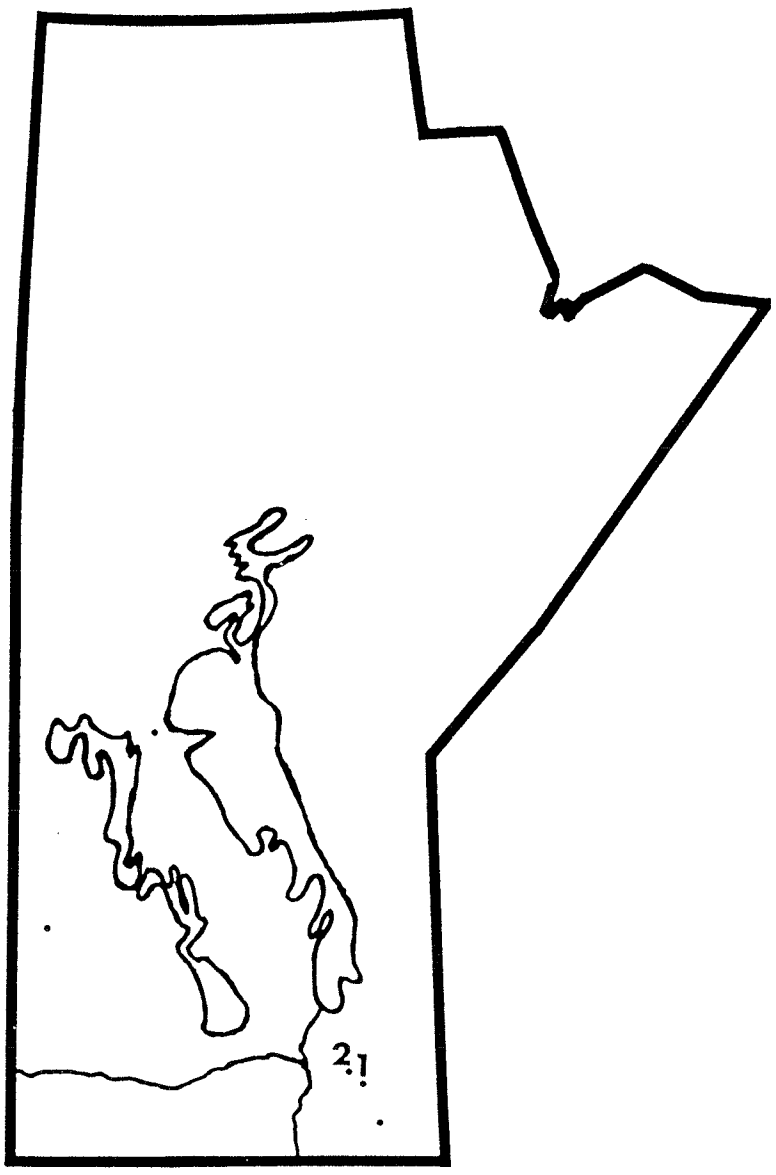
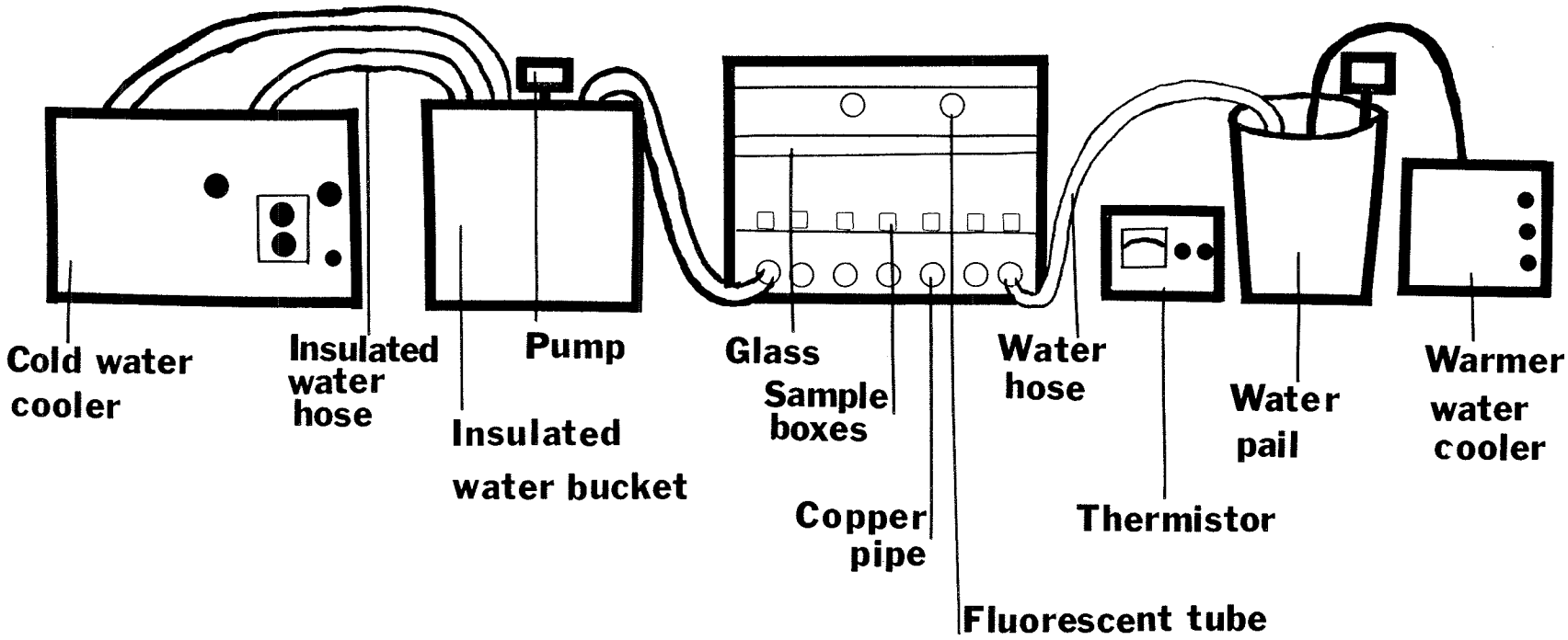


Fig. 1 . Out line map of Manitoba showing collection sites.

of which three were covered with foil. Fifteen and twenty seed were placed in each dish for the first trial and the second trial respectively. The samples were placed at random in the different regimes. The dish arrangement was changed each time the germination count was taken. The rate and percent germination was noted under low indirect light every one or two days.

A further temperature trial was conducted using a copper plate temperature gradient (Fig. 2). The seed were illuminated 24 hours a day by four warm white fluorescent tubes giving a light intensity of 47 ± 7 microE.m^{-2}.sec^{-1} measured with a Li-Cor quantum sensor sensitive in the 400 to 700 nm range or 9.1 ± 1.5 W.m^{-2} measured with a Li-Cor pyranometer sensitive in the 400 to 1100 nm range, measured on a Li-Cor model Li-185A Quantum/Radiometer/Photometer. The temperatures used on the gradient were 3.8°, 9.5°, 15°, 18°, and 20°C measured with an Atkins thermistor model 3L51J. The temperatures normally varied plus or minus 2°C except when the equipment malfunctioned for one day and the temperature dropped 5 to 8°C after four days into the experiment. The temperatures on the copper plate were regulated by two water baths. The cold water bath controlled the cooler part of the gradient while the warmer bath controlled the warmer part of the gradient. The temperatures in the middle of the gradient were influenced by both baths. Plastic rectangular covered sample containers were placed parallel to the copper pipes

FIGURE 2. Diagram of the experimental set-up used for thermogradient studies.


Cold water cooler
Insulated water hose
Pump
Insulated water bucket
Glass
Sample boxes
Copper pipe
Fluorescent tube
Water hose
Thermistor
Water pail
Warmer water cooler

running underneath the copper plate to the separate water baths. Five replicates of 20 seed per sample were used in the trials. One ml of distilled water was initially placed on the filter paper. Whatman #1 filter paper was cut to fit the boxes. Additional water was added as the paper dried. Due to time restrictions, temperatures of 15°, 18°, and 20°C only were used for the second trial. The first trial was up to 9 weeks with germination counts taken periodically. The second trial was up to 3 weeks in length. Germinated seed were removed when the germination counts were made.

Low Light Intensity Study

To determine if light can affect the seed germination of C. coccinea, experiments were conducted to determine the wavelengths responsible for promoting or inhibiting germination. The filters, light sources, and exposure times were as follows:

Light	Filter	Light source	Exposure times
red	Roscolux #27	4x15 W flourescent warm white tubes	5 or 60 mins.[1] 2x30 mins.[2], 1 day or 2 days[3]
blue	Roscolene #85	as above	as above
far red	2x#27 & 2x#85	2x60 W incandescent	as above
control	none	see note[4]	as above
green	3x Roscolux #90	1 8 W fluorescent cool white tube	not applicable

[1] Exposure times for the first trial.
[2] Seeds were given a 24 hour dark period between 30 min. exposures.
[3] Exposure times for the second trial.
[4] For the red and blue lights the control treatment used the fluorescent tubes but used the incandescent for the far red treatment.

The light transmitted by the filters, as indicated by the manufacturers, are given in Appendices 1a, 1b and 1c. The light intensity was 65 ± 10 micro $E.m^{-2}.sec^{-1}$ as measured by a Li-Cor model Li-185A Quantum/Radiometer/Photometer for the control treatment with the fluorescent lights. The Petri dishes were covered initially with aluminum foil to exclude light immediately after the seed were placed inside the dishes, and after exposure to the various light treatments. One week of germination time was allowed after the final treatment. The green light was used as a safe light when working with the seed. The seed were allowed to imbibe water either 8 or 24 hours prior to exposure to light in the first trial and 12 hours in the second trial. The experimental setup is given in Appendices 1B and 1C.

High Light Intensity Study

The inefficiency of low light intensities to promote germination led to studies using higher light intensities. The intensities of the light as measured by a Li-Cor model Li-185A Quantum/Radiometer/Photometer transmitted through the filters and the control treatment are as follows:

Treatment	Intensity
Control	295 ± 15 micro $E.m^{-2}.sec^{-1}$ 72.0 ± 5.0 $W.m^{-2}$
Blue Filter Roscolene #85	8 ± 1 micro $E.m^{-2}.sec^{-1}$ 9.2 ± 1.0 $W.m^{-2}$
Red Filter Rosculux #27	32 ± 2 micro $E.m^{-2}.sec^{-1}$ 17.0 ± 1.5 $W.m^{-2}$

After water was imbibed by the seed on filter paper in Petri dishes in the dark for 8-10 hours, the samples were exposed to 1, 2 or 3 day light treatments. For each one day period the seed received eight hours of dark at 18°C and 16 hours of light at 23°C. A similar experiment with continuous illumination was also set up in the header house but was unsuccessful due to the highly variable temperature conditions and therefore will not be included.

Three replicates of 20 seed each were used for each treatment. The experiment was repeated three times. The dishes were wrapped in heavy duty foil when not undergoing the light treatments. One week of germination time was allowed after the final treatment. The experimental setup was similar to that of the low intensity studies with the exception that six Sylvania very high output Gro-lux WS fluorescent tubes plus six 40 W cool white fluorescent tubes were used. During the second trial the light bank was inadvertently raised five inches higher than necessary thus lowering the expected light intensities by more than 20 $W.m^{-2}$.

Host Specificity

To determine the host specificity of the C. coccinea, an experiment involving several common plants was designed. C. coccinea was also grown separately to determine if survival was possible without hosts. The plants chosen for the study were:

Ageratum houstonianum Mill. 'Blue Danube',

Castilleja coccinea (L.) Spreng,

Castilleja coccinea forma lutescens Farw.

Chrysanthemum parthenium aureum (L.) Bernh. 'Golden Moss',

Senecio vira-vira Hieron. 'Silver Dust',

Tagetes patula L. 'Yellow Boy',

Viola cucullata Ait.

Three plants were grown from seed collected in the wild. The C. coccinea seed was collected from site 1 while the Viola seed was from site 3. The remaining plants were grown from commercially available seed. The C. coccinea were germinated initially in Petri dishes but seedlings that died off in the first two months of the experiment were replaced with seed germinated in a media of peat, sand, and soil (1:2:1 by volume). All plants were transplanted into the medium in four inch clay pots for the duration of the experiment. The design setup for the experiment was such that each plant was grown separately as a control, with the red form C. coccinea, and with the yellow form C. coccinea forma lutescens. A randomized complete block design with three blocks was utilized. Measurements of the height and width of host plants, and the width of the rosettes of the parasites were taken periodically. Flower number and height of the flowering parasites were noted. Final dry weight of the hosts and parasites was determined.

RESULTS AND DISCUSSION

Seed Germination Trials

The preliminary trials of Castilleja seed germination at various temperatures in the light and dark revealed highly significant differences (Table 1). Germination percent in the light was significantly higher than in the dark. There were no significant differences in germination percent among the temperature treatments in the light, except that there was significantly lower germination at 2 ± 2°C than all other treatments. In the dark, there was a significantly lower germination percent at 2±2°C than 22/15°C, the best germination treatment. Otherwise there were no differences among the temperature treatments in the dark. Also, there were no significant differences between the highest germination in the dark and the lowest germination in the light. There were no significant interactions between temperature and the light and dark. The seed will germinate in the dark and at temperatures just above freezing, but at a very low percentage compared to the more optimum conditions of light and temperatures in the range of 17 to 22°C for the day and 11 to 18°C for the eight hour night.

The rate of germination is indicated in Figure 3. The rate of germination is approximately the same for all regimes. All significantly follow a quadratic regression although the maxima for 17/11°C is lower than the others.

TABLE 1: The mean data of the effect of light and dark and various temperature regimes on the germination of Castilleja.

Temperature	Mean % Germination	
	Dark	Light
2 ± 2°C[1]	0[3] a[4]	31.50 c
17/11[2]	3.85 b	71.90 d
20/15	4.15 b	70.85 d
22/15	12.50 b	76.10 d
22/18	4.40 bc	76.10 d

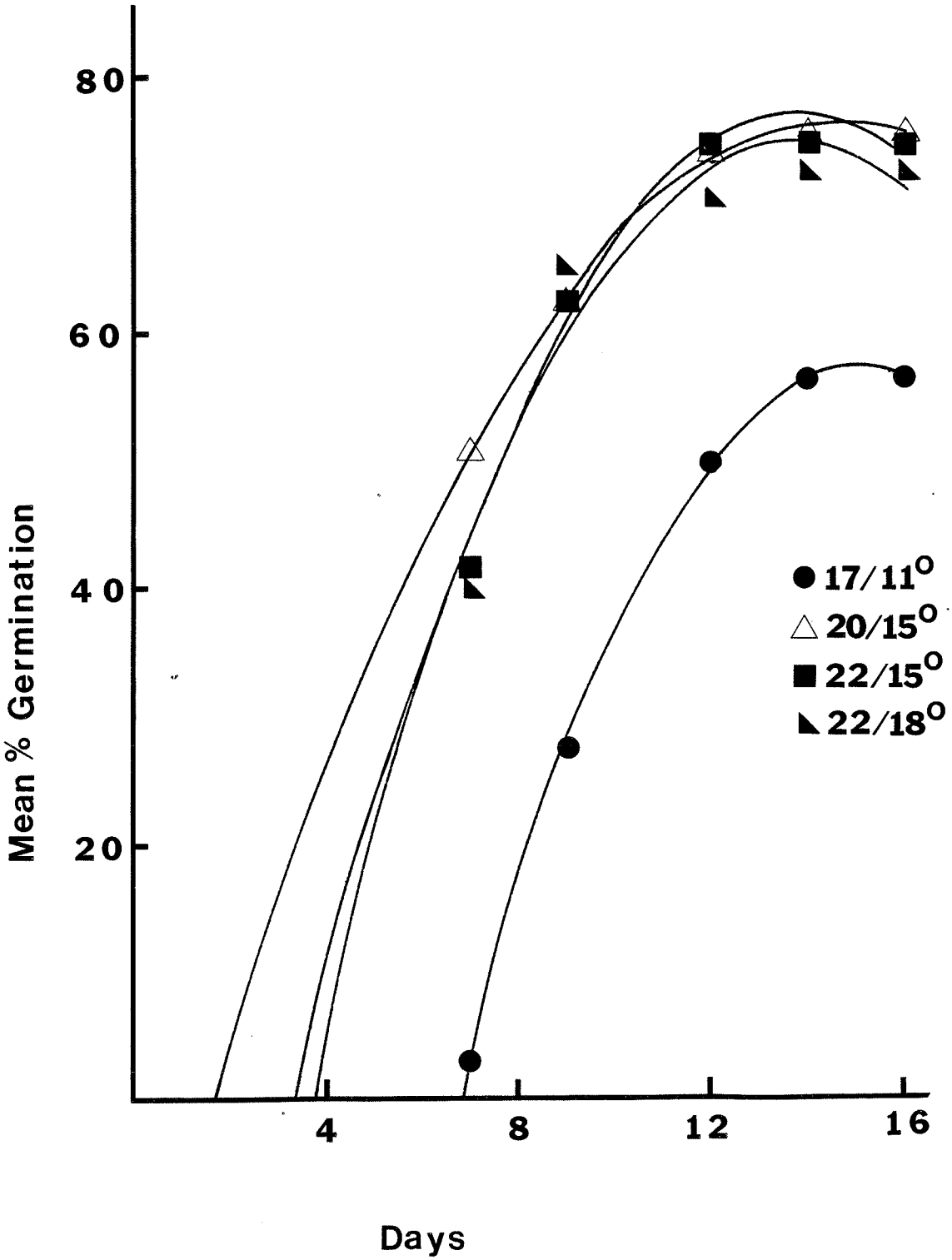
[1] Constant temperature.

[2] Alternating day/night temperature.

[3] Values are the mean % of two samples transformed by arc sin square root for analysis.

[4] Values followed by the same letter do not differ significantly by Duncan's Multiple Range Test, 99% level.

FIGURE 3. Rate of germination of C. coccinea seed under various temperature regimes.


80
60
40
20
Mean % Germination
4
8
12
16
Days
17/11°
20/15°
22/15°
22/18°

The data in Table 2 indicates no significant differences in germination percent among temperatures ranging from 3.8 to 20°C. However, the seed germinated more slowly at the cooler temperature. Two weeks into the experiment the germination was complete at 18 and 20°C. At 15°C the germination was complete after three weeks. Germination was only complete after six weeks for 9.5°C and eight weeks for 3.8°C.

Low Light Intensity Study

The light requirement of Castilleja was studied to determine the wavelengths and intensities of light required for germination. Under low light intensities (65 ± 10 micro $E.m^{-2}.sec^{-1}$) with exposure times of less than one hour, independent of filter, temperature or inbibition time, germination was approximately zero (Table 3). This indicates a requirement for longer exposure times and/or greater light intensities.

Therefore Castilleja does not exhibit the typical low energy phytochrome system found in most germinating seed where seed can be responsive to light as low as one foot candle (Jones and Bailey, 1956).

Increasing the exposure time under low light intensities from two 30 minute exposures 24 hours apart to 48 hours of exposure, significantly increased germination (Table 4). This indicates a dependence of duration of exposure to light, typical of the high energy reaction (Hartmann, 1966).

TABLE 2: The germination of C. coccinea seed on a thermogradient plate.

Temperature (°C)	Mean Germination	
	Trial 1	Trial 2
3.8 ± 2[1]	2.8[2] a[3]	-
9.5	6.6 a	-
15	5.6 a	4.4 a
18	5.6 a	6.4 a
20	7.2 a	5.4 a

[1] Daily temperature fluctuations of ±2°C except under one malfunction of -5 to 8°C temperature decrease.

[2] Values are means of five samples of 20 seed transformed by $\sqrt{x+.5}$ for analysis.

[3] Data followed by the same letter do not differ significantly by Duncan's Multiple Range Test, 95% level.

TABLE 3: Germination of Castilleja under low light intensities with various temperatures, filters, exposure and inbibition times.

Filters	Time of Inbibition	Response Time	Mean % Germination 13°[1]	23/18°[2]	25°
Red	8 hrs.	5 mins	0	0	0
		60 mins	0	1.67[3]	0
	24 hrs.	5 mins	0	0	0
		60 mins	0	0	0
Blue	8 hrs.	5 mins	0	0	0
		60 mins	0	0	0
	24 hrs.	5 mins	0	0	0
		60 mins	0	0	1.67
Far-Red	8 hrs.	5 mins	0	0	0
		60 mins	0	0	0
	24 hrs.	5 mins	0	0	0
		60 mins	0	0	1.67
Control	8 hrs.	5 mins	0	0	0
		60 mins	0	0	0
	24 hrs.	5 mins	0	0	0
		60 mins	0	0	0

[1] Constant temperature.

[2] Alternating day/night temperature.

[3] Values are the means of three samples of 20 seed.

TABLE 4: The effect of increased exposure time of low intensity light on the germination of Castilleja.

	Mean Germination		
Filter	Exposure Time 2x30 minutes	24 hours	48 hours
Red	0[1]	1.0	1.0
Blue	0.33	0	0.67
Far-Red	0	0	0.67
Control	0.33	0.44	1.56
Mean	0.22 a[2]	0.39 a	1.12 b

1 Values are the means of three samples of 20 seed transformed by $\sqrt{x+.5}$ for analysis.

2 Values followed by the same letter do not differ significantly as determined by Duncan's Multiple Range Test, 95% level.

Although the germination is higher under longer exposures, the total mean germination is much lower than achieved during the preliminary experiments (54.12 percent) under growth room lights.

High Light Intensity Study

When the light intensity was increased to 295 ± 15 micro $E.m^{-2}.sec^{-1}$ from 65 micro $E.m^{-2}.sec^{-1}$ for the control treatment, the overall mean increased from 3 percent to 50 percent. This increase indicates a dependence on light intensity for germination. This contradicts the statement by Malcolm (1962) that the light requirement for Castilleja is satisfied by low light intensities. The intensity dependence indicates a high light energy reaction (Hartmann, 1966 and Schneider and Stimson, 1972).

Filtering the light with red or blue filters significantly affected germination (Table 5). The red filter significantly reduced germination over the blue filter and control (no filter) indicating that the red light inhibits germination of C. coccinea. Jose and Vince-Prue (1977) found blue light inhibited hypocotyl growth in lettuce and radish, while red light both promoted and inhibited separate parts of the apex at certain stages of development, thereby nullifying the effect of red light. Borthwick et al. (1952) found blue light at an irradiance of 90 x 10^{-3} $joules.cm^{-2}$ could promote germination of 'Grand Rapids' lettuce.

TABLE 5: The germination of Castilleja seed under high light intensity with various filters.

Light Filters	Mean Germination			
	1 Day	2 Days	3 Days	Mean
Blue	6.67[1]	12.33	14.33	11.11 **[2]
Red	5.67	5.00	7.00	5.89 *
Control	10.67	13.00	16.00	13.22 **
Mean	7.67 a[3]	10.11 ab	12.44 b	

1 Values are the mean of nine samples of 20 seed transformed by $\sqrt{x+0.5}$ for analysis.

2 Values followed by the same symbol in the same column do not significantly differ by Duncan's Multiple Range Test, 99% level.

3 Values within the same row followed by the same letter do not differ significantly by Duncan's Multiple Test, 99% level.

Also, as under low light intensities, increasing exposure times significantly increased germination. The mean adjusted germination for the three day exposure time was significantly higher than the one day exposure time. Therefore the duration of exposure is important in both high and low light intensity experiments.

Host Specifity

The survival of C. coccinea and C. coccinea forma lutescens was poor. Less than one-half of either form was alive at the termination of the experiment (Figure 4). There were no significant differences in terms of survival of either parasite. There was no significant effect of the parasite on host survival up to one year (Table 6). This confirms the report by Heckard (1962) that the effect is negligible if water is abundant, as in this study.

There were no significant differences among the hosts for supporting growth of either of the Castilleja (Table 7). Both forms of C. coccinea survive as well growing without a host as with a host. The results support the findings by Heckard (1962) but disagree with Malcolm (1962 and 1964) who states Castilleja cannot reach maturity unless host contact is made. With larger sample sizes differences may appear among the hosts. Viola did not support the growth of C. coccinea or C. coccinea forma lutescens. Parasites grew relatively well with the other form of parasite but not well

FIGURE 4. The total survival of C. coccinea and C. coccinea forma lutescens over time.

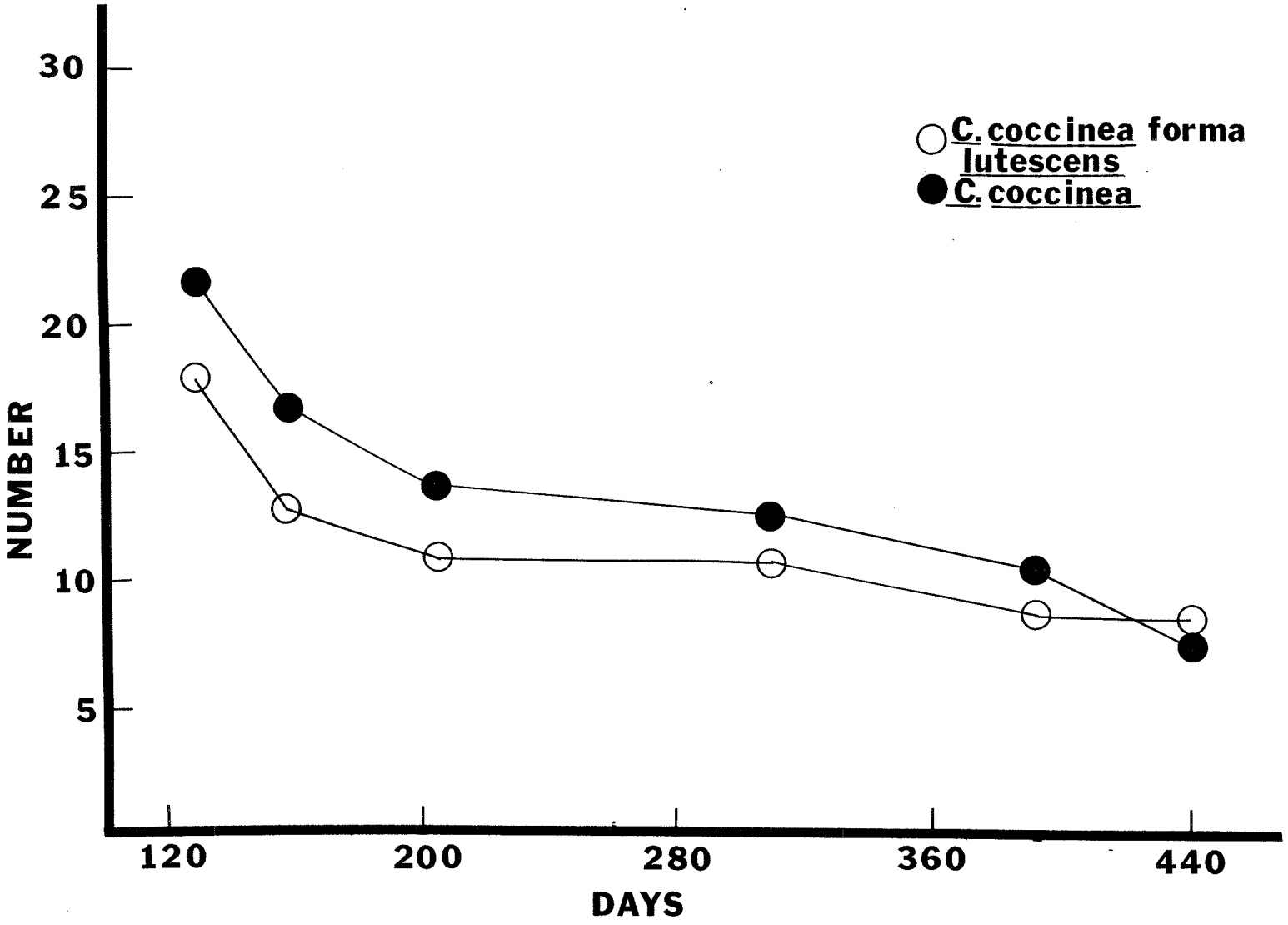

30
25
20
15
10
5
NUMBER
120
200
280
360
440
DAYS
C. coccinea forma lutescens
C. coccinea

TABLE 6: Effect of Castilleja on host survival.

Hosts	% Survival		
	Plants Grown With C. coccinea	Plants Grown With C.c. forma lutescens	Plants Grown Alone
Viola	33.3	66.6	100
Chrysanthemum	100	66.6	100
Tagetes	0	33.3	100
Senecio	100	100	100
Ageratum	0	0	33.3
Castilleja[1]	33.3	50	66.6
Castilleja[2]	66.6	33.3	33.3

[1] C. coccinea

[2] C.c. forma lutescens

TABLE 7: Effect of hosts on *Castilleja* survival.

Hosts	% Survival	
	C. coccinea	*C. coccinea* forma *lutescens*
Ageratum	0.0	33.3
Castilleja[1]	33.3	16.7
Castilleja[2]	50.0	66.7
Control[3]	33.3	33.3
Chrysanthemum	100	33.3
Senecio	0.0	66.7
Tagetes	66.7	0.0
Viola	0.0	0.0

[1] Grown with same form of *Castilleja*.

[2] Grown with other form of *Castilleja*.

[3] Grown alone.

with the same form. In combination with C. coccinea forma lutescens, two C. coccinea flowered even though the former had died approximately 245 days before. The only C. coccinea forma lutescens to flower was in combination with an Ageratum which had died 150 days previous. Two C. coccinea also flowered with Chrysanthemum and one with Tagetes. The height of the flowering plants varied from 11.9 cm to 49.5 cm. Flower number ranged from three to 50.

Several other Castilleja were alive at the termination of the experiment and could possibly have flowered if the experiment had continued. All but three of the remaining 17 plants were larger than four centimeters in rosette diameter, a mature plant according to Malcolm (1962b). Plants in the experiment flowered when as small as 3.3 cm in rossette diameter.

The effects of various hosts on the dry weights of Castilleja are presented in Figures 5 and 6. Analysis of variance and subsequent T tests revealed significant differences among the dry weights of C. coccinea when hosted by Viola and C. coccinea versus Chrysanthemum or C. coccinea forma lutescens. There were no significant differences in the effect of the hosts on C. coccinea forma lutescens but trends were apparent. For both forms of Castilleja the weights of the control (grown alone) were not the lowest. This again signifies that both forms of C. coccinea can grow without a host, thus contradicting Malcolm (1962 and 1964).

FIGURE 5. The effect of various hosts on the dry weight of *C. coccinea*.

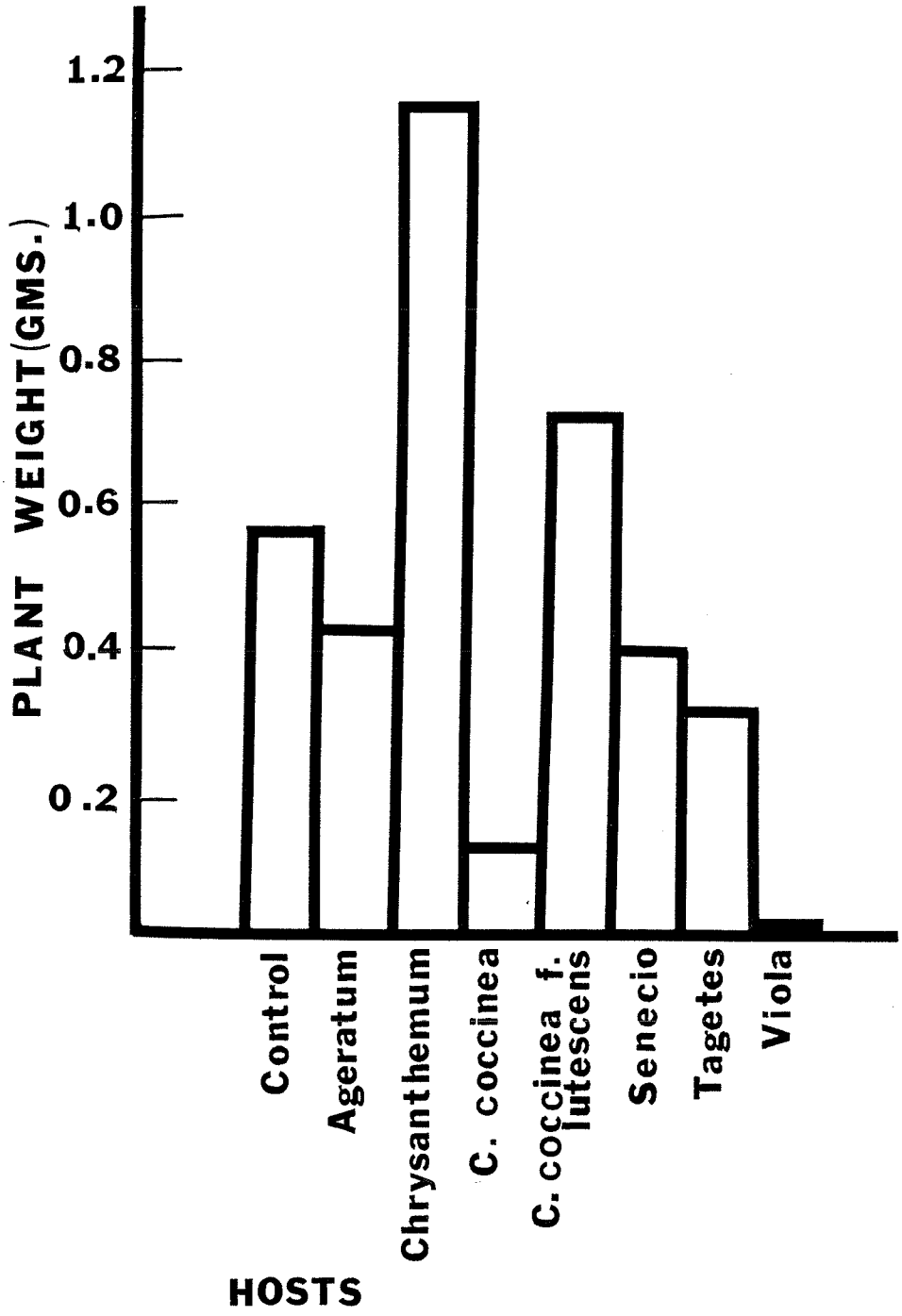
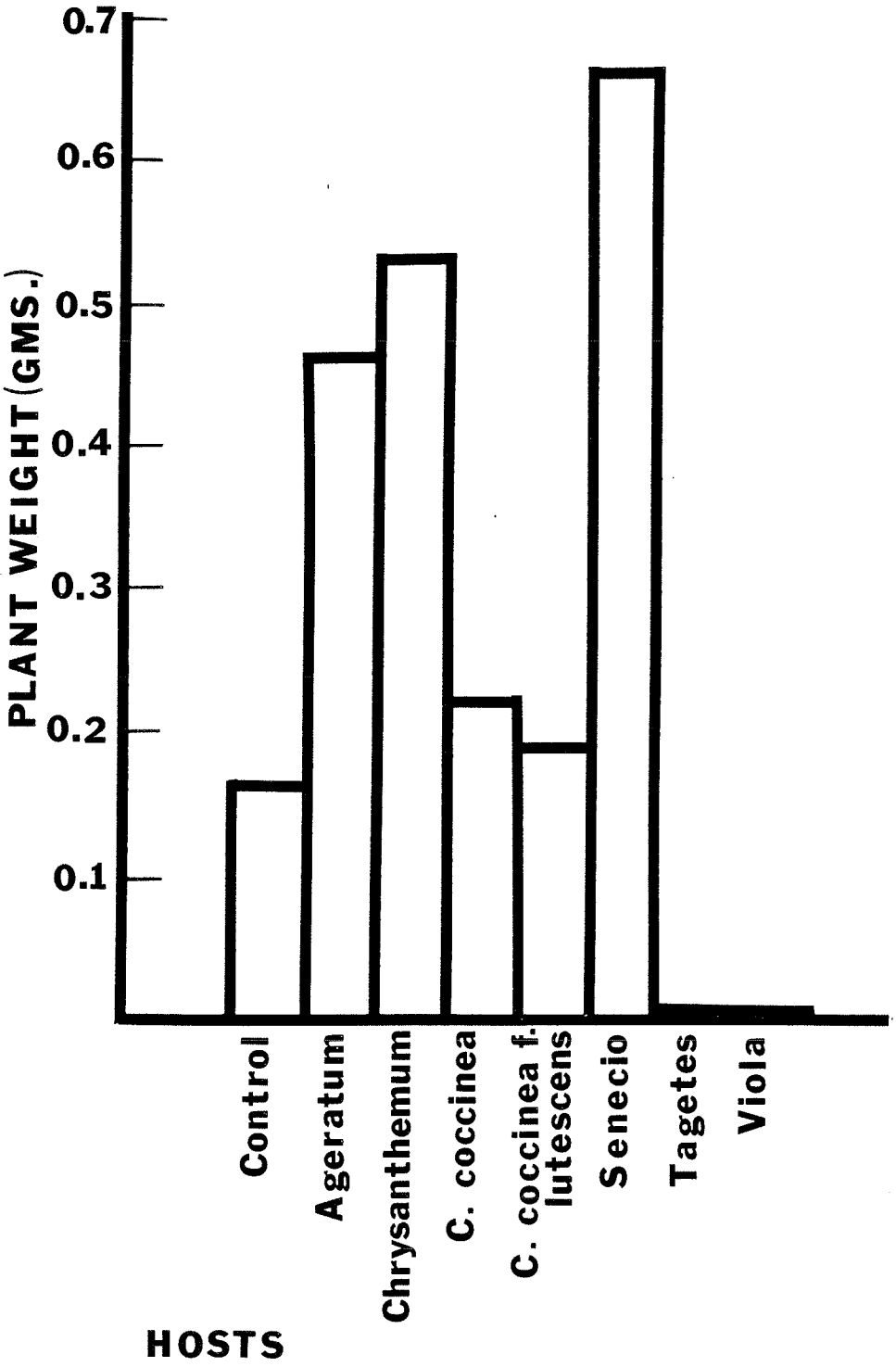

PLANT WEIGHT(GMS.)
1.2
1.0
0.8
0.6
0.4
0.2
Control
Ageratum
Chrysanthemum
C. coccinea
C. coccinea f. lutescens
Senecio
Tagetes
Viola
HOSTS

FIGURE 6. The effect of various hosts on the dry weight of *C. coccinea* forma *lutescens*.


PLANT WEIGHT (GMS.)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Control
Ageratum
Chrysanthemum
C. coccinea
C. coccinea f. lutescens
Senecio
Tagetes
Viola
HOSTS

While both forms survived with Senecio vira-vira, C. coccinea forma lutescens grew best with this while C. coccinea grew best with Chrysanthemum. Tagetes supported poor growth in C. coccinea and no growth of C. coccinea forma lutescens. Figures 5 and 6 also indicate that Castilleja can parasitize other Castilleja. Both forms grew better when grown with the other form rather than the same form of Castilleja. The findings tend to support the research by Malcolm (1962b) and Piehl (1962) that the root parasites in the family Scrophulareacea are not host specific. Malcolm (1962b) speculates that the best hosts have the densest root systems. This could account for the lack of growth of Castilleja with Viola which produces a small loose root system but not for the poor growth with Tagetes patula 'Yellow Boy' which produces a dense root system.

The effects of the hosts on the size of Castilleja is depicted in Table 8. Analysis of variance and T tests indicate that the growth of C. coccinea with Viola is significantly lower than any other combination. The combination of C. coccinea with Chrysanthemum was significantly better than any other combination of the red flowering form. For C. coccinea forma lutescens the combinations of Chrysanthemum and Senecio with the yellow flowering form were significantly different than all but the Ageratum combination.

The dry weights of the hosts were not significantly affected by the parasites (Figure 7) again supporting the

TABLE 8: The effect of various host on the size of *Castilleja*.

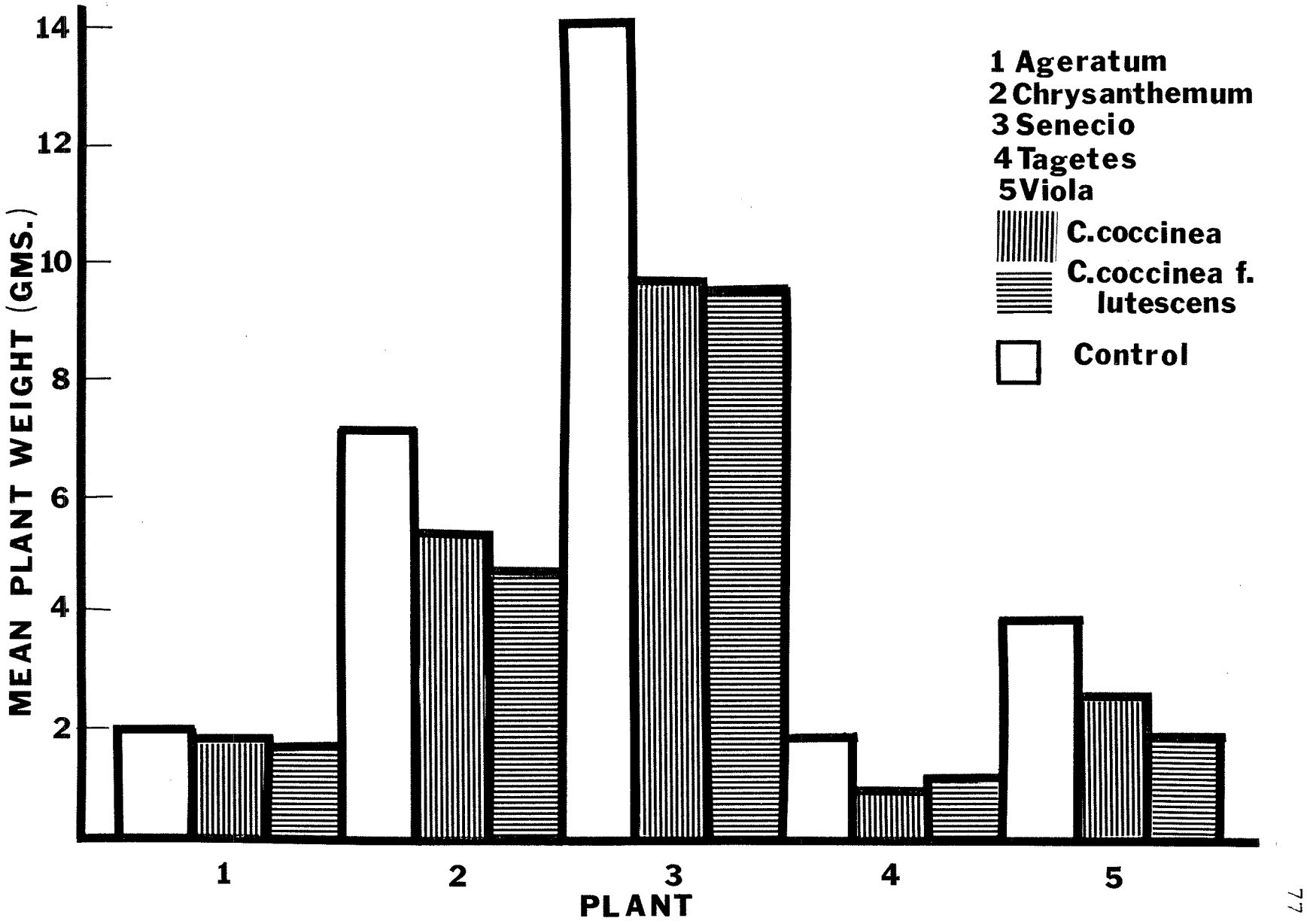
Hosts	Rosette diameter (cm)	
	C. coccinea	*C. coccinea* forma *lutescens*
Viola cucullata	0.33[1]	0.58
Chrysanthemum	7.97	6.43
Tagetes patula	3.57	0.23
Senecio vira-vira	3.80	6.07
Ageratum	3.93	4.20
Castilleja[2]	1.88	1.45
Castilleja[3]	4.00	1.27
Control	3.07	2.40

[1] Values are the mean of three samples.

[2] Grown with the same form of *Castilleja*.

[3] Grown with the other form of *Castilleja*.

FIGURE 7. The effect of Castilleja on the dry weights of the host plants.


14
12
10
8
6
4
2
MEAN PLANT WEIGHT (GMS.)
1 Ageratum
2 Chrysanthemum
3 Senecio
4 Tagetes
5 Viola
C.coccinea
C.coccinea f. lutescens
Control
1
2
3
4
5
PLANT

research by Heckard (1962). However, trends were apparent. All the hosts weighed less when parasitized by Castilleja, with C. coccinea forma lutescens usually having a greater effect. The analysis of the cross sectional area of the hosts (Table 9) indicated approximately the same trends but the values were too variable to be valid. The cross sectional area of Viola varied from 35.04 cm^2 to 166.6 cm^2 when parasitized by C. coccinea because the measurement depended on the spread of the leaves. As only a few leaves were present at one time, the measurement was very variable. There were similar problems in calculating the cross sectional area of other plants.

TABLE 9: The mean cross sectional area of hosts grown alone or with C. coccinea or C. coccinea forma lutscens.

Hosts	Control (cm^2)	Grown with C. coccinea	Grown with C. coccinea forma lutescens
Viola cucullata	146.67	91.83	99.30
Chrysanthemum	102.45	96.60	129.62
Tagetes patula	93.31	80.88	66.21
Senecio vira-vira	202.37	159.16	151.37
Ageratum	99.15	99.87	78.00

CONCLUSIONS

The following may be concluded on the basis of the research conducted on Castilleja coccinea.

1. Alternating day/night temperatures of 17 to 22°C and 11 to 18°C, and constant temperatures between 3.8 and 20°C, there is no significant difference in germination percent. Seed germination is inhibited at 2±2°C.
2. The germination percent of seed under low light intensities independent of exposure or inbibition time, filter or temperature is lower than the germination percent under high light intensities.
3. Under high light intensities, red filtered light inhibited germination and blue light promoted germination.
4. Increasing exposure time under both low and high light intensities increased germination percent.
5. Although both C. coccinea and C. coccinea forma lutescens can grow with a variety of hosts including C. coccinea and C. coccinea forma lutescens, different hosts appear to support better growth.
6. C. coccinea and C. coccinea forma lutescens can survive without host contact.
7. Hosts were not significantly affected by Castilleja but host plant weight and volume is generally reduced.

Summary

On the basis of the research conducted, it is summarized that while C. coccinea can be grown and will flower indoors, growth is slow and the parasitic habit would tend to make it difficult to handle in the seed and florist trade.

CHAPTER III

CONTROLLED ENVIRONMENT PROPAGATION AND CULTURE OF *ANEMONE PATENS* VAR. *WOLFGANGIANA* (BESS.) KOCH

Introduction

The need to study and conserve native plants has long been a goal in North America. The cities and farms encroaching on natural habitats has led to the interest of not only conserving native plants but also turning them into a commercial asset. Today only two species out of approximately 150,000 species of native flowering plants in North America are used extensively in the seed and florist trade (Tessene, 1980). The floral emblem of Manitoba, *Anemone patens* var. *wolfgangiana* (Bess.) Koch was selected as a suitable species to introduce into the florist trade as a potted flowering plant.

A. patens var. *wolfgangiana*, the Prairie crocus or anemone, is a member of the family Ranunculaceae. The herbaceous perennial has large solitary purple flowers which emerge in early spring prior to the appearance of the leaves (Wildeman and Steeves, 1982). The Prairie crocus inhabits open fields, hillsides and overgrazed pastures (Budd, 1979, Dudley, 1930 and Taylor and Hamblin, 1976).

Appropriate cultural and propagation practices are essential for successful competition on the commercial market. Seed propagation requires a suitable combination of

water, temperature, gases and light (Hartmann and Kester, 1975). The rudimentary embryos often found in the family Ranunculaceae require a post-harvest after-ripening period for maturation of the seed (Horowitz et al., 1975). Exogenous application of gibberellic acid is unsuccessful in shortening the germination lag period of Anemone (Bullowa et al., 1975). The water-repellent hairy seed coats of Anemone may also be involved in the lag time prior to germination (Bullowa et al., 1975). After-ripening by moist refrigeration was reported by Nichols (1934) to decrease the time necessary for germination. The effect of light on germination of Anemone is unclear. Bullowa et al. (1975) noted that A. coronaria seed had a slower and reduced rate of germination in the light. Mitchell (1926) found that A. virginiana germination required light. Horowitz et al. (1975) recommend captan as a fungicide to prevent fungal infestations and damping-off of seed and seedlings.

A good medium and fertilization program is essential for plant growth. The medium can be composed of various combinations of soil, sand, peat, sphagnum moss, vermiculite and perlite in order to achieve a good balance of aeration, moisture holding capacity and nutrition (Hartmann and Kester, 1975). The overall health and appearance of the plant depends directly on the nutrition the plant receives. Essential macro and micro nutrients work together in forming plant enzymes, hormones, proteins and structures (Hartmann

and Kester, 1975). Deficiency symptoms occur if an element is unavailable in sufficient quantities (Epstein, 1972).

In addition to seed dormancy, perennial plants often undergo periods of bud dormancy as protection against unfavorable conditions (Bidwell, 1979, and Salisbury and Ross, 1969). Dormant plants will not grow even though subjected to suitable temperature, moisture and oxygen conditions. According to Wildeman and Steeves A. patens floral bracts appear in late May or June and continue to develop until early fall when the plants become dormant. Meiosis occurs during the following spring. The temperature and length of time required to overcome the bud dormancy was studied.

MATERIALS AND METHODS

Seed Collection and Storage

Ripe seed of A. patens was collected from native plants on the following dates (Figure 8):

Site 1 on May 31, 1981,

Site 2 on June 8, 1981,

Site 3 on June 18, 1982,

Site 4 on June 15, 1982.

The seed were allowed to air dry at 20°C for four to 10 days before they were sorted and the immature seed discarded. The long hairy styles were cut off for ease of handling at a later date. The seed were stored dry at 9°C in Globe brand manila envelopes or brown paper bags. Seed collected in 1981 was used in late 1981 and early and mid 1982. Seed collected in 1982 was used in the latter part of 1982.

Seed Germination

Preliminary Study

To determine the germination characteristics of A. patens, preliminary trials were conducted at day/night alternating temperatures at 17/11, 22/15, 22/18°C. The seed was placed in Pyrex Petri dishes on three pieces of Whatman #1 filter paper with 5 ml of distilled water. The Petri dishes were placed individually in plastic bags to reduce moisture loss. To determine whether or not a light requirement

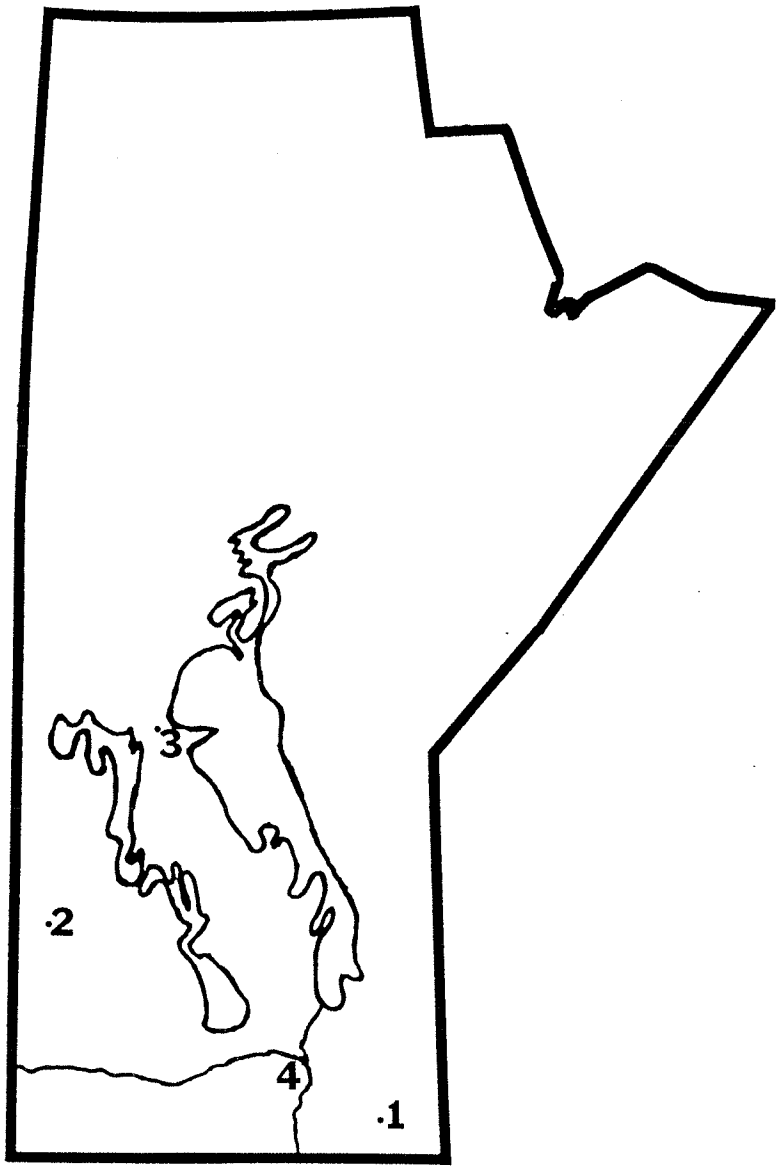


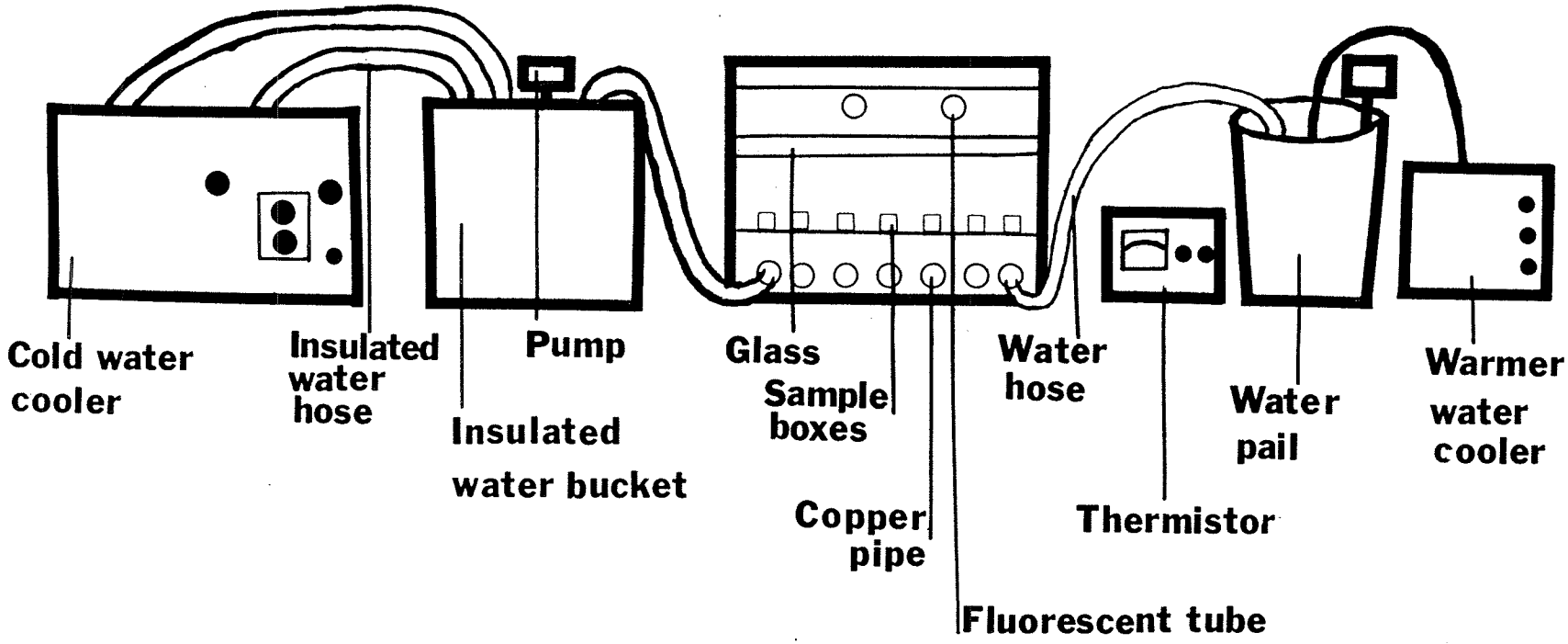
Fig. 8. Outline map of Manitoba showing collection sites.

existed, half of the samples in each regime were covered with aluminum foil to exclude light. For the first trial, started on January 11, 1982, the sample size was ten seed. In the second trial, started on April 29, 1982, the sample size was 20 seed. Six replicates were used for each temperature treatment, three of which were covered with foil. The replicates were placed at random in the various regimes. The arrangement was changed each time the germination count was taken at two to three day intervals.

Thermogradient Study

Another germination trial was conducted in a range of temperatures which were maintained by a copper plate (Figure 9). The temperatures used were 2.2°, 10°, 17.5°, and 21°C and were measured with an Atkins Thermister model 3L51J. Plastic rectangular, covered sample containers were placed on a flat temperature controlled copper plate. The temperature was controlled by water filled copper pipes running beneath the copper plate to separate warm and cold water baths. The temperatures normally varied ± 2°C except when experimental malfunctions and excess heat load caused the temperatures to rise 5 to 8°C on two separate days near the end of the experiment. The seed were illuminated 24 hours a day by four warm white flourescent tubes producing a light intensity of 47 ± 7 microE.m^{-2}.sec^{-1} and 9.1 ± 1.5 W.m^{-2} measured with a Li-Cor model Li-185A Quantum/Radiometer/Photometer. Four

FIGURE 9. Diagram of the experimental set-up used for thermogradient studies.


Cold water cooler
Insulated water hose
Pump
Insulated water bucket
Glass
Sample boxes
Copper pipe
Fluorescent tube
Water hose
Thermistor
Water pail
Warmer water cooler

replicates of 20 seed per sample were used in the trial. The seed were treated to reduce damping-off by a preplant soaking in a slurry of Later's Benomyl 50 percent W.P. at the concentration of 180g/L distilled water for twenty minutes. The seed were allowed to dry for one hour before being placed on pieces of Whatman #1 filter paper cut to fit the containers. One mL of distilled water was added initially to the containers. Additional distilled water was added as the filter paper dried. The duration of the experiment was 53 days. The germination count was taken periodically. The germinated seed were removed after each count.

Seed Fungicide Treatments

Seed and seedling damping-off of A. patens during the germination trials led to designing an experiment using several common methods for reducing damping-off. The treatments are as follows:

Treatment	Ingredient	Concentration
Control	Water	
Orthocide slurry[1]	50% Captan W.P.	360g/L water
Orthocide dry[2]	50% Captan W.P.	1 tsp./seed lot
Later's Benomyl[1]	Benomyl 50% W.P.	180g/L water
Later's Benomyl[2]	Benomyl 50% W.P.	1 tsp./seed lot
Hot water[3]		
No Damp[1]	Oxine Benzoate 2.5%	15 mL/L water
Ethanol[4]	Ethyl Alcohol	20% in water

[1] 10 minute soak
[2] Shaken together in manila envelope
[3] 50 mL of 40 to 50°C water for 20 minutes
[4] 5 minute soak

The seed were treated prior to being placed in the dark at 15°C in Petri dishes. Each treatment consisted of three replicates of 10 seed. The germination count was recorded every 2 to 3 days.

Seed Treatments and Media

A further study to reduce damping-off employed several different fungicides as well as media. The various combinations of media are as follows:

Media	Proportion
Sand	
Pasteurized soil, sand, peat[1]	1:1:1
Pasteurized soil, sand, peat	1:2:1
Soil, sand, peat	1:1:1
Soil, sand, peat	1:2:1
Sand and peat	1:1
Vermiculite	
Vermiculite and peat	1:1
Perlite	
Perlite and peat	1:1

[1] Steam pasteurization for 1 hour

The fungicide treatments were as follows using the manufacturers recommended rate of commonly available fungicide:

Treatment	Ingredient	Concentration
Orthocide	Captan 50% W.P.	5g/L distilled water
Later's Benomyl	Benomyl 50% W.P.	.5g/L distilled water
No Damp	Oxine benzoate 2.5%	15mL/L distilled water
Control	Water	

Prior to treatment the seed were placed in the selected media in 2 1/2 x 3 1/2 inch plastic pots at 22/18°C in a lighted growth room. The pots were placed in a completely randomized

design with three replicates and three locations. The seed were only treated once at the beginning of the experiment. The percent survival of the seed and seedlings was noted.

After-ripening

The effect of after-ripening on A. patens was studied using untreated seed and seed treated with No Damp (Oxine Benzoate, 2.5 percent), a fungicide commonly used to reduce damping-off. The treated seed were soaked with No Damp at the rate of 15 $mL.L^{-1}$ distilled water for one hour to reduce damping-off during the low temperature storage period. The seed were placed in Pyrex Petri dishes which were individually wrapped in plastic bags to reduce moisture loss. Five mL of distilled water was added initially with an additional 2 to 3 mL added after the dishes were removed from storage. Each treatment consisted of three replicates of 20 seed. The seed were removed from the low temperature storage of 9°C at two week intervals. The rate and percent germination was checked two to three times each week. The experiment duration was up to 16 weeks of cold storage. Two months germination time was allowed at 23/16°C.

Plant Study

Fertilizer Requirement Study

An experiment was conducted using six month old seedlings to determine the type and quantity of fertilizer needed

by A. patens to produce the maximum amount of visually attractive foliage. The treatments are as follows:

Fertilizer	Recommended Rate	Amount	ppm N	ppm P	ppm K
Control	0	0	0	0	0
10-52-10	1/2 X	12.75g.3.8L^{-1} water*	224	98	186
10-52-10	1 X	25.50g.3.8L^{-1} water	448	196	372
10-52-10	2 X	51.00g.3.8L^{-1} water	884	391	742
20-20-20	1/2 X	4.25g.3.8L^{-1} water	335	761	278
20-20-20	1 X	8.50g.3.8L^{-1} water	670	1523	556
20-20-20	2 X	17.00g.3.8L^{-1} water	1342	3050	1114

* 8.5g.3.8L^{-1} water is approximately equal to 1 tablespoon/gallon

The plants were treated once a week for 26 weeks with 50 mL of the assigned fertilizer treatment and grown in a growth room at 23/18°C with a sixteen hour day. The plants were watered daily. At the end of six months (27 weeks), leaf number, leaf colour, plant dry, and plant fresh weight were determined.

Dormancy

Trials were conducted to determine what effects time and low temperature have on flowering and leaf formation of A. patens. An initial trial of 45 plants was conducted at a temperature of 2° ± 1°C with an eight hour day (for up to 20 weeks). Nine plants were removed from the low temperature storage at two week intervals and placed in a growth room at 22/18°C day/night temperatures with a sixteen hour day. The growth was monitored for two months. For the second trial with 40 of the same plants the temperature was reduced from

22/18°C to 0/-1°C over a four week period to slowly harden the plants and prevent freezing injury. The temperature was reduced as follows:

1. 15/10°C for one week,
2. 10/5°C for the next week,
3. 5/0°C for the next week, and
4. 0/-1°C for the duration of the 2 to 24 weeks.

For the first three cooling down weeks, the lights were on in the growth cabinet for an eight hour day. The final temperature regime was eight hours for the -1°C and 16 hours for the 0°C, all in the dark. At two week intervals, two plants were removed either to 2° ± 1°C with an eight hour day or to 22/18°C with a sixteen hour day. The number of leaves and flowers displayed was noted for up to six months.

RESULTS AND DISCUSSION

Seed Germination

Preliminary Studies

The germination of A. patens is not significantly different in the light or dark (Table 10) or if germinated at alternating day/night temperatures of 17/11°C, 22/15°C, or 22/18°C. Thus, unlike Bullowa et al. (1975) reported for A. coronaria, and Mitchell (1926) for A. virginiana, A. patens is apparently not sensitive to light during germination. As depicted in Table 1, the percent germination was between 33 to 42 percent. All but a few of the seed died due to damping-off prior to or just following germination.

Thermogradient Study

If the seed is germinated on a thermogradient, a significant decrease in germination is noted at low temperatures. Figure 10 indicates a highly significant positive linear relationship for the germination of Anemone between temperatures of 2.2° and 21°C. The coefficient of determination r^2 is 98 percent indicating that 98 percent of the variability is accounted for by the linear relationship of temperature and the adjusted germination (arc $\sin\sqrt{\text{percent}}$) of A. patens.

Seed Fungicide Treatments

The serious damping-off problem noticed during the

TABLE 10: The effect of light conditions and various temperature regimes on the mean germination of *Anemone patens* var. *wolfgangiana* seed *in vitro*.

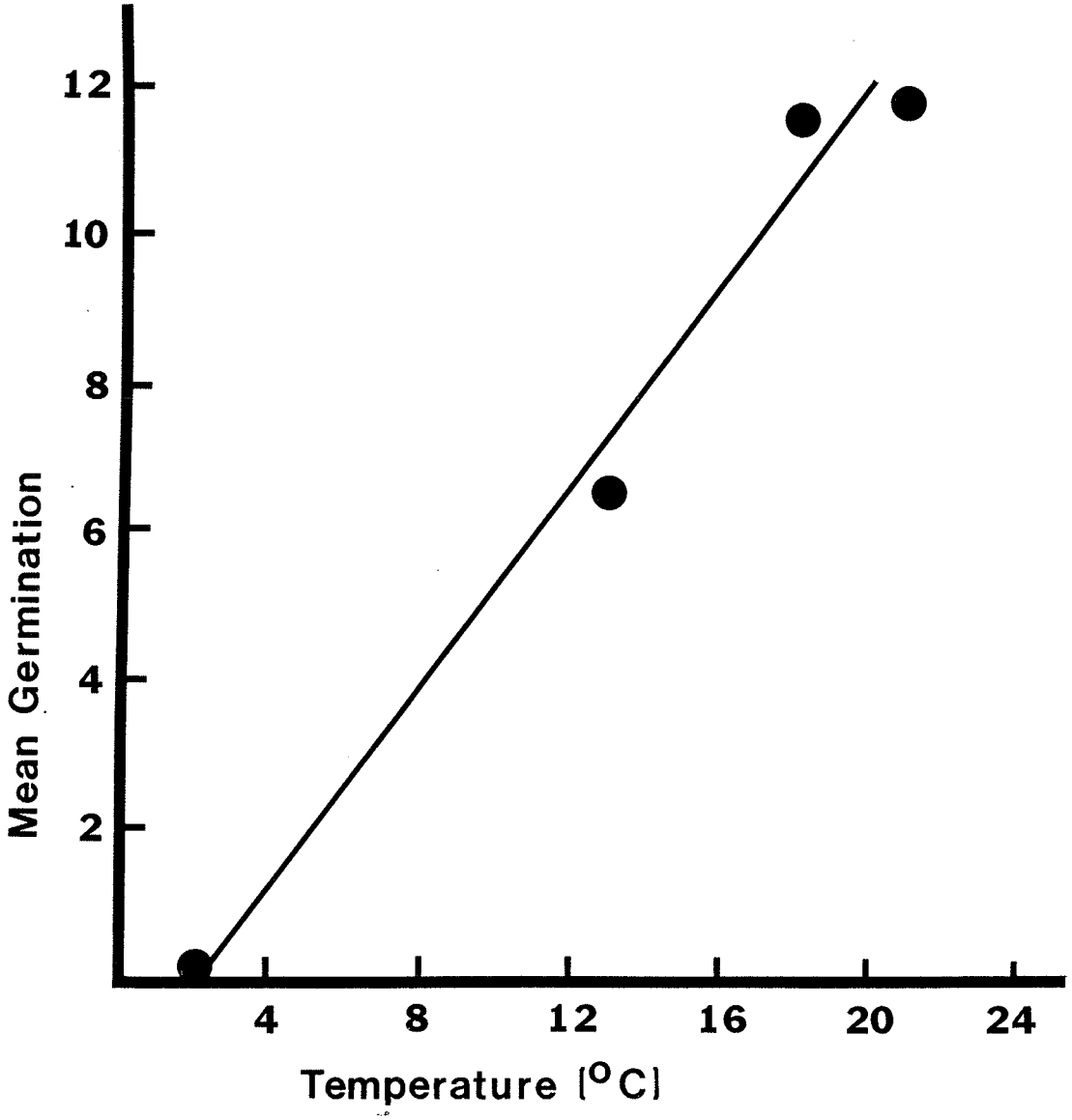
Temperature (°C)	% Germination	
	Dark	Light
17/11[1]	39.95[2]	41.60
22/15	40.80	34.10
22/18	34.95	33.30

[1] Alternating day/night temperatures.

[2] Data transformed by arc sin $\sqrt{\text{percent}}$ for analysis.

FIGURE 10. The effect of temperature on the mean germination[1] of Anemone patens var. wolfgangiana seed in vitro.

1 Values are means of four replicates of 20 seeds.


12
10
8
6
4
2
Mean Germination
4
8
12
16
20
24
Temperature [°C]

initial germination trials was reduced through the use of several commonly available fungicides. Although the damping-off was reduced, the seed was adversely affected by all the fungicidal treatments except dry benomyl (Table 11). A significant decline in germination was noted. The dry benomyl adhering in small clumps to the seed coat hairs did not fully control the damping-off but was much superior to no treatment. A low germination count occurred with the benomyl slurry recommended by Strider (1980) for Zinnia, possibly indicating that large amounts of benomyl adhering to the seed would decrease the germination whether the benomyl was in a slurry or dry.

The captan, recommended by Horowitz et al. (1975) for Anemone, resulted in low germination both as a slurry or dry. This suggests that while the damping-off may be controlled by the use of fungicidal seed treatments the chemicals cause detrimental effects within the seed.

Seed Treatments and Media

The data in Table 12 indicates that the media type has no significant effect on the germination of A. patens. Within experimental error, seed germinated equally well in all the media tested. The media does however have an effect on the future survival of the seedlings (Table 12). Ninety days following the seeding of Anemone, significant differences were noted among the treatments. Sand, vermiculite and

TABLE 11: The effect of various fungicides on the germination of Anemone patens var. wolfgangiana in vitro.

Treatment	% Germination
Control	80.0[1] e[2]
Orthocide captan slurry	36.6 bc
Orthocide captan dry	20.0 a
Later's benomyl slurry	30.0 ab
Later's benomyl dry	70.0 de
Hot water	50.0 c
No Damp	66.6 d
Ethanol	50.0 c

[1] Values are the data transformed by arc sin $\sqrt{\text{percent}}$ for analysis.

[2] Values followed by the same letter do not differ significantly as determined by Least Significant Difference Test, 95% level.

TABLE 12: The effect of various media on the germination and survival of Anemone patens var wolfgangiana.

Media	% Germination	% Survival 95 Days	% Survival 205 Days
Sand	75.0[1] a[2]	58.3 a[2]	25.0 ab[2]
Past. soil, sand, peat 1:1:1	75.0 a	100.0 b	75.0 c
Past. soil, sand, peat 1:2:1	75.0 a	83.3 ab	58.3 bc
Unpast. soil, sand, peat 1:1:1	58.3 a	91.7 ab	91.7 c
Unpast. soil, sand, peat 1:2:1	58.3 a	100.0 b	83.3 c
Sand, peat 1:1	41.7 a	91.7 ab	66.7 c
Vermiculite	50.0 a	58.3 a	8.3 a
Vermiculite, peat 1:1	83.3 a	83.3 ab	83.3 c
Perlite	41.7 a	58.3 a	16.7 a
Perlite, peat 1:1	66.7 a	83.3 ab	75.0 c

[1] Values are a mean percent of 12 samples.

[2] Values do not differ significantly within the column as determined by Chi-squared, 95% level.

perlite when used as the sole component of the medium resulted in reduced seedling survival (58.3 percent). The combination of various components, similar to those recommended by Hartmann and Kester (1975), resulted in higher seedling survival indicating that the combination provided sufficient aeration and nutrition for seedling development. After 205 days the difference between the single component media and the combinations of components was more definite. Seedling survival ranged from as low as 8.3 percent for vermiculite alone to 91.7 percent for 1:1:1 unsterilized soil, sand and peat.

The data in Table 13 of the germination of Anemone in media treated with fungicides indicates significant differences among the treatments. Captan and No Damp significantly reduce germination compared to benomyl and the control treatment. Seed or seedling damping-off was not a problem in the control treatment. As indicated in Table 13, the fungicidal seed treatments have no lasting detrimental effect on seedling survival as the seed that does germinate survives 77 to 90 percent of the time.

After-ripening

As indicated by the data in Table 14, short periods of moist after-ripening of untreated seed has no significant effect on germination. As reported by Horowitz et al. (1975) a post-harvest after-ripening period for maturation of

TABLE 13: The effect of various fungicides on the germination and seedling survival of Anemone patens var. wolfgangiana.

Treatment	% Germination	% Plant Survival
Control	86.67[1] b[2]	76.70 a
Later's benomyl	73.33 b	80.00 a
Orthocide captan	43.33 a	76.70 a
No Damp	46.67 a	90.00 a

[1] Data transformed by arc sin $\sqrt{\text{percent}}$ for analysis.

[2] Values followed by the same letter in the same column do not differ significantly as determined by Chi-squared, 95% level.

TABLE 14: The effect of short periods of after-ripening on Anemone patens var. wolfgangiana.

Weeks of Storage	Mean Germination
0	3.0[1]
2	3.3
4	5.7
6	3.7
8	2.0

[1] Values are means of three replicates of 20 seed transformed by $\sqrt{x + .5}$ for analysis.

rudimentary embryos is required. This study found that 12 to 14 days elapse before visible germination occurs. Germination was complete after 20 to 30 days. At similar temperatures, Bullowa et al. (1975) found lag periods for A. coronaria of 8 to 10 days. Horowitz et al. (1975) found lag periods of wild plants of A. coronaria of 15 to 33 days at 14°C while the lag periods of cultivated A. coronaria 'de Caen' were 8 to 36 days depending on the age of seed. For A. virginiana germination took 29 to 75 days in the light, with no germination in the dark (Mitchell, 1926).

Longer periods of after-ripening, as indicated in Figure 11, result in significant differences in germination. A significant negative linear component of the germination of treated and nontreated seed combined, indicates that the germination decreases as the period of after-ripening increases. The germination decreases up to six weeks of after-ripening, increases slightly and then decreases again indicating a possible cyclic variation of the effects of after-ripening.

The analysis of variance for longer periods of after-ripening indicates highly significant differences between the seed treated with No Damp and the control seed. There were no significant interactions between treatments and weeks of after-ripening. Overall, the germination of the treated seed is higher than the untreated seed (Figure 12). This information seems to contradict the results from the previous studies where No Damp significantly reduced germination. The

FIGURE 11. The effect of length of after-ripening on the mean germination[1] of Anemone patens var. wolfgangiana seed.

1 Values are means of three replicates of 20 seed.

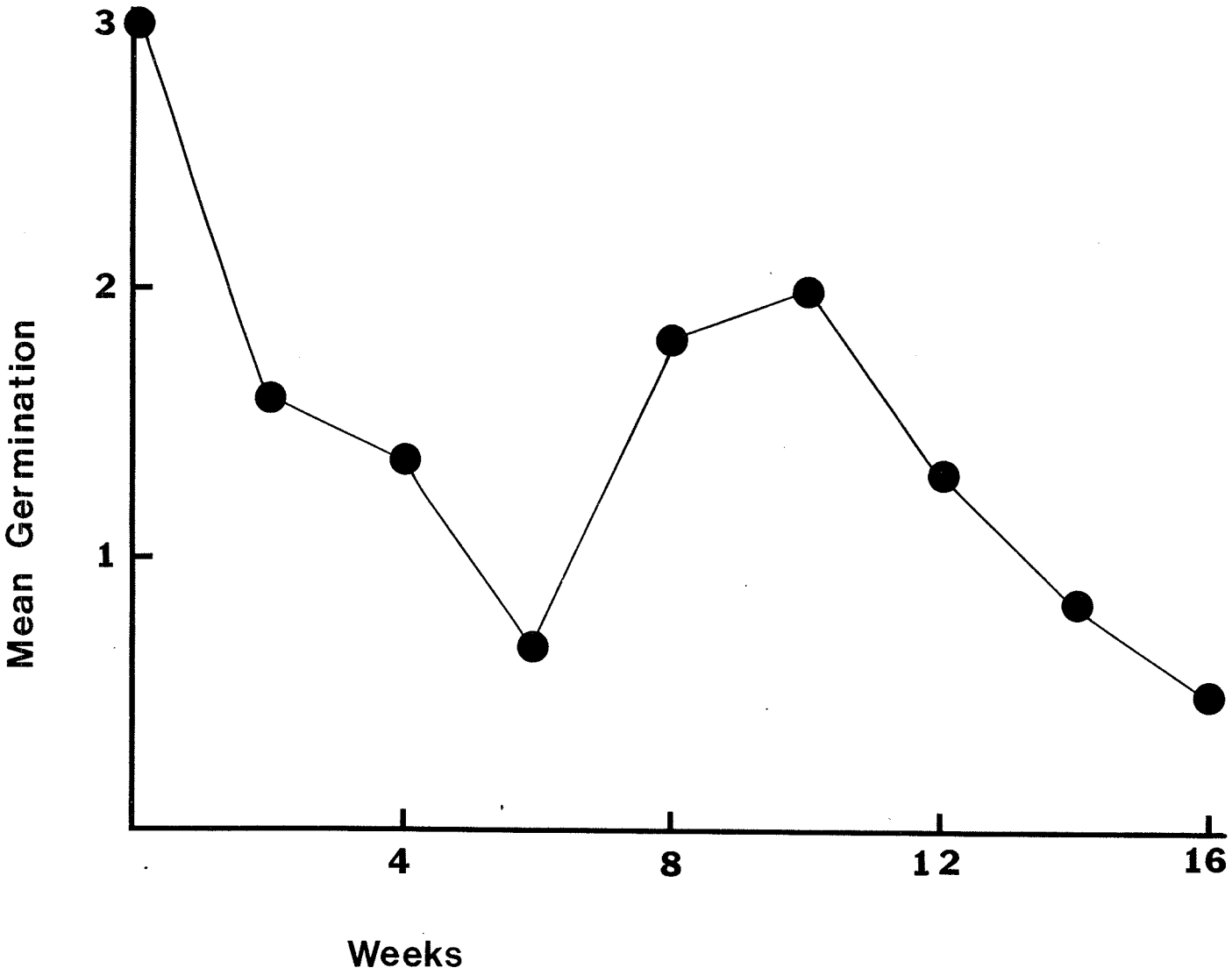
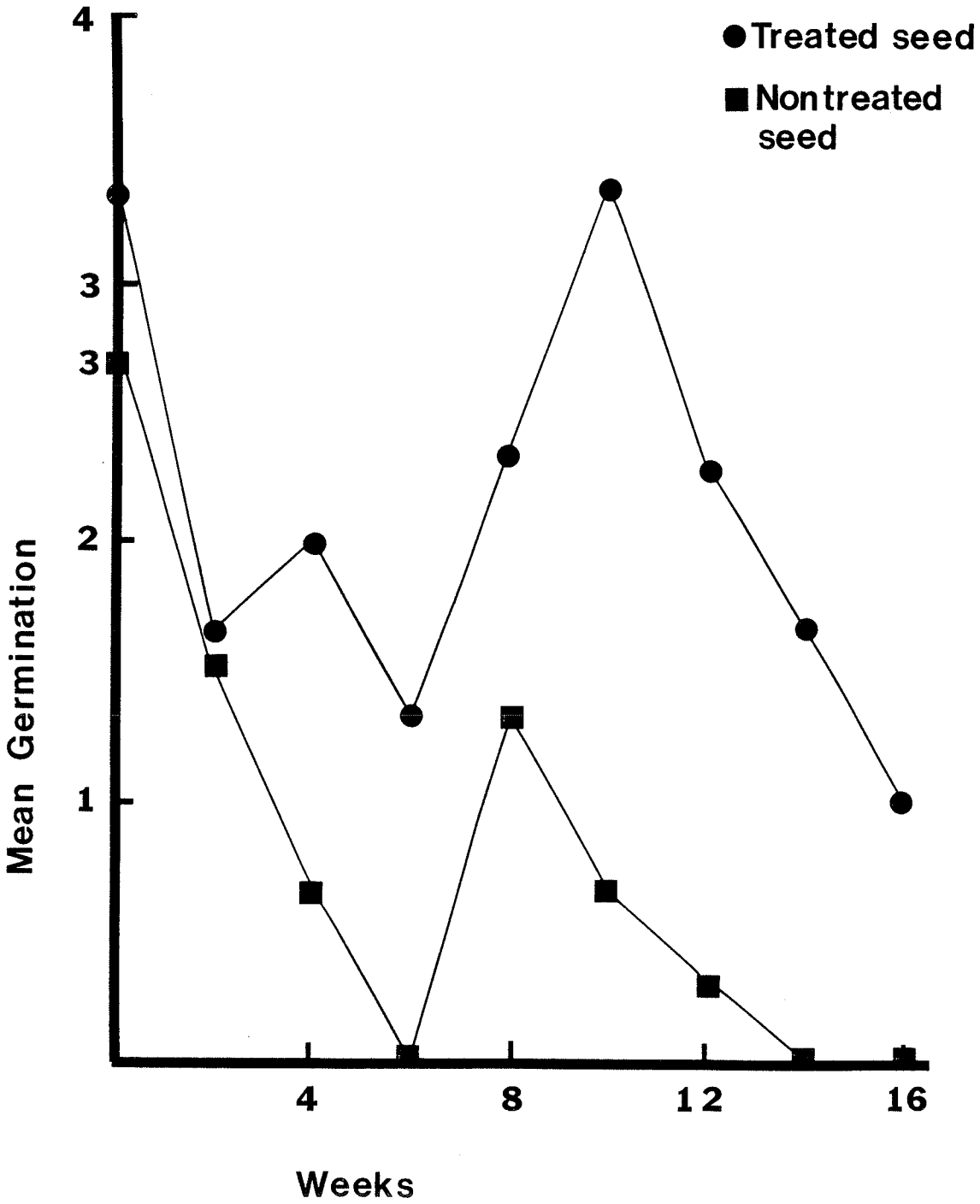

3
2
1
Mean Germination
4
8
12
16
Weeks

FIGURE 12. The effect of after-ripening on the mean germination[1] of Anemone patens var. wolfgangiana seed[2].

1 Values are means of three replicates of 20 seed.

2 Non-treated seed and seed treated with No Damp.


Treated seed
Non treated seed
Mean Germination
4
3
3
2
1
4
8
12
16
Weeks

author suggests that due to the extended length of time over which the experiment occurred, the control seed were severely affected by damping-off thereby greatly decreasing the germination. Severe damping-off occurred with untreated seed from six weeks and on, while the treated seed had little or no visible signs of damping-off.

Vegetative and Reproductive Growth

Fertilizer Requirement Study

The data for the fertilizer studies indicate that the amount of nitrogen (N), phosphorus (P), and potassium (K) applied significantly affects the growth of A. patens. The data in Table 15 indicates significant differences among leaf number with different levels of N, P, and K. The lowest leaf numbers correspond to the control and to the highest amount of nutrients added. The low leaf number in the former treatment may indicate a deficiency of the nutrients while the latter indicates an excess of nutrients. The highest leaf numbers were associated with intermediate rates of N, P, and K. The explanation by Moorby and Besford (1983) of the typical relationship between yield and nutrient supply applies. Yield increases with nutrient supply up to a point where a plateau is reached. At higher nutrient levels, the yield decreases.

A nutrient imbalance, or development of a deficiency may

TABLE 15: The effects of various fertilizer treatments on Anemone patens var. wolfgangiana.

Treatment (ppm)			Mean Leaf Number	Mean Volume (cm^2)	Leaf Colours	Comments
N	P	K				
0	0	0	1.556[1] ab[2]	98.239 a	146B, 146C[3]	tip and general necrosis
224	196	186	9.333 b	144.502 a	146A, 154A	59A leaf tips, necrotic spotting
448	196	372	10.889 b	178.861 a	146A, 147A	tip and spot necrosis mainly on older leaves
894	391	742	8.333 bc	188.989 a	146A, 147A	tip and spot necrosis, 151D tips and bases
335	761	278	12.444 c	162.889 a	146A, 146B, 147A, 147B, 148B	necrotic tips and spots, 59A tips on new leaves
670	1523	556	11.444 c	176.500 a	146A, 146B 146C, 147A	severe tip and general necrosis, 71A, 59A and 63B leaf tips
1342	3050	1114	3.11 a	96.194 a	146D	leaf veins 146A, 66.7% dead

1 Values are the mean of 9 samples.
2 Values in the same column followed by the same letter do not differ significantly at L.S.D., 95% level.
3 Colours determined by Royal Horticultural Society of London colour chart.

occur due to excess nutrients such as nitrogen (Mills and Jones, 1979). The application of one nutrient may depress the concentration and content of another nutrient by impairing the function of the nutrient at the site of action (Robson and Pittman, 1983) thus affecting the growth and development of the plant.

Multiple regression analysis indicates a significant positive relationship between N and leaf number if K is held constant. A negative relationship exists between K and leaf number if N is held constant. P adds no predictive ability to the multiple regression equation that already contains N and K.

The data in Table 15 indicates no significant differences in plant volume at different rates of N, P, and K. However, there is a definite trend towards smaller plants at the lowest and highest N, P, and K rate once again indicating a deficiency and excess respectively.

The visual appearance of the plants also differed with the fertilizer treatments (Table 15). The numbers 146 through 151 are in the yellow-green group of the Royal Horticultural Society of London colour guide (1966). Increasing numbers represent increasing yellow colour. A through D represent a descending order of saturation. The colours 59, 71 and 63 represent different shades of reddish-purple. The greatest difference in the yellow-green colour is noted in the control and highest N, P, and K treatment when the leaves

were noticeably a lighter hue than the other plants. The amount of necrosis varied from plant to plant but was most evident in the last two treatments where N, P, and K were high. The surviving plants of the highest N, P, and K treatment were strikingly different from the other plants. These plants had prominent 146A leaf veins on a lighter 146D blade in contrast to the other plants that produced a uniform overall colour. The reddish-purple colours noted by Epstein (1972) and Roberts and Boothroyd (1971) are signs of phosphorus deficiency. This deficiency may be due to an excess of N and K in most treatments except in the treatment with 98 ppm P where this amount may be insufficient to promote healthy plant growth.

The fresh and dry weights of the entire plants are listed in Table 16. The analysis of variance reveals significant differences among the treatments in terms of rates of N, P and K. The dry weights of the plants grown under the lowest and highest rate, 0 ppm N, P, and K and 1342 ppm N, 3050 ppm P and 1114 ppm K respectively are significantly lower than all other treatments. Multiple regression analysis on both the dry and fresh weights failed to reveal any trends of N, P or K. All three components led to an insignificant F-ratio and T test for the analysis of regression.

When the dry weights of the roots and shoots of A. patens are taken separately the data indicates not only

TABLE 16: The effects of various fertilizers on the dry and fresh weights of the roots and shoots of *Anemone patens* var. *wolfgangiana*.

Treatment (ppm)			Mean Weight (gms.)				
N	P	K	Fresh Weight Entire Plant	Dry Weight Entire Plant	Dry Weight Shoots	Dry Weight Roots	Dry Weight Root/Shoot
0	0	0	9.504[1] ab[2]	3.91 a	1.046 a	2.864 ab	2.79 d
224	98	186	15.898 bc	7.32 b	2.752 bc	4.564 bc	1.811 bc
448	196	372	24.256 d	9.67 b	3.392 c	6.282 c	1.911 c
894	391	742	15.739 bc	6.99 b	2.938 bc	4.053 b	1.642 bc
335	761	278	23.467 cd	9.34 b	3.417 c	5.920 c	1.723 bc
670	1523	556	16.898 bcd	7.55 b	3.780 c	3.768 b	1.029 ab
1342	3050	1114	5.319 a	2.40 a	1.503 ab	0.900 a	0.199 a

1 Mean of 9 values.

2 Values within the same column followed by the same letter do not differ significantly with Least Significant Difference Test, 95%.

significant differences among the rates of N, P and K, but also significant multiple regression trends (Table 16). As in the entire plant weight, the roots and shoots weights of the lowest and highest rates of N, P, and K are the lowest. Multiple regression of the shoot weights reveals that all three elements N, P and K are important in prediction, accounting for approximately 98 percent of the variability. The N component indicates that dry weight of the shoot will increase with increasing N if P and K are constant. This finding agrees with Bunting and Drennan, after Puckridge (1983), that increasing N increases leaf area, thus shoot weight, in wheat plants. P and K on the other hand resulted in decreased shoot weight with increasing P and K if the other two components are held constant, thus contradicting Langer (1983) who found that increasing P and K resulted in increasing leaf area of cereals. Asher and Longeran (1967) also state that increasing P increases the dry weights of various species. However, they do state that in some species at high levels of P, the dry weight starts to decrease and severe necrosis of the leaves occurs.

For the roots, only the P component was important, accounting for 45 percent of the variability. An inverse relationship also exists for P in relation to this experiment on the effects of nutrients on the dry weights of the roots.

The root/shoot ratio also indicated significant trends. The ratios of the lowest and highest rates of N, P, and K

were significantly higher or lower respectively than most of the intermediate rates ratios. Overall, as the amounts of fertilizer increase, the root/shoot ratio decrease, signifying either an increase in shoot dry weight or a decrease in root dry weight. Multiple regression analysis reveals that only the inverse relationship between P and root/shoot ratio is significant. As both the previous multiple regressions on root and shoot dry weight indicate inverse relationships between P and dry weight, the root/shoot ratio thus reveals relatively greater reduction in root dry weight as P increased. Wild et al. (1974) found similar relationships of K and the four species tested. The roots and shoots responded in a similar fashion to K but as in this experiment, the root/shoot ratios were highest at low levels of K.

The author suggests further experiments be carried out with lower rates of N, P, and K to reveal the optimum amounts required and possibly different relationships between the nutrients and dry weight.

Dormancy Induction and Termination

A. patens stored at temperatures of 2±1°C retained green leaves for the entire storage period of up to 20 weeks and did not produce new leaves or flowers when removed from the low temperature storage and placed at 22/18°C for two months. This indicates that the temperature was not cold enough and/or the length of exposure time was insufficient to

cause leaf senescence. The plants were not visually harmed by the treatment but the dormancy was not terminated since no new leaves or flowers were displayed. One control plant not exposed to any temperature or illumination changes flowered six months following the initiation of the low temperature storage.

The data from the second low temperature treatment at 0/-1°C (Table 17) indicates no significant differences among treatment weeks for flower numbers displayed after the low temperature storage. However, the plants did flower upon removal to growing conditions of 22/18°C and 2±1°C in contrast to the former trial where only one plant flowered. There were no significant differences in flower numbers between the growing temperatures. This result indicates a requirement for low temperatures at/or below 0°C in order to remove dormancy.

Production of new leaves following low temperature storage was significantly affected by the temperature after storage and the length of time in storage. As indicated by the analysis of variance, growing the plants in 2±1°C after storage rather than 22/18°C results in significantly higher leaf numbers. This response corresponds to the cool spring temperatures when the Anemone emerges and begins growth. Figure 13 demonstrates a highly significant curvilinear relationship between mean leaf number for after storage temperatures and weeks of low temperature storage. The

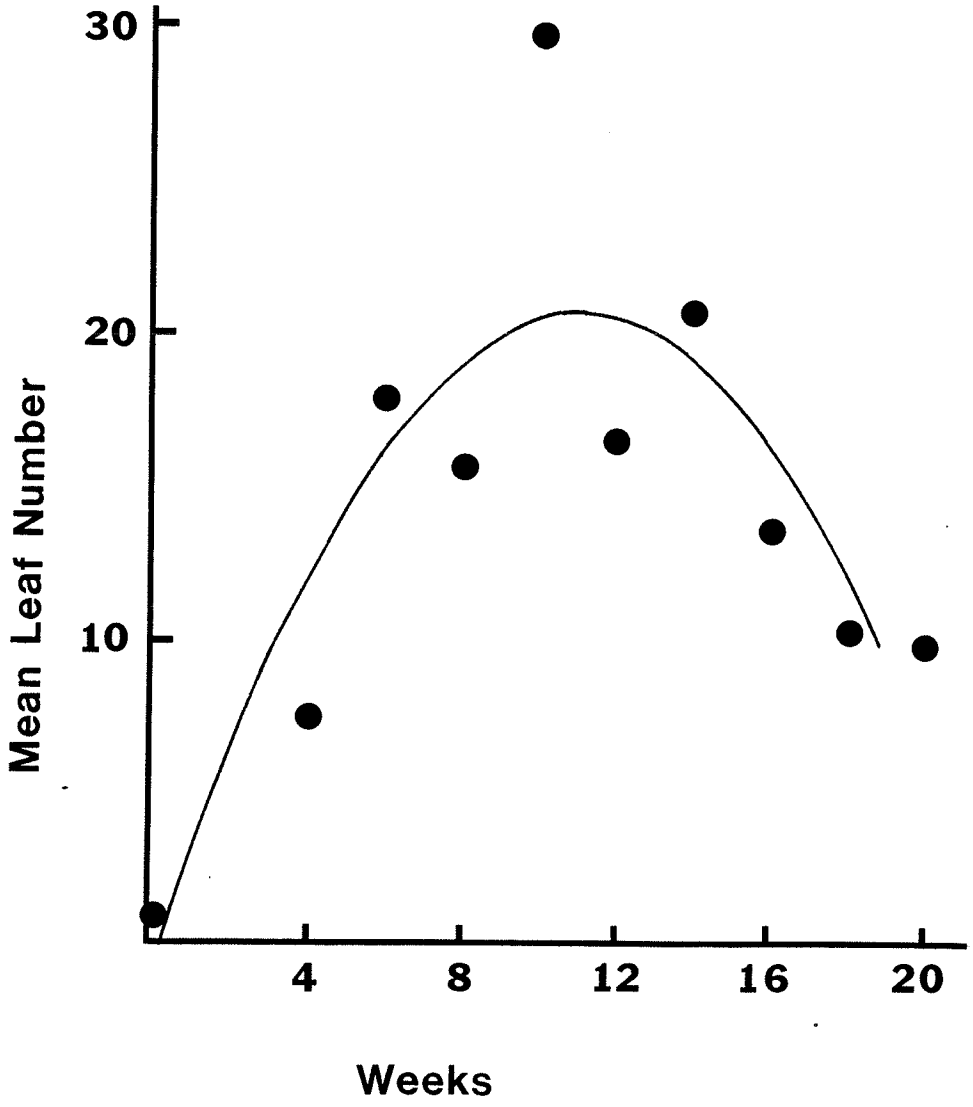
TABLE 17: The effect of length of time of low temperature storage (0/-1°C)[1] on the flowering of Anemone patens.

Weeks of Low Temperature	Total Flower Number
0	0[2]
4	2
6	2
8	3
10	1
12	5
14	4
16	3
18	0
20	0

[1] Alternating 16 hours 0°, 8 hours -1°C.

[2] Values are the total number of flowers from four plants grown in either 2±1°C or 22/18°C.

FIGURE 13. The effect of length of low temperature storage on leaf number of Anemone patens.


30
20
10
Mean Leaf Number
4
8
12
16
20
Weeks

results indicate that the optimum length of low temperature storage was 6 to 16 weeks with a significant decrease in leaf number below or above the optimum storage times.

CONCLUSIONS

The following may be concluded on the basis of the research conducted on *Anemone patens* var. *wolfgangiana*.

1. The germination percent increases with temperatures within the range of 2.2° to 21°C.
2. Light is not required for germination.
3. Damping-off was a problem with *in vitro* germination but not in media.
4. The damping-off of seed and seedlings *in vitro* was reduced, without reducing germination, only with dry benomyl. The fungicides captan (wet or dry), benomyl slurry, ethanol, hot water, and No Damp, significantly reduced germination.
5. Media type does not affect seed germination but is important for seedling survival. Combinations of several components provide the highest survival percents.
6. Moist after-ripening of seed resulted in a decrease in germination. No Damp was successfully used to reduce damping-off of the seed *in vitro*.
7. The rates of the nutrients nitrogen, phosphorus and potassium are important in determining visual appearance, leaf number, and plant dry and fresh weight.

   In general, increased amounts of N, P, and K resulted

in: nitrogen increased leaf numbers and dry shoot weights, phosphorus decreased dry shoot and root weights and root/shoot ratio, and potassium decreased leaf numbers and dry shoot weights.

8. Low temperature storage at temperatures at/or below 0°C from 6 to 16 weeks is essential for obtaining satisfactory termination of dormancy and subsequent leaf and flower display.

Summary

On the basis of the research conducted on Anemone patens var. wolfgangiana it is concluded that the successful greenhouse culture and propagation is possible and practical. At the termination of the fertilizer experiments, the year old plants appeared mature and ready for low temperature treatment. Further research is required to increase flower number and to select for the most vigorous plants.

CHAPTER IV

CONTROLLED ENVIRONMENT PROPAGATION AND CULTURE OF *CYPRIPEDIUM CALCEOLUS* VAR. *PARVIFLORUM* (SALISB.) FERN

Introduction

The approximately 65 native orchid species of Canada vary from large showy to diminutive delicate plants (Sherk, 1965). In North America, approximately 11 species of the largest flowering native orchid, the *Cypripedium*, grow wild. There are also varieties and hybrids (Luer, 1975 and Marshall *et al.*, 1966). The terrestrial *Cypripedium calceolus* L. has three highly variable varieties: *C. calceolus* L. var. *pubescens* (Willd.) Correll, the Large yellow lady's slipper, *C. calceolus* var. *parviflorum* (Salisb.) Fern, the Small yellow lady's slipper, and *C. calceolus* var. *planipetalum*, the Flat-petalled lady's slipper. While the Large and Small lady's slipper may be found over a large range of provinces, the Flat-petalled lady's slipper is believed to be restricted to southern Quebec and Newfoundland (Luer, 1975). The three varieties vary in flower size and colour, the colour and orientation of the sepals and petals, and overall plant size. *C. calceolus* var. *pubescens* produces a large pale yellow pouch 35 to 60 mm in length. The sepals and petals are greenish brown or yellow in colour and slightly twisted. The plant is densely pubescent and grows to heights of 80 cm. The pouch of *C. calceolus* var. *parviflorum* is deep

yellow, 20 to 50 mm long with reddish brown to purple strongly twisted sepals and petals. A fragrance may be present. The less pubescent C. calceolus var. parviflorum grows to heights up to 70 cm. C. calceolus var. planipetalum is much smaller than the other two varieties, growing to heights of only 20 cm. The yellow lip is 20 to 30 mm in length. The flat short sepals and petals are greenish yellow (Bailey and Bailey, 1976, Budd, 1979, Luer, 1975 and Marshall et al., 1966).

The habitats and growth requirements of C. calceolus var. pubescens and C. calceoleus var. parviflorum are similar. In nature the plants usually grow in moist deciduous woods often near swamps or bogs (Bailey and Bailey, 1976, Budd, 1979 and Luer, 1975). According to Reynolds (1979) C. calceolus var. pubescens prefers some direct sun while C. calceolus var. parviflorum prefers very little direct sun. Both prefer soil with neutral, not too acid pH. For growing in the greenhouse or garden, researchers recommend several different media. Sherk (1965) successfully grew C. reginae and C. calceolus in deep flats in the greenhouse for over three years. The medium was rich in humus and neutral in pH. C. calceolus required a moderate amount of moisture while C. reginae needed constant moisture. Both required some shade for part of the day. Whitlow (1977) developed two media to be used in wood or styrofoam containers. The first medium was acid in pH and consisted of brown sphagnum, sand,

loam, and partially rotted pine needles. The second medium was more neutral in pH and consisted of loam, black peat, a portion of acid medium and dried grass clippings. He recommended that the media be allowed to dry before rewatering. Luer, as quoted by Case (1967), stated that moving terrestrial native orchids "is essentially hopeless" due to the fact that the plants exist in a "very delicate balance in only the right kind of soil in the right degree of acidity and moisture, and often in association with certain fungi". Case, on the other hand, felt that the native terrestrials could be grown by skillful gardeners if the needs of the plants were met.

C. calceolus can be propagated by dividing the rhizome. Holman (1975) and Reynolds (1979) found that the division of the Cypripedium stimulates the growth of new stems. Holman suggests that the division can be done at any time of the growing season while Muick (1978) and Taylor and Hamblin (1976) suggest spring is the best time. However, Whitlow (1977) determines that since root growth appears to be most active in fall, the plants should be transplanted and divided in late August to September. The rhizomes are divided by cutting in sections with at least one crown per section. Muick (1978) recommends that only two or three offshoots of Cypripedium be taken off yearly, making sure that all divisions have roots. Divisions as small as one pip or crown can grow and multiply (Holman, 1975). The crowns are commer-

cially called pips (Bailey, 1975).

Vernalization is often required by perennial plants to produce flowers. The process is usually thought of as the chilling period necessary to acquire or speed up the ability of the plant to flower (Chouard, 1960). Chouard states that oxygen and the appropriate length and temperature of chilling are required before vernalization can be complete. In the artificial bog developed by Holman (1975) for growing C. reginae the temperature five cm below the soil surface was 0° ± 0.5°C. He also found that plants refrigerated at +1.5°C upon removal from the frozen bog began to grow. Plants refrigerated at -0.5°C remained dormant until moved to warmer greenhouse temperatures where flowering occurred in one month. Sherk (1965) also recommends temperatures around -0.5° but Case (1980) feels that the plants should not be allowed to freeze and should be kept at +3°C for the cold treatment.

In order that the proper greenhouse culture and propagation of the C. calceolus be established, media, plant division techniques and length of cold storage were studied.

MATERIALS AND METHODS

Plant Collection and Preparation

C. calceolus used in the experiments were collected from site 1 (Figure 14) in a shaded moist grassy thicket during October of 1981, after the shoot growth of the plants had died back for the winter. The plants were dug and the excess soil surrounding the roots removed, retaining sufficient soil on the roots to prevent dehydration. The plants, with the root side down, were placed into plastic flats, packed with moist peat moss, and placed in storage at 7 ± 1°C in the dark. The plants were watered in storage up to 25 weeks as needed to prevent total drying out.

Vernalization of Unpotted Plants

To determine the effect of vernalization on unpotted plants the C. calceolus were placed in cold storage of 7 ± 1°C for up to 25 weeks. Five clumps with various plant numbers were removed prior to the beginning of the treatment to serve as controls and placed immediately in 22/18°C alternating day/night temperatures. Ten plant clumps were removed at each of 14, 17, 18, 19, 20, 22, 23, 24 and 25 weeks to the warmer temperatures. After removal to warmer temperatures the plants were immediately potted up in 4 or 6 inch clay pots with a soil, sand, and peat mix, 1:2:2 by volume. The stand, flower number, flower size, and plant height were

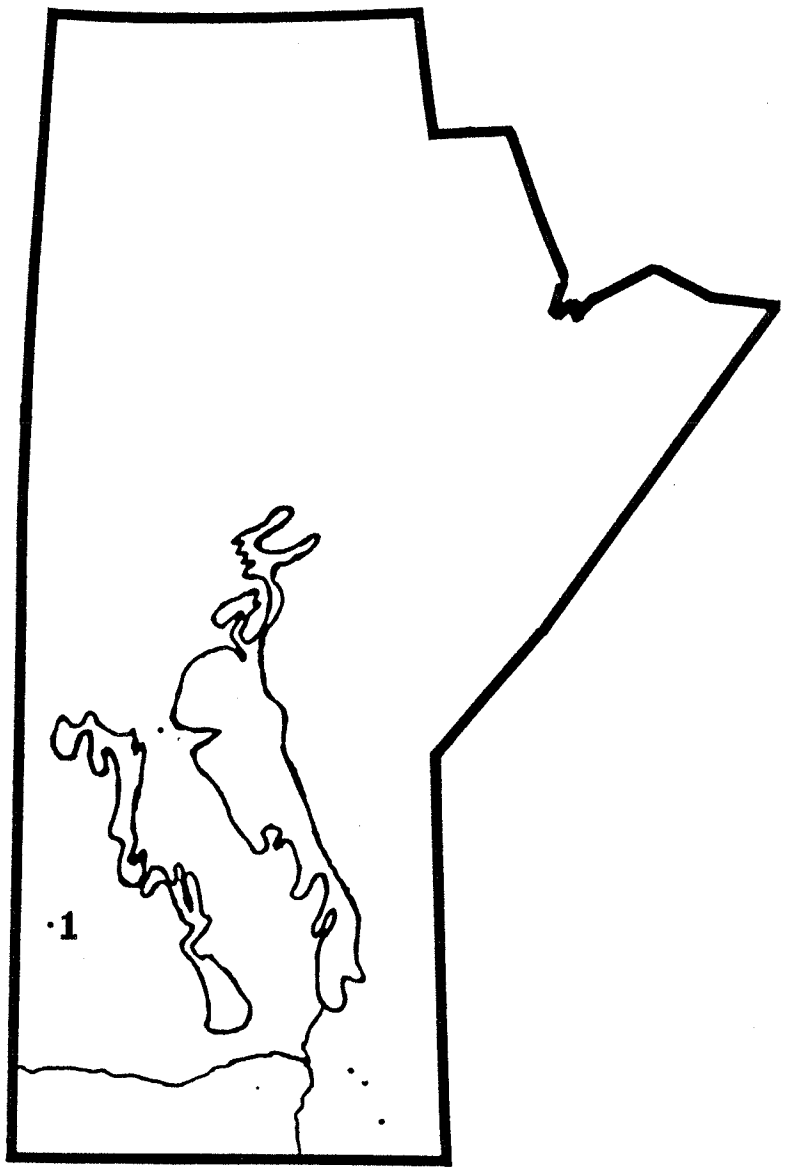


Fig. 14. Out line map of Manitoba showing collection site .

recorded. Measurements were taken when the plants and flowers were at maximum size.

Vernalization of Potted Plants

To determine the effect of cold storage on potted plants the individual plants obtained from divisions of the clumps (Propagation Section) were placed at 7 ± 1°C for up to 25 weeks. The soil was sprinkled with 1/2 teaspoon of Later's Benomyl for each pot prior to storage to decrease rot in storage. Five pots were removed to 23/16°C at each of 0, 10, 11, and weekly from 14 to 25 weeks. In a second trial, the individually potted plants were placed in alternating temperatures of 16 hours at -1°C and 0°C for 8 hours each day. The plants remained in the dark for both cold storage trials. In the second trial, five pots were removed at 0, 10, 14, 16, 18, 20 and 22 weeks to 28/16°C in the growth room. Final plant height and flower size were noted.

Propagation

In order to remove the complicating effect of varying plant stands per pot, and to find a practical method to increase plant numbers, several plant groups were divided up and potted up as individual plants. The divisions were made by cutting the rhizomes with a sharp knife between two dormant crowns being sure to leave several healthy roots attached to each crown (Fig. 15). The effects from the initial

FIGURE 15. Root and crown structure of Cypripedium calceolus var. parviflorum.

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VEUILLEZ ECRIRE A L'AUTEUR POUR LES RENSEIGNEMENTS OU VEUILLEZ CONSULTER L'EXEMPLAIRE DONT POSSEDE LE DEPARTEMENT DES ARCHIVES ET DES COLLECTIONS SPECIALES, BIBLIOTHEQUE ELIZABETH DAFOE, UNIVERSITE DU MANITOBA, WINNIPEG, MANITOBA, CANADA, R3T 2N2.

1. Rhizome
2. Multiple crown
3. Root

division of the plants during collection and the second planned division of the plants were noted.

RESULTS AND DISCUSSION

Dormancy and Vernalization of Unpotted Plants

The unpotted Cypripedium stored at 7±1°C for up to 25 weeks displayed significantly different plant heights for each treatment when grown at 22/18°C. The stands of the plant groups varied from zero to seven with an average stand of 1.52. Stand was measured by counting the living crowns. Analysis of covariance using $\log_{10}$ (height + 12.8) revealed very highly significant differences among treatments. The average height was 22.356 cm. Regression analysis on the mean values indicated a highly significant quadratic regression (Figure 16).

Analysis of variance for flower size using mean lip length for each replicate indicated no significant differences among treatments. Approximately 63 percent of all the plants flowered. Using Chi-squared to test percent flowering per treatment revealed very highly significant differences (Table 18).

The question of whether or not vernalization or termination of plant dormancy was involved was not determined at this time. The results indicated that low temperature was essential for flowering but it was unclear whether or not this was due to the terminating of dormancy or the plant requiring the cold temperature to initiate or accelerate flowering, thus fulfilling the definition of vernalization by

FIGURE 16. The effect of length of low temperature storage (7±1°C) on the height of C. calceolus var. parviflorum.

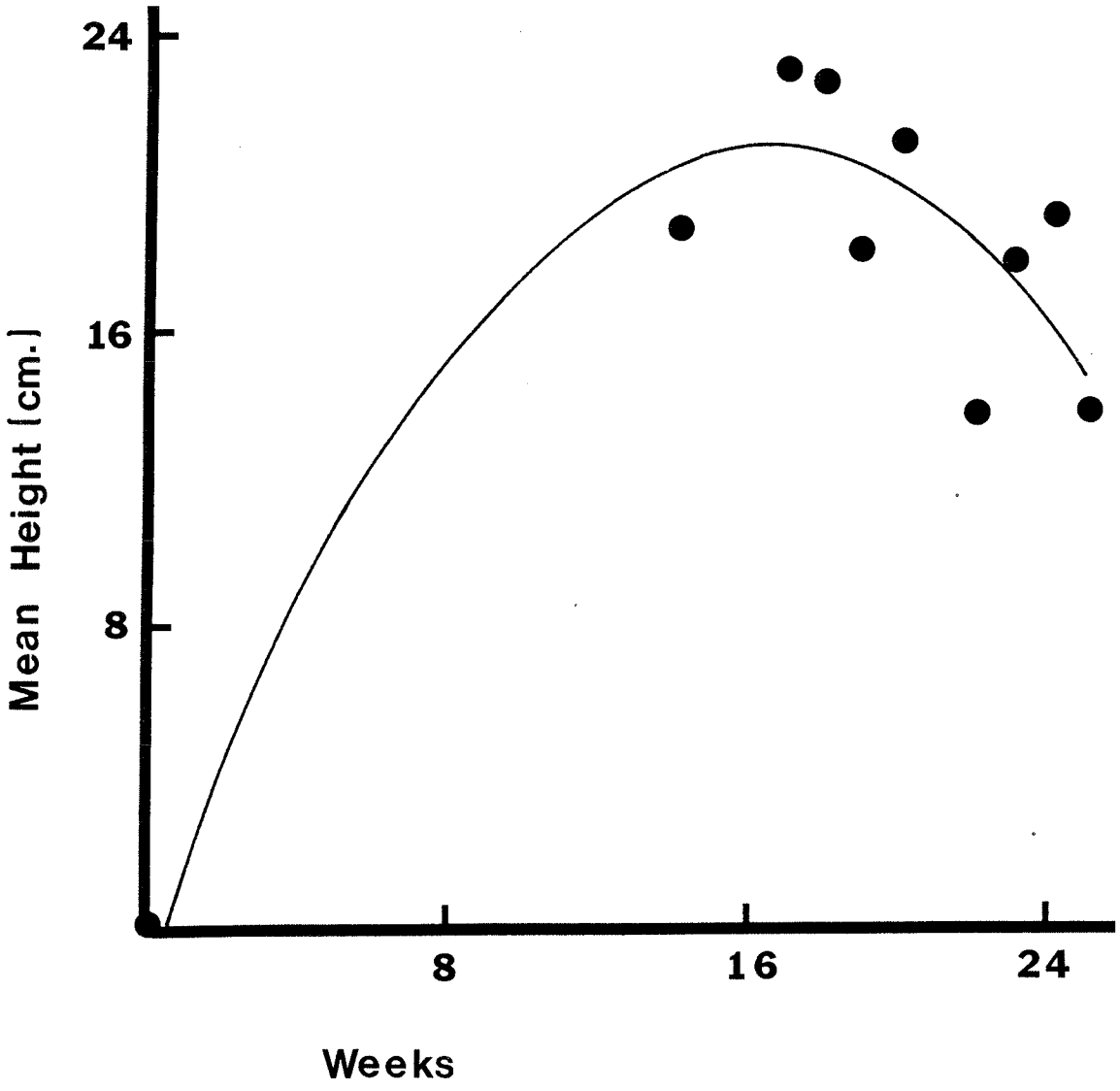

24
16
8
Mean Height (cm.)
8
16
24
Weeks

TABLE 18: The effect of length of low temperature storage (7±1°C) on the flowering of Cypripedium calceolus var. parviflorum.

Treatment Weeks	Flowering Percent
0	0 a[2]
14	78.57 e
17	77.78 e
18	88.24 f
19	54.55 c
20	77.78 e
22[1]	55.56 c
23	69.23 d
24	76.47 de
25	42.11 b

[1] Cold room breakdown occurred two weeks previous.

[2] Values followed by the same letter do not differ significantly as indicated by Chi-squared, 99.5% level.

Chouard (1960). Poincelor (1980) stated that temperate biennial and perennial seed or bulbs can undergo vernalization. Hartmann et al. (1981) defined vernalization as "any temperature treatment that induces or promotes flowering" but also states that "few require low temperature for flower induction ... but low temperatures are required to promote flowering once the flower has formed within the bulb". Whereas dormancy is a "state of suspended growth and metabolism" (Bidwell, 1979) and the plant will not grow when placed under favourable conditions (Salisbury and Ross, 1969). Periods of low temperature are usually required to complete both conditions and in this case not clearly distinguishable.

The colours of the flowers varied from 12A to 12C (Royal Horticultural Society of London, 1969) in the yellow group. Individual flowers had different patterning of the 183B reddish purple stripes on the sepals, petals and interior of the lip. Flowers also differed slightly in the shape of the convolutions on the top 'neck' of the lip. The leaves were all within the 138B green group. The colouring and plant height indicate the variety to be C. calceolus var. parviflorum as outlined by several authors (Bailey & Bailey, 1976, Budd, 1979, Luer, 1975, and Marshall et al. 1966).

Vernalization and Dormancy of Potted Plants

Upon removal from 7±1°C and growing at 23/16°C, the singly potted crowns turned green and began growth. The over-

all mean height of the plants was 9.536 cm, 12.82 cm lower than the previous trial. Analysis of variance revealed significant differences in height among the treatments. The control treatment was significantly different than all but the 20 week treatment. The 20 week treatment was not significantly different than any other treatment.

Regression analysis revealed a highly significant quadratic regression (Figure 17) although the data indicated no significant regression when the control treatment is excluded.

Analysis of variance for lip length among treatments indicated no significant differences. The mean lip length was 2.831 cm. The percentage of plants flowering was 14.7, a large decrease over the 60 percent achieved in the previous trial.

The days to flowering decreased as the period of low temperatures storage increased (Figure 18). If the infinite days to flowering of the control was considered, the trend would be more evident. The data also seem to indicate a low of 17 weeks and a gradual increased number of days to flower thereafter. More replicates would be necessary to determine the exact patterns. This overall decrease in days to flower as low temperature storage time increases possibly indicates that vernalization is occurring because vernalization is partially defined as the acceleration of flowering by a chilling treatment (Chouard, 1960). The gradual increase in

FIGURE 17. The effect of length of low temperature storage (7±1°C) on the height of potted C. calceolus var. parviflorum.

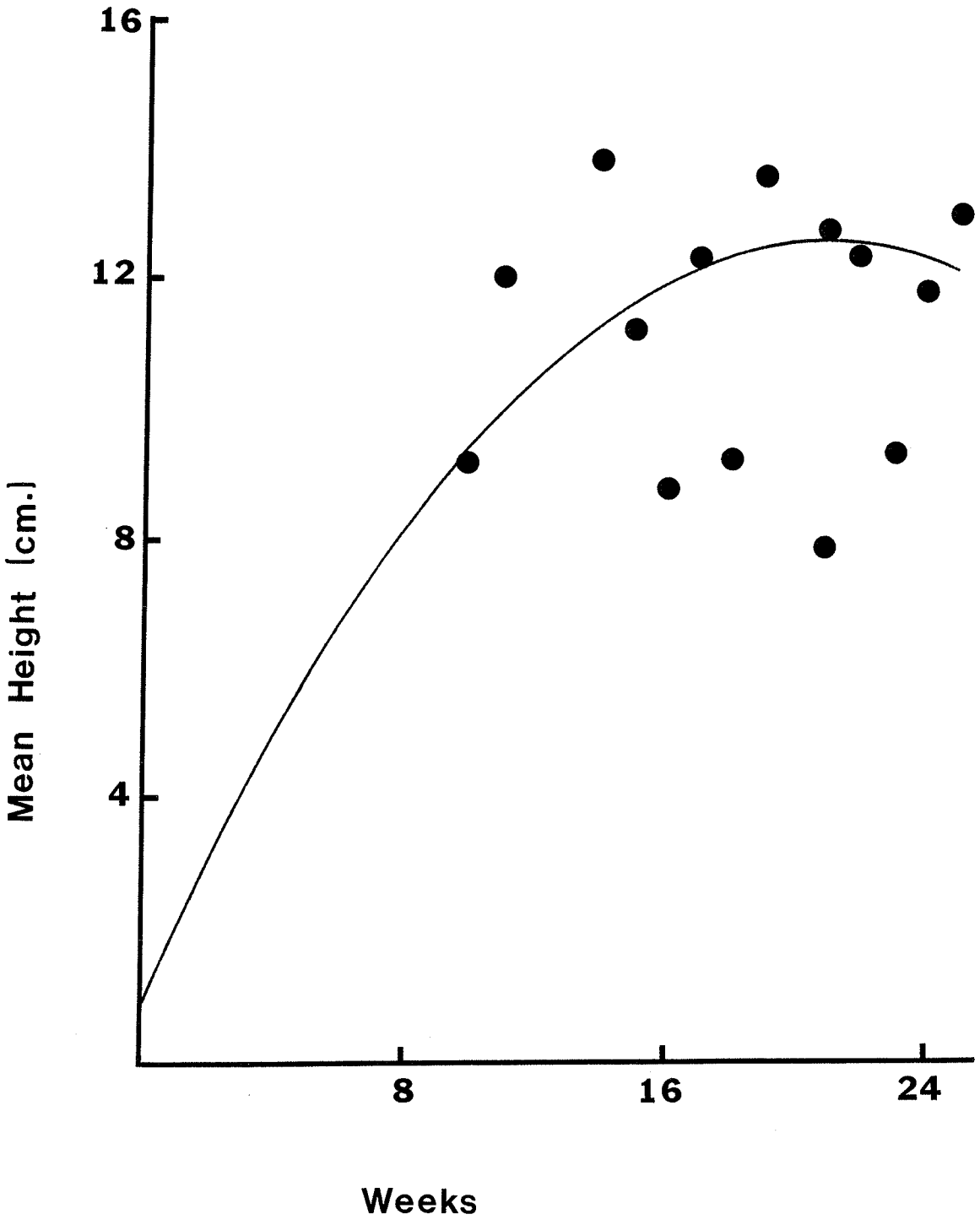
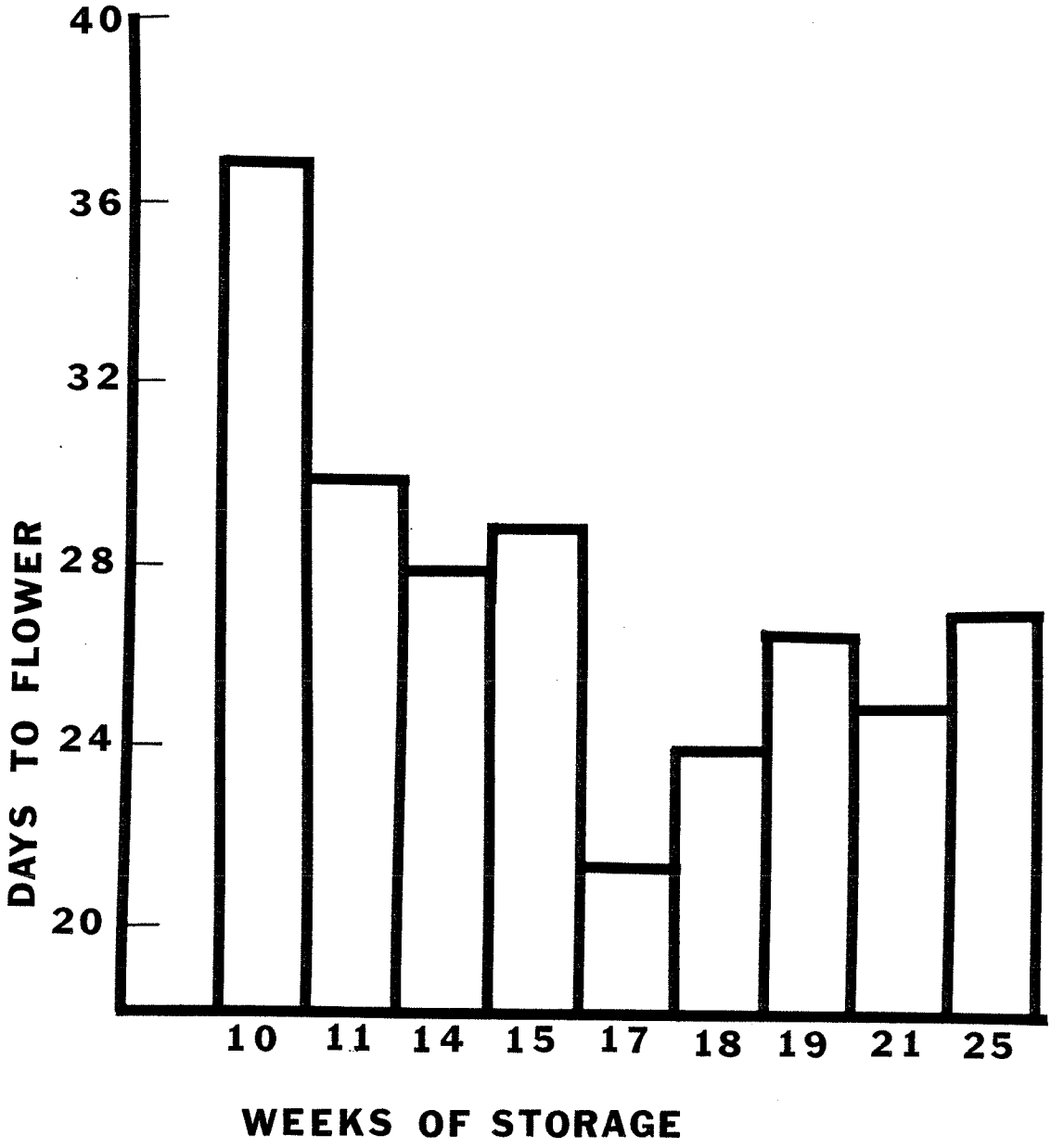

16
12
8
4
Mean Height (cm.)
8
16
24
Weeks

FIGURE 18. The effect of length of low temperature storage (7±1°C) on the days to flowering of C. calceolus var. parviflorum.


40
36
32
28
24
20
DAYS TO FLOWER
10
11
14
15
17
18
19
21
25
WEEKS OF STORAGE

flowering time following 17 weeks may be due to over-vernalization. Salisbury and Ross (1969) state that a "slight drop in the level of vernalization attained by the plant occurs with extremely long vernalization time (several months)".

There was no visible growth of one cold-treated crown. However, this plant was not dead because when checked 4.5 months after the termination of the experiment, the crown had divided and produced three visually healthly crowns. In addition, one crown of the control treatment, after the experiment was terminated, became exposed to light due to repeated waterings washing away the soil from the crown. This plant spontaneously flowered without elongation of the leaves or stem. The flower was normal in terms of colour and correct number of parts, but was distorted in the shape of the inflated lip.

The second low temperature storage trial of the potted plants, where the plants were stored at -1/0°C, appeared to weaken the plants further. The overall mean height of the plants decreased to 3.606 cm. The lip length of the plants remained stable at a mean length of 2.617 cm. Neither of the analyses of variance on height or lip length indicated any significant differences among treatments. The percent flowering declined to 11.4 percent. More than 50 percent of the plants, not including the controls, failed to grow.

Propagation

The initial collection and division of the plants stimulated a crown increase from 59 to 100, indicating the plants can be successfully dug, stored, transplanted and grown indoors for at least one year. This supports statements by Holman (1975) and Reynolds (1979) that division stimulated the growth of new crowns. Holman found that transplanted divided crowns increased by 27 percent while undisturbed plants increased only three percent. An increase of 70 percent occurred with the Cypripedium in this experiment, a much greater increase than noted by Holman. However, his plants grew in an artificial bog outdoors while the plants in this experiment were kept in controlled environment rooms.

The plants used in the first potted low temperature storage trial were uprooted and counted 4.5 months after completion of the study. Analysis of variance indicated significant differences among the number of previous storage weeks and crown numbers (Table 19). The greatest crown increases occurred with the intermediate treatments while the poorest occurred with the control treatment which received no chilling treatment.

The previous height of the plant significantly affects the increase in number of crowns (Table 20). There was no significant increase or decrease in number crowns for the smallest plants according to the Chi-squared test at 95 percent level. Medium and large plants displayed significant

TABLE 19: The effect of low temperature storage (7±1°C) time on the increase in crown numbers of Cypripedium calceolus var. parviflorum.

Previous Treatment Weeks	Crown Number	
0	0[1]	c[2]
10	0.8	abc
11	2.4	ab
14	2.2	ab
15	1.4	abc
16	0.4	ab
17	2.2	ab
18	3.0	a
19	2.8	a
20	2.6	ab
21	2.2	ab
22	2.2	ab
23	0.4	ab
24	1.8	ab
25	2.4	ab

[1] Transformed data $\sqrt{x + 0.5}$ for analysis.

[2] Values followed by the same letter do not differ significantly as indicated by Duncan's Test, 95% level.

TABLE 20: The effect of previous plant height on the increase of crown numbers of Cypripedium calceolus var. parviflorum.

Height Groups	Crown Numbers		
	Original	Final	Total
H < 6.0 cm	19	17	36
6.0 < H < 14.8	39	71	110
H > 14.8	17	46	63
	75	134	209

1 Plant height transformed by $\log_{10}$ (H + 12.8) for analysis.

increases in plant crown numbers. This indicates that the larger plants were healthier and readily produce crown divisions. A certain minimum plant height may be required for plant survival and increase. Further experiments are required to determine the smallest plant height that can survive and multiply.

Also noted during the experiment was that the plants that previously flowered had relatively large increases in plant numbers while nonflowering plants had only a moderate increase in plant numbers. The flowered plants increased in number from 11 to 31, an increase of 180 percent, while the plants which did not flower increased only 61 percent, indicating that flowered plants were healthier and more capable of increase.

CONCLUSIONS

The following may be concluded on the basis of the research conducted on Cypripedium calceolus:

1. The plants used in this experiment were Cypripedium calceolus var. parviflorum.
2. The plants do not grow or flower without a chilling treatment.
3. The height of the plant increases with increasing time of low temperature storage up to an optimum level of 17 to 20 weeks after which there is a slow decrease.
4. Flower size is not affected by the length of time or temperature of low temperature storage.
5. Days to flower decreases with increasing length of low temperature storage up to 17 weeks and then increases slowly.
6. Plant number and size, and flower number decrease over successive experiments indicating conditions were not ideal.
7. Transplanting and division of the plants stimulated an increase in plant numbers.
8. Plants which flower and are of a certain minimum size are more likely to survive and increase in numbers.

Summary

On the basis of the research conducted, it is concluded that Cypripedium calceolus var. parviflorum can be grown indoors. Further research is required to establish methods of prolonging the life of plants indoors and selecting the most desirable plants.

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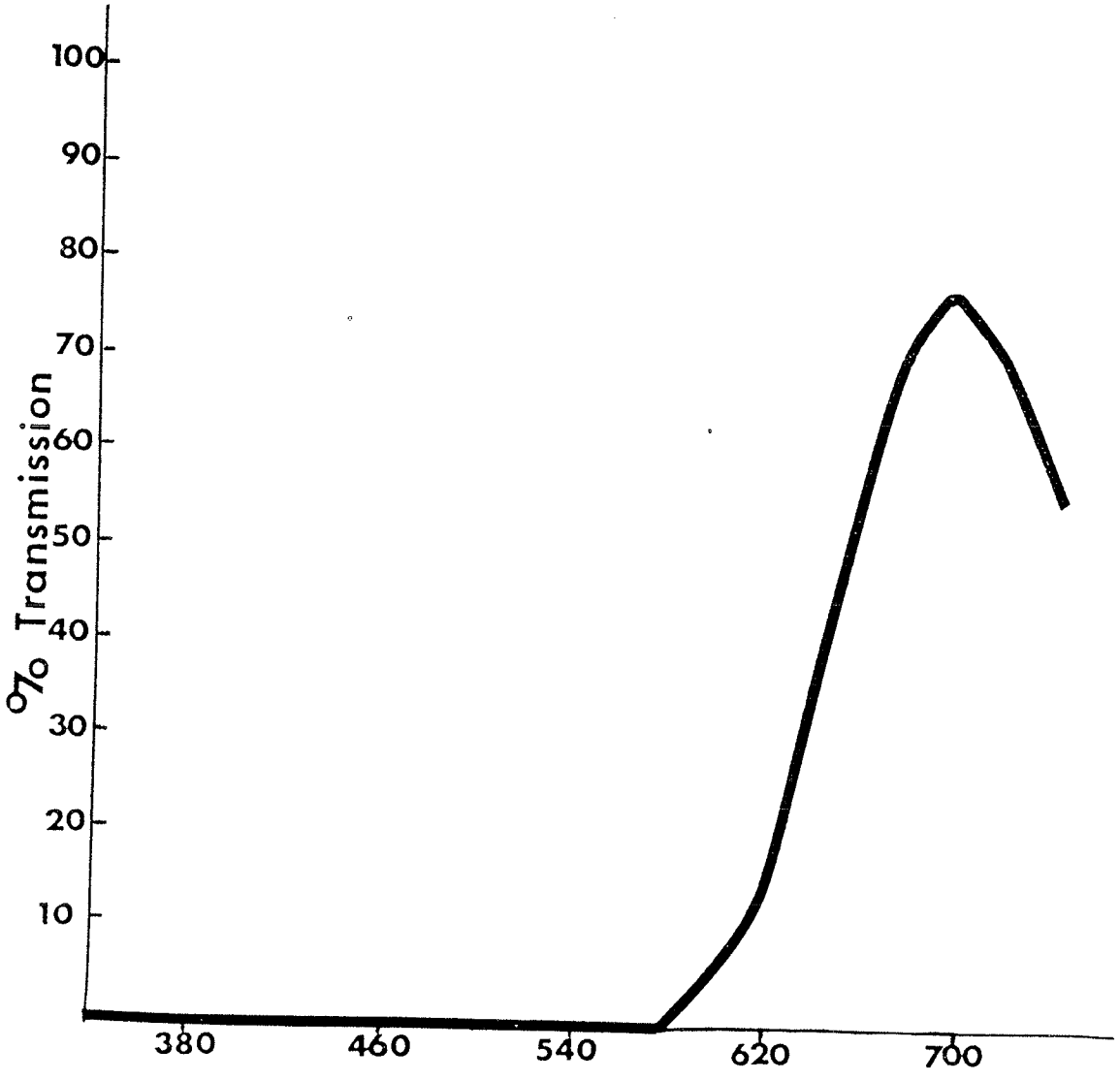
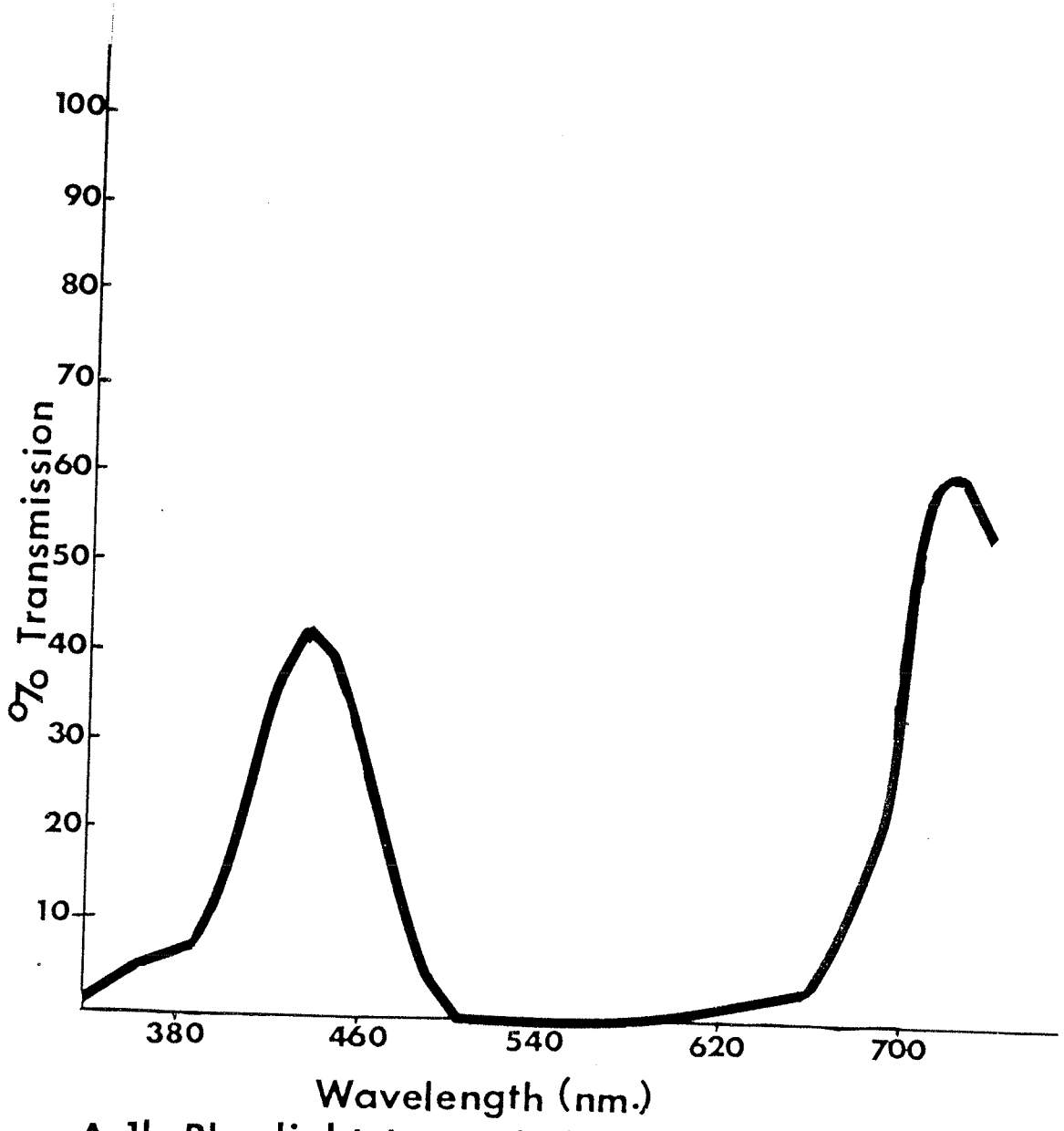
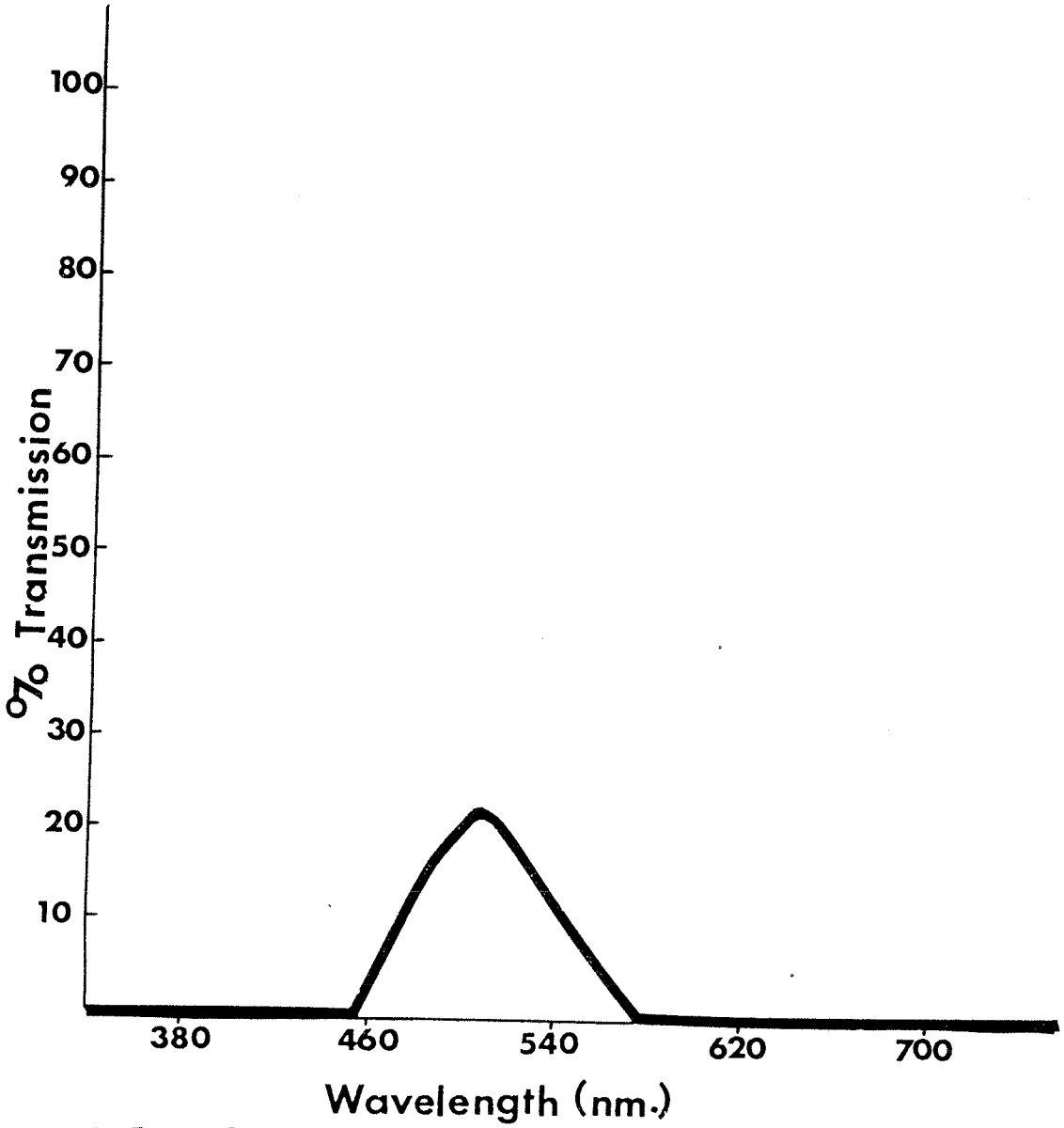
APPENDIX



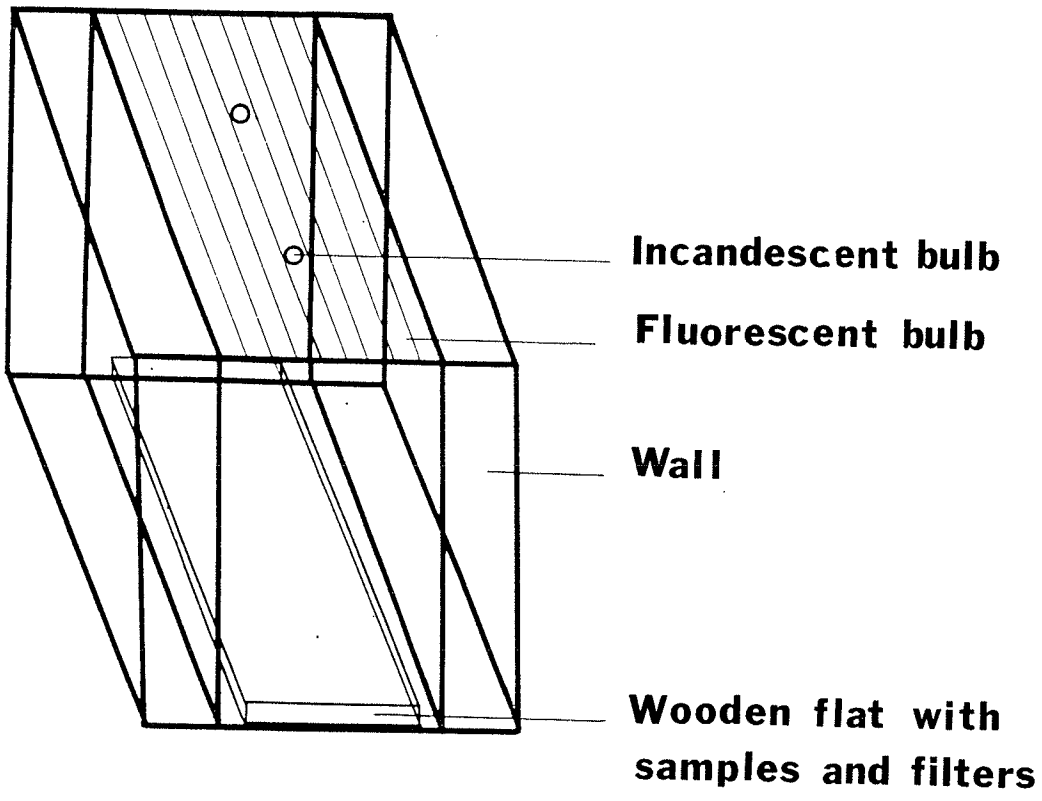
A. 1a Red light: transmission spectrum of Roscolux #27 filter



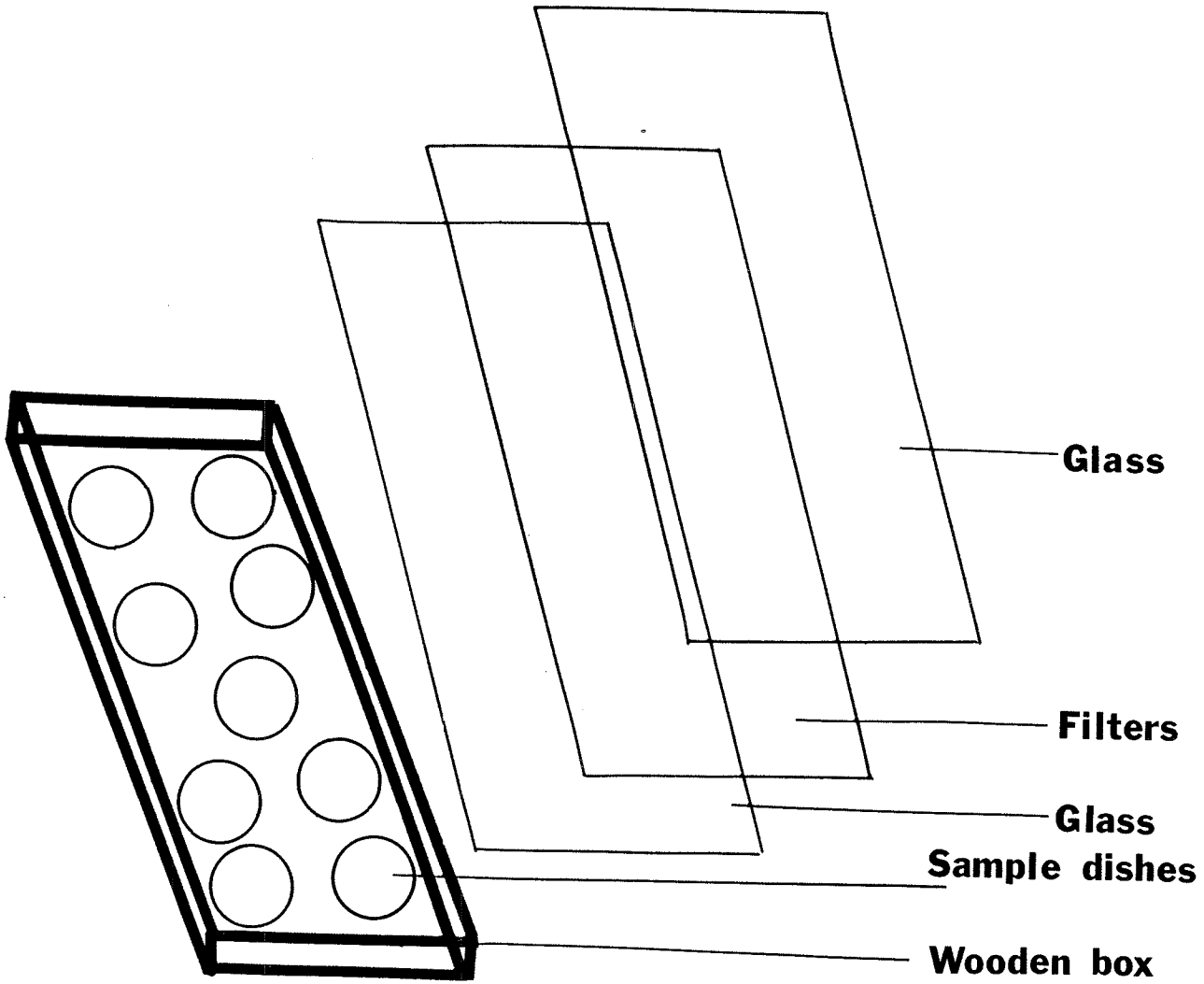
A.1b Blue light: transmission spectrum of Roscolene #85 filter



A.1c Green light: transmission spectrum of Roscolux #90 filter



APPENDIX 1d. Experimental set-up for light experiments



APPENDIX 1e. Exploded view of light experiment set-up

APPENDIX 2. Analysis of variance for the germination of *Castilleja* seed in the light and dark at various temperatures.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Among treatments	9	10,798.386	1,199.821	50.887[xxx1]
temperatures	4	1,330.754	332.689	14.110[xxx]
light conditions	1	9,303.750	9,303.750	394.562[xxx]
interaction	4	163.882	40.971	1.738[ns]
Error	10	235.779	23.578	

1 [xxx] significance level of $P < .001$

APPENDIX 3. Analysis of variance of *Castilleja* seed germination on a thermogradient.

1. TRIAL #1

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Between treatments	4	3.362	0.841	2.541[ns]
Within treatments	20	6.618	0.331	

2. TRIAL #2

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Between treatments	2	0.403	0.202	1.981[ns]
Within treatments	13	1.327	0.102	

APPENDIX 4. Analysis of variance for *Castilleja* seed germination under low light intensity with various exposure times and filters.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Treatments	11	2.354	0.214	2.404[x1]
filters	3	0.460	0.153	1.719[ns]
exposures	2	1.458	0.729	8,191[xx]
interaction	6	0.436	0.073	0.820[ns]
Error	42	3.756	0.089	

1 [x] significance level of $P < .05$
[xx] significance level of $P < .01$

APPENDIX 5. Analysis of variance for Castilleja seed germination under high light intensity with various exposure times and filters.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Times (T)	2	3.289	1.644	6.292 xxl
Filters (F)	2	5.943	2.971	11.368 xx
Exposures (E)	2	2.760	1.380	5.281 xx
TxF interaction	4	0.672	0.168	0.643 ns
TxE interaction	4	0.784	0.196	0.750 ns
FxE interaction	4	0.874	0.218	0.836 ns
TxFxE interaction	8	1.916	0.240	0.916 ns
Error	54	14.114	0.261	

1 xx significance level of $P < .01$

APPENDIX 6a. Analysis of the effect of hosts on the size of Castilleja coccinea.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Between treatments	7	108.414	15.488	3.42 xl
Within treatments	22	99.560	4.526	

APPENDIX 6b. Analysis of variance of the effect of hosts on the size of C. coccinea forma lutescens.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Between treatments	7	135.415	19.345	2.91 x
Within treatments	22	146.521	6.660	

APPENDIX 6c. Analysis of variance of the effect of hosts on the dry weight of C. coccinea.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Between treatments	7	3.260	0.466	2.05 s
Within treatments	22	5.002	0.227	

APPENDIX 6d. Analysis of variance of the effect of hosts on the dry weight of C. coccinea forma lutescens.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Between treatments	7	1.312	0.187	1.22 ns
Within treatments	22	3.383	0.154	

APPENDIX 6e. Chi-square test of effect of hosts on the survival of C. coccinea.

Host	Dead	Alive
Ageratum	3	0
C. coccinea	4	2
C.c.f. lutescens	3	3
Chrysanthemum	0	3
Control	2	1
Senecio	3	0
Tagetes	1	2
Viola	3	0
	$x^2 = 12.055^{ns}$	

APPENDIX 6f. Chi-square test of effect of hosts on the survival of C. coccinea forma lutescens.

Host	Dead	Alive
Ageratum	2	1
C. coccinea	2	4
C.c.f. lutescens	5	1
Chrysanthemum	2	1
Control	2	1
Senecio	1	2
Tagetes	3	0
Viola	3	0
	$x^2 = 5.27^{ns}$	

APPENDIX 6g. Analysis of variance of the effect of C. coccinea and C. coccinea forma lutescens on the dry weight of the hosts.

1. Ageratum houstonianum 'Blue Danube'

Source of Variation	D.F.	S.S.	M.S.	F-ratio
treatments	2	0.202	0.101	0.24 ns
error	6	2.484	0.414	

2. Chrysanthemum parthenium aureum 'Golden Boy'

Source of Variation	D.F.	S.S.	M.S.	F-ratio
treatments	2	6.94	3.47	0.461 ns
error	6	45.04	7.51	

3. Senecio vira-vira 'Silver Dust'

Source of Variation	D.F.	S.S.	M.S.	F-ratio
treatments	2	38.66	19.33	2.44 ns
error	6	47.52	7.92	

4. Tagetes patula 'Yellow Boy'

Source of Variation	D.F.	S.S.	M.S.	F-ratio
treatments	2	1.876	0.938	1.02 ns
error	6	5.517	0.920	

5. Viola cucullata

Source of Variation	D.F.	S.S.	M.S.	F-ratio
treatments	2	5.80	2.90	1.39 ns
error	6	12.55	2.09	

APPENDIX 6h. Analysis of variance of the effect of C. coccinea and C. coccinea forma lutescens on the volume of hosts.

1. Ogeratum houstonianum 'Blue Danube'

Source of Variation	D.F.	S.S.	M.S.	F-ratio
treatments	2	925.85	462.93	0.37 ns
error	6	7,534.56	1,255.76	

2. Chrysanthemum parthenium aureum 'Golden Boy'

Source of Variation	D.F.	S.S.	M.S.	F-ratio
treatments	2	1,862.61	931.31	0.91 ns
error	6	6,129.21	1,021.53	

3. Senecio vira-vira 'Silver Dust'

Source of Variation	D.F.	S.S.	M.S.	F-ratio
treatments	2	4,528.32	2,264.16	1.30 ns
error	6	10,439.70	1,739.95	

4. Tagetes patula 'Yellow Boy'

Source of Variation	D.F.	S.S.	M.S.	F-ratio
treatments	2	1,104.68	552.34	0.24 ns
error	6	13,586.39	2,264.40	

5. Viola cuculatta

Source of Variation	D.F.	S.S.	M.S.	F-ratio
treatments	2	5,306.88	2,653.44	1.35 ns
error	6	11,761.60	1,960.27	

1 s significance level of P < .1
x significance level of P < .05
xx significance level of P < .01

APPENDIX 7. Analysis of variance for the germination of Anemone patens var. wolfgangiana seed in light and dark at various temperatures.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Light conditions	1	0.43	0.43	0.015[ns]
Temperatures	2	13.31	6.66	0.232[ns]
Replicates	1	5.69	5.69	0.198[ns]
Interactions	2	33.77	16.89	0.589[ns]
Error	5	143.45	28.69	

APPENDIX 8. Analysis of variance for the germination of Anemone patens var. wolfgangiana seed on a thermogradient.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Between treatments	3	19.836	6.612	11.41[xxx1]
Within treatments	12	6.954	0.580	

1 significance level of P < .01

APPENDIX 9. Analysis of variance for the germination of A. patens var. wolfgangiana seed in vitro treated with various fungicides.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Treatments	7	3500.567	500.081	5.22 [xx1]
Replicates	2	82.535	41.268	0.43 [ns]
Error	14	1342.538	95.896	

1 significance level of P < .01

APPENDIX 10. Analysis of variance of the effect of after-ripening on the germination of A. patens var. wolfgangiana seed.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Treatments	4	1.356	0.339	3.459 ns
Replicates	2	0.368	0.184	1.878 ns
Error	7	0.685	0.098	

APPENDIX 11. Analysis of variance of the effect of longer periods of after-ripening on the germination of treated and untreated A. patens var. wolfgangiana seed.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Seed treatments	1	3.422	3.422	63.37 xxx1
Weeks	8	3.974	0.497	9.20 xxx
Replicates	2	0.126	0.063	1.17 ns
Interactions	8	0.897	0.112	2.07 ns
Error	32	1.730	0.054	

1 significance level of P < .001

APPENDIX 12a. Analysis of variance of the effect of various fertilizer treatments on the leaf number of A. patens var. wolfgangiana plants.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Locations (L)	2	155.556	77.778	6.667 x1
Error a	6	70.000	11.667	
Replicates over Locations	8	225.556		
Treatments (T)	6	611.969	101.995	3.191 x
LxT interaction	12	575.555	47.963	1.502 ns
Error	36	1149.333	31.926	

1 significance level of P < .05

APPENDIX 12b. Analysis of variance on the effect of various fertilizer treatments on the cross sectional area of A. patens var. wolfgangiana plants.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Locations (L)	2	13,070.428	6,535.214	1.30 ns
Error a	6	30,119.165	5,019.861	
Replicates over Locations	8	43,189.593		
Treatments (T)	6	79,414.704	13,235.784	1.662 ns
LxT interaction	12	58,650.336	4,887.528	0.614 ns
Error	36	286,745.895	7,965.164	

APPENDIX 12c. Analysis of variance on the effect of various treatments on the fresh weights of A. patens var. wolfgangiana.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Locations (L)	2	1,248.409	624.205	30.64xxx1
Error a	6	122.219	20.370	
Replicates over Locations	8	1,370.628		
Treatments (T)	6	2,528.527	421.421	6.005xxx
LxT interaction	12	1,685.690	140.474	2.002 ns
Error	36	2,526.511	70.181	

1 significance level of P < .001

APPENDIX 12d. Analysis of variance of the effect of various fertilizer treatments on the total dry weights of A. patens var. wolfgangiana.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Locations (L)	2	251.34	125.67	36.11xxx1
Error a	6	20.86	3.48	
Replicates over Locations	8	272.20		
Treatments (T)	6	433.36	72.23	8.14xxx
LxT interaction	12	234.88	19.57	2.21x
Error	36	319.263	8.87	

1 x significance level of P < .05
xxx significance level of P < .001

APPENDIX 12e. Analysis of variance of the effect of various fertilizer treatments on the dry weights of the shoots of A. patens var. wolfgangiana.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Locations (L)	2	14.669	7.335	6.656 [x1]
Error a	6	6.612	1.102	
Replicates over Locations	8	21.281		
Treatments (T)	6	57.590	9.560	4.145[xxx]
LxT interaction	12	28.956	2.413	1.042 [ns]
Error	36	83.359	2.316	

1 [x] significance level of P < .05
[xxx] significance level of P < .001

APPENDIX 12f. Analysis of variance of the effect of various fertilizer treatments on the dry weights of the roots of A. patens var. wolfgangiana.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Locations (L)	2	111.678	55.839	24.465[xxx1]
Error a	6	13.694	2.282	
Replicates over Locations	8	125.372		
Treatments (T)	6	181.369	30.228	6.880[xxx]
LxT interaction	12	134.836	11.236	2.557[x]
Error	36	158.175	4.394	

1 [x] significance level of P < .05
[xxx] significance level of P < .001

APPENDIX 13. Analysis of variance of the effect of length of low temperature storage and growing temperature on flower number of A. patens var. wolfgangiana

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Temperatures	1	16.00	1.60	1.22 [ns]
Times	9	7.00	0.78	0.60 [ns]
Interactions	9	9.40	1.04	0.79 [ns]
Replicates	1	3.05	3.05	2.32 [ns]
Error	19	24.95	1.31	

APPENDIX 14. Analysis of variance of the effect of low temperature storage on the leaf number of A. patens var. wolfgangiana.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Temperatures (T)	1	5.725	5.725	6.312[xl]
Weeks (W)	9	47.804	5.312	6.114[xxx]
WxT interactions	9	6.099	0.678	0.745 [ns]
Replicates	1	1.243	1.243	1.371 [ns]
Error	18	17.236	0.907	

1 [x] significance level of $P < .05$
[xxx] significance level of $P < .001$

APPENDIX 15a. Analysis of covariance of the effect of length of storage on the height of Cypripedium calceolus unpotted during storage.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Weeks	9	2.402	0.266	19.92 [xxx]
Replicates	9	0.216	0.024	1.79 [ns]
Stand	1	1.769	1.769	132.01 [xxx]
Error	80	1.072	0.013	

APPENDIX 15b. Analysis of variance of regression analysis of the mean height of unpotted Cypripedium calceolus.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Due to regression	2	356.355	178.178	23.637 [xxx]
Due to residual	7		52.767	7.538
				r = 0.871

APPENDIX 15c. Chi-square analysis of effect of length of storage on amount of flowering of Cypripedium calceolus.

Weeks of Storage	Flower Number	Plant Number
0	0	10
14	11	14
17	14	18
18	14	15
19	12	22
20	14	18
22	10	18
23	9	13
24	13	17
25	8	19
		$x^2 = 32.96$ xxx1

1 xxx significance level of $P < .001$

APPENDIX 15d. Analysis of variance of effect of length of storage on flower size of Cypripedium calceolus.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Between weeks	8	0.00243	0.00030375	1.454 ns
Within weeks	63	0.01316	0.00020892	

APPENDIX 16a. Analysis of variance of effect of length of storage on height of potted Cypripedium calceolus.

1. TRIAL #1

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Weeks	14	0.426	0.030	2.24 ns
Error	60	0.816	0.136	

2. TRIAL #2

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Weeks	6	0.261	0.043	2.01 ns
Error	28	0.605	0.022	

APPENDIX 16b. Analysis of variance of regression analysis of height of potted Cypripedium calceolus.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Due to regression	2	106.246	53.123	9.39 xxx1
Due to residual	12	67.961	5.660	
				r = 0.610

APPENDIX 16c. Analysis of variance of length of storage on flower size of potted Cypripedium calceolus.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Between weeks	9	0.000613362	0.000068151	8.45 ns
Within weeks	12	0.000016127	0.0000080635	

1 significance level of P < .001

APPENDIX 17. Analysis of variance of the effect of number of weeks storage on crown numbers of Cypripedium calceolus.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
Weeks	14	7.918	0.586	2.14 x1
Error	60	15.891	0.265	

1 significance level of P < .05