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FACTORS AFFECTING THE GERMINATION OF ORCHID SEEDS[1]

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INTRODUCTION

The Orchidaceae is one of the largest and most diverse of all plant families, consisting of 500–800 genera and 20,000–30,000 species (Garay 1960, Schultes and Pease 1963). It is also among the most widely distributed. Its representatives may be found from the Arctic to the Antarctic; in bogs, deserts, valleys, plains, hills, mountains, and even below ground (Hatch 1953).

Sizes of orchid plants vary from a few (3–4) mm. to several meters, whereas flowers may range from 2–3 mm. to 15–20 cm. or more in diameter. Some flowers are beautiful, e.g., *Cattleya* Lindl. and *Phalaenopsis* Blume, but

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others are grotesque, e. g., *Gongora* Ruiz & Pav. and *Stanhopea* Frost & Hooker. They may be of almost any color from pure white to almost pitch black (although none are pure black).

These wide variations necessitate numerous adaptive characteristics. Growth habits and forms are often the result of such adaptations; for example, certain orchids may contain little or no chlorophyll (Senn 1927) and are therefore saprophytic or parasitic (Burgeff 1932, Hamada 1939, Campbell 1962, 1963, Hamada and Nakamura 1963). The specialized flower structure has resulted in very specific pollination mechanisms (Darwin 1888) which include pseudocopulation (Ames 1948), self pollination (Knudson 1956, Summerhays 1951), and cross pollination (Dunsterville and Garay 1959). Extremely close relationships between flowers, their structures, and their pollinators have evolved. (Dunsterville and Garay 1959, Dodson and Frymire 1961, Dodson 1962). The pollinating agents may be insects *(Lepidoptera, Diptera,* and *Hymenoptera),* birds, and possibly bats (Dunsterville and Garay 1959, Dodson and Frymire 1961, Dodson 1962).

Perhaps the most interesting adaptive features of the Orchidaceae are those occurring in the physiology of their seed germination (Constantin 1917). Unfortunately, the evolutionary origins of these features are lost in antiquity and are now merely matters for speculation (Ames 1948). The subtle complexity of conditions required by germinating orchid seeds may best be appreciated by recalling that more than 2,000 years elapsed between the original description of what Dioscorides assumed to be an orchid (Ames 1948, Schultes and Pease 1963) by Theophrastus and the first published description of an orchid seedling (Salisbury 1804). Ninety-five more years elapsed before it was discovered almost by accident that in nature orchid seeds will germinate only if infected by a fungus (Bernard 1899). Finally, 20 additional years had to pass before orchid seeds were germinated asymbiotically on a medium containing inorganic salts and sucrose (Knudson 1921, 1922). At present, quantities of orchid seeds are easily germinated asymbiotically and yet our knowledge of their exact requirements is still limited.

At least three distinct periods may be delineated in the investigations dealing with orchid seed germination. During the initial period, investigations were at first limited to observation and later, following the discovery of orchid mycorrhiza, to studies of symbiotic relationships (Bernard 1899, 1900, 1903, 1904a, b, 1905, 1906a, b, 1908, 1909, Ramsbottom 1922a, b, 1929, Wolff 1923a, Lami 1927a, Cordemoy 1904, Burgeff 1936, 1959). In the early phases of the second period, which began with Knudson's publications, the merits of symbiosis and asymbiosis were argued, sometimes violently, by their partisans (Constantin 1925, 1926, Constantin and Magrou 1922, Knudson 1924, 1925, 1927, 1929, 1930, Burgeff 1936, 1959, Bultel 1924-1925, 1926). Later the effects of various ions (LaGarde 1929, Wynd 1933a), sugars (Quednow 1930, Wynd 1933b), vitamins (Noggle and Wynd 1943, Meyer 1943, Mariat 1944, 1948, 1952, Bahme 1949), hormones (Mariat 1952, Withner 1959a), and other complex organic additives (Knudson 1922, Quednow 1930) were examined. At present investigators are attempting the study of the physiology of orchid seed germination in

the light of modern physiological, biochemical, and molecular biological concepts (Raghavan and Torrey 1963, 1964, Arditti 1965a, c, In Press a, In Press c).

THE ORCHID SEED

The structure and size of the orchid seed are among the most striking characteristics of the Orchidaceae. Orchid seeds are minute, weigh from 0.3 to 14 μg. (Burgeff 1936, Harley 1951), and measure from 0.250 to 1.2 mm. in length (Knudson 1922, Hoene 1940, 1949) and 0.090 to 0.270 mm. in width (White 1927, 1939, Knudson 1929, Davis 1946, Scott and Arditti 1959, Kupper and Linsenmaier 1961). They are produced in large numbers, a supposed characteristic of higher groups (Rolfe 1962), ranging from 1,300 to 4,000,000 seeds per capsule (Darwin 1888, Anon. 1890, Harley 1951, Tournay 1960, Arditti 1961). The information in Table I, although approximate and by no means complete, provides an idea of the sizes, weights, colors, and numbers of some orchid seeds. It should be remembered, however, that often only one seed of a vast number may germinate and grow to blooming size (Anon. 1965).

The seed, which may at times be apomictic (Stoutamire 1964b), has been compared to fern spores (Went 1949) and consists of a small spherical embryo suspended within a membranous, often transparent, but at times pigmented, seed coat (Burgeff 1936, Darwin 1888, Anon. 1923a, c, Dymes 1923, Knudson 1929, Hoene 1940, 1949, Vacin 1950a, Scott and Arditti 1959, Kupper and Linsenmaier 1961, Werblin 1963). Di- and polyembryonic seeds have also been reported (Wallace 1951, Ito 1961b, Stoutamire 1964b, Poddubnaya-Arnoldi 1964).

Structurally, two major groups of orchid seeds may be distinguished. A small number of species may have relatively differentiated embryos including a rudimentary cotyledon, as for example *Sobralia macrantha* Lindl. and *Bletilla hyacinthina* Rchb. f. (Burgeff 1936, Harley 1951); these are somewhat easier to germinate. The great majority of species have relatively undifferentiated seeds, no cotyledons (Harley 1951, Maheshwari and Narayanaswami 1952), and no endosperm. This has been described as being a characteristic of higher groups (Rolfe 1962).

In the latter group the embryo is reportedly attached to the seed coat by means of several fine strands or cells. Upon removal of the seed coat these strands remain attached to the embryo (Carlson 1940). The embryo itself consists of relatively undifferentiated, mostly isodiametric cells (Knudson 1929, Ramsbottom 1929, Burgeff 1936, Davis 1946, 1948, Scott and Arditti 1959, Werblin 1963), at times as few as ten in number (Stoutamire 1964b), with dense granulated cytoplasm and conspicuous nuclei. Upon close examination two distinct regions may be discerned. The posterior region consists of large, at times vacuolated, cells. The anterior region, on the other hand, consists of decidedly smaller and denser cells. It eventually develops into the apical meristem of the young seedling (Carlson 1935, 1940, Burgeff 1936). The suspensor, consisting of very large elongated and probably dead cells (Burgeff 1936), can be seen attached to the posterior end of the embryo.

In *Vanda,* this embryo differentiates early in its development into three

regions: parenchymatous, meristematic, and suspensor. The suspensor degenerates early, leaving the parenchymatous region to serve as a nutritive tissue for the meristem (Alvarez 1963). Recent reports regarding "green pod" cultures (Sagawa 1963, 1966, Sauleda 1965, Davidson 1965) have indicated that orchid embryos become viable and are capable of normal development prior to being fully ripe. The connection, if any, between embryo differentiation and its ability to germinate appears open to conjecture at this time.

Whereas the embryo itself almost always retains its spherical or globular shape, that of the seed coat may vary a great deal, being elliptical, fusiform, rounded, globular, or butterfly shaped. Seed coats may be much larger than the embryo or only as large as the embryo; may be stubby or slender; angular or rounded; long or short; transparent, translucent, or opaque (Godfrey 1923, Knudson 1922, 1929, Ramsbottom 1929, Burgeff 1936, Davis 1946, 1948). They normally consist of dry membranous material interlaced with heavily thickened cell walls which in *Cattleya* seeds, absorb and hold safranin well. The seed coats of *Selenipedium* Rchb. f. are sclerotic, whereas those of *Cypripedium* L., *Paphiopedilum* Pfitzer, and *Phragmipedium* Rolfe are thin with reticulate coats (Rosso 1966).

Longevity of orchid seeds is highly variable. Some may lose their viability within nine months (Humphreys 1960), two months (Brummitt 1962), or less (Hey 1963, Lindquist 1965), but others may remain viable for long periods (up to 18 years) if permitted to dry and if stored in a desiccator and refrigerated at 0° C. (32° F.) (Kano 1965), 4.5°-8° C. (40°-46° F.), or even 10° C. (50° F.). However, most will lose their viability fast if kept at room temperatures (21°-22° C.) (Fehlandt 1960, Humphreys 1960, Blowers 1963, Leuschner 1963, Stoutamire 1946b, Kano 1965, Davidson 1966). This has been amply demonstrated in a number of species (Wolff 1924, Knudson 1933, 1940, Watkins 1948, 1956, Lowe 1960). Seeds of certain species may survive comparatively short exposures to nearly 0° C. (Poddubnaya-Arnoldi and Selezneva 1957), and appear generally resistant to dry cold (Hey and Hey 1966). Quick freezing and storage of seeds in the frozen condition for lengthy periods are also not detrimental (Fehlandt 1960). However, developing *Cattleya* seeds in ripening capsules, if subjected to 40° F. for prolonged periods, may suffer a partial loss of viability (Stephens 1966).

No information is available on the upper limits of temperature tolerance by orchid seeds except that soaking of up to four hours at 39° C. (98° F.) did not reduce their germination (Thomasen 1964).

The major food reserves in orchid seeds appear to be lipids (Anon. 1922a, Poddubnaya-Arnoldi and Zinger 1961). Chemical analysis of *Cymbidium* seeds has shown them to contain 32 per cent fat, 1 per cent sugar, and no starch. These figures have been confirmed (Arditti, unpublished results; Knudson 1929). Microchemical tests of *Cypripedium parviflorum* Salisb. seeds (Carlson 1940) have shown that the seed-coat cell walls contain lignin and cellulose deposited unevenly on the walls in strips or bars. No cutin was detected. In some species the seed coats may be impregnated with substances capable of inhibiting or slowing down germination (Withner 1955). The epidermal cells of the

embryo appear to be non-lignified and to have no cuticle. Oil droplets were observed in some embryo cells (Alvarez 1963), including the large posterior cells, but no starch, nitrates, or sugars could be detected (Carlson 1940).

Mineral content of seeds has been determined by a number of investigators but none of their findings appear to have been published (Wheeler and Ramos 1965, McAlpine 1966, Wheeler 1966). Some of the available information is summarized in Table II. All determinations were by semi-quantitative emission spectroscopy with unknown limits of accuracy (Wheeler 1966).

Because of their structure orchid seeds are very light and buoyant. As a result they may travel long distances by air (Summerhays 1951) or water currents (Ames 1948), but some are transported by ants (Anon. 1915b) and birds (Anon. 1923b). Probably due to this lightness and wind dispersal of their seeds, orchids were among the pioneering plants on Krakatoa following that famous explosion (Ames 1922b, Went 1949). Rates of seed descent in the atmosphere and sinking in water have been carefully investigated and timed (Burgeff 1936).

Despite their minuteness and seeming frailty, orchid seeds can resist and survive comparatively harsh chemical treatments (Beeman 1947, Redlinger 1961, Jordan 1965). Experiments with decontaminating agents have shown that they may withstand at least 6 per cent H_2O_2 for up to 10 minutes (Breddy 1953); 1:32 Clorox solution for up to 15 minutes (Northen and Northen 1948, Liddell 1948); Wilson's (1915) calcium hypochlorite solution up to 48 hours (Knudson 1948); 36 hours in 10 per cent potash (Bouriquet and Boiteau 1937); short exposures to 1:2500 solution of bichloride of mercury (Willoughby 1950); and 10 minutes in toluene followed by 30 minutes in 90 per cent ethanol and 30 minutes in calcium hypochlorite (Withner 1955). In contrast, ammonia gas has proved harmful (Breddy 1953) as have 1:10 Clorox solution (Liddell 1948) and ethyl, propyl, and isobutyl vanillate (McAlpine 1947, Knudson 1947)). It has also been suggested that soaking the seeds in 5 per cent Clorox for 10 minutes actually stimulates germination (Rao and Avadhani 1963) (Clorox contains 5.25 per cent sodium hypochlorite by weight) as would 1.5 hours in Wilson's calcium hypochlorite solution (Kano 1965). Orchid seeds seem to be able to withstand up to 24,000 r. of X-ray radiation without a reduction in their germination percentage (Kano 1965).

ORCHID MYCORRHIZA AND SYMBIOSIS

Orchid germination resembles the development of a dormant bud more than it resembles a germinating seed. Chlorophyll may or may not be formed following the initial swelling of the embyro. The germinating embryo swells further and bursts out of the seed coat (Fig. 1), and a cone-shaped or spherical seedling is soon formed. This has been named the protocorm stage (Bernard 1909). On the flat upper surface, the first leaf primordium becomes evident as a small bulge. Absorbing hairs appear around the periphery on the underside and the entire protocorm grows in diameter. Soon the first miniature leaves appear. This is followed shortly by the appearance of the first root, resulting in a slowly developing miniature plant. With minor modifications germination in most orchids proceeds as described above (Knudson 1922, Carlson 1935, Burgeff 1936,

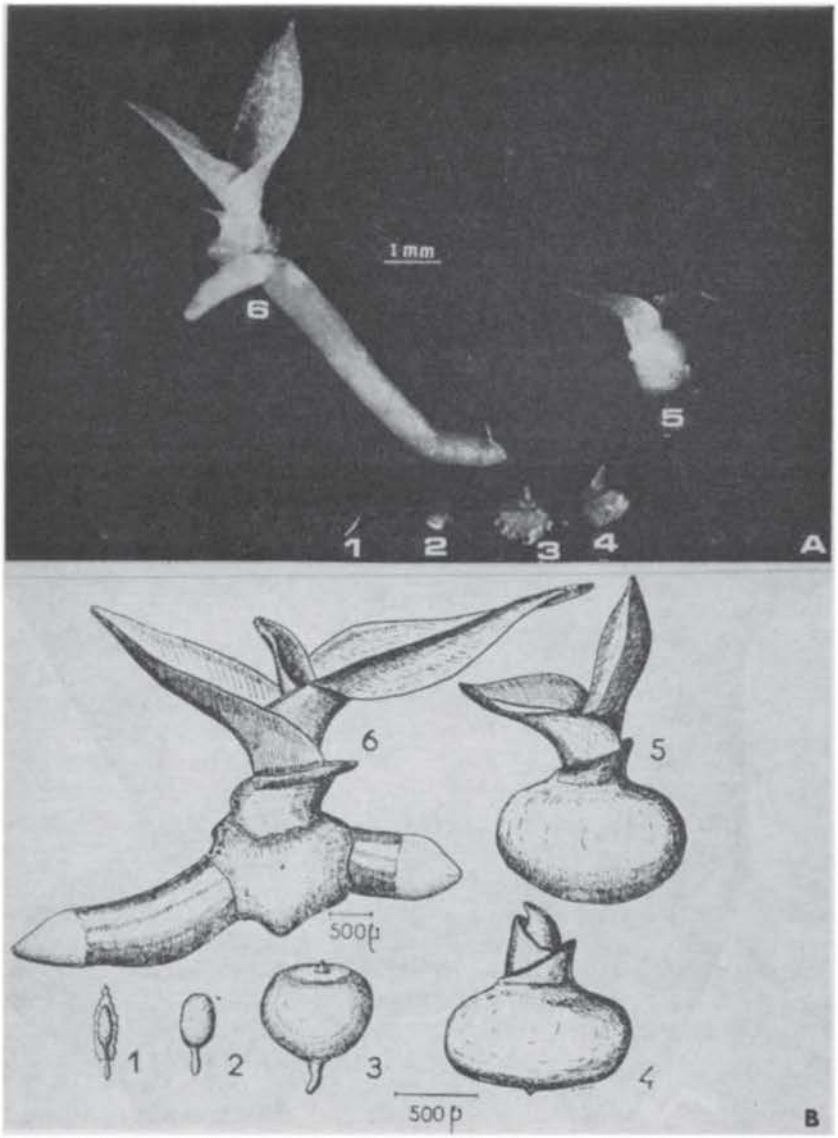

Figure 1. Stages in the germination of an orchid embryo and seedling development.
A. *Laeliocattleya* × Ibbie selfed.
B. *Cattleya* [(Drawings by F. Mariat reproduced with permission of the author) from Rev. Gén. Bot., Vol. 59 (1952)].
1. Embryo swollen and green but still covered with its testa.
2. Embryo swollen some more forming a spherule, seed coat broken and lost.
3. Early protocorm showing a pointed vegetative apex.
4. Discoid late protocorm showing leaflets.
5. Plantlet showing two spreading leaflets.
6. Plantlet showing two or more leaflets as well as one or more roots.

1959, Curtis 1943, Poddubnaya-Arnoldi and Selezneva 1957, Arditti and Bils 1965). However, this comparatively simple process escaped detection for some time. For many years it was believed that orchid seeds are not viable (Constantin 1913a, Bouriquet 1947) or at least are incapable of germination and that orchids multiply by means of bud or gemma-like structures which undergo a series of metamorphoses prior to the formation of another mature plant (Fabre 1855, 1856, Moran 1890).

The first germinating orchid seeds to be described were apparently those of *Bletilla* Rchb. f., *Orchis morio* L., and *Limodorum verecundum* Prodr. (Salisbury 1804, Burgeff 1909). This was followed by descriptions of germinating seeds and seedlings of *Goodyera procera, Eulophium maculatum* (Lindl.) Pftz., and *Angraecum maculatum* Lindl. by Link in 1840 (Prillieux and Rivière 1856); *Miltonia* Lindl. (Prillieux 1860); *Platanthera chlorantha* Rchb. f. in 1850, *Orchis militaris* L. and *Sobralia macrantha* in 1853 by Irmish (Burgeff 1909, Reinke 1873); *Pleurothallis* R. Br. in 1854 by Schacht (Burgeff 1909); *Orchis variegata* All. and *Sarcanthus rostratus* Lindl. by Beer in 1863 and possibly earlier (Beer 1854, Burgeff 1909); *Zygopetalum* Hook., *Epidendrum, Laelia,* and *Cattleya* by Pfitzer around 1900 and *Odontoglossum* H.B.K. by Ledien in 1907 (Burgeff 1909). Original descriptions were followed by a considerable number of detailed descriptions of germinating seeds and seedlings of many species (Mercier 1826, Prillieux 1860, Veitch 1887-1894, Burgeff 1909, 1911, Ames 1922a, Ramsbottom 1922b, Anon. 1922b, 1923b).

Orchids, therefore, were shown to produce viable seeds capable of germination. However, the factors connected with orchid seed germination remained obscure. The fungal infection which always accompanies seed germination in nature remained unnoticed despite the fact that the fungus itself was seen but not recognized as early as 1824 by Link (Link 1824). In 1840 Link presented somewhat unclear graphical evidence of fungi in *Goodyera procera* (Ker. Gawl.) Hook. root cells (Ramsbottom 1922a). The nature of the "bysoid substance" found in roots of *Monotropa hypopitys* L. was apparently recognized by Reissek in 1842 (Ramsbottom 1922a), and by 1846 he suggested the presence of a fungus in the roots of *Neottia nidus-avis* Rich., *Gymnadenia viridis* Rich., *Orchis morio* L., and *Platanthera bifolia* Rich. (Wahrlich 1886, Anon. 1922a). The fungus in orchid roots was also noticed, but apparently not recognized, by such an eminent botanist as Schleiden in 1849 (Wahrlich 1886, Magnus 1900). Other reports of the presence of fungi in orchid roots or seedlings followed. Despite the increase in number and detail of such descriptions the role of the fungus was not appreciated at first (Prillieux 1856, 1860, Reinke 1873, Mollberg 1884, Dangeard and Armand 1897, MacDougal 1899a, b, Magnus 1900). The universal occurrence of orchid mycorrhiza was firmly established following careful examination of the roots of 500 orchid species from all parts of the world (Wahrlich 1886).

The term *mycorrhiza* (meaning root fungus) was conceived by Frank in 1885 (Frank 1892, Ramsbottom 1922a) who also suggested the possibility of a symbiotic relationship. The effect of the fungus on clump formation within root cells was investigated (Dangeard and Armand 1897) but its correct and

important role remained hidden even to MacDougal (1899a, b) although he did suggest that the fungus is decidedly useful to the plant.

The real significance of the fungus and its cardinal importance were first recognized by Noel Bernard in 1899 who apparently made his discovery upon stumbling on a large mass of *Neottia* L. seedlings during a stroll in the forest. He noted that all seedlings were infected by a fungus and conceived the possibility that the fungal infection was a requisite for germination. Combining brilliant reasoning with skillful experimentation he succeeded in proving his deduction by isolating the fungi and devising a practical method for orchid seed germination (Bernard 1899, 1900, 1903, 1904a, b, 1905, 1906a, b, 1909, 1923). Subsequent observations showed that tropical epiphytic orchids also require infection by a fungus for germination (MacDougal 1899a, b, Cordemoy 1904, Constantin 1912, Mercier 1926). However, it has been reported that some orchids can apparently germinate in clay pits without fungal aid (Richardson 1956).

These observations were soon followed by investigations of the fungi *per se* and their effect on orchid seeds, seedlings, and mature plants (Burgeff 1909, 1911, 1932, 1936, 1959). The fungi were first assumed to comprise a separate group and were given the name *Orcheomyces* (Burgeff 1909, 1911, 1932, 1934, 1936). Subsequent studies of the orchid-fungus relationships in orchids native to Scotland (Downie 1940, 1941, 1943a, b, 1949a, b, 1957, 1959a, b), the U.S. (Curtis 1936, 1937, 1939b, 1943), and other parts of the world (Knudson 1922, Campbell 1962, 1963, 1964, Nakamura 1964) have shown this not to be so. The fungi isolated from orchid roots include species of *Rhizoctonia* (Bernard 1909, Burgeff 1909, 1959, Duperrex 1961), *Corticium* (Derx 1937, Downie 1957), *Armillaria* (Kusano 1911, Farmer 1915, Hamada 1939, Burgeff 1959, Campbell 1962), *Fomes* (Burgeff 1959, Campbell 1964), *Hymenochaete* (Hamada and Nakamura 1963), and others (Catoni 1929, Mollison 1943, Campbell 1963). Species of *Phytophthora* (isolated from Easter Lilies) (Knudson 1929), *Penicillium, Aspergillus,* and *Trichoderma* have also been reported to initiate and promote germination (Curtis 1939a).

The specificity of the orchid-fungus relationship is still open to question. Investigators have found that best germination is obtained following infection with fungi isolated from the seed's parent plant or from the same species (Bernard 1904a, 1904b, 1905, 1906a, 1909, Burgeff 1909, 1936, 1959, Derx 1937, Downie 1941). This was taken as an indication of a very specific relation between the plant and the fungus. However in other instances no evidence in support of specificity could be obtained. Germination took place in the presence of fungi isolated from a variety of orchid species and some non-orchidaceous plants (Knudson 1929, Curtis 1937, 1939a, Downie 1959a). Certain other fungi were not capable of inducing germination and even killed the seed (Bernard 1904a, b, 1905, 1906a, 1909, Burgeff 1909, 1932, 1936, 1959, Ramsbottom 1922a, 1929, Knudson 1925, Derx 1937, Curtis 1937, 1939a, Downie 1959a, Nuesh 1963, Hyatt 1965). Variations in fungal strains and virulence may account in part for these apparently contradictory results (Ramsbottom 1922a). In addition, seed age and viability (Wolff 1923a, b, 1925) as well as the ability of the

orchid plant to check fungal growth, without destroying it (Bernard 1905), through its defense mechanism, may also play an important role in the plant-fungus relationship (Bernard 1909, Farmer 1915, Burgeff 1936, Nuesh 1963). Several reviews on mycorrhiza discuss that aspect of the Orchidaceae to the extent it is currently understood (Harley 1959, 1963).

Until the middle part of the 19th Century European growers had no method for the germination of orchid seeds. Plants could not be raised from seed, no hybrids could be obtained, and all new plants had to be imported. Mention of seed germination can not be found in major works on orchids as late as 1849 (Hooker and Lyons 1849). A report on germinating *Sobralia macrantha* (one of the few orchids to have a rudimentary cotyledon) (Beer 1854) is accompanied by a statement that very little is known about seed germination. It appears that John Haris, a surgeon in Exeter, was first to note that orchid seeds scattered at the base of the mother plant will germinate (Anon. 1893). He apparently taught his method to Mr. Dominy, chief grower at the Veitch nursery, who is reported to have sown some seeds in 1852 and 1854 (Veitch 1887-1894, Anon. 1893, Constantin 1913b). Neumann, at the Paris Museum, Rivière, also in France, and Moore in Dublin, Ireland, utilized identical methods at approximately the same time (Constantin 1913a). It is not clear whether they arrived at their methods independently or learned them from Haris and Dominy. Until Bernard's work (Anon. 1890) and even later (Williams and Williams 1894, Burberry 1895, O'Brien 1911, Bultel 1920), similar methods (Anon. 1911b, c, 1912, 1914, 1915a, b, 1962, Harrison 1914, Curtis 1910, Grover 1953, Lawrence 1958) were the only means for the germination of orchid seeds both in this country and in Europe. Such procedures were advocated following the advent of the asymbiotic method (Anon. 1924b, 1925b, d, e, Owen 1925, Gratiot 1934) and even at present (Tsuchiya 1959, Graves 1954, Goldsmith 1956, Brummitt 1962, Boyd 1963, Slade 1963, Blowers 1964, Rothwell 1966), although germination under such conditions may in some instances require up to two years (Humphreys 1960). Symbiotically germinated seedlings have been reported as generally being more robust, less subject to fungal attack, and having a better chance to survive (Blowers 1966). These reports are supported by the discovery that orchid plants when infected by a fungus are capable of a so-called defense reaction which culminates in the production of relatively non-specific fungus inhibitors (Arditti 1966c). It is reasonable, therefore, to assume that a symbiotically germinated seedling would contain these inhibitors and be protected against fungal attacks.

As a result of Bernard's work a method for seed germination was developed (subsequently known as the symbiotic method) in which culture tubes were inoculated both with seeds and fungi (Bernard 1904a, b, 1905, 1906a, b, 1909, Anon. 1909, 1910, 1911a, Burgeff 1909, 1911, 1932, 1934, 1936, 1954, 1959, Schmidt 1925, Irpa 1965). This was an elaborate procedure (Gratiot 1934); germination in tubes containing isolated symbionts was much better and more consistent than on pot surfaces, but the method left much to be desired (Harley 1951). It is reported that in 15,000 trials successful germination was observed only in a few hundred (Bernard 1909). Shortly before his death in

1911, Bernard (1909) had initiated experiments which may have led to an asymbiotic method of orchid seed germination (Duperrex 1961).

The effect of the fungus on the seeds and seedlings is a very decided one. It has been investigated by several workers but its exact nature and details are as yet unknown. The fungus penetrates the embryo through the suspensor cells or the epidermal hairs (Burgeff 1936, 1959, Harley 1951, 1959, 1963, Ramsbottom 1922a, Knudson 1922). Profound changes occur in the embryo following infection. Oil droplets begin to disappear and no longer seem to press on the nucleus (Burgeff 1936). The early suggestion that the fungus may affect starch content (Reinke 1873) was eventually substantiated when it was shown that following infection starch first appears in the embryo, but later disappears again (Burgeff 1936, 1959). In asymbiotically grown seedlings starch may accumulate; this has been interpreted by some as proof of the "abnormalcy" of these plantlets (Knudson 1927). Often infection may cause the nuclei to become very distinct (Burgeff 1936, 1959), although not all cells of the embryo are invaded by the fungus. In some cells special storage hyphae are formed *(Eiweisshyphen)* which are eventually digested by the plant (Wilson 1906, Burgeff 1959, Summerhays 1951). In certain saprophytic orchids these hyphae may be the only source of food (McLennan 1959). Some have compared the fungal infection to a chronic disease which somewhat inhibits growth at first (Wilson 1906, Pavillard 1912, Constantin 1913a) and is of benefit to the plant only later. The growth and spread of the fungus is kept in careful check by the defensive action of the plant, involving the production of a fungistatic compound (Nuesch 1963, Arditti 1965c). The outcome is a symbiotic relationship which renders both plant and fungus better able to exist and compete with other organisms.

The physiochemical effects of the fungus have long been recognized (Pavillard 1912) although their exact nature remains a mystery. Since the endophytic fungus can invert sugar (i.e., hydrolyze sucrose) in culture solutions it has been theorized that the fungus acts by increasing osmotic concentration both within and outside the cell (Bernard 1909). The increased osmotic concentration was compared in its effect to the action of the male gamete in fertilization. It has also been suggested that the fungus assists with the nitrogen and mineral nutrition of the orchid plant (Magrou 1944).

There is little doubt at present that the factor or factors contributed by the fungus are not merely food factors or enzymes. Evidence has accumulated to suggest that a specific compound, possibly a vitamin, or precursors or derivatives thereof, may be contributed by the fungus (Constantin 1925, Burgeff 1936, Schaffstein 1938, 1941, Shopfer 1943, Noggle and Wynd 1943, Downie 1949a, b, Bahme 1949, Mariat 1952, Arditti 1965a). This may be an important contribution since in several instances seeds have failed to germinate in the presence of sugar without the addition of fungal extract (Downie 1943a, 1949a, b) or a vitamin (Noggle and Wynd 1943). No germination occurs in the presence of certain vitamins and growth factors in sugarless media (Arditti 1965a), but seeds have germinated fungus-free in clay pits (Richardson 1956); therefore, vitamin deficiency may be only one limiting factor in the germination of orchid seeds.

ASYMBIOTIC INVESTIGATIONS

Proceeding on the assumption that orchid tubers contain all that is required by germinating orchid seeds, successful attempts were made to germinate *Cattleya* × *Laelia* hybrid seeds on salep (a preparation made of ground *Ophrys* L. tubers) (Bernard 1909). Increased concentrations of salep were found to improve germination. This was regarded as further proof that increased osmotic concentration and pressure initiated germination. Bernard's early and premature death in 1911 prevented him from further work along those lines. Burgeff (1909, 1911), who had been working independently of Bernard along the same lines, did not pursue the matter much further either.

Due to his work on the influence of carbohydrates on green plants, Lewis Knudson (1916) also became interested in orchid seed germination (Knudson 1921). He analyzed salep (Knudson 1922) and found it to consist of 48 per cent mucilage, 27 per cent starch, 5 per cent protein, some sugars and soluble minerals. From this and from reports that the fungus could invert cane sugar (Bernard 1909) he (Knudson 1922, 1929) concluded that the fungus stimulated germination by breaking down starch, pentosans, and nitrogen substances and possibly also by producing growth-stimulating compounds. He then proceeded to show that *Cattleya, Laelia,* and *Epidendrum* seeds germinate freely on a sugar- and mineral-containing agar medium (Knudson 1921, 1922, Anon. 1922a, 1923c) and stated that the fungus *per se* was not required for germination (Anon. 1925c, Knudson 1925). His statements were vigorously challenged on the grounds that a seedling grown asymbiotically was not "normal" (Constantin and Magrou 1922, Constantin 1926) and that in drawing conclusions about biological subjects one should only consider "naturally" occurring evidence (Ames 1922a, Ramsbottom 1922b, 1929, Burgeff 1934, 1936, 1959), thus implying the asymbiotically grown seedlings were somehow un-natural. Some went as far as to state that Knudson's discovery was no real contribution and that "to a mycologist an orchid seedling without its fungus is like 'Hamlet' without the Prince of Denmark" (Ramsbottom 1922b). Others reported success in the germination of seedlings on Knudson's medium (Smith 1932) and, after comparisons between symbiotically and asymbiotically grown seedlings, maintained that the latter were normal indeed (Bultel 1920, 1921, 1924-1925, 1926). Some workers without divulging the nature of their culture medium also reported successful germination and normal seedlings of various species on asymbiotic media (Ballion and Ballion 1924, 1928, Anon. 1924a, 1925a, b, c, Clement 1925). The asymbiotic method of orchid seed germination is widely used at present and premixed media are easily available (B., J. W. 1961).

In response to another line of criticism (Ramsbottom 1922b), Knudson proceeded to show that *Odontoglossum, Cypripedium, Phalaenopsis, Ophrys,* and *Dendrobium* Swartz seeds can also germinate asymbiotically. The dispute between Knudson and his dissenters became rather heated (Knudson 1927) and in the hope of obtaining a conclusive answer a *Laeliocattleya* hybrid was raised from seed to flower asymbiotically (Knudson 1930). However, the culture flask eventually became contaminated with a species of *Chlorella,* a small colony of moss, and a fungus resembling *Penicillium.* Despite the lack of fungal infection

in the orchid roots it is possible that the contaminants may have released substances into the medium which the orchid plant could have taken up. Thus, while this experiment did prove that an orchid can germinate, grow, attain maturity, and flower without fungal infection, it did not demonstrate that an entirely aseptic plant can produce blooms. However, since then it has been reported that *Oncidium pusillum* (L.) Reichb. f. (Livingston 1962) and *Dendrobium* Thwaitesiae (Tanaka 1957) seedlings can bloom in culture flasks under totally aseptic conditions. Following his initial work Knudson devoted attention to the development of better culture media (Knudson 1946a, 1951, 1952) and to the germination of native American orchids (Knudson 1941) and *Vanilla* seed (Knudson 1950). As a whole, Knudson has probably contributed more to Orchidology than any other man, both by his work and by providing methods and stimuli for others.

Although basically utilizing his methods, many investigators have attempted to improve Knudson's culture media and methods or adapt them to the special requirements of various species (Robinson 1941, Sampolinski 1965). The effects of various ions (Wynd 1933a, b, c) and carbohydrates (LaGarde 1929, Quednow 1930, Ernst 1966a,b) have been investigated and compared. Numerous simplified methods and media for orchid seed germination and seedling culture have been suggested as a result. Many of them are based on Knudson's work (Wolff 1923a, Burgeff 1936, 1954, Fagundes 1938, Meyer 1944a, 1945a, Withner 1947, 1959a, b, Vacin 1950a, Sideris 1950, Breddy 1953, Kriechbaum 1953, Seeman 1953a, b, Hager 1954, Kofranek 1957, McEwan 1961, Farrar 1963, Yamada 1963a, b, Arditti 1964b, Lawrence and Arditti 1964a). Attempts have been made to determine the specific requirements of various species by varying culture media components and by tissue analysis (Curtis 1936, Tienken 1947, Wheeler and Ramos 1965, Wheeler 1966). Special culture media or procedures have been suggested for seeds of *Cypripedium* (Cappelletti 1935a, b, Liddell 1944, 1953a, b, 1955, Thomale 1951, 1954, Kache 1952, Hegarty 1955, Withner 1953, Tsukamoto, Kano, and Katsuura 1963, Stoutamire 1965), *Cymbidium, Cattleya,* and *Dendrobium* (Vacin 1950a, b, Tsukamoto, Kano, and Katsuura 1963). The requirements of *Vanilla* have been studied closely (Bouriquet and Boiteau 1937, Bouriquet 1947, 1948a, b, Knudson 1950, Tonnier 1954a, b, Lugo-Lugo 1955a, b). Efforts have been made to devise methods for sterile addition of nutrients to aseptic culture flasks containing very young seedlings (Morrison 1963), or for other manipulations (McEwan 1960). Such methods may be utilized in experimental work designed to test the effect of nutrients and other substances on seedlings at various stages of development. Most findings are readily available to research workers and practical growers in a number of works (Burgeff 1936, 1954, White 1945, Meyer 1944b, Watkins 1948, 1956, Northen 1950, Anon. 1953, Butterfield 1955, Holttum 1957, Poddubnaya-Arnoldi 1959, Poddubnaya-Arnoldi and Selezneva 1957, Kofranek 1958, Withner 1959b).

Embryological studies (Carlson 1940, Johansen 1950, Poddubnaya-Arnoldi 1960, 1964, Poddubnaya-Arnoldi and Zinger 1961) have resulted in increased knowledge of the requirements of developing orchid embryos. Application of this knowledge has brought about ovule culture techniques which provide the

plant physiologist with yet another research technique (Withner 1943, 1955, Tsuchiya 1954a, b, Ito 1955, Ayers 1960, Choon 1962, Israel 1963, Sagawa 1963, 1966).

The requirements of shoot meristems in aseptic cultures have been shown to be little different from the requirements of germinating orchid seeds (Morel 1960, 1964, Wimber 1963), thus suggesting the possibility of introducing tissue culture as an additional tool in investigations of orchid physiology (Murashige 1961).

Utilizing such aseptic culture techniques, various requirements and responses of germinating orchid seed and developing seedlings have been investigated and the information currently available is summarized and reviewed below.

Mineral nutrition

Following the development of the first media for orchid seed germination (Knudson 1921, 1922, 1925, 1946a) a considerable number of other media have been devised (La Garde 1929, Wynd 1933a, b, Burgeff 1936, Yates and Curtis 1949, Vacin and Went 1949a, Vacin 1950a, b, Sideris 1950, Kriechbaum 1953, Withner 1959a, b, Kano 1965). Certain of these culture media are only slight modifications of Knudson's B or C (1922, 1946a) media whereas others include considerable changes and represent an effort to improve seed germination and seedling growth, especially in species which are difficult to germinate. Attempts have also been made to devise simple media utilizing commercial premixed fertilizers (Kano 1965). Hyponex, a commonly used preparation (total N not less than 7 per cent, nitrate N not less than 5.8 per cent, ammoniacal N not less than 1.2 per cent, available phosphoric acid not less than 6 per cent, water soluble potassium not less than 6 per cent), at 3 g./l. has been shown to support growth of *Dendrobium, Cattleya, Cymbidium,* and *Paphiopedilum* better than Knudson C medium (Kano 1965). In general most media provide adequate amounts of all macronutrients (Table III). Moreover, it appears that orchid seeds and seedlings can adapt easily to a wide variety of inorganic salt combinations and concentrations from as low as 102 p.p.m. total salt content to many times that amount (Curtis 1947c, Yates and Curtis 1959, Withner 1959a). In nature the nutrient solutions available to orchids may be very dilute (Curtis 1946) and the osmotic pressure of their sap may be exceedingly low, ranging from 2.6 to 7 atmospheres as against an average of 10.41 for mesophytes (Harris 1934, Curtis 1946).

A systematic investigation of the effects of various ions has revealed that best germination and development occurred on media low in phosphate irrespective of whether the calcium or potassium salts were used (Wynd 1933a). Inasmuch as insoluble ferrous phosphate [$Fe(PO_4)_2$] may be formed in the presence of phosphate during autoclaving (Vacin 1950b), it is possible that at a low phosphate concentration sufficient iron remains present in available form. There is no evidence available at present to indicate a direct effect of phosphate *per se.* Variations in the proportions of other ions had little effect although anions do appear to be more influential than cations in the germination of orchid seeds (Wynd 1933a, b). Best growth was obtained on media containing 0.0025

M KH_2PO_4, 0.0049 M $Ca(NO_3)_2$, 0.0123 M $MgSO_4$ or 0.0097 M K_2SO_4, 0.0019 M Ca $(H_2PO_4)_2$, 0.039 M Mg $(NO_3)_2$ (Wynd 1933a, c). No comparisons were made with growth on more conventional media and the results are therefore somewhat difficult to evaluate. Nevertheless, it appears that the effects of altering the ratios of various salts depend on the total concentration. No comparisons of specific ion effects were possible in solutions differing in total salt concentrations (Wynd 1933c, Withner 1959a).

Optimum salt concentrations may vary with the species (Yates and Curtis 1949) and the same may be true of ionic ratios. Growth rates of several orchids on some of the more common seed culture media show considerable variations. Various species show marked preferences regarding culture medium composition (Wynd 1933a, b, Withner 1942, Breddy 1953) and improved growth on specially designed media may be due to these preferences (Knudson 1922, 1925, LaGarde 1929, Burgeff 1936, Yates and Curtis 1949, Vacin and Went 1949a, Vacin 1950a, b, Sideris 1950, Liddell 1953a, b, Seeman 1953a, Kriechbaum 1953, Withner 1959a, b). Seeds of *Vanilla planifolia,* for example, germinated best on one tenth the normal concentration of Knudson's solution B. Increases of the concentration to twice normal greatly inhibited germination (Lugo-Lugo 1955a, b). Variation in the concentrations of most components of the solution produced no appreciable effect on germination. Nitrogen content, however, proved to be an exception (Lugo-Lugo 1955a, b) and will be discussed later.

Iron deficiencies may occur in orchid seed cultures due to the ease with which this metal may precipitate (Sideris 1950, Vacin 1950b, Yamada 1963a, b). In attempts to increase iron availability several iron salts have been used. Ferrous sulfate appears to be superior to ferrous phosphate in this respect (Knudson 1946a, b, Vacin 1950 b, Sideris 1950).

Various organic or inorganic acids have been used in an effort to assure iron availability. These include tartaric acid (Vacin 1950b, Sideris 1950), citric acid (Burgeff 1936, Sideris 1950, Liddell 1953a, b), malic acid (Sideris 1950), metaphosphoric acid (Sideris 1950), and versene (Stoutamire 1964b). Tomato and pineapple juice, potassium tartarate, and sodium hexametaphosphate (Calgon) are also suggested as good iron stabilizers (Sideris 1950). Comparative studies have shown that sodium hexametaphosphate is the "best means of controlling chlorosis resulting from iron deficiencies in seedling cultures" (Storey *et al.* 1947). It has been suggested that any of the newly available chelated iron preparations could be used successfully (Yamada 1963a, b). "Complexed" iron has been reported as perfectly adequate for plants in culture (Withner 1959a).

Addition of manganese to orchid seedling cultures has improved growth and development (Noggle and Wynd 1943, Knudson 1946a, b, Yates and Curtis 1949, Sideris 1950, Vacin 1950b). Additions of 50 and 100 p.p.m. manganese to *Epidendrum nocturnum* Jacq. cultures have enhanced seedling color to a richer green than controls and markedly stimulated root growth, but had little or no effect on shoot growth. Higher concentrations of manganese (500 p.p.m. and 1000 p.p.m.) have caused marked chlorosis (Yates and Curtis 1949). Sodium hexametaphosphate (Storey *et al.* 1947) and citrate (Liddell 1953a, b) have been employed for the improvement of manganese availability. An iron to man-

ganese ratio of five to two has been generally recommended for orchid cultures (Knudson 1946a, Young 1952, Griffith and Link 1957).

Calcium may at times precipitate as calcium phosphate but this is not detrimental (Storey *et al.* 1947) possibly due to the fact that orchid tubers and seeds contain very little calcium (Tienken 1947, Wheeler 1966) and may therefore have a low calcium requirement.

Potassium may play an important role in the germination of *Galeola septentrionalis* Rchb. f., a terrestrial orchid lacking chlorophyll (Nakamura 1962, 1963, 1964). Soaking the seeds of this species in solutions of several potassium salts has increased germination 3-3.5-fold. Of the salts used, KH_2PO_4 did not improve germination whereas K_2CO_3, KI, and KCOOH inhibited it. Concentrations of 5×10^{-1} M and higher of KCl in culture media were found to be supraoptimal, whereas concentrations of 5×10^{-4} M and lower were suboptimal. Soaking seeds of *Cymbidium virescence* Willd., following sterilization, for five hours in 0.01, 0.1, and 1.0 per cent of KCl has failed to increase germination. Soaking for longer periods and/or in higher KCl concentration has inhibited germination (Kano 1965). Of the other alkali metals tested, LiCl severly inhibited germination and cesium eliminated it completely. Rubidium brought about only a twofold increase in germination. Germination of sodium-chloride-treated seedlings was equal to that of the controls. Very good germination was obtained on a commercial brand of tomato juice (Libby's); "according to the label (it) is not too highly seasoned except with salt," implying the presence of sodium (Ito 1951). Immature seeds develop well on a medium containing a total of 16.9 mg. sodium (Tsuchiya 1954a, b). It appears, therefore, that orchid seedlings are not very sensitive to sodium.

The need for microelements in orchid seed cultures has been questioned since enough may be present in the agar, sugars, and salts used (Knudson 1950). Nevertheless, several microelement solutions have been used for orchid seed cultures. Boron, copper, molybdenum, and zinc are normal components of such solutions. Manganese and iron may be added in instances where these elements are not present in the macronutrient solution (Withner 1942, 1947, Breddy 1953, Tsuchiya 1945a, b, Arditti 1964b, 1965a, b, c, 1966 b, In Press a, In Press b). Iodine has been recommended for immature seed cultures (Tsuchiya 1954a). In a comparative study, increased growth was obtained by the addition of boron, iron, molybdenum, copper, manganese, and zinc to a number of iron- and manganese-lacking basal media (Breddy 1953). Extreme caution should be exercised in the use of copper since orchids are very sensitive to this metal (Sheehan 1962).

Inasmuch as hydrochloric acid is frequently employed to adjust pH of culture media (Scott and Arditti 1959) and several chlorides are used as culture media components, it appears safe to assume that orchid seeds and seedlings are fairly resistant to this ion at least in the concentrations used (Table III). On the other hand, practical growers have noted that orchid seedlings irrigated with water high in chlorine become inhibited and may develop leaf tip burn.

Immature seeds or ovules seem to have the same mineral requirements as mature seeds since they grow and develop well on standard basal media pro-

vided certain vitamins and growth factors are added (Withner 1943, 1955, Tsuchiya 1954a).

The fertilization of young seedlings in culture flasks with sterile fertilizer solutions has stimulated seedling growth but may cause some leaf burn (Morrison 1963).

In summary, it may be stated that the mineral requirements of germinating orchid seeds and developing young seedlings are not substantially different from those of most other flowering plants.

Nitrogen nutrition

The requirement for appropriate sources of nitrogen during germination of orchid seeds has long been recognized. It has been suggested that an important role of the mycorrhizal fungus is to assist with nitrogen nutrition (Magrou 1944) and association with nitrogen fixing bacteria is of importance to certain orchid species (McLennan 1959). In general, it appears that low concentrations of nitrogen stimulate germination of orchid seeds whereas high concentrations are inhibitory. The germination of *Vanilla planifolia* seed was greatly improved following a tenfold reduction in nitrogen content of Knudson's B medium, but was totally inhibited if concentrations were doubled (Lugo-Lugo 1955a, b). Best growth and development of *Epidendrum nocturnum* was recorded at 5 and 10 mM of NH_4NO_3 (Yates and Curtis 1949).

Both nitrate and ammonia ions may be utilized by orchids (Curtis and Spoerl 1948) and are apparently absorbed at approximately the same rates (Sideris 1950). Nevertheless, data from a number of experiments show that ammonium nitrogen is required by *Paphiopedilum* seeds (Burgeff 1936); is less inhibitory than nitrate for *Vanilla planifolia* seeds (Lugo-Lugo 1955a, b); is "superior" for *Cymbidium* seeds, only slightly better for *Cattleya,* and clearly inferior for *Vanda* (Curtis and Spoerl 1948). No interaction between nitrogen source and pH could be detected (Curtis and Spoerl 1948). On the other hand, in comparisons with organic nitrogen sources, best germination, growth, and development of *Laeliocattleya* have been recorded on ammonium sulfate (Burgeff 1936, Magrou, Mariat, and Rose 1949), and of *Phalaenopsis* and *Dendrobium* on potassium nitrate and ammonium sulfate (Schaffstein 1941). Ammonium tends to favor the growth of the more heterotrophic and saprophytic species whereas the more autotrophic species when grown in the light are favored by nitrate (Burgeff 1936). It has been shown, recently, that *Cattleya labiata* seeds will germinate readily on ammonium nitrogen but are drastically inhibited by nitrate nitrogen (Raghavan and Torrey 1963, 1964). Attempts to grow seedlings in the presence of nitrate as the only source of nitrogen by changing the pH or by additions of ascorbic acid, kinetin, or molybdenum were without success. However, following an initial period of growth of 60 days or more on NH_4NO_3, seedlings could grow normally solely in the presence of $NaNO_3$. The ability of the seedlings to utilize nitrate was paralleled by the appearance of nitrate reductase (Raghavan and Torrey 1963, 1964). Arginine, ornithine, and urea, all of them compounds related to the ornithine cycle, were capable of replacing NH_4NO_3 and could induce rapid growth. Proline and *gamma*-aminobu-

tyric acid were only moderately good sources of nitrogen (Raghavan and Torrey 1964). Phenylalanine, citrulline, tyrosine, aspartic acid, glutamic acid, glutamine, asparagine, and phenylurea were unable to replace NH_4NO_3 as nitrogen sources, but did not inhibit the utilization of the latter. Germination and subsequent growth were inhibited by glycine, *alpha*-alanine, valine, *alpha*-amino butyric acid, leucine, phenylglycine, hydroxyproline, canavanine, and thiorea. This inhibition was only slightly or not at all overcome by NH_4NO_3 (Raghavan 1964, Raghavan and Torrey 1964). It therefore appears that orchid seedlings undergo not only morphological-structural differentiation but also a rather complex biochemical-physiological differentiation which converts the seemingly saprophytic very young seedling to an autotrophic mature plant (Knudson 1924, Withner 1959a, Raghavan and Torrey 1963, 1964).

Urea, when used as a nitrogen source in orchid seed cultures, has given contradictory results. The growth of *Cymbidium* seedlings on urea was reported to be both markedly inhibited (Cappelletti 1933) and promoted (Burgeff 1936). The growth of *Dendrobium phalaenopsis* Fitzgerald and *Phalaenopsis* sp. was inhibited (Burgeff 1936) whereas development of *Laeliocattleya* (Magrou, Mariat, and Rose 1949), *Vanilla planifolia* (Lugo-Lugo 1955a, b), *Cattleya* (Curtis 1947a, Raghavan 1964), and *Vanda* (Burgeff 1936) was enhanced by urea.

Experiments with nucleic acids and related compounds are difficult to interpret owing to differences in experimental procedures. A further complication is the possibility of chemical changes during autoclaving. For example, most of the experiments were carried out prior to the discovery of phytokinins, therefore no attempts were made to separate the effects of nucleic acids *per se* and possible autoclave derivatives of this nature. Compared to the growth promoting effect of $(NH_4)_2SO_4$ or NH_4NO_3, nucleic acid has enhanced the germination and growth of *Phalaenopsis, Vanda, Cymbidium,* and *Laeliocattleya* in one series of experiments (Burgeff 1936) but had little or no effect (Withner 1951) or was inhibitory (Schaffstein 1941, Magrou, Mariat, and Rose 1949) in others. "Sodium nucleinate" inhibited the germination and growth of *Phalaenopsis* (Burgeff 1936, Schaffstein 1941), *Dendrobium* (Schaffstein 1941), and *Cattleya* (Curtis 1937) but had no appreciable effect on *Vanda, Cymbidium,* and *Laeliocattleya* (Burgeff 1936). "Nucleinate" and "Nucleotide" (Curtis 1947a) produced growth equal to or better than the control in *Paphiopedilum insigne* Wallich, *P. hirsutissimum* Lindl., and *Phaius grandiflorus (P. grandifolius* Lindl.?).

Almost all amino acids have been investigated for their ability to promote growth and provide nitrogen. The resulting data are variable. Aspartic acid can not serve as a carbon or a nitrogen source for some orchid embryos (the reference here is apparently to germinating fully mature seeds) (Knudson 1932) or replace NH_4NO_3 for *Cattleya* (Raghavan and Torrey 1964), but it did act as a good nitrogen source for *Cattleya trianei* fully mature seeds (Spoerl 1948). Glutamic acid behaves in a similar fashion (Raghavan 1964), did not affect growth in *Cattleya trianei* (Spoerl 1948), enhanced *Dendrobium* seedlings at concentrations of $10^{-5} - 10^{-3}$ M (Kano 1965), but reduced germination

by approximately one half in *Galeola septentrionalis* (Nakamura 1962). Leucine has been reported as being incapable of supplying carbon requirements but able to replace inorganic nitrogen to a limited extent for some orchid seedlings (Knudson 1932). It reduced germination of *Galeola septentrionalis* (Nakamura 1962) and inhibited the growth of *Phalaenopsis, Vanda, Cymbidium, Cattleya,* and *Laeliocattleya* (Burgeff 1936, Spoerl and Curtis 1948, Raghavan and Torrey 1964, Raghavan 1964). Cystein and cystine gave better growth in the dark in experiments with *Cattleya* (Spoerl 1948), but the latter inhibited the growth of *Phalaenopsis, Vanda, Cymbidium,* and *Laeliocattleya* (Burgeff 1936). Arginine has stimulated the growth of ripe and unripe seeds of *Cattleya trianei* (Spoerl 1948, Raghavan and Torrey 1964, Raghavan 1964) and *Vanilla* as has lysine (Withner 1955). Asparagine promoted growth of seedlings of European terrestrial orchids (Burgeff 1954), enhanced *Cymbidium* growth slightly (Burgeff 1936), had no effect on *Vanda* and *Laeliocattleya* seeds (Burgeff 1936), slightly inhibited the germination of *Galeola septentrionalis* (Nakamura 1962) and the development of *Phalaenopsis* (Schaffstein 1941, Burgeff 1936), *Laeliocattleya* (Magrou, Mariat, and Rose 1949), and *Cattleya* ×'Mollie' (Curtis 1947a), but it can replace NH_4NO_3 for *Cattleya* (Raghavan and Torrey 1964). It completely prevented growth of *Dendrobium* and *Phalaenopsis* under certain conditions (Schaffstein 1941). Histidine had no effect on *Galeola septentrionalis* germination; hydroxyproline, methionine, and tryptophan reduced it by one third, whereas serine and alanine exhibited drastic inhibition (Nakamura 1962). Hydroxyproline was also inhibitory to *Cattleya* (Raghavan 1964). Ornithine, proline, *gamma*-aminobutyric acid, phenylalanine, tyrosine, citrulline, glutamine, and phenyl urea were all good nitrogen sources for *Cattleya* (Raghavan 1964, Raghavan and Torrey 1964). Glycine, *alpha*-alanine, valine, *alpha*-aminobutyric acid, phenylglycine, and canavanine inhibited *Cattleya* embryos (Raghavan 1964). All other amino acids are either inhibitory or without effect on the germination and growth of seeds of *Brassolaeliocattleya, Cattleya, Zygopetalum* (Spoerl 1948, Kano 1965). *Dendrobium, Dendrobium nobile, Vanda, Phalaenopsis* (Schaffstein 1941, Kano 1965), and other species (Burgeff 1936).

The effects of trytophan deserve special attention due to its role in IAA and niacin metabolism. Very young (70-day-old) *Cattleya* seedlings were inhibited by concentrations of 40.6 μM, 81.2 μM, and 162.4 μM of this amino acid. Older (130-day-old) plantlets were inhibited only by concentrations of 162.4 μM. No inhibition was noted with any of these concentrations in 190-day-old *Cattleya* seedlings (Arditti 1965a, In Press c). Similar results were obtained with 182-day-old *Brassolaeliocattleya* and 156-day-old *Dendrobium* seedlings grown on 10 and 100 μM of l-trytophan. However, a concentration of 1×10^{-3} M did prove inhibitory for both species (Kano 1965). Interestingly enough, one-year-old seedlings of *Phalaenopsis* were considerably enhanced by a concentration of 10 μM dl-tryptophan (Ernst 1966b).

Among the nitrogen bases, guanine and guaninehydrochloride have inhibited the growth of *Phalaenopsis, Vanda, Cymbidium,* and *Laeliocattleya* (Burgeff 1936). Adenine has been reported to promote *Cattleya* seed germina-

tion and seedling growth both separately and in combination with ribose and pyruvate (Withner 1942, 1943, 1951) (Table XI). Others, however, have reported that adenine has had no growth-enhancing effect and may have even inhibited germination and growth (Kano 1965, Arditti 1965a, 1965c, In Press a, Ernst 1966b) (Table XI).

A great deal remains to be elucidated in the nitrogen nutrition of germinating orchid seeds and young seedlings. Nevertheless, it appears that in general orchid seedlings do not differ greatly in their nitrogen metabolism from other green plants, whereas germinating seeds may have special requirements.

THE EFFECTS OF SUGAR AND OTHER CARBOHYDRATES

Several investigators have tested the ability of germinating orchid seeds and young seedlings to utilize various sugars and other carbohydrates. Some of the results are summarized in Tables IV, V, VI, and VII.

It appears that in most instances a wide variety of sugars may act as carbon sources for germinating orchid seeds and young seedlings, whereas organic acids are of little value. A closer examination of the data in Tables IV, V, VI, and VII shows that certain species, although able to utilize many different sugars, do show some preferences. Other species will not germinate at all unless specific sugars or combination of sugars are present. It is possible that these differences could be due to specific requirements for selected sugars, although it is also possible that they may be a result of impurities in the sugars used. In one instance where chemically pure sugar was used (Noggle and Wynd 1943) there was no germination. A number of species native to Scotland require the presence of both a sugar and a water extract of the endophytic fungus for germination (Downie 1940, 1941, 1943a, 1949a, b). Recently, the ability of two genera to utilize a variety of mono-, di-, and trisaccharides and sugar alcohols has been investigated. The rather interesting results are listed in Tables V, VI, VII (Ernst 1966a).

The inability of orchids to utilize galactose is not very surprising, since this sugar is less common in plants than the six carbon sugars capable of supporting good growth. It is interesting to note that lactose, primarily a sugar of mammalian origin, can be utilized by *Vanilla* (Bouriquet 1947). Autoclaving does not seem to render any of the sugars unusable by orchids (Berggren 1962).

An additional point of interest is the ability of orchids to utilize disaccharides such as sucrose, cellobiose, maltose, and lactose and a polysaccharide such as starch. Whereas cell membranes may be permeable to disaccharide molecules, they are probably impermeable to starch. It is possible that in order to make use of starch the plant would have to produce and secrete a starch-hydrolyzing enzyme. Unfortunately this has not been investigated. Should certain orchids be able to produce such enzymes the question of the role of the endophytic fungus (Knudson 1929) would require further examination. It is conceivable that the fungus simply contributes a growth factor. An investigation of the enzymes of carbohydrate metabolism should prove very interesting.

Dr. E. A. C. L. E. "Ted" Schelpe, Professor of plant taxonomy at the University of Cape Town, has humorously suggested that the relation between the

confusion in the taxonomy of any plant and its popularity with growers is a direct one. A corollary to this axiom may perhaps state that this also holds true of misconceptions regarding physiology. Orchids are a prime example of this and the germination of their seeds has been subject to some rather muddled thinking, poor reasoning, and total ignorance. Suffice to mention here that an amateur grower writing in a very respected journal has attempted to explain lack of germination on sugar-containing media as being due to the *total* destruction of the sugar during autoclaving (Sleap 1962a). Statements of this type are complete nonsense and have been discredited experimentally (Williams 1962).

THE EFFECTS OF VITAMINS

While sugars may be necessary as an energy source for the germination of orchid seeds, additional factors may also be required and the mycorrhizal fungus may indeed supply them (Knudson 1922, 1924, 1925, Cappelletti 1933). It has been suggested that such a factor may be a vitamin (Constantin 1925, Schaffstein 1938, 1941, Schopfer 1943, Harley 1951, Burgeff 1959). Vitamins have been shown to promote growth of plant tissues and organs *in vitro* (Bonner 1937, Schopfer 1943). Since supposedly pure agar (Robbins and White 1936, Hawker 1936, 1939, Robbins 1939) and sugar (Withner 1942, Noggle and Wynd 1943, Knudson 1952) contain a variety of vitamins and inhibitors as impurities, demonstration of vitamin requirements by orchid seedlings and seeds has been difficult (Withner 1959a, Arditti 1963, 1965a), because supposedly vitamin-free culture media in many instances may have contained sufficient amounts of vitamins. Satisfactory evaluation and interpretation of the available data are therefore difficult.

The apparently contradictory results may also be due to physiological differences between various species and genera of the Orchidaceae (Burgeff 1934, 1936, Harley 1951). Indeed, it has been shown that in mature *Cypripedium, Calanthe* R. Br., and *Dendrobium* seeds no vitamin C is to be found. Yet *Cypripedium* ovule sacs are much richer in this vitamin than *Dendrobium* and *Calanthe* ovule sacs (Poddubnaya-Arnoldi and Selezneva 1957, Poddubnaya-Arnoldi 1960, Poddubnaya-Arnoldi and Zinger 1961).

A number of workers have investigated the effects of vitamins on germination and growth of orchid seeds (Table VIII). From these data it appears that specific requirements for certain vitamins may be exhibited by some species. These requirements may be due to full or partial metabolic blocks in the biosynthesis of such vitamins as thiamin (Mariat 1952), for example. Orchid embryos, seeds, and seedlings may therefore provide the plant physiologists and plant biochemists with an excellent opportunity for investigations of vitamin biosynthesis in the higher plants since vitamin-requiring Angiosperms are comparatively rare (Redei 1965). However, it should be remembered that a general trend in vitamin requirements can not be demonstrated except for niacin where a growth-enhancing effect has been reported in most instances (Arditti 1965a).

THE EFFECTS OF HORMONES

The discovery of various plant hormones has led to their utilization in attempts to promote orchid seed germination and seedling growth (Tables IX, X, and XI).

In interpreting the results of growth studies with auxin it should be remembered that only traces of auxin have been found in *Cypripedium* seeds and none at all in *Calanthe* and *Dendrobium* seeds (Poddubnaya-Arnoldi 1960, Poddubnaya-Arnoldi and Zinger 1961).

No growth-promoting effects were observed following the addition of thyroid hormone to *Cattleya* cultures (Withner 1951) and of "female hormone" to various other species (Schopfer 1943).

It is interesting to note that whereas tryptophan is inhibitory, two of its metabolites, niacin and indoleacetic acid, do stimulate seedling growth. The biosynthetic pathways of IAA and niacin as well as that of tryptophan degradation in orchid seedlings should prove interesting (Arditti 1965a).

The decreasing differences between treated and control plants which become evident as the plants develop and grow older may be interpreted as yet another indication that biochemical-physiological differentiation in orchids appears to parallel the more evident morphological-anatomical differentiation. Possibly few or no hormones are produced during the early stages of germination and growth, but production is initiated and increased as the seedlings grow older and leaves as well as roots are formed. The varying responses to different tryptophan concentrations seem to support this contention and are worthy of note (Arditti 1965b, Kano 1965, Ernst 1966b). Comparatively little information is currently available on the effect of gibberellins (Table X). In general this group of plant hormones appears to have a negative effect on germinating orchid embryos (Sisa and Sawa 1963, Kano 1965, Ernst 1966b). Phytokinins and related substances (Table XI) in general are without any beneficial effects and at times may be inhibitory (Raghavan and Torrey 1963, 1964, Kano 1965, Arditti 1965a, c, Ernest 1966b). Further studies of hormone production by orchid seedlings may prove to be most rewarding.

THE EFFECTS OF COMPLEX ADDITIVES

On the premise that orchid seeds may require supplements other than a carbon source and some of the known growth factors, various investigators have tested the effects of complex additives on the germination of orchid seeds and the development of the young seedlings (Table XII). It is easily apparent that a very wide variety of substances are capable of stimulating germination and growth, and that the germination promoting factor(s), if any, are not limited to any one group of organisms. Since certain of the additives used contain little or no sugar or minerals, it is possible that their main effect is due to the presence of certain specific growth factor(s). The finding that ashed tomato juice has no enhancing effect on germination and in some instances only the addition of fungal or other extracts could initiate it seems to support

this view. Although tomato juice was originally described as an excellent culture medium for orchids (Meyer 1944a, c, Watkins 1945, Vacin and Went 1949b, Ito 1951), recent, more careful investigations, have shown it to be unsatisfactory and even inhibitory (Kano 1965, Arditti 1965a, b, 1966b, In Press b) unless used in low concentrations (Lawrence and Arditti 1964a,b, Ernst 1966b). The contradictions are probably more apparent than real and due mostly to the peculiar effect this additive has on orchid seeds and the methods used in evaluating growth. Germination percentages on tomato juice media are high, but so is mortality; of the protocorms that survive very few grow and develop normally at a very fast rate. The rest fail to differentiate, forming instead very large masses of undifferentiated tissue possessing multiple meristems. Early growth-evaluation methods were based either on visual estimation of seedling development or on measurements of protocorm diameters. With both methods the effect of tomato juice would appear to be favorable. However, when the parameters used to evaluate growth measure protocorm survival and normal development its true effects become apparent. The growth-index method (Spoerl 1948, Mariat 1952, Arditti 1965a) and direct seedling measurements (Ernst 1966b) provide such parameters and are therefore capable of providing more accurate information.

A recently developed method for the propagation of orchids has been the culture of excised apical meristems (Morel 1960, Wimber 1963, Ilsley 1966). The original explants form protocorm-like structures which are sectioned after they reach a certain size and subcultured. The subcultures are treated in a similar fashion. Since excessively large protocorms with an increased number of meristems would be advantageous in this instance, tomato juice media may prove to be of value for such cultures. The consistency with which pineapple juice and banana homogenates have enhanced orchid seed germination and seedling growth would seem to suggest that these preparations may contain certain growth factor(s) worthy of further investigation.

THE EFFECTS OF TEMPERATURE

Despite the numerous and varied orchid seed culture techniques and their wide use, little specific information is available regarding the effects of temperature. This may be due to the ease with which orchid seeds germinate on well formulated culture media and to the apparent lack of highly specific temperature requirements by most species. Little direct research on the effects of temperature on germinating orchid seeds has been carried out. In two instances the temperature requirements of difficult-to-germinate species have been investigated. Comparative data are therefore available for *Vanilla* (Bouriquet 1947, Knudson 1950) and *Galeola septentrionalis* (Nakamura 1962, 1964) (Table XIII). In addition, the temperature requirements of germinating *Cattleya* × 'Luegeae' seeds have been investigated (Northen and Northen 1949) (Table XIII).

General information concerning temperature requirements of germinating seeds of additional species is available as a byproduct of other research or from the experience of practical growers. Since experimental conditions as well as

culture techniques and media vary considerably valid conclusions are difficult to draw. Therefore, the data in Table XIII are presented "as is" and in some detail.

It is possible to state only that orchid seeds seem to germinate best at the 20-25° C. range but can germinate and develop at temperatures ranging from 6° to 40° C. or even higher.

THE EFFECTS OF LIGHT

Orchid seeds vary considerably in their requirements for light. Some require light for germination (Darnell 1952) whereas others do not and may germinate in the dark (Bouriquet and Boiteau 1937, Knudson 1950). *Vanilla* seems to germinate well in the dark (Bouriquet and Boiteau 1937, Knudson 1950) but not under a light intensity of approximately 800 foot candles (Knudson 1950). *Cypripedium* seeds germinate well in darkness and the young seedlings seem to survive better if kept for up to 90 days in the dark (Yamada 1963b), or under light intensities as low as 0.8 - 4.5 foot candles (Hegarty 1955). The young seedlings should be removed only very gradually to higher light intensities (Yamada 1963b). It has been suggested that they be kept at 3 - 9 foot candles for a while, prior to being given higher light intensities (Hegarty 1955).

Certain species may germinate both in the light and dark but the seedlings grown in the light will differ from those grown in the dark. Seedlings of *Cattleya, Epidendrum,* and *Oncidium* are reported to vary greatly in their ability to grow in the dark (Yates and Curtis 1949). Actually, under some conditions *Cattleya* seeds may not even germinate in the dark (Burgeff 1909). *Cattleya loddigesii* Lindl. germination in the dark was greatly reduced and seedling growth inhibited. Other *Cattleya* seedlings grown in the dark are generally etiolated (Knudson 1924, Yates and Curtis 1949), form no roots (Yates and Curtis 1949), but may develop up to two leaves in 90 days (Knudson 1924). The utilization of amino acids by *Cattleya* seedlings grown in the dark differs from that by seedlings grown under light intensities of 50 foot candles and an 18-hour photoperiod (Spoerl 1948).

Cymbidium seeds germinate well in the dark (Kohl 1962) but form small protocorms, scale-like leaves, and no roots (Yates and Curtis 1949). When individual protocorms were transferred to complete darkness they lost their chlorophyll but produced shoots readily. These shoots were greatly etiolated (Kohl 1962).

Seedlings of *Oncidium* seem to require no light for both shoot and root formation. Seedlings grown in the dark appear normal but growth is more extensive in the light (Yates and Curtis 1949). Similar results were recorded for other species (Burgeff 1936).

Subdued, diffuse sunlight generally has been recommended for *Cattleya* (Bernard 1909, Mariat 1948, 1952, Bahme 1949), *Laelia* (Bernard 1903), and other species (Schaffstein 1938). Practical growers commonly germinate their seed under comparatively low light intensities. Photoperiods, light intensities,

and light qualities which have proved satisfactory in various experiments or practical culture are presented in Table XIV.

In experiments designed to determine the effect of 18-hour photoperiods (with light supplied by a 200-watt unshielded electric light bulb in the ceiling of the germinating chamber) the results varied with the genus (Table XV) (Storey *et al.* 1947).

Seedlings of *Cattleya* under very long photoperiods or continuous light tend to be somewhat lighter in color than seedlings grown under photoperiods of 12–15 hours. In general, most orchid seedlings are grown under natural light and photoperiods with occasional supplementary illumination. Such illumination may be by incandescent light bulbs as well as cool white, daylight, and Gro-Lux fluorescent bulbs.

As previously indicated, interpretation of the data is difficult due to the varied and often inconsistent techniques or constituents of the culture media used. Several instances of germination (or lack of it) both in the light and darkness could very well have been influenced by unsuspected factors present in the media, or by poor techniques.

THE EFFECTS OF pH

The need for an appropriate pH in the germination of orchid seeds was fully appreciated as early as 1924. However, arguments and contradictory statements in the literature make it difficult to determine priority of discovery (Knudson 1924, 1925, 1927, Clement 1924a, b, 1926, 1929). Owing to the selective uptake of ions by orchid seedlings (Sideris 1950) and the poor buffering capacity of some of the culture media used, pH often changes radically during the culture period. This has led to the development of buffered media (Burgeff 1936) or supposedly improved media (Vacin and Went 1949a, Vacin 1950a, Sideris 1950, Knudson 1951). It appears, however, that pH may be critical only during the early stages of germination and the seedlings themselves are less sensitive to differences of pH (Knudson 1951).

Few comparative studies on the effect of pH are available. (Quednow 1930, Nakamura 1962, Kano 1965, Kotomori and Murashige 1965). However, information on the most suitable pH for various species may be gathered from a number of sources (Table XVI). The data can not be interpreted or evaluated completely owing to the many variables present and the frequently poor presentation (Sleap 1962b) or unusual terminology (Wherry 1918, 1920, 1921, 1927). The effects of pH may possibly depend on the substances present in the culture medium, and, as previously mentioned, in many instances the true composition of the culture media used is not entirely clear. It is also difficult to determine whether the effect of pH on embryos and seedlings is direct or indirect and due to its effect on culture media components and their availability.

THE EFFECTS OF THE ATMOSPHERE AND ITS COMPONENTS

Due to the sterile culture techniques employed in orchid seed germination the question of proper gas exchange and the composition of the atmosphere inside the culture vessels has been considered by several investigators.

It appears that germination of seeds in airtight containers is at least equal to (Quednow 1930) or superior to (Meyer 1948, Anon. 1952, Kano 1965) that of seedlings in non-airtight culture vessels. On the other hand it has been observed that seeds which do become buried in the agar or find their way below the agar surface germinate well but do not differentiate so well as those on the surface. Up to one third of the buried protocorms lacked chlorophyll, appeared to develop in a manner similar to that of the chlorophyll-containing ones, but at a much slower rate (Arditti 1964a). These observations are in agreement with other (Leuschner 1963) reports that seeds on soft agar (Kano 1965, Kotomori and Murashige 1965, Morton 1965) or submerged in liquid media germinated well (Kohl 1962), but some protocorms lacked chlorophyll whereas others proliferated abnormally, forming up to 100 growing points. When the cultures became very crowded, leafy shoots developed but no adventitious roots could be observed (Knudson 1946b, Kohl 1962). This appears to indicate that in the absence of air, root, but not leaf, initiation may be inhibited.

Tests with a variety of culture container plugs, plug material, and stoppers seem to indicate that in most instances the amount and nature of gas exchange are satisfactory (Liddell 1944, Breddy 1953) or that germinating orchid seeds can adapt to a variety of atmospheric compositions, and rates of gas exchange.

Some reports and observations by growers seem to indicate that fertilization with sterilized air or carbon dioxide may improve seedling growth (Frackowiak 1933, Withner 1959a, Bean 1965, Borg 1965, Hurd 1965, Tennant 1965), but other observations do not lend support to these reports (R. J. Scott and J. Arditti, unpublished).

The germination and improved growth (Kano 1965) in airtight culture vessels is difficult to explain and is very interesting. Seedlings grown on sugar-containing media may derive most or all of their carbon from the culture medium and carry out little or no photosynthesis. In fact, some seedlings do not form chlorophyll during the first month and obviously can not photosynthesize (Stoutamire 1963, 1964b). Such seedlings will have almost no need for CO_2 and may therefore be able to germinate well in an airtight culture vessel where the original supply of atmospheric gasses is not being replenished. However, in the absence of gas exchange and a possible lack of photosynthesis the usually large number of seedlings present in each flask would soon exhaust all available oxygen despite the very low respiration rates of orchid seedlings (Bahme 1949). A 500 cc. culture flask containing 125 cc. of culture medium will have 375 cc. of atmosphere, approximately 75 cc. (20 per cent) of it oxygen (ignoring the presence of water vapor). Such a flask would normally contain several hundred (up to 1000) seeds. Even allowing for a low rate of aerobic respiration this still is a rather low amount of oxygen. Thus an interesting question arises in respect to the respiratory pathways of germinating orchid seeds: do they perhaps respire anaerobically? It is possible, of course, that even in the presence of a carbohydrate source in the culture medium a certain amount of photosynthesis does take place and replenishes the oxygen supply. Nevertheless, this would only extend the use of whatever oxygen may be present in the

flask and a shortage would eventually develop. Inasmuch as orchid seedlings are removed from their original flasks after six months to a year it is possible that there is no time for such a shortage to develop. Still, the respiratory pathways of germinating orchid seeds present a challenging problem.

THE EFFECTS OF MOISTURE

Water is of the greatest importance in the germination of orchid seeds; without it they remain dormant. Because of the specialized structure of the embryo and seed coat, orchid seeds are not easily wettable. As long as the seed is dry it does not sink, but floats easily for prolonged periods, and may be carried considerable distances by streaming water. Most seeds will become thoroughly wet and sink only following vigorous shaking (at least 10 - 15 minutes). Wettability of orchid seeds has been studied in great detail (Burgeff 1936).

Seed germination in the laboratory is most often performed on agar media and rarely on sand impregnated with an appropriate solution (Clark 1959). Since these media have a high water content the effect of moisture levels is difficult to assess, despite reports that variations in water content may affect germination seriously (O'Brien 1911, Goldsmith 1956, Boyd 1963, Slade 1963).

Young seedlings of *Dendrobium* and *Vanda* grow well on several potting media consisting of cinder and tree-fern root mixtures provided moisture levels are sufficient (Beaumont and Bowers 1953). It is generally agreed among practical growers that orchid seedlings require constant moisture and should never be subjected to moisture stress. This may be due to the large leaf area, few roots, and lack of water storage tissues and organs in the young seedling.

Relative humidity of at least 50 and preferably 70-80 per cent appears to be most favorable for all orchid seedlings (Logan and Cosper 1950).

THE EFFECTS OF MISCELLANEOUS FACTORS

The suitability of various culture containers and types of glass has been investigated and it appears that most produce satisfactory results (Breddy 1953). In some instances where "cheap glass" bottles have been used, however, washing with hydrochloric acid has led to improved germination and growth, possibly due to removal of certain toxic elements which may be present in the glass (Knudson 1946b, Arditti 1965a). Growth appears to be better in larger culture vessels (Knudson 1922, Kano 1965).

It has been reported that soaking *Paphiopedilum* seeds in water up to a month and other seed for 48 hours in saturated sugar solution has resulted in greatly improved germination, possibly due to leaching of a water soluble inhibitor (Burgeff 1936, Withner 1953, Hegarty 1955, Hey 1966a). *Vanilla* seeds may also contain inhibitors but of a different nature since their germination has been improved by washing with organic solvents (Withner 1955). Immersion of seeds of certain native American orchids in a 1:2000 solution of colchicine is reported to have improved seedling growth (Liddell 1944). Whether this is due to leaching of inhibitors or to induced polyploidy which would result in more robust seedlings is not clear at present.

Gravity disorientation experiments have indicated that gravity does not play a large part in seedling development (Kohl 1962). On the other hand seeds failed to germinate on a "wrist action" shaker, whereas seedlings transferred to such a shaker proliferated in an abnormal manner or died (Kohl 1962).

Following mutilation by "cutting, slicing, crushing and mashing," protocorms and protocorm fragments developed new protocorms and "seedlings" when plated on standard agar (Kohl 1962). This may be interpreted as an indication of the embryonic nature of protocorm cells. It is also suggestive of a propagation method, for instance, where only few seeds or protocorms of a very valuable hybrid or species are available. Moreover, it presents a method for the production of large clones of genetically identical chlorophyllous tissue for experimental purposes. Seedlings of *Vanda tricolor* grown on culture media containing barbiturates such as sodium ethyl-(1-methyl-butyl) barbiturate, sodium cyclopentylallyl barbiturate, and phenyl ethyl barbituric acid have proliferated into masses of undifferentiated tissue. On the other hand, esculin, a known plant growth inhibitor, when applied to *Phalaenopsis* seedlings at 10 p.p.m. of esculin hemihydrate failed to exert much inhibition (Ernst 1966b).

THE NATURE OF THE FACTORS REQUIRED BY GERMINATING ORCHID SEEDS

It is clearly evident from the information presented in this review that orchid seeds are unique in their requirements for certain factors both during germination and subsequent development. The available experimental evidence concerning the nature of these requirements is difficult to evaluate owing to the great number of species and various culture media employed in these investigations. In addition, the degree of purity of these chemicals has varied widely. Nevertheless, it appears certain that one or possibly more growth factors are required for germination.

The growth-stimulating factor in question can be obtained from a variety of plant and animal material (Table XII). Algae and mosses are possible sources while fungi, higher plants, and fish are proven sources. Thus the growth factor(s) and/or precursors must be of wide occurrence in both plants and animals. It appears, therefore, that there is no strictly specialized "orchid factor."

Since the germination-promoting capacity of various complex media is apparently not destroyed by autoclaving, it is possible to conclude that the factor(s) in question are not heat labile (Burgeff 1936, Schaffstein 1941, Vacin and Went 1949b). The results of fractionation studies of the endophytic fungus (Burgeff 1936, Downie 1940, 1943a, 1949a, b), *Vicia* (Schaffstein 1941), tomato (Arditti 1965a, b, 1966b), and yeast (Nakamura 1962) have indicated that they are: (1) very soluble in water and alcohol (Burgeff 1936, Schaffstein 1941, Nakamura 1962, Arditti 1965a) but insoluble in absolute alcohol (Schopfer 1943); (2) soluble in acetone (Burgeff 1936, Arditti

1965a), but with some difficulty (Schaffstein 1941); (3) slightly soluble in benzene (Schaffstein 1941); (4) insoluble in ether (Burgeff 1936, Schaffstein 1941, Schopfer 1943, Nakamura 1962) and chloroform (Schaffstein 1941); (5) easily adsorbed on charcoal and partially so on bole (Schaffstein 1941) but not on filter paper or Fuller's earth (Schopfer 1943); (6) able to permeate a cellophane membrane (Nakamura 1962); (7) precipitated by phosphotungstic acid but not by lead acetate, mercuric chloride, silver nitrate, and alcoholic barium solution (Schaffstein 1941); (8) resistant to ultraviolet irradiation and perhydrol oxidation (Burgeff 1936, Schopfer 1943).

Two physiological aspects of germinating orchid seeds stand out. Seeds and protocorms developing on a purely mineral medium show a green color (which is indicative of the presence of chlorophyll) but are apparently incapable of normal photosynthesis (Curtis and Clark 1950, Withner 1959a). Seedlings placed on media containing highly purified agar and maltose germinate poorly and the seedlings are unable to develop normally, in effect, cell division ceases, starch accumulates, and many of them die (Noggle and Wynd 1943, Schopfer 1943). This would appear to indicate that the seedlings are possibly unable to manufacture and/or utilize sugar or at least that they may encounter major difficulties in doing so. It has been suggested that this may be due to a vitamin deficiency (Burgeff 1936, Schaffstein 1938, 1941, Schopfer 1943, Noggle and Wynd 1943, Bahme 1949, Harley 1951, Mariat 1952).

Of various amino acids tested as substitutes for the required factor only histidine and possibly aspartic acid, tryptophan, and arginine appear to have had any activity (Spoerl 1948, Nakamura 1962, Arditti 1965a, Kano 1965, Ernst 1966b) (see section on Nitrogen Nutrition). Only niacin among the vitamins has consistently improved germination and promoted growth. The effect of other vitamins is still open to question (see section on The Effects of Vitamins). It has therefore been suggested that the substance in question is niacin (Harley 1951, Burgeff 1959) or a derivative thereof (Schaffstein 1941, Schopfer 1943, Harley 1951). Should the factor indeed be niacin, it is possible that orchid seedlings may not be able to produce or utilize sufficient amounts of it (Harley 1951).

The growth-promoting effects of adenine (sometimes called the "leaf growing factor") and ribose (Withner 1942, 1943, Kano 1965, Arditti 1965a,c, Ernst 1966b) also require comment. Both compounds are constituents of the NAD and NADP molecules, as is niacin, and it has indeed been suggested that the requirements of germinating orchids are for a component of the "codehydrase" molecule (Harley 1951). However, application of exogenous NAD and NADP singly and in combination has sharply inhibited germination and growth (Arditti 1965a, c, In Press a).

In summation, it appears that the currently available information is not sufficient for a positive identification of the factor or factors required by germinating orchid seeds (something referred to as the "orchid factor" (Schaffstein 1938) and "Vandophytin" (Schaffstein 1941)). Much additional

research is needed for that and for a more complete understanding of orchid seed germination.

A NOTE ON THE LITERATURE

Although many authors, starting with Confucius (551-479 B.C.) and Theophrastus (370-285 B.C.), have written about orchids, it appears that the English botanist Richard Salisbury was the first to publish a description of germinating orchid seeds, in 1804. Since then numerous articles on orchid seeds and their germination have appeared in a Babel of languages and a very large number of publications, some of them obscure, by professional, well-trained botanists and horticulturists as well as by amateurs whose degree of botanical training has varied from excellent to exceedingly poor. These publications, when taken together, represent the sum-total of our current knowledge on the subject of orchid seeds and their germination. In preparing this review article, the author was faced with the task of separating worthwhile contributions from the "chaff." This has not been always easy, for several reasons, the most common of which are listed below.

1. Articles may often be short, cryptic, or poorly written and therefore difficult to evaluate.
2. Experimental procedures, if any, are not always fully described, making a critical evaluation difficult if not impossible.
3. Reagent purity at times may be a matter of conjecture, since it is not given, or is due to the fact that standards have changed through the years. It is difficult, therefore, to determine the role of possible impurities.
4. Terminology has changed and at times older terms or values are difficult to convert into present day terminology. Wherry's method of reporting acidity is a prime example of meticulously done and carefully reported work which is difficult to understand because of his apparent refusal to use the term pH.

A strict elimination of any publication which did not meet the very highest standards could have resulted in the elimination of some worthwhile papers. The author has, therefore, adopted a more liberal approach and included in this review papers which, although not of present-day highest standards, appear to contain valuable information. Hopefully, this has resulted in a more inclusive review incorporating the majority of significant works on the subject. Nevertheless, it is possible that some worthwhile papers may have been omitted and insignificant ones included. Should this be so, one can only apologize and hope to be corrected.

TABLE I

SIZE, WEIGHT, COLOR, AND NUMBER OF SEEDS PER CAPSULE IN SOME ORCHID SPECIES

Species	Seed Size, in mm.	Seed Weight 10^{-6} g.	Color	Seeds per Capsule	Reference
Acanthophippium sylhetense Lindl.	.19 × .07	0.66			Burgeff 1936
Acropera Lindl.				371,257 (74,000,000 per plant)	Poddubnaya-Arnoldi & Selezneva 1957
Anacamptis pyramidalis (L.) L. C. Rich				1,935	Tournay 1960
Angraecum Bory spp.			reddish-brown		Hey 1966a, b
Angraecum eburneum Thouars. × *A. sesquipedale* Thouars.		0.70			Burgeff 1936
Anguloa ruckeri Lindl.		0.39		3,900,000	Burgeff 1936, Poddubnaya-Arnoldi & Selezneva 1957
Bulbophyllum barbigerum Lindl.			golden-yellow		Kupper & Linsenmaier 1961, Hey 1962
Caladenia cardiochila Tate	.43 × .13				Stoutamire 1963
C. carnea R. Br.	.8 × .3				Stoutamire 1963
C. patersonii R. Br.	.47 × .12				Stoutamire 1963
Calanthe R. Br.			white		Poddubnaya-Arnoldi & Selezneva 1957

Calochilus campestris R. Br.	.33 × .2				Stoutamire 1963
Calopogon pulchellus R. Br.	.075 × .02				Stoutamire 1964b
Calypso Salisb.			cream		
Catasetum macrocarpum L. Rich ex Kunth	.58 × .15				Burgeff 1936
Cattleya sp.	0.250 × 0.075		white, cream, yellowish, and tan		Knudson 1921, Davis 1946
Cattleya sp.	0.420 × 0.090			1,000,000 to 6,000,000	Davis 1940, Poddubnaya-Arnoldi & Selezneva 1957
Cattleya sp.				500,000 (approx.)	Leuschner 1960, 1963
Cattleya sp.	0.150 × 0.090				Knudson 1929
Cattleya aurantiaca (Batem. ex. Lindl.) P. N. Don				256,000	Tournay 1960
Cattleya gigas Linden et Andre	.250 × .075			500,000 to 700,000	White 1927, 1945
Cattleya labiata Lindl.		2.5		929,000	Hager 1952
Cattleya lobata Lindl.	0.910 × 0.130				Hoene 1940, 1943
Cattleya trianae Linden et Rchb. f.	0.560 × 0.110				Hoene 1940, 1943
Cephalanthera damasonium (Mill) Druce				6,020	Tournay 1960

TABLE I (continued)

SIZE, WEIGHT, COLOR, AND NUMBER OF SEEDS PER CAPSULE IN SOME ORCHID SPECIES

Species	Seed Size, in mm.	Seed Weight 10^{-6} g.	Color	Seeds per Capsule	Reference
Cephalanthera grandiflora S. F. Gray				6,020	Darwin 1888
Cephalanthera pallens Rich.		2.0			Burgeff 1936
Cocleanthes			yellow		Hey 1966a, b
Coeloglossum viride (L.) Hartm.				1,330	Tournay 1960
Corallorhiza innata R. Br.	0.200 x 0.100 (embryo) 0.700 long (testa)				Downie 1943a
Cycnoches ventricosum Batem. var. *chlorochilon* (Klotzsch) P. H. Allen		3.6		4,000,000	Arditti 1961
Cymbidium Swartz	0.255 x 0.146 (450 cells) 1.57 x 0.27		yellowish or tan	1,500,000	Scott and Arditti 1959, Leuschner 1960, Kupper & Linsenmaier 1961
Cymbidium traceyanum Hort.				850,000	White 1927, 1945 Davis 1946
Cypripedium L.			dark brown		Leuschner 1960
Cypripedium aucale Ait.	.15 x .026	2		54,180	Stoutamire 1964b

C. reginae Walt.	.093 × .03				Stoutamire 1964b
Dactylorchis fuchsii (Druce) Verm.				3294 to 6200	Tournay 1960
Dendrobium attenuatum Lindl.		6.5			Ames 1922b, 1948
Dendrobium autumnalis		5.65			Burgeff 1936
Dendrobium Swartz spp.			yellow		Hey 1966a, b
Dendrobium nobile Lindl.					
embryo	0.181 × 0.081				Ito 1955
whole seed	0.428 × 0.102				Ito 1955
Didymoplexis pallens Griff.		0.45			Burgeff 1936
Diuris sulphurea R. Br.	.4 × .13				Stoutamire 1963
Epidendrum radicans Pavon ex Lindl.		6.0			Burgeff 1936
Epidendrum L.			greenish white		Leuschner 1960
Epipogium mutans Rchb. f.	.23 × .15				Burgeff 1936
Galeola altissima (Bl.) Rchb. f.	1.000 × 2.000	17.0		18,000	Hamada & Nakamura 1963
Galeola lindleyanna Rchb. f.		14.0			Burgeff 1936
Galeola septentrionalis Rchb. f.	0.840 × 0.350 × 0.024	24.0	dark brown, nearly black	16,000	Nakamura 1962, 1964
Goodyera oblongifolia Raf.	.3 × .16				Stoutamire 1964b
G. pubescens (Wild.) R. Br.	0.93 × .22				Stoutamire 1964b

TABLE I (continued)

SIZE, WEIGHT, COLOR, AND NUMBER OF SEEDS PER CAPSULE IN SOME ORCHID SPECIES

Species	Seed Size, in mm.	Seed Weight 10^{-6} g.	Color	Seeds per Capsule	Reference
G. pubescens × *G. repens*	0.74 × .11				Stoutamire 1964b
Habenaria Willd.			light brown		
H. blephariglottis (Willd.) Torr.	.3 × .05				Stoutamire 1964b
H. blephariglottis × *H. lacera*	.04 × .015				Stoutamire 1964b
H. blephariglottis × *H. leucophaea*	.05 × 0.23				Stoutamire 1964b
H. blephariglottis × *H. psycodes*	.133 × .084				Stoutamire 1964b
H. cilliaris R. Br.	.12 × .05				Stoutamire 1964b
H. cilliaris × *H. blephariglottis*	.12 × .05				Stoutamire 1964b
H. ciliaris × *H. leucophaea*	.04 × .018				Stoutamire 1964b
H. ciliaris × *H. psycodes*	.045 × .018				Stoutamire 1964b
H. hookeri Torr.	.67 × .15				Stoutamire 1964b
Haemaria discolor (Ker) Lindl.	1 × .09				Burgeff 1936
Laelia Lindl. sp.	0.250 × 0.075		yellowish or tan		Knudson 1922, Leuschner 1960

Limodorum abortivum Sw.	1 × .23			Godfery 1933
Liparis loeseli Rich.	.12 × 0.42			Stoutamire 1964b
Listera ovata (L.) R. Br.	0.125 × 0.093			Downie 1949a
Listera cordata (L.) R. Br.	.37 × .11		376 (2,860 per plant)	Stoutamire 1964b
Lycaste Lindl. sp.	0.280 × 0.100	yellow		Davis 1946, Poddubnaya-Arnoldi & Selezneva 1957
Malaxis monophyllos (L.) Sw.			1,572 (19,490 per plant)	Stoutamire 1964b
Maxillaria Ruiz & Pav. sp.		yellow, yellowish-tan	1,756,440 (10,000,000 per plant)	Tournay 1960, Poddubnaya-Arnoldi & Selezneva 1957
Microtis unifolia Rchb. f.	.37 × .1			Stoutamire 1963
Odontoglossum HBK.		orange or brown		Kupper & Linsenmaier 1961, Leuschner 1960, Hey 1966a,b
Oncidium Swartz		yellowish or tan		Leuschner 1960
Orchis maculata L.			6,200	Darwin 1888
Paphiopedilum curtisii Pftz.	1.16 × 0.13			Hoene 1940, 1949
Paphiopedilum parishii Pftz.	0.5 × 0.16			Hoene 1940, 1949
Paphiopedilum specierum Rchb. f.	0.7 × 0.16			Hoene 1940, 1949

TABLE 1 (continued)

SIZE, WEIGHT, COLOR, AND NUMBER OF SEEDS PER CAPSULE IN SOME ORCHID SPECIES

Species	Seed Size, in mm.	Seed Weight 10^{-6} g.	Color	Seeds per Capsule	Reference
Phalaenopsis sp.	0.40 x 0.08 0.41 x 0.1		brown or white to cream		Gratiot 1934, Davis 1946, Poddubnaya-Arnoldi & Selezneva 1957, Leuschner 1960
Pogonia ophioglossoides (L.) Ker.	.115 x .018				Stoutamire 1964b
Pterostylis boormanii Rupp.	.25 x .16				Stoutamire 1964b
P. falcata R. S. Rogers	.6 x .16				Stoutamire 1963
P. furcata R. Br.	.7 x .13				Stoutamire 1963
P. nana R. Br.	.7 x .1				Stoutamire 1963
P. nutans R. Br.	.6 x .1				Stoutamire 1963
P. pedunculata R. Br.	.6 x .1				Stoutamire 1963
Serapias cordigera	.66 x .22				Stoutamire 1963
Sobralia macrantha Lindl.		6.3			Burgeff 1936
Spiranthes cernua Rich.	.05 x .01				Stoutamire 1964b
Stanhopea oculata (Lodd.) Lindl.		3.0			Kerner 1895

S. wardii Lodd. ex Lindl.		pale yellow	Hey 1966a,b
Taeniophyllum aphyllum (Makino) Makino	.279 x .075		Mutsuura, Ito, & Nakahira 1962
Thunia Rchb. f.		yellow	Poddubnaya-Arnoldi & Selezneva 1957
Vanda sp. Jones	0.25 x 0.11	brown, reddish-brown	Davis 1946, Leuschner 1960, Werblin 1963, Hey 1966a,b
Vanda tricolor Lindl.	0.197 x 0.077 (120 cells)		Curtis 1947a, Curtis & Nichol 1948
Vanilla Swartz spp.	0.312 x 0.26	dark brown, nearly black	Bouriquet & Boiteau 1937, Knudson 1950
Vanilla planifolia Andr.	0.29 x 0.25		Lugo-Lugo 1955a
Vanilla planifolia Andr.	max. diam. 0.313		Tonnier 1954a

TABLE II

MINERAL CONTENT OF *CORALLORHIZA* R. BR. SEEDS (WHEELER 1966)

	Amount		
Element	p.p.m. plant dry weight	p.p.m. in ash	% plant dry weight
Potassium		23	
Calcium		5.4	
Magnesium		5.5	
Sodium		1.5	
Phosphorus		8.8	
Nitrogen	Not determined	Not determined	
Silicon	466		
Lead	8		
Iron	22		
Nickel	0.5		
Barium	Trace		
Aluminum	7		
Boron	13		
Copper	2		
Titanium	16		
Strontium	2		
Manganese	48		
Ash			1.37

TABLE III

COMPOSITION OF SOME COMMON SEEDLING CULTURE MEDIA (WITHNER 1959a) AND ORCHID-NURTURING TREE TRUNK EFLUATE (CURTIS 1946). AMOUNTS EXPRESSED IN MILLIMOLES.

Ion	Sladden modif. Burgeff	Knudson C	Burgeff Eg-1	Burgeff N_3f	Thomale GD	Tree Trunk Efluate
Nitrate	8.40	8.40	8.40	8.40	9.45	0.0025
Ammonium	10.50	7.60	3.80	3.80	5.50	0.0880
Phosphate	2.94	1.80	3.20	1.40	2.20	0.0105
Sulfate	6.50	4.80	2.90	2.90	0.45	0.0052
Chloride				3.40		0.1430
Potassium	2.94	1.80	4.60	6.20	6.20	0.0770
Magnesium	1.20	1.00	1.00	1.00	0.43	0.1770
Calcium	4.80	4.20	4.20	4.20		0.0250
Citrate	1.89			0.43		
Iron	0.67	0.09	0.07	0.07	0.07	0.0073
Manganese		0.034				
Sodium						0.1310

TABLE IV

THE EFFECTS OF VARIOUS CARBOHYDRATES ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

The > and = signs indicate relative effectiveness.

Investigator	Species	Sugars and other carbohydrates	Remarks
Bernard 1909	*Laelia*	Sucrose	Germination
Knudson 1922	Epiphytic genera	Sucrose > fructose > glucose	Satisfactory germination
LaGarde 1929	*Cattleya trianei*	Maltose > fructose > glucose > sucrose	Satisfactory germination
Quednow 1930	Epiphytic genera and two sp. of *Orchis* L.	Glucose > fructose > sucrose > maltose > mannite > galactose > lactose	Satisfactory germination
		Citric, malic, tartaric, oxalic, and succinic acids	No effect
Smith 1932	Seedlings	Sucrose = glucose = fructose = glucose plus fructose	Good growth
Wynd 1933a	*Cattleya trianei*	d-Mannose > d-glucose > maltose > d-fructose > sucrose > raffinose	d-mannose particularly good
		d-Galactose, l-arabinose, l-rhamnose, l-xylose	No growth
Burgeff 1936	*Cymbidium*	Glucose, fructose, sucrose	Largest on glucose
	Vanda	Glucose, fructose, sucrose	Largest on glucose, fasciated
	Paphiopedilum	Glucose and fructose	

Schaffstein 1938	*Phalaenopsis* *Dendrobium*	10 g. glucose, 10 g. levulose 10 g. glucose, 10 g. levulose	Good germination and growth
Withner 1942	*Cattleya* hybrids	Sucrose > dextrose > brown sugar plus dextrose in equal amounts	Brown sugar alone is toxic
		Ribose	Good germination
		Pyruvate, malate	Possible favorable effect
Downie 1943a	*Corallorhiza innata* R. Br.	Glucose solution	No asymbiotic growth
Bouriquet 1947	*Vanilla*	Sucrose, glucose (20 g./l.), lactose, in the presence of *Vanilla* plant decoction. Starch.	Good germination, best germination on glucose
		Mannite and maltose	No germination
Noggle & Wynd 1949	*Cattleya* hybrids	Maltose (unpurified)	Excellent germination and growth
		Maltose (purified)	No germination. Slow development. Only traces of chlorophyll
		Maltose (purified) plus niacin	Normal growth and development
Yates & Curtis 1949	*Cattleya trianei*, *Epidendrum nocturnum*	Sucrose	High sucrose concentration, increased shoot growth (see text)
Downie 1949a	*Goodyera repens* (L.) R. Br.	Mixture of glucose, fructose, and ribose	Effective only in the presence of water extract of fungus
Downie 1949b	*Listera ovata* (L.) R. Br.	Glucose	No asymbiotic growth

TABLE IV (continued)

THE EFFECTS OF VARIOUS CARBOHYDRATES ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Sugars and other carbohydrates	Remarks
Bahme 1949	*Brassocattleya* hybrid, *Cattleya*, *Vanda*	Sucrose	Improved growth in the presence of niacin
Knudson 1950	*Vanilla planifolia*	Sucrose	Good growth if temperature is at least 32°C.
Mariat 1951	*Cattleya*	Sucrose 10 g., plus glucose 15 g.	Poor germination in the presence of coconut milk and/or copra
Vacin 1950b	*Cymbidium*	Sucrose	Good germination and growth
Ito 1951	*Dendrobium*, *Cattleya*, *Vanda*, *Phalaenopsis*, *Epidendrum*	Corn starch (Kingsford's)	Used in mixture with tomato juice. Good germination and growth
Mariat 1952	*Cattleya*, *Bletilla hyacinthina*	Sucrose	Good germination and growth
Withner 1953	*Cypripedium aucale*	Sucrose	Good germination

Breddy 1953	*Odontoglossum* *Cattleya* *Cymbidium* *Miltonia* *Vanda* *Phalaenopsis* *Dendrobium*	All genera were grown on media containing sucrose, glucose, fructose, maltose, mannose, singly, or in combination.	Maltose or sucrose best Sucrose or glucose best Sucrose best Sucrose or maltose best Glucose or sucrose best Glucose or sucrose best Maltose best Data obtained in a series of experiments
Liddell 1953b	*Cypripedium*	2 g. sucrose plus 18 g. glucose	Improved germination
Tonnier 1954a	*Vanilla madagascariensis* Rolfe	Glucose	Germination
Nakamura 1962	*Galeola septentrionalis*	Cane sugar	Good germination and growth
Tsukamoto *et al.* 1963	*Dendrobium*, *Cattleya*, *Cymbidium*, *Paphiopedilum*	Sucrose	In mixture with a variety of complex organic additives. Good germination and growth
Stoutamire 1963	Terrestrial, native Australian	Sucrose; glucose and fructose in mixture	Germination
Stoutamire 1964b	Terrestrial, native American	Sucrose; glucose and fructose in mixture	Germination
Israel 1963	*Dendrobium* (immature ovaries)	Sucrose	In mixture with NAA. Good growth
Rao 1963	*Vanda*	Sucrose	Good germination. Some proliferation

TABLE IV (continued)

THE EFFECTS OF VARIOUS CARBOHYDRATES ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Sugars and other carbohydrates	Remarks
Raghavan & Torrey 1964	*Cattleya labiata*	Sucrose	Good germination and growth on ammonium-containing media
Arditti 1965a, b, c, 1966b, In Press a, b	*Cattleya*	Maltose Ribose	Good germination and growth Unsatisfactory
Kano 1965	*Dendrobium, Brassolaeliocattleya*— 120 days old	Sucrose	Best germination and development on 4% concentration
	Dendrobium, 8 mo.	Sucrose	2-4% best following transflasking
	Brassocattleya, 12.5 mo.		4% most suitable
	Brassavola nodosa (L.) Lindl., 10 mo.		4% most suitable
	Paphiopedilum callosum Rchb.f. 51 days		4% most suitable
	Cymbidium hybr. 195 days		2-4% most suitable
Lindquist 1965	*Disa grandiflora* L. *(Disa uniflora)*	"Grape sugar"	

TABLE V (from Ernst 1966a)

EFFECT OF CARBOHYDRATE SELECTION ON THE GROWTH RATE OF FRESHLY GERMINATED *PHALAENOPSIS* ('ELINOR SHAFFER' × 'DORIS') SEED

Carbohydrate 2% anhydrous basis:	No. of Carbons	Leaves av. number of:	Leaves av. length of:	Leaves av. width of:	Roots av. number of:	Roots max. diameter of:	Seedlings av. weight in mg.:	
Monosaccharides:								
D-xylose, C.P.	5	3.65	13.700 mm.	7.000 mm.	4.70	2.00 mm.	194.110	
D-ribose	5	2.05	4.575	3.325	1.10	1.25	24.575	
α-D-glucose, C.P.	6	4.20	12.400	5.625	4.00	2.00	162.900	
β-D-glucose, C.P.	6	3.95	14.300	6.450	4.50	2.00	190.500	
D-fructose, C.P.	6	3.80	15.275	7.825	4.15	2.50	211.900	
D-mannose, C.P.	6	3.80	12.950		4.60	2.00	172.000	
Disaccharides:								
Sucrose, C.P.	12	4.00	13.200	6.550	4.75	2.00	163.800	
Maltose, C.P.	12	3.80	11.100	6.450	4.05	1.75	138.120	
Cellobiose, C.P.	12	3.35	12.500	6.350	3.20	2.00	118.800	
Trehalose, C.P.	12	3.50	11.250	5.875	3.65	1.50	113.915	
α-Melibiose, C.P.	12	2.88	9.833	4.944	2.33	1.00	66.722	(only 9 survived)
Turanose, C.P.	12	2.45	10.500	6.000	2.60	1.75	135.640	
Trisaccharides:								
Raffinose, C.P.	18	3.05	9.475	5.900	3.00	1.50	95.865	
Melezitose, C.P.	18	2.95	11.000	5.075	2.85	2.00	82.025	
Sugar Alcohols:								
D-Arabinitol	5	2.25	7.325	4.325	1.70	1.50	46.390	
Ribitol, C.P.	5	1.30	4.675	2.950	0.90	1.00	20.500	
Xylitol	5	2.90	9.575	4.750	2.20	2.50	89.275	
Sorbitol, C.P.	6	3.20	9.825	5.175	3.40	1.50	95.065	
D-Mannitol, C.P.	6	3.65	13.750	6.700	3.95	2.00	136.200	

TABLE VI (from Ernst 1966a)

CARBOHYDRATES FAILING TO PROMOTE GROWTH WITH FRESHLY GERMINATED *PHALAENOPSIS* ('ELINOR SHAFFER' × 'DORIS') SEED

Carbohydrate 2%, anhydrous basis:	Number of Carbons	Remarks:
Monosaccharides:		
D-arabinose, C.P.	5	All spherules died within 30 days
L-arabinose, C.P.	5	All spherules died within 30 days
D-xylose, C.P.	5	All spherules died within 30 days
L-xylose, C.P.	5	All spherules died within 150 days
D-galactose, C.P.	6	All spherules died within 30 days
L-glucose	6	All spherules died within 30 days
L-mannose	6	All spherules died within 150 days
L-sorbose, C.P.	6	Approximately 95% of the spherules died within 30 days. All perished within 100 days
Sedoheptulosan	$(7)_n$	42 spherules survived, but only 2 seedlings differentiated. 1 seedling with 2 leaves, 4 mm. in length, 3 mm. in width, and 2 roots. 1 seedling with one leaf, 3 mm. in length, 2 mm. in width, and 1 root. Balance of spherules with maximum diameter of 2 mm. Color, pale green.
Disaccharide:		
D-lactose, C.P.	12	All but 3 spherules died within 30 days. 3 spherules survived and showed leaf point
Deoxy Sugars:		
2-Deoxy-D-glucose	6	All spherules died within 30 days
D-fucose	6	All spherules died within 60 days, approximately 10% survived for 50 days
L-fucose	6	All spherules died within 30 days
L-rhamnose, C.P.	6	48 spherules survived for 50 days, 22 for 100 days, 5 for 150 days, 4 for 200 days, and only 3 remained alive after one year. Of these, 2 showed 2 leaves, with a maximum length of 4 mm. and one had produced 1 leaf and a single root. Weight of 3 seedlings was 4.47 mg.
Sugar Alcohols:		
meso-erythritol, C.P.	4	Approximately 90% of the spherules died, with 10% still green after one year. All failed to differentiate
L-arabinitol, C.P.	5	Approximately 80% of the spherules survived, but failed to differentiate
Galactitol, C.P.	6	Essentially all spherules survived, but failed to differentiate. Spherules measured 1 to 4 mm. in diameter after one year. Color, pale olive green
myo-inositol, C.P.		Essentially all spherules survived, but failed to differentiate. One spherule showed emerging leaf. Average weight of 20 spherules was 1.77 mg.

TABLE VII (from Ernst 1966a)

GROWTH OF *DENDROBIUM PHALAENOPSIS* × SELF DURING ONE YEAR, WITH VARIOUS CARBON SOURCES

Carbohydrate: 2%, anhydrous basis:	Leaves		Roots	Seedling
	average number	average length	average number	average weight, mg.
Monosaccharides:				
D-xylose, C.P.	4.40	9.0 mm	2.35	13.85
L-xylose, C.P.	No survivors			
D-glucose (alpha), C.P.	5.35	28.2	3.95	83.25
L-glucose	No survivors			
D-fructose, C.P.	5.50	33.65	4.45	117.295
D-mannose, C.P.	5.60	27.55	4.05	77.775
L-mannose	No survivors			
D-galactose, C.P.	No survivors			
Disaccharides:				
Sucrose, C.P.	4.95	26.8	3.75	56.875
Maltose, C.P. hydrate	4.90	14.3	2.80	37.35
Cellobiose, C.P.	4.35	8.675	1.85	16.775
Trehalose, C.P., dihydr.	4.95	15.65	2.8	43.50
Melibiose (alpha), C.P.*	6.3	8.0	2.0	17.30
Lactose, C.P., hydr. **	4.285	6.8	2.07	18.428
Trisaccharide:				
Raffinose, C.P. pentah. ***	4.583	8.333	2.08	16.325
Deoxy Sugars:				
L-Rhamnose, C.P. hydrate	No survivors			
L-fucose, C.P.	No survivors			
Sugar Alcohols:				
Sorbitol, C.P. hydrate	5.70	22.1	3.1	43.25
D-mannitol, C.P.	3.65	7.65	2.0	11.40
myo-inositol, C.P.		Failed to differentiate		

* Only 3 seedlings survived
** Only 14 seedlings survived
***Only 12 seedlings survived

TABLE VIII

THE EFFECTS OF VITAMINS ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Supplement	Remarks
Burgeff 1934	*Cattleya*	Vitamin preparation from yeast (same fraction found effective in rats). Vitamins A, B_1, and B_2	No effect
Burgeff 1936	*Vanda* group	"Bios-II like material"	Present in dead or live fungus. Slow development in its absence.
	Various	Vitamin "Harris"	Limited effect
Schaffstein 1938	*Phalaenopsis*	Neither biotin, vitamins B_1, B_2, A, or D, nor esterone, nor bios, nor lecithin	Obtained from the fractionation of *Vicia faba*, yeast, and various seeds
Bonner & Greene 1938	Hybrid *Cattleya*	Vitamin B_1	Satisfactory effect
Pollacci & Bergamaschi 1940	*Cattleya autumnalis* Veitch, *Oncidium pulvinatum* Lindl.	Vitamin C	Increased germination and growth
Downie 1940	*Goodyera repens*	Vitamins B_1 and C	No germination
Evers 1940	*Cattleya mossiae* Hook.	Vitamin B_1	No outstanding differences between treated and untreated plants. A few of the treated plants may have been somewhat greener

Schaffstein 1941	*Dendrobium nobile, Vanda, Phalaenopsis*	"Vandophytin"	Favorable effect. Niacin derivative, but not identical with niacin or niacinamide
	Dendrobium nobile	Vitamins B_1, C, niacin, pantothenic acid. Adermin (Vitamin B_6)	No effect
	Phalaenopsis hybrids, *Vanda coerulea* Griffith	Niacin, niacinamide	Growth promoting effect
Withner 1942	*Cattleya* hybrids *Epidendrum tampense* Lindl.	Vitamins C, B_1, B_2, B_6, calcium, pantothenate, biotin, niacin, inositol	No effects observed
Downie 1943a	*Corallorhiza innata*	Vitamins B_1 and C	Not very successful
Withner 1943	*Epidendrum cochleatum* L.	Vitamins B_1, B_6, niacin, adenine	Used in ovule culture. Good growth of ovules on Knudson's medium
Meyer 1943	*Rodriguezia* Ruiz & Pav. spp., *Cattleya harrissoniae* Paxt.	Vitamin B_1	Stimulating
Noggle & Wynd 1943	*Cattleya* hybrids	Applied the following vitamins in a medium containing purified maltose:	
		B_1, C, calcium pantothenate	Not effective
		B_2	Slightly effective
		B_6	Good germination, poor subsequent growth
		Niacin	Good germination, good subsequent growth

TABLE VIII (continued)

THE EFFECTS OF VITAMINS ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Supplement	Remarks
Schopfer 1943	Various	Vitamins A, B_1, B_2, D	Inactive
Magrou & Mariat 1945	*Cattleya*	Vitamin B_1	No effect on germination. Accelerated differentiation
Bouriquet 1947	*Vanilla planifolia*	Vitamin C	Some germination. Protocorms died soon thereafter
Storey *et al.* 1947	Various	Vitamins of the B complex	No conclusive results
Mariat 1948	*Cattleya* hybrids	B_1 = pyrimidine = pyrimidine plus thiazole Thiazole alone	Favored germination and differentiation Inhibition noted
Downie 1949a	*Goodyera repens*	Thiamine, calcium, pantothenate, riboflavin, niacin, amino-benzoic acid, inositol, pyridoxine hydrochloride, folic acid. Used singly or together	Did not stimulate asymbiotic germination. Germination and growth did occur in the presence of fungal extract
Mariat 1949	*Cattleya* seedlings	Niacin	Effective
Bahme 1949	*Cattleya* hybrids	Mixture of vitamins, B_1, B_6, C, calcium pantothenate, niacin, and B_2 Niacin	Effective The only additive shown effective when used singly
Harley 1951	Various	Niacin derivative	The growth factor required may be a niacin derivative
Withner 1951	*Cattleya*	Thiamine, biotin, folic acid, "10 B vitamins," glutathione	No noticeable effect over control

Mariat 1952	*Cattleya*	B_1, pyrimidine, niacin	Effect reaffirmed
		Biotin	Stimulating
		B_6	Favorable in high doses
		B_2 and B_6	Helpful in the differentiation of plants already at the leaf point stage
		Calcium pantothenate	Not effective
		Thiamine	Pyrimidine portion of molecule is as effective as the whole molecule of this vitamin
		Niacin	Most effective
		Inositol, folic acid, para-amino benzoic acid	May stimulate germination
Young 1952	Various	Vitamin B_1	Better growth
Burgeff 1954	European terrestrial species	Vitamin B_1	Promoted growth
Withner 1955	*Vanilla planifolia* ovules	Mixture of vitamins B_1, B_6, niacin, five amino acids, and indolebutyric acid (IBA)	Promoted growth, inconsistent
		Vitamin B_{12} singly	Similar results
		Vitamins and IBA	Similar results
Hegarty 1955	*Cypripedium*	Vitamins B_1 and niacin in mixture with indole propionic acid (or IBA) and peptone	Stimulated germination
		"Vitamins"	Did not improve growth of seedling in the absence of sugar
Burgeff 1959	*Vanda* *Phalaenopsis*	Niacin	Improved germination
		Niacinamide	Stimulated growth, suggests that Vandophytin is identical with niacin

TABLE VIII (continued)

THE EFFECTS OF VITAMINS ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Supplement	Remarks
Ito 1960, **1961a,** 1964	*Dendrobium nobile:* pollen	Vitamins B_1 B_6, and tocopherol acetate mixture	Enhanced germination percentage and tube length
	ovary *in vitro*	Vitamin B_1 (0.1 p.p.m.)	Excellent for fruit growth
		Vitamin B_6 (1 p.p.m.)	Excellent for fruit growth
		Tocopherol acetate	Inhibits fruit growth, improves seed fertility
		Mixture of all three vitamins	Fruit growth not improved, increase in seed size
Lawrence & Arditti 1964a	*Cattleya*	Niacin in mixture with other factors	Stimulated growth and development
Arditti & Bils 1965	*Cattleya*	Niacin	Stimulated germination and growth
Arditti 1965a	*Cattleya, Laeliocattleya*	Niacin and its tryptophan pathway precursors	Only niacin, kynurenine, 3-hydroxyanthranilic acid, and quinolinic acid enhanced germination and the growth of seedlings of all ages. Tryptophan was inhibitory to germination and young seedlings
Arditti 1965c, In Press a	*Cattleya*	Niacin	Enhanced growth alone and various combinations with ribose and adenine
Arditti 1966a	*Laeliocattleya*	(See Arditti 1965, above)	(See Arditti, 1965 above)

Arditti, In Press c	*Cattleya*	Niacin	The only additive, among those tried, to stimulate germination and growth when added alone
Kano 1965	*Dendrobium*	Thiamin, 1 mg./l. Panvitan (Vit. A 5000IU, Vit. D_2 500IU, Vit. B_1 2mg., Riboflavin 3mg., Nicotinamide 20 mg., Pyridoxine HCl 2 mg., Na Pantothenate 5 mg., Vit. B_{12} 2 mg., Ascorbic acid 75 mg., L-lysine monohydrochloride 25 mg.): 0.5 mg./l. 1.0 mg./l.	Slight enhancement Enhancement Slight enhancement
Luning 1966	*Disa grandiflora (D. uniflora)*	Thiamin	Considerable enhancement

TABLE IX

THE EFFECT OF AUXINS ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Compound	Remarks
Burgeff 1934	*Cattleya*	Auxin from orchid pollen	No effect
Burgeff 1936	Various	IBA	No effect
Downie 1943a	*Corallorhiza innata*	Beta-indolyl-acetic acid	Simulated growth
Meyer 1945a, b	*Cattleya harrissoniae, Epidendrum faustum* Rchb. f., *Odontoglossum, Miltonia, Cattleya labiata, Oncidium varicosum* Lindl., *Laeliocattleya, Laelia tenebrosa* Rolfe, *Laelia anceps* Lindl., *Oncidium marshallianum* Rchb. f.	Hortomone A—sodium alpha naphthalene acetic acid (NAA)	Hormone added to tomato juice medium and Knudson's, Burgeff's, and Sladden's media. Stimulated germination. Stimulated seedling growth both before and after transplanting
Meyer & Pelloux 1948	*Cattleya warneri* Moore	Gamma-indolbutyric acid (IBA) (NAA)	Stimulated germination and growth Stimulated germination and growth
Yates & Curtis 1949	*Epidendrum nocturnum*	NAA	Stimulated root and shoot growth
Withner 1951	*Cattleya*	Indoleacetic acid (IAA), IBA, NAA	Slightly effective in promoting seedling growth

Mariat 1952	*Cattleya gigas* × *Cattleya fabiana*	Indole-beta-acetic acid	Promoted growth
Hegarty 1955	*Cypripedium*	Indolebutyric acid (IBA)	Somewhat better germination. Used in mixture with thiamine, peptone, and niacin
	Vanilla planifolia	IBA	Improved germination. Used in mixture with coconut milk, thiamine, niacin, and peptone
	Cypripedium	IBA	Used in mixture with coconut milk. Seedlings developed faster than control, but in six months differences between treated and control plants became less pronounced
	Cattleya, Cymbidium, Oncidium, Phalaenopsis	IBA	Excellent germination. Used in mixture with coconut milk
Withner 1955	*Vanilla planifolia* embryo culture	IBA	Low concentration stimulated growth while higher concentration was inhibitory
Israel 1963	*Dendrobium* × 'Jaquelyn Thomas' (ovaries)	NAA	Death occurred in the absence of NAA. No marked correlation between growth and NAA concentration within the concentrations used
Hamilton 1965	*Cymbidium*	IBA, "Dash" of 2% powder	In Chang's medium (Chang 1953) with pineapple juice and blue whale. Enhanced growth

TABLE IX (continued)

THE EFFECT OF AUXINS ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Compound	Remarks
Kano 1965	*Dendrobium*		
	122-day-old	IAA, 1 p.p.m.	Slight inhibition
	117-day-old	IBA, 0.1, 1 p.p.m.	No effect
		10 p.p.m.	Slight inhibition
	Laeliocattleya	IAA, 1 p.p.m.	Enhancement
	122-day-old		
	Brassolaelio-	IBA, 0.1, 1 p.p.m.	No effect
	cattleya	10 p.p.m.	Inhibition
	178-day-old		
Brown 1966		NAA, 0.1% lanolin paste	Increased seed production in ovaries
Ernst 1966b	*Phalaenopsis*	Indole-3-acetic acid,	Increase in fresh weight of seedlings.
	'Elinor Shaffer'	10 p.p.m.	No other noticeable effects
	× 'Doris'		

TABLE X

THE EFFECT OF GIBBERELLINS ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Compound	Remarks
Moir 1957	Various hybrids	Gibrel (potassium gibberellate)	Increased growth. Results visible 5 days after treatment. Chlorosis
Smith 1958	*Laeliocattleya, Cymbidium*	Potassium gibberellate	Results visible 7 days after treatment. General chlorosis. Leaf elongation. Effect primarily on early growth. In late growth control have caught up or have exceeded treated plants
Blowers 1958	*Cattleya*	Gibberellin	Accelerated seedling growth
Humphreys 1958	*Cattleya, Cypripedium, Cymbidium, Odontoglossum*	"Gibberellic acid 0.008" (units not given)	Germination rapid, overly swelled pale protocorms which died in 104 days. Seedlings: fast growth, little root action
Hirsh 1959	*Cattleya*	"Gibberellates"	Stimulant
Harbeck 1963	*Orchis maculata* L., *Leucorchis albida* E. M., *Orchis morio* L.	Gibrel (potassium gibberellate)	Small protocorms, no roots, elongated leaves
Ernst 1966b	*Phalaenopsis* 'Elinor Shaffer' × 'Doris', one year old	Gibberellic acid (GA_3) 10 p.p.m.	Increased leaf length, but decreased width and average number. Decreased root number and fresh weight of seedlings

TABLE X (continued)

THE EFFECT OF GIBBERELLINS ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Compound	Remarks
Kano 1965	*Bletilla striata* (Thumb.) Rchb. f.	Gibberellic acid, 0.1 and 1.0 p.p.m.	No effect
	100 days old	10 p.p.m.	Inhibition. Reduced leaf number, width and length. Root growth suppressed
	210 days old	1, 10, 100 p.p.m.	No effect when added to 90- and 172-day-old seedlings. Generally leaf number and width reduced, length increased. Roots suppressed
	Dendrobium	GA, 0.5, 5, 50 p.p.m.	80-day-old seedlings transplanted on Knudson's C plus 10% apple juice and GA. Root growth and initiation inhibited
	Brassolaelio-cattleya, 100 days old	GA, 0.5,50 p.p.m.	Inhibition
Hyatt 1965	*Phalaenopsis* 'Serenity' × *Phal. manii*	Gibberellic acid, no conc. given	Seedlings died

TABLE XI

THE EFFECTS OF PHYTOKININS AND RELATED SUBSTANCES ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Compound	Remarks
Arditti 1965a, c, In Press a	*Cattleya* 75, 135, 195 days old	Adenine 8.12 μM	No effect
Ernst 1966b	*Phalaenopsis* 'Elinor Shaffer' × 'Doris', one-year-old	1, 3 Diphenylurea, 10 p.p.m.	Slight increase in leaf length, slight decrease in root number
		Adenine sulphate	No effect
Kano 1965	*Dendrobium* hybrid, 122 days old	Adenine, 40 p.p.m.	Inhibition
		Kinetin, 0.5 p.p.m.	Inhibition when added singly or in combination with IAA or IAA and adenine
	Laeliocattleya hybrid, 122 days old	Adenine, 40 p.p.m.	Enhancement
		Kinetin, 0.5 p.p.m.	Root growth totally inhibited both when added singly and with IAA. Very slight enhancement when added in combination with IAA and adenine
Raghavan & Torrey 1963, 1964	*Cattleya*	Kinetin	No improved growth on $NaNO_3$ as the only nitrogen source
Withner 1942, 1943, 1951	*Cattleya*	Adenine	Enhancement

TABLE XII

THE EFFECT OF COMPLEX ADDITIVES ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Supplement	Remarks
Bernard 1909	*Laelia*	Salep	Germination improved with increase in concentration
Knudson 1921, 1922	*Cattleya*	Canna, peat, carrot, and beet preparations	Good development
Lami 1927b	*Vanda, Phalaenopsis*	Peptone	Rapid normal growth and development
Quednow 1930	*Laeliocattleya* 'Colman' × *Cattleya gaskelliana* Rchb. f.	Potato, barley and wheat extract	No effect on germination. Improved growth (at 1/10 concentration)
		Beet extract	Inhibits germination. Improved growth (at 1/10 concentration)
Burgeff 1934, 1936	Several	Dead hyphae; fungal extracts	Activity same as that of live hyphae
Curtis 1936	Native Wisconsin orchids	Extracts of carrot and sphagnum	No germination
Schaffstein 1938	*Vanda*	Seed, leaf, root, and yeast extracts	Stimulating effect
Downie 1940	*Goodyera repens*	Yeast extract	Stimulated germination
Meyer 1944a, c	*Laelia, Cattleya, Oncidium, Encyclia, Epidendrum, Rodriguezia*	Tomato juice	Enhanced germination and growth
Watkins 1945	Various	Tomato juice	
Bouriquet 1947	*Vanilla*	Peptone; *Vanilla* plant decoction	Improved germination

Curtis 1947a	*Paphiopedilum, Phaius, Vanda tricolor, Cattleya* × 'Mollie'	Peptone	Improved growth
Mariat 1948	*Cattleya* spp.	Peptone	Improved growth
Downie 1949a	*Goodyera repens, Cymbidium*	Water extract of endophyte	Stimulated seedling development
Vacin & Went 1949a	*Cymbidium, Epidendrum o'brienianum* Rolfe	Fresh tomato juice	Very good germination and development
Vacin 1950b	*Cymbidium*	1% Protein hydrolysate "Prominogen"	2% concentration was toxic
Ito 1951	*Dendrobium, Cattleya, Phalaenopsis, Epidendrum*	Tomato juice and starch	Germinated and grew well. *Vanda* seeds germinated well but were slow in differentiating
Mariat 1951	*Cattleya lawrenceana* Rchb. f.	Coconut milk Water extract of copra	Inhibitory Poor growth
Liddell 1953b	*Cypripedium*	Peptone, yeast extract, meat extract	Improved germination
Chang 1953	*Dendrobium*	Fish emulsion	Improved growth
Alberts 1953	*Cattleya, Dendrobium*	Fish emulsion plus peptone	Good germination and development
Thomale 1954	*Phalaenopsis*	Honey, salep	Improved germination
Tonnier 1954a	*Vanilla madagascariensis*	Boiled *V. fragrans* leaves, stems and roots in mixture with boiled soil, coconut milk, and yeast	Improved germination

TABLE XII (continued)

THE EFFECT OF COMPLEX ADDITIVES ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Supplement	Remarks
Ann 1955	*Dendrobium*, *Vanda*, *Arachnis* Blume	Fish emulsion	Greatly improved germination, growth and development
Withner 1955	*Vanilla*	Fresh banana	Good growth
Ito 1955	*Dendrobium*	Onion extract, yeast extract	Seed from immature pods. Good development
Hegarty 1955	*Paphiopedilum* *Vanilla* *Cattleya*	Coconut milk Coconut milk Coconut milk	Stimulating effect Stimulating effect Stimulating effect
Griffith & Link 1957	*Cattleya skinneri* Batem.	Orange juice, tomato juce	Inhibitory. Germinated and grew well
	Cattleya mossiae Parker	Cotton seed meal Fish emulsion	Poor growth Adequate for germination and growth
Burgeff 1959	Various	Medium which previously supported fungus growth	Good germination
Rossiter 1960	*Cattleya*	Sauerkraut juice (canned)	Good germination and growth
Hoyt 1961	Various	Pineapple	Lowers pH, requires adjusting before autoclaving
Nakamura 1962	*Galeola septentrionalis*	Yeast preparation	Improved germination
Bracey 1963	*Cattleya*	Fresh banana	Good germination and growth

Ilsley 1966	*Cattleya* and others	Fresh banana, pineapple juice, potato peelings	Good germination and growth
Tsukamoto *et al.* 1963	*Dendrobium, Cymbidium, Paphiopedilum*	Fresh-strained apple juice (10-20%)	Successful
		Difco-Bacto tryptone	Successful
		Difco-Bacto peptone or tryptone	Successful
Harbeck 1963	European terrestrial spp.	Peptone; fish emulsion	Improved germination
Wimber 1963	*Cymbidium* meristems	Tryptone	Good growth and development
Yamada 1963a, b	*Cypripedium* and others	Coconut milk	Improved germination
Stoutamire 1964b	Terrestial, native American	2 g. peptone/liter	
Lawrence & Arditti 1964a, b	*Cattleya*	A mixture of tomato and pineapple juices plus banana, coconut milk, and vitamins	Excellent growth
Arditti 1965a, b, 1966b, In Press b	*Cattleya*	Tomato juice and some of its extracts	Unsatisfactory
Anderson 1965	*Cattleya*	Pineapple juice, emulsified banana, fish emulsion	Satisfactory growth
Kotomori & Murashige 1965	*Dendrobium*	Coconut milk 15%, 30%, 45%	Germination unaffected, growth inhibited; one-year-old seedlings stimulated
Morton 1965	*Sophrolaeliocattleya*	Pineapple juice	Good growth

TABLE XII (continued)

THE EFFECT OF COMPLEX ADDITIVES ON GERMINATING SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Supplement	Remarks
Kano 1965	*Dendrobium* (90, 100, 122, 125, 191 days old)	Tomato juice (15%, 50%)	Considerable enhancement in low mineral content media
		Tomato juice (15%, 30%)	Slight enhancement
		Tomato juice (10%)	No enhancement
		Apple juice (5%, 10%, 15%, 20%, and 30%)	Considerable enhancement
		Cybidium juice (30%)	Slight enhancement
		Fresh yeast (0.5%)	Very slight enhancement
		Yeast extract (2 g./l.)	Inhibition
		Dried apple (10, 20 g./l.)	Enhancement, in some instances considerable
		Casein (0.2%)	Slight enhancement
		Peptone (0.2%)	Slight enhancement
		Tryptone (0.2%)	Slight enhancement
	Dendrobium grantii C.T. White	Mashed banana (5% v/v); onion juice (10%); dried apple fruit (5 g./l.), fresh apple juice (10%)	"Best growth on fresh apple juice, followed by that with onion juice." "As a whole germination promoted by additions"
	Brassocattleya 191-day-old	Apple juice (5%, 15%)	Slight inhibition
		Tomato juice (15%, Meyer's solution)	Considerable inhibition
	Brassolaelio-cattleya, 140, 191, 230 days old	Tomato juice (15%, 30%)	Slight to heavy inhibition
		Apple juice (5%, 15%, 30%)	Decreased plant height, increased % of rooting
		Cymbidium juice (30%)	Rooting totally inhibited, reduced top growth
		Peptone (0.2%)	Slight enhancement
		Tryptone (0.2%)	Inhibition
		Yeast extract (0.2%)	Marked inhibition
		Casein (0.2%)	Slight inhibition

Laeliocattleya 90, 111, 122 days old	Tomato juice (50%)	No enhancement. In some cases slight inhibition
	Tomato juice (30%)	Inhibition
	Tomato juice (10%)	Inhibition
	Apple juice (30%, 20%)	No enhancement, growth equal to that of control
	Apple juice (10%)	Considerable enhancement
	Cymbidium juice (30%)	Inhibition
	Yeast (0.5%)	Inhibition
Bletilla striata (Thumb.) Rchb. f. 60, 120, 140 days old	Tomato juice, apple juice, cymbidium juice (30%)	Least growth on apple and cymbidium media
Cypripedium reginae	Tomato, apple juices	No germination on any in two years
	Tryptone, peptone	Some enhancement
Cymbidium (hybrid), 272 days old	Apple juice (10%)	Marked inhibition
	Peptone (0.2%)	Marked enhancement
	Tryptone (0.2%)	Marked enhancement, no effect on older seedlings
Cymbidium virescence immature ovules, 123, 168, 704 days old	10% apple juice, peptone, tryptone	Some enhancement
Paphiopedilum callosum, 267 days old; transplanted, 151 days	Peptone	Enhancement
	Tryptone	Enhancement on Hyponex medium, none on Knudson C
	Apple juice (10%)	Inhibition, no rooting
P. insigne immature ovules	Peptone, tryptone	Enhancement

TABLE XII (continued)

THE EFFECT OF COMPLEX ADDITIVES ON GERMINATING SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Supplement	Remarks
Kano 1965 (continued)	*Calanthe discolor* Lindl., 704 days old	Peptone, tryptone	No effect on Knudson C medium, some enhancement on Hyponex medium
	Brassavola nodosa (L.) Lindl., 253 days old	Apple juice (5%)	No effect
		Peptone (0.2%)	Slight enhancement
		Tryptone (0.2%)	Slight inhibition
Ernst 1966b	*Phalaenopsis* 'Elisa' × 'Best Girl' 'Ruby Lips' × 'New Era'	Banana (15%)	"400-500% of blank"
		Pineapple (15%)	"400-500% of blank"
		Brown Turkey Fig (15%)	"200-300% of blank"
		Tomato (15%)	"200-300% of blank"
		Mango, *Psalliota campestris* mushroom, coconut milk (each at 15%)	"each about 150% of blank"
		Papaya (15%)	"about equal to blank"
		Kiwi fruit, Grapes, Raspberries	each "inferior to blank and showing signs of toxic effects." With raspberries "90% of the population have died."
		Tryptone, peptone	"each about 200% of blank"
		autolyzed fish	"about equal to blank"
Hey 1966a, b	Various, rare	Banana	Marked enhancement
Lindquist 1965	*Disa grandiflora (D. uniflora)*	Fresh sphagnum beds	*S. palustre* and *S. magellanicum* are preferable
Yeoh 1966	Various	Malaysian beer	Most remarkable additive of all

TABLE XIII

THE EFFECTS OF TEMPERATURE ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Temperature	Remarks
Alberts 1953	*Cattleya labiata*	21°C. (approx.)	
Anderson 1965	*Cattleya*	26.5°-29.5°C.	
Arditti 1965a, b, c, 1966b	*Cattleya*	21°-26°C.	
Bahme 1949	*Cattleya, Vanda*	28°C.	Good germination and growth
Bernard 1903	*Cattleya mossiae* Parker, *Laelia purpurea*	28°C.	Good germination and growth (symbiotic)
Borg 1965	*Cymbidium*	25°C. (70°F.)	Satisfactory
Bouriquet 1947	*Vanilla planifolia*	27°C. 35°C.	Seeds germinated in 55 days Medium dries faster. Recommends 27°C. as being most appropriate
Bouriquet & Boiteau 1937	*Vanilla planifolia*	27°C.	Good germination. First record of *Vanilla* seed germination. Germination also occurred when temperature varied between 20° and 35°C.
Burgeff 1936	*Vanda, Vandanthe* (*Vanda* × *Euanthe* Slchter.), *Vandopsis* Pfitz., *Laeliocattleya, Odontoglossum, Cymbidium, Bifrenaria* Lindl. × *Lycaste* Lindl.	18°-20°C.	Good germination and growth on proper media
Curtis 1947a	*Calanthe, Cattleya* spp., *Cymbidium* spp., *Epidendrum* spp., *Paphiopedilum* spp., *Vanda tricolor, Zygopetalum mackayi* Hook., and others	26°C.	Good germination on proper media

TABLE XIII (continued)

THE EFFECTS OF TEMPERATURE ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Temperature	Remarks
Curtis 1947b	*Vanda tricolor*	30°C.	
Curtis & Nichol 1948	*Vanda tricolor, Cymbidium*	28°C.	A study of proliferating orchid embryos. Temperatures varied between 26° and 30°C.
DeMartini 1963	Various	68°F. (N), 85°F. (D)	
Downie 1949a	*Goodyera repens*	16°C.	
Downie 1949b	*Listera ovata*	16°C.	
Ernst 1966a	*Dendrobium, Phalaenopsis*	20°-27°C.	
Griffith & Link 1957	*Cattleya skinneri* Batem., *Cattleya mossiae* Parker	22°-30°C. 18°-22°C.	During the photoperiod During the dark period
Hager 1954	*Cattleya*	27°-30°C. 20°-21°C.	Day Night
Hamilton 1965	*Cymbidium*	70°-90°F.	
Hegarty 1955	*Cypripedium* *Vanilla*	21°C. 21°C., 25°C. 30°C., 37°C.	 No growth at 37°C. on any medium No growth in Knudson C medium at any temperature
Hey 1966a	Various, rare	75°-80°F.	
Hill 1961b, 1965	Various	62°-85°F.	Following transflasking
Humphreys 1961a	*Cypripedium calceolus* L.	70°F.	No germination. Reasons may not be with temperature alone

Ilsley 1966	Various	22°C. (70°F.) 32°C. (90°F.)	Night, minimum Day, maximum
Israel 1963	*Dendrobium* ovularies	25°C.	
Ito 1955	*Dendrobium nobile* seeds	6°-40°C.	Growth on proper media
Kano 1965	*Cymbidium, Laeliocattleya, Brassolaeliocattleya, Cypripedium, Brassocattleya, Brassavola, Bletilla, Paphiopedilum, Dendrobium, Cymbidium*	15°-30°C.	
Knudson 1921	*Cattleya*	25°-30°C.	Good growth and germination
Knudson 1924	*Cymbidium, Odontoglossum, Phalaenopsis, Dendrobium, Ophrys*	25°-30°.C	Good germination and growth
Knudson 1925	Various	20°-30°C.	No constant temperature. Lower temperatures at night. Marked temperature departures during the spring and fall
Knudson 1950	*Vanilla fragrans* Salisb. *Vanilla fragrans* × *V. pompona* Schiede	32°C. 34°C.	No germination at lower temperatures This is a minimum requirement
Kofranek 1957	Various	21°-24°C.	
Kohl 1962	*Cymbidium*	21°C.	
Kotomori & Murashige 1965	*Dendrobium*	85°F.	Constant
Liddell 1953b	*Cypripedium*	22°C.	
Lindquist 1965	*Disa grandiflora (D. uniflora)*	6°-20°C.	
Lugo-Lugo 1955a, b	*Vanilla planifolia* Andrews	32°C.	

TABLE XIII (continued)

THE EFFECTS OF TEMPERATURE ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Temperature	Remarks
Mariat 1948	*Cattleya* spp.	18°-20°C.	
Mariat 1952	*Vanda, Cattleya, Laeliocattleya, Bletilla hyacinthina* Rchb. f.	20°C.	
Meyer 1943	*Rodriguezia, Cattleya harrissoniae* Paxt.	25°C.	
Meyer 1944a	*Cattleya schilleriana* Rchb. f., *Laelia purpurata* Lindl. ex Paxt.	18°-22°C.	
	Cattleya citrina Lindl. × *Lc. Blackii*	18°-22°C.	
Nakamura 1962, 1964	*Galeola septentrionalis*	30°C.	
Northen 1952	Various	21°C. (70°F.)	Constant
Northen 1953	*Phalaenopsis*	20°C.	Minimum
Northen & Northen 1949	*Cattleya* × 'Luegeae'	21°C.	Growth at 21°C. was decidedly better than at 18°C. while growth at 24°C. was slightly better than at 21°C.
Poddubnaya-Arnoldi 1959	*Dendrobium nobile*	20°-25°C.	
Poddubnaya-Arnoldi & Selezneva 1957	Various	20°-25°C.	
Ratclife 1963	*Cypripedium*	18°-21°C. (65°-70°F.)	Constant

Rothwell 1966	*Cymbidium*	15.5°C. (60°F.)	Seeds sown on compost among moss in pot containing a mature plant
Schaffstein 1938, 1941	*Phalaenopsis, Vanda, Dendrobium*	25°C.	
Scott & Arditti 1959	*Cymbidium*	21°C. 29°C.	Night temperature Day temperature (seldom exceeded)
Spoerl 1948	*Cattleya*	25°C.	
Stoutamire 1963	Terrestrial, native Australian	16.5°-32°C. (60°-90°F.)	20°C. (68°F.) best
Stoutamire 1964b	Terrestrial, native American	20°C.	
Vacin & Went 1949a, b	*Epidendrum o'brienianum* *Cymbidium*	22°C. 27°C.	Night temperature Day temperature
Wimber 1963	*Cymbidium* meristem	22°C.	In liquid culture
Withner 1959a	*Vanilla*	20°-34°C.	Mostly around 25°-27°C.
Wynd 1933a, b, c	*Cattleya trianae*i Linden & Reichb.	20°-25°C.	Occasionally temperature reached 30°-35°C.
Yamada 1963a, b	Various	Room temperature	
Yates & Curtis 1949	*Epilendrum nocturnum* Jacq., *Cattleya* p., *Oncidium* sp.	Room temperature	

TABLE XIV

THE EFFECTS OF PHOTOPERIOD, LIGHT QUALITY, AND LIGHT INTENSITY ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Light Intensity	Light Quality	Photoperiod
Anderson 1965	*Cattleya*	80 watt	Gro-Lux (Wide spectrum)	
Arditti 1964a, Arditti & Arditti 1964, Lawrence & Arditti 1964a, b, Arditti 1965a, b, c, 1966b, In Press a, b	*Cattleya*	450-500 micro-watts	Gro-Lux Lamps (rich in blue and red)	10 hours
Bahme 1949	*Vanda*, *Cattleya* hybrid	Diffuse sunlight		Natural daylength
Bernard 1903	*Cattleya*, *Laelia*	Diffuse sunlight		Natural daylength
Borg 1965	*Cymbidium*		Gro-Lux (best), Warm white, Daylight (Finland)	
Curtis 1947a	Various	50 ft. cdls.	Provided by 80 watt mercury-vapor fluorescent lamps	12 hours
Curtis 1947b	*Vanda tricolor* Lindl.	not given	Provided by Cooper-Hewitt fluorescent lights	Constant
Curtis & Nichol 1948	*Cymbidium*, *Vanda*	50 ft. cdls.	Provided by Cooper-Hewitt fluorescent lights	18 hours

DeMartini 1963	Various	750-1000 ft. cdls.	Daylight	Normal (San Francisco area)
Dungal 1951	Various	100 watt bulb 12-16 ft. from flasks	Incandescent	
Ernst 1966a	*Dendrobium phalaenopsis* Fitzgerald	600-1000 ft. cdls.	Daylight	
Griffith & Link 1957	*Cattleya skinneri, Cattleya mossiae*	675-725 ft. cdls.	Provided by three 40 watt warm white fluorescent bulbs and three 40 watt white, inside-frosted incandescent light bulbs	12 hours. Between 6 P.M. and 6 A.M.
Hager 1954	*Cattleya*	200 ft. cdls.	Gradually increased following germination to 400 ft. cdls.	16 hours
Halpin & Farrar 1965	*Cattleya, Laeliocattleya, Brassocattleya, Vanda*	40 watt lamps, 16 inches from plants	Wide spectrum Gro-Lux, Gro-Lux, warm white, cool white	16 hours
Hamilton 1965	*Cymbidium*		Gro-Lux	
Hey 1966a	Various, "rare"		Warm white fluorescent	
Hill 1961a, b, 1965	Various	150-200 ft. cdls. at first, 300-400 ft. cdls. following germination		
Ilsley 1966	Various	900-1000 ft. cdls.	Natural daylight	Natural daylength
Kano 1965	*Paphiopedilum callosum*		Dim light better than darkness	

TABLE XIV (continued)

THE EFFECTS OF PHOTOPERIOD, LIGHT QUALITY, AND LIGHT INTENSITY ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Light Intensity	Light Quality	Photoperiod
Kofranek 1957	Various	500 ft. cdls.	Given as a maximum figure	Natural daylength
Koopowitz 1966	*Disa racemosa* L.	Total darkness for three days		
Kotomori & Murashige 1965	*Dendrobium Johnsonii* × *D. ionoglossum*	200 ft. cdls.	Fluorescent	
Kranz & Kranz 1961	*Dendrobium, Cattleya, Odontoglossum, Cypripedium, Odontioda, Cymbidium*	80 watts	Cool white fluorescent	12-18 hours.
Mariat 1948, 1952	*Bletilla, Cattleya*		Subdued light	
Northen 1952	Various	200 ft. cdls.		
Northen & Northen 1949	*Cattleya,* × 'Luegeae'	35 ft. cdls.	Provided by fluorescent lights	Continuous
Page 1965	*Cattleya dowiana* Bateman, *Brassocattleya, Laeliocattleya, Sophrolaelio-cattleya*	80 watt	Gro-Lux (superior to "blue-white" and natural)	16 hrs. photoperiod. (Accelerated seedling development, but too long for flower induction.)

Quednow 1930	*Cattleya loddigesii* Rchb.	not given	Diffuse daylight, 90% germination, good growth. Green, 60% germination, growth of about 60% of that of control. Infrared, 30% germination, growth inferior to that under green light. Ultraviolet, 20% germination, growth inferior to that under infrared. Darkness, 30% germination, growth somewhat superior to that under ultraviolet light, but inferior to that under infrared light	not given
	Cattleya labiata × *Brassavola digbyana* Lindl.		No data given on germination Data on development parallels that given for *Cattleya loddigessii*	
	Cattleya 'Dusseldorf' × *Cattleya intertexta*, *Oncidium varicosum* Lindl., *Laelia pumila* Rchb. f., *Cyrtopodium punctatum* Lindl.		Parallel to *C. loddigesii*	
Ratcliffe 1963	*Cypripedium*	Darkened case		
Scott & Arditti 1959	*Cymbidium*	about 1500 ft. cdls.	Daylight	Natural daylength
Spoerl 1948	*Cattleya*	50 ft. cdls.	Provided by fluorescent lights	18 hours

TABLE XIV (continued)

THE EFFECTS OF PHOTOPERIOD, LIGHT QUALITY, AND LIGHT INTENSITY ON GERMINATING ORCHID SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	Light Intensity	Light Quality	Photoperiod
Stoutamire 1963, 1964a	Terrestrial, Australian native	300-700 ft. cdls.	Fluorescent	12-hour photoperiods
Stoutamire 1964b	Terrestrial, native American		Fluorescent	12-hour photoperiods, continuous light may be inhibitory in some instances
Tennant 1965	*Cymbidium*		Gro-Lux	16-hour photoperiods
Vacin & Went 1949a	*Cattleya, Epidendrum o'brienianum*	150-500 ft. cdls.	Daylight	Natural daylength
Wimber 1963	*Cymbidium* meristems	100 ft. cdls. or less	Not given	Constant
Withner 1955	*Vanilla* (Immature seed)	Not given	"Provided by banks of red, blue and white 40 watt fluorescent lights about 10 inches from the culture"	Constant

TABLE XV

THE EFFECT OF 18-HOUR PHOTOPERIODS ON GERMINATION OF ORCHID SEEDS AND DEVELPING SEEDLINGS

Genus	Germination	Growth of Early Protocorm	Growth of Late Protocorm
Cattleya	accelerated	accelerated	accelerated
Dendrobium	inhibited	retarded	accelerated
Laelia	accelerated	accelerated	accelerated
Phalaenopsis	inhibited	retarded	accelerated
Spathoglottis Blume	inhibited	retarded	accelerated
Vanda	inhibited	retarded	accelerated

TABLE XVI

THE EFFECT OF pH ON GERMINATING SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	pH	Remarks
Alberts 1953	*Cattleya labiata* 'Rex'	5.0	Medium contained fish emulsion
Arditti 1965a, etc.	*Cattleya*	5.0	Various media
Bahme 1949	*Brassocattleya, Cattleya, Vanda*	5.0	
Bouriquet 1947	*Vanilla*	5.8	
Breddy 1953	*Cattleya, Cymbidium* *Phalaenopsis*	4.8 5.2 - 6.0	Seeds will not germinate below pH 5.2
Cappelletti 1933	*Cymbidium*	3.0 - 6.0	
Clement 1924b	*Odontoglossum*	6.5 - 6.8	
Clement 1926	*Miltonia*	6.5 - 6.8	
Curtis 1936	Various, native to Wisconsin	4.7 - 5.0	
Curtis & Spoerl 1948	*Cattleya* 'Mollie' *Cymbidium* *Cattleya trianei* *Vanda tricolor*	4.6 - 5.0 5.0 4.8 4.8	
Downie 1940	*Goodyera repens*	3.6 - 7.6	pH seems to have little effect; no germination in the absence of fungal extract
Ernst 1966a, b	*Phalaenopsis, Dendrobium*	5 ± .2	
Griffith & Link 1957	*Cattleya mossiae, Cattleya skinneri*	4.9 - 5.1	On media containing a variety of organic supplements

Hegarty 1955	*Cypripedium, Vanilla* Other genera	6.1 5.2	
Hoyt 1961	Various	6.4	Caused plants to turn brown
Humphreys 1961b	Various	4.8 - 5.1	La Garde's medium
Israel 1963	*Dendrobium,* immature ovularies	5.5	Immature seed in tissue culture
Ito 1955	*Dendrobium nobile*	5.0	Immature seed
Kano 1965	*Brassolaeliocattleya* *Dendrobium*	4.55 4.95	Acceptable range: 4.55 - 5.65 Acceptable range: 4.25 - 6.02
Knudson 1927	Various	close to 5.0	At pH 5.5 seedlings are yellowish and at pH 6.6 they become white
Knudson 1930	*Laeliocattleya*	6.0	Plant bloomed in flask
Knudson 1941	*Goodyera pubescens* (Wild.) R. Br.	5.0	
Knudson 1946a	*Cattleya mossiae*	4.3 - 5.2	Best results obtained at pH 5.0
Knudson 1950	*Vanilla,* several spp.	5.0 - 5.5	
Knudson 1951	*Cattleya skinneri*	5.0	pH below 4 will kill the seed, no germination at pH 4.5. Only initial pH is of importance. Changes down to pH 3.63 subsequent to germination are not deleterious. Best growth recorded at pH 4.35
Knudson 1952	*Phalaenopsis* *Cattleya*	 4.8 - 5.0	Killed at pH 4.8 Killed at pH 4.5; yellow-green color at pH 5.5; no chlorophyll at pH 6.0
Kotomori & Murashige 1965	*Dendrobium*	4.8 - 5.4	pH 4.6 best

TABLE XVI (continued)

THE EFFECT OF pH ON GERMINATING SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	pH	Remarks
LaGarde 1929	*Cattleya*	5.4	
Liddell 1944	Native American	4.2 - 4.5 5.2	Nutrient solution Nutrient agar
Liddell 1953a, b	*Cypripedium*	5.0	
Liddell 1955	*Cypripedium*	5.2 - 5.3	
Mariat 1948	*Cattleya*	5.5	
Mariat 1951	*Cattleya lawrenceana* Rchb. f.	5.5	
Mariat 1952	*Cattleya, Vanda, Laeliocattleya, Bletilla hyacinthina*	4.8 - 5.2	
Meyer 1943	*Rodriguezia, Cattleya harrissoniae*	4.8 - 5.0	Media contained vitamin B_1
Meyer 1944a	*Laelia, Cattleya, Oncidium, Epidendrum, Encyclia* Hook., *Rodriguezia*	4.8 - 5.0	Tomato juice agar
Morton 1965	*Sophrolaeliocattleya*	4.5	
Nakamura 1962	*Galeola septentrionalis*	4.6 - 5.0	
Noggle & Wynd 1943	*Cattleya*	5.0	On maltose as carbohydrate
Northen & Northen 1948	*Cattleya* 'Luegeae'	5.0	

Quednow 1930	*Cattleya trianei* × *Cattleya* 'Perrin'	4.7	Best germination was obtained at pH given in series of detailed experiments
	Cattleya loddigesii	4.4 - 4.8	
	Oncidium amabilis Rchb. f.	4.7	
	Cyrtopodium punctatum Lindl.	4.7	
	Laelia pumila Rchb. f. var. 'Praestans'	4.7 - 5.3	
	Dendrobium nobile	4.7 - 5.3	
	Cattleya 'Emperor Frederik'	4.7	
Raghavan & Torrey 1964	*Cattleya labiata*	5.5	
Rossiter 1960	*Cattleya* and allied bigeneric hybrids, *Cymbidium*	5.3	In a solution containing sauerkraut juice
Schaffstein 1938	*Vanda, Phalaenopsis, Dendrobium*	4.8 - 5.0	
Scott & Arditti 1959	*Cymbidium*	5.0	
Smith 1932	*Cattleya*	4.2 - 5.0	
	Phalaenopsis, Epidendrum, Renanthera Loureiro	4.5	
	Broughtonia R. Br.	6.0	
Stoutamire 1963	Terrestrial, native Australian	5.0 - 5.5	
Tsuchiya 1954a, b	Various, ovule culture	5.5	
Tsukamoto *et al.* 1963	*Dendrobium*	approx. 5.0	Apple juice-containing medium
	Cattleya	approx. 5.0	
	Cymbidium	approx. 5.0	Peptone-containing medium
	Paphiopedilum	approx. 5.0	Peptone- or tryptone-containing medium.

TABLE XVI (continued)

THE EFFECT OF pH ON GERMINATING SEEDS AND DEVELOPING SEEDLINGS

Investigator	Species	pH	Remarks
Vacin 1950a	*Cymbidium*	5.5	
Vacin & Went 1949a	*Epidendrum o'brienianum*	5.46	
Vacin & Went 1949b	*Epidendrum o'brienianum*, *Cymbidium*	5.5	Tomato juice medium
Withner 1942	*Cattleya* hybrids	4.9 - 5.1	
Withner 1947	Various	5.1 - 5.2	Suggests that pH of media raises 0.1 - 0.2 pH units during autoclaving
Wherry 1918, 1921	Many native American terrestrials	4.5 - 8.0	
Wherry 1927	*Corallorhiza maculata* Raf.	4.5 - 6.0	These are only approximations since the author used his own peculiar system for indicating acidity
	C. odontorhiza (Willd.) Nutt.		
	C. trifida Chatelain	4.5 - 7.0	See note above
	C. wisteriana Conrad.	6.0 - 6.5 to 7.5	See note above
	C. striata Lindl.	6.5 - 7.5	See note above
	C. macrantha Schltr.	6.5 - 7.5	See note above
	Hexalectris spicata (Walt.) Barnhart	6.0 - 6.5 to 7.5 - 8.0	See note above
Wynd 1933a	*Cattleya trianei*	4.8 - 5.1	
Yates & Curtis 1949	*Epidendrum nocturnum*, *Oncidium*, *Cattleya labiata*	4.8	

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