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Title PHYSICAL FACTORS AFFECTING LONGEVITY AND GERMINATION OF SEED OF WESTERN DWARFMISTLETOE (ARCEUTHOBIUM CAMPYLOPODUM ENGELM. F. CAMPYLOPODUM)

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Longevity and germination of seed of western dwarfmistletoe (Arceuthobium campylopodum Engelm. f. campylopodum) of ponderosa pine (Pinus ponderosa Laws.) was investigated to determine: 1) the influence of humidity and temperature on seed viability and deterioration during storage; 2) the physiology of seed dormancy; 3) the composition of seed reserve food at intervals during dormancy, and 4) the influence of temperature, moisture and light on seed germination.

Standard procedures were used for chemical analyses and paper chromatography; moisture conditions during germination were controlled over gradients of sulfuric acid, while light

intensities and temperatures were maintained in standard growth chambers.

Seed were collected in paper bags and stored both in shelters in the field and in the laboratory refrigerator. Viability determinations were made with one percent triphenyltetrazolium chloride or three percent hydrogen peroxide whereas the criterion for seed germination was radicle emergence.

Western dwarfmistletoe seed, after expulsion in the fall, remain dormant for approximately six months. Preliminary investigations suggested dormancy is regulated by a chemical inhibitor associated with the endocarp. Initial seed viability varied from one infected stand to another, whereas retention of viability was correlated with temperature. Seed stored at 1.5°C retained initial viability levels for 10 months; after 10 months seed began to significantly deteriorate. In some cases, however, viability was observed after a prolonged storage period of 48 months.

Western dwarfmistletoe seed germinate over a temperature range from 1.5 to 31°C. The optimum constant temperature lies between 15 and 20°C, whereas various combinations of alternating night-day temperatures revealed a combination of 5 to 15°C to be most favorable for germination. Absorption of liquid moisture is essential for germination and germination readily occurs at reduced levels of aeration. Germination also occurs in total darkness;

however, light intensities between 200 and 1000 foot candles in conjunction with favorable temperatures significantly enhance germination. Increases in photoperiod up to 24 hours progressively increased germination percentages. Red light was slightly more effective in seed germination than far-red light. Black light (near ultraviolet) was injurious to seed when levels were greater than 120 foot candles and exposures exceeded 12 hours.

Unfavorable temperatures, moisture, and light during the 30 days following seed discharge appeared to be the most contributory factors toward low seed viability and accompanying low infection potentials.

PHYSICAL FACTORS AFFECTING LONGEVITY AND GERMINATION OF SEED OF WESTERN DWARFMISTLETOE (ARCEUTHOBIUM CAMPYLOPODUM ENGELM. F. CAMPYLOPODUM)

by

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INTRODUCTION

Species of Arceuthobium (dwarfmistletoe) are parasitic on coniferous trees in the temperate regions of North America, Europe and Asia. Several species of Arceuthobium are indigenous to the coniferous forests of western North America; however, the present investigation concerns only Arceuthobium campylopodum Engelm. f. campylopodum on Pinus ponderosa Laws. in Oregon.

This parasitic plant reduces tree growth, wood quality and production of tree seed and is reported to decrease host resistance to disease and insect attack (10, p. 312; 25; 27; 48; 63). It is often fatal in its own right.

Dwarfmistletoe as a major forest disease has come to rank along with the heart rots since ever more effective fire control has reduced natural control of dwarfmistletoe (58), and the cutting of virgin timber has lessened the importance of decay. Therefore it is important to understand the behavior of dwarfmistletoe in order to establish effective control of the parasite.

Various investigators of dwarfmistletoe, cited later in the literature review, have studied species distribution, taxonomic

relationships, ecological implications, growth impact, and chemical and silvicultural control. However, little has been done on the physiology of seed germination or functional host-parasite relationships.

The objectives of this dissertation were 1) to establish the humidities and temperatures which limit seed deterioration during storage; 2) to investigate the physiological mechanisms related to seed dormancy; 3) to analyse the composition of seed reserve food metabolites during dormancy, and 4) to determine the influence of temperature, moisture and light on seed germination. The work reported here also relates laboratory findings to field observations to provide information particular to the epidemiology of western dwarfmistletoe of ponderosa pine.

LITERATURE REVIEW

The mistletoes as parasites were recognized in ancient times as "something apart from the branches of the host tree" (25). Mythology presupposes that the European missel thrush, a bird very fond of the mistletoe berries and a "messenger of the gods", is responsible for transmitting the mistletoe to earth through the adherence of seed to its feet. Others believe the word mistletoe may have been derived from the Anglo-Saxon mistle-tan meaning "a different twig" (54).

There are two categories of mistletoe per se--the true mistletoe and the dwarf, both of which are in the family Loranthaceae. The true or "leafy" mistletoes, belonging to several genera, are widely distributed around the world; whereas the dwarfmistletoes, having only scale-like leaves, are more limited in distribution. Genera of true mistletoe are common in Australia (Amyema), Africa and Asia (Loranthus), Europe (Viscum), and in North America (Phoradendron); while there is but one genus of dwarfmistletoe (Arceuthobium), and it is found primarily in North America. Single species are reported from south central Asia and from the Mediterranean area (10, p. 310).

There are five species of Arceuthobium commonly recognized in the United States: 1) A. pusillum Peck on black spruce; 2)

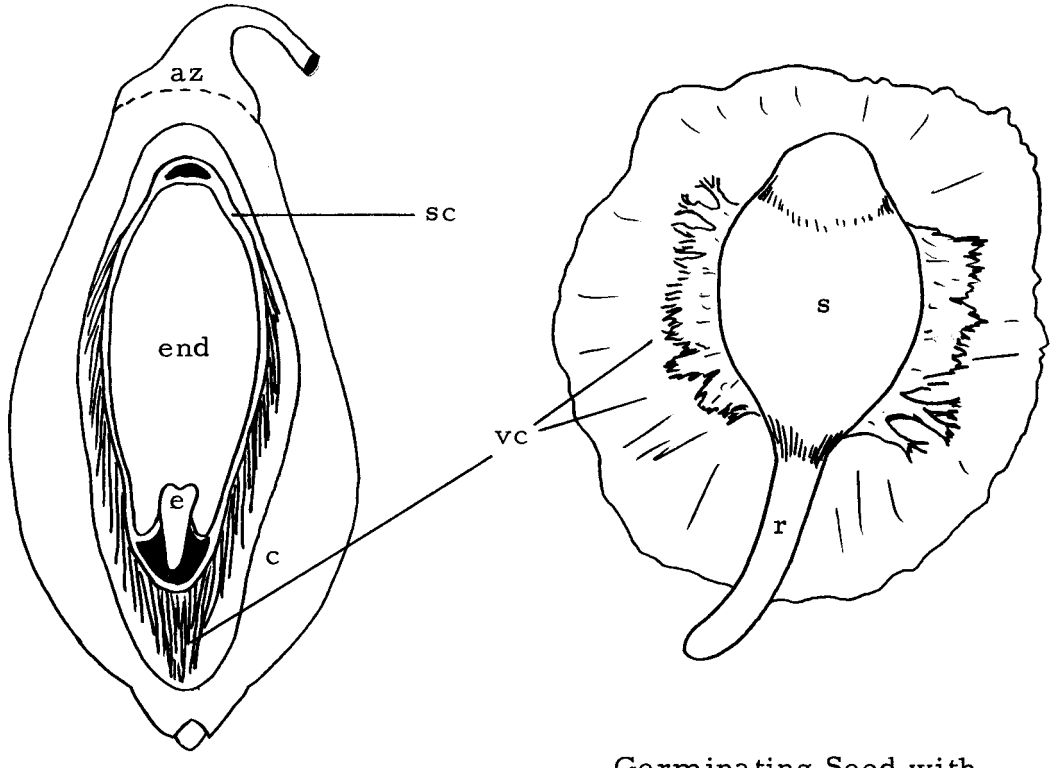
A. americanum Nutt. on lodgepole and Jack pine; 3) A. douglasii Engelm. on Douglas fir; 4) A. vaginatum (Willd.) Presl on three-needle pines, and 5) A. campylopodum Engelm. on ponderosa pine, spruce, true fir, larch, and hemlock (10, p. 311; 27).

A. campylopodum Engelm. f. campylopodum, the western dwarfmistletoe, is widespread throughout Washington, Oregon and California and the northern Rocky Mountains. This species is principally parasitic on ponderosa pine among its pine hosts but may also be found on Coulter, digger, Jeffrey and Monterey pines (10, p. 311).

The western dwarfmistletoe is dioecious. Pollination takes place between July and August. By the following year between September and October, the pistillate plants produce large clusters of mature berries ready for discharge (43). The mature berry containing a single seed varies in color from blue to olive green (24, p. 135). The fleshy, ovoid to oblong fruit is attached to its shoot by a recurved pedicel. Figure 1 illustrates the anatomical structures of the fruit and the seed of western dwarfmistletoe. The use of the word "seed" (the small projectile which is ejected as a naked endosperm and embryo encased in the endocarp of the fruit) in this report of western dwarfmistletoe conforms with previous citations by Gill (24, p. 137), Wagener (72), and Kuijt (47, p. 340).

At maturity, pressure builds up on the pericarp so that it breaks away at the base of the fruit and the seed is shot out through

Figure 1. Illustration of anatomical structures of fruit and germinating seed of western dwarfmistletoe.



Longitudinal section of Fruit

Germinating Seed with Viscin Cells Spread

az = abscission zone between capsule and pedicel

sc = seed coat which surrounds endosperm and embryo

vc = viscin cells which are attached to seed coat

end = endosperm

e = embryo

c = capsule or exocarp of fruit

sc + vc = endocarp of fruit

s = seed which is the projectile ejected from fruit at maturity

r = radicle of germinating seed

Redrawn from Kuijt 1960.

the base with considerable force (34, p. 59-62; 63). A sticky mucilaginous covering of "viscin" enables the seed to adhere to objects it strikes. The forward portion of the seed (the end opposite the radicle end known as the polar end) is a dome-like structure void of viscin cells. The viscin cells are hydrophilic in nature, absorb free moisture, and expand in size. As they dry they spread over the contacted surface and form a firm attachment. The first rains in autumn place the seed at the fascicle region in the pines (59). Germination usually occurs in the spring.

Germination is recognized by the emergence of a "radicle" outside of the endocarp (24, p. 139). The term radicle is reserved for that portion of the dwarfmistletoe embryo which arises from the radicular pole (12). Use of the term radicle is debated by various students because the endophytic part of the dwarfmistletoe plant has not been accepted by all as a root system.

Dowding (18) reports that seed of A. americanum germinate the same autumn that expulsion occurs; however, penetration of the host tissue does not occur until the following spring. Cannon (11) observed germination of Phoradendron villosum and P. californicum in great abundance during the spring which suggests a period of dormancy follows dispersal. Crocker (14, vol. 2, p. 794) reports that in Viscum album, germination is possible within one month following discharge if the optimum light and temperature requirements are

fulfilled. In A. vaginatum, germination occurs within several weeks after the seed is expelled (1, 26, 33). Seed of A. vaginatum which do not germinate by the end of September, or in other words remain dormant for more than two months, fail to germinate (26). Weir (74, p. 4) stated that "seed are capable of germination some two weeks before they are normally discharged from the capsule." Kimmey and Graham (44) concur with Gill, Andrews, and Hawksworth regarding A. vaginatum but believe that in most species of dwarfmistletoe, germination is probably delayed until spring. Scharpf and Parmeter (61) working with A. campylopodum parasitizing Pinus sabiniana in California concluded that the seed requires no period of after-ripening for germination, merely favorable temperature. The author concluded after working with A. campylopodum on ponderosa pine in Oregon that germination normally occurs in the spring following a dormancy period of five to six months.

An extensive review of the literature by Gill and Hawksworth (28) pertaining to the mistletoes revealed a number of investigations concerning the factors affecting germination, particularly of the European mistletoes (14, 22, 23, 29, 38, 39, 40, 46, 50, 70). Kuijt (47, p. 342) claims that no particular unusual conditions are necessary for the germination of Arceuthobium seed. Peirce (53) stated that the seed will germinate on anything, if it will germinate at all. Others (24, p. 139; 27) concur that germination is dependent

only on proper moisture and temperature and is not dependent on stimuli from the substrate. Peirce worked with A. occidentale (A. campylopodum Engelm.) and reported observing germination on dead and live pine needles, trees and shrubs, branches, and fence boards whenever the air was moist and warm. His laboratory attempts at germination were not successful and his temperature data were not quantitative. Heinricher (41) working with A. oxycedri in Austria concluded that light was essential for germination, however, he also lacked quantitative data for determinations of optimum effect. Weir (73) considered the amount of light not a critical factor in germination. Glimcher (29) working with Viscum cruciatum determined that germination was favored by high light intensity but that once the seed received sufficient illumination, germination would continue in darkness. Others working with Viscum album have found light to be necessary for germination (37, p. 578-581; 76, p. 145-153). Rigby (57) reports that light is a regulating factor in the germination and development of mistletoes of the genera Muellerina and Lysiana as well as with several species of the genus Amyema.

From the previous investigations, it appears that: 1) the optimum temperature range for the germination of temperate zone species is between 15 and 20°C (28, p. 10); 2) minimal temperature on the basis of minimal monthly averages for germination of Viscum album is 3.8°C (37, p. 581-602); 3) moisture requirements vary

considerably--tropical forms germinate readily in free water and temperate forms germinate in high humidities. The latter condition however is instrumental in the development of injurious fungi and bacteria (28, p. 10); 4) illumination requirements for germination vary with the particular species investigated (29, 73), and 5) negative phototropism of the radicle has been observed (33; 46, p. 589)--see Figure 24.

With few exceptions (38, 39, 40, 41, 53, 60, 61), most investigations of factors affecting seed germination of the mistletoes have been outside the genus Arceuthobium. Those investigations of Arceuthobium that have been conducted have been limited in scope or lacking in quantitative data.

MATERIALS AND METHODS

Collection of Seed

Seed was collected in the autumn of 1961, 1962, and 1963, in central Oregon, along the Metolius River near Camp Sherman, on the slopes of Pringle Butte (Deschutes Co.), and in the Pole Creek area near the town of Sisters. Throughout the three years, seed from over 2,000 plants was collected from stands of young ponderosa pine growing beneath overstories containing infestations of western dwarfmistletoe (see Figure 2). The estimated yield per plant was 300 to 500 seed.

Modifications of the techniques of Sayre (60, p. 15) and Scharpf and Parmeter (61) were used to collect the seed: 1) a kraft paper bag was placed around the female plant, and fastened to the infected branch with a spring-type clothes pin. Several weeks later, after the majority of the seed had discharged naturally, the bag with adhering seed was retrieved; or 2) a bag was placed around a mature plant and then the branch was shaken or severely rapped causing the seed to discharge; or 3) a portion of a branch or stem containing a female plant was pruned from the tree and placed in a bag. The bag was closed, shaken several times, opened and turned upside down to drop out the trash residue leaving only the seed attached to the sides of the bag. A statistical analysis of seed viability determined that

Figure 2. Typical collection site showing young ponderosa pine beneath overstory infected with western dwarfmistletoe.

there was no significant difference between the above three methods of seed collection.

The first method of collection was discontinued after 1961, because rain washed some of the seed away and favored fungi development. Seed once wetted were very hard to remove from the bags. The bags were sometimes destroyed by rodents.

Storage of Seed

Seed was stored in both field and laboratory. A portion of the bags, after seed collection, were placed in cardboard boxes and stored in sheds near the collection sites. Others were refrigerated at 1.5 ± 1°C and a relative humidity of 34-74 percent.

Determination of Seed Viability

The standard triphenyltetrazolium chloride test was utilized for seed viability determinations (2, p. 121; 21; 61). The principle of the tetrazolium (TZ) test is based on reduction of the colorless triphenyltetrazolium chloride to red formazan by the activity of respiratory enzymes in living tissue.

Seed were incubated in one percent TZ solution (room temperature) for three days; permitted to dry on filter paper; cut longitudinally with a razor blade, and examined for the red reaction in the embryo and endosperm. Any detection of red in either the embryo

or endosperm was considered an indication of viability. Differences usually were striking and easily observed.

Seed also were incubated in three percent hydrogen peroxide solution (room temperature) for 30 days to determine the "germination readiness" or state of dormancy of the seed.

Surface Sterilization of Seed

The high incidence of fungus and bacterial colonization of the seed in the absence of precautionary measures necessitated seed surface sterilization. Either soaking seed in a 0.1 percent solution of Arasan (tetramethylthiuram disulfide) for one hour or in 1:20 Clorox (sodium hypochlorite):distilled water for five minutes proved successful without reducing germinability. Both solutions supressed fungi and bacterial development for approximately 30 days. The latter treatment was utilized with greater regularity because of its non-residual character. Triple washings with sterile distilled water were used to remove the sterilant and thereby prevent seed damage from prolonged exposure.

Determination and Regulation of Moisture

Humidity requirements for longevity and germination of seed were investigated over sulfuric acid of graded concentrations (66, 79). Sulfuric acid solutions have several advantages over other

materials used for this purpose: 1) homogeneous solutions varying from 1 to 100 percent water are obtainable; 2) sulfuric acid solutions rapidly come to equilibrium with the surrounding atmosphere; 3) sulfuric acid exerts little if any vapor pressure; 4) the percentage of moisture in the surrounding atmosphere is quickly and accurately determined by measuring the density of the solution, and 5) the relative vapor pressure varies only slightly with wide ranges in temperature.

Figure 3 shows two variations in chambers used. Chamber A was converted from a Pyrex baking dish by grinding the upper edge flush with a 3/8-inch plate glass. Addition of lubricant grease along the dish edge provided a seal between the transparent lid and the dish. The sample material was elevated above the solution by a glass rod frame. Chamber B consisted of a 500-ml beaker, a circular piece of 1/4-inch wire mesh, and a cedar block with retention wells. The wire mesh was held in position over the beaker by wire clamps and the block was coated with three layers of lacquer to prevent moisture absorption. The four retention wells, 3/4 inch deep, served to seat glass vials containing seed, while a wire handle served to remove the block from the chamber.

Determination of the specific relative humidity in each chamber was made by measuring the density of the solution prior to and immediately following each experiment. Chambers of both types were

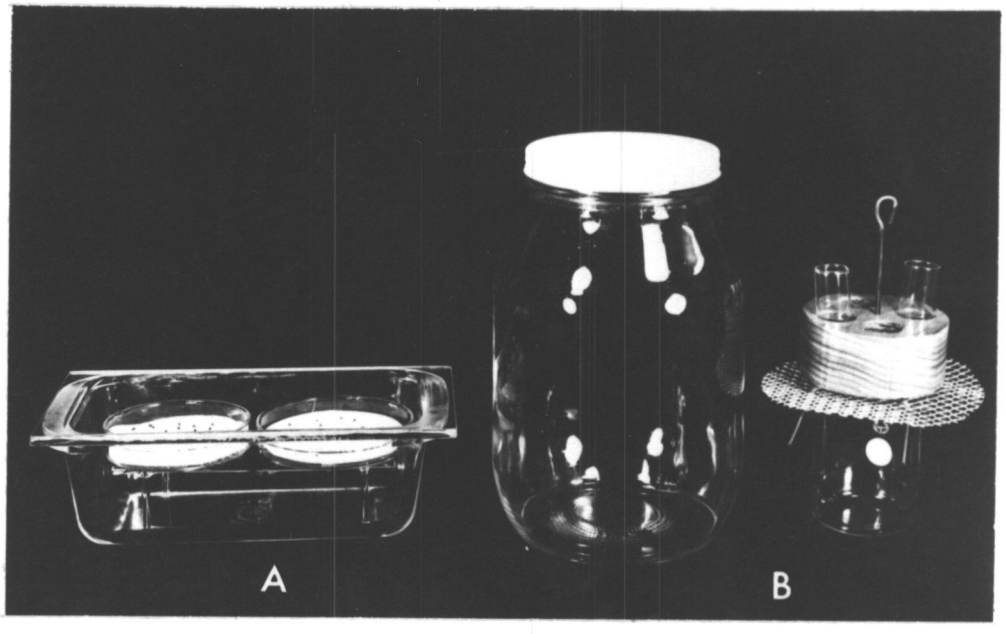


Figure 3. Relative humidity chambers used to regulate seed moisture. A) Loaf dish chamber. B) Glass jar chamber with seed containers and supports.

maintained at ± two percent of the desired humidity.

Determination and Regulation of Temperature

Controlled environmental growth chambers as well as standard drying ovens and incubation chambers were utilized to regulate temperature. One controlled growth chamber consisted of a standard compressor and refrigeration system capable of regulating alternate temperatures for day and night with rheostats providing selectivity from 30-100 ± 1°F. Another controlled growth chamber provided similar thermal programming but was used more specifically for light investigations. All temperatures in the controlled growth chambers were maintained at ± 1° of variation.

Temperatures surrounding seed in the field were measured with water-calibrated thermocouples. The thermocouples were attached to a branch so that the tip of the thermocouple was in a position at the twig-fascicle commonly occupied by a dwarfmistletoe seed. Groups of three thermocouples were used to represent the average temperature at one of three regions of the crown (upper, middle, and lower). For purposes of comparison the generally prevailing atmospheric temperatures were recorded by thermocouples concurrently with readings from within the tree crowns. Measurements were made periodically during seed dormancy with a Leeds Northrup Company (catalog No. 8693) dual range potentiometer.

Determination and Regulation of Light

Light quantity and quality were regulated by changing the number, type, and distance of fluorescent or incandescent lamps in the growth chambers or special illumination boxes. Light in one growth chamber was from 15 Sylvania F 96-inch T 12/GRO/VHO grolux, 15 Sylvania 96-inch T 12 standard cool white, four G.E. 48-inch T 12 standard cool white fluorescent lamps, and twenty-two 60 watt G.E. incandescent lamps providing a maximum illumination of 3,600 foot candles. Use of standard filtration methods provided illumination reductions to six foot candles.

The phytochrome phenomenon (9; 68,. p. 316) was investigated in a red light box containing four 15 watt AC or DC rapid starting delux warm white fluorescent lamps (Figure 4A). Two layers of red cellophane filtered out all light but the red, while a false bottom provided conditions of total darkness. Figure 4B illustrates the far-red box containing six 40 watt incandescent lamps. Heat from the light source was absorbed by a glass tray of water, while double layers of red and blue cellophane filtered out all the spectrum except far-red.

Paragon time switches (Paragon Electric Company model No. 4001-0) were used in conjunction with the growth chambers, while Tork time switches (Tork Time Controls, Inc. No. 4100) were used to control the interval and duration of light programmed for the special illumination boxes.

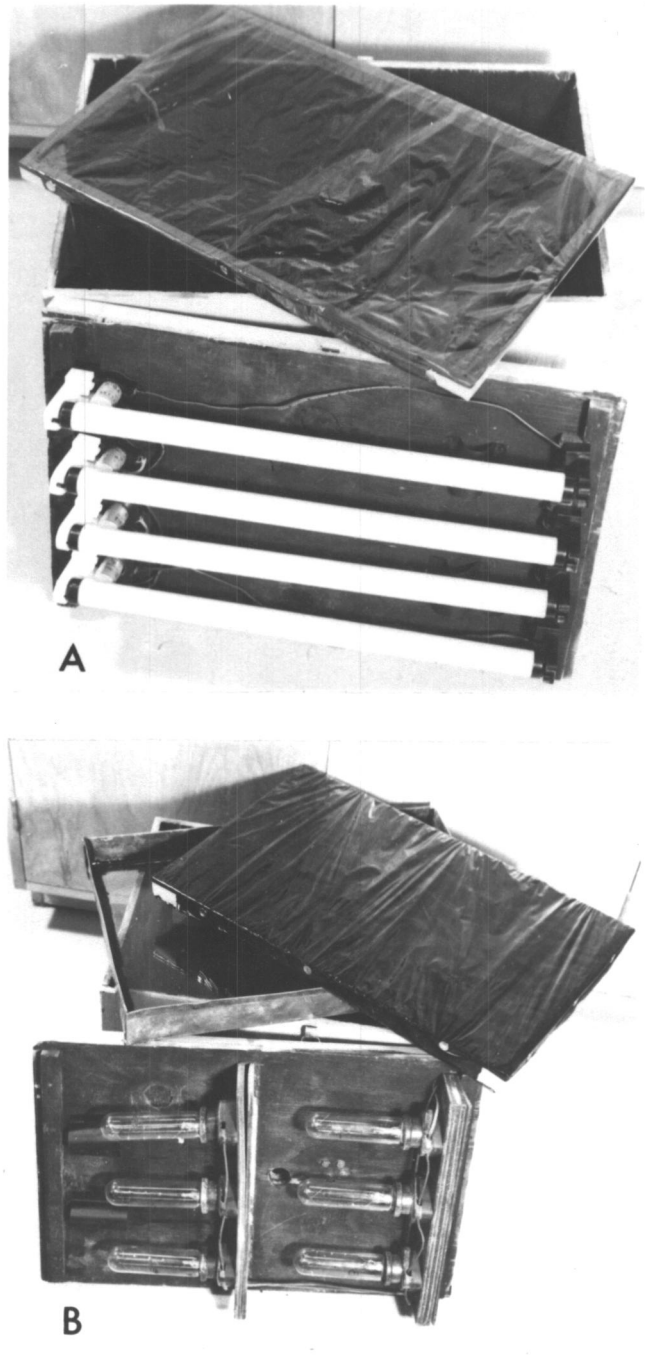


Figure 4. Special illumination boxes. A) Apparatus used for red light. B) Apparatus used for far-red light.

All determinations of illumination intensification were made with a Weston illumination meter (Daystrom Inc. Quartz Filter model No. 756) which had three separate scales to provide greater sensitivities at low, medium, and high intensities--having a maximum capacity of measuring 12,000 foot candles.

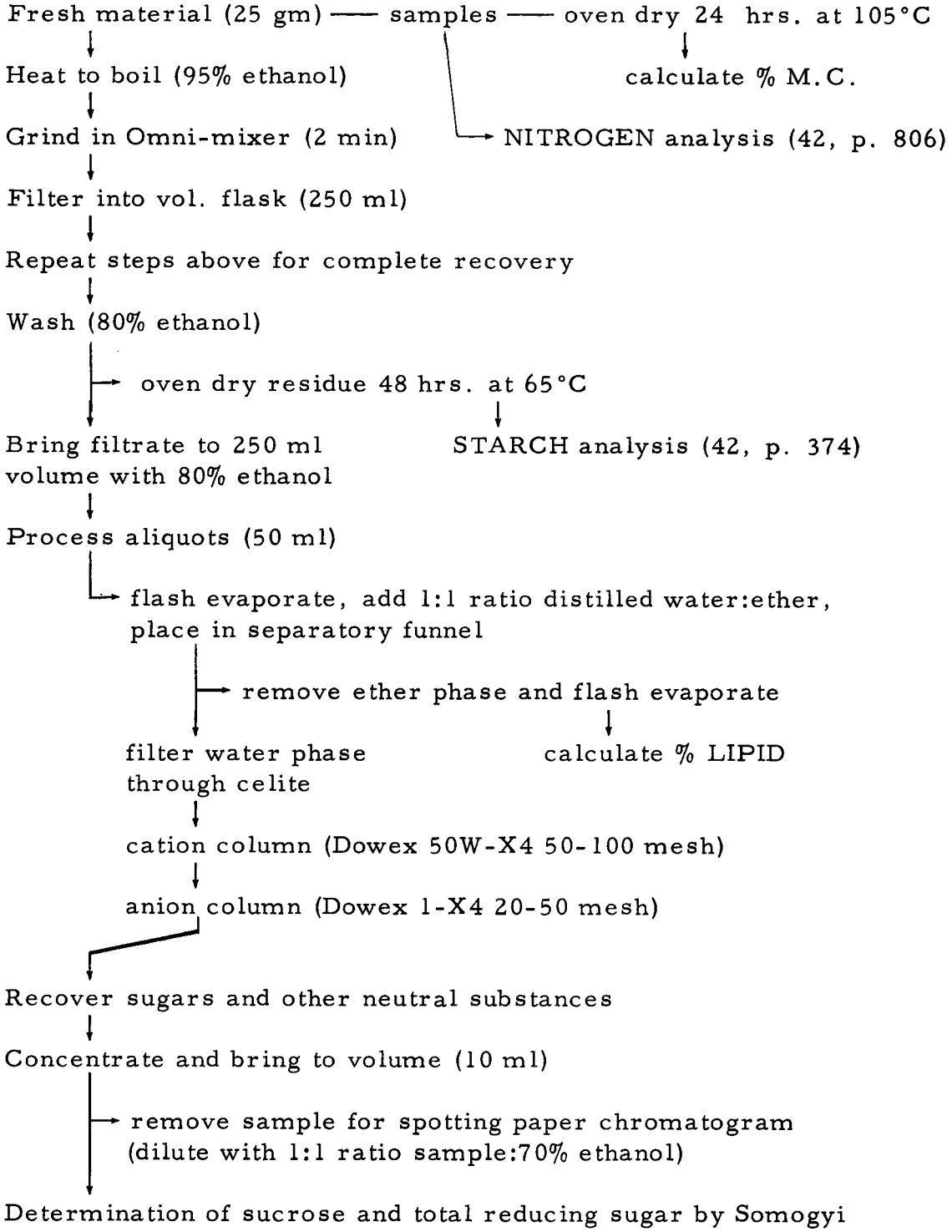
Chemical Analyses of Seed Constituents

Seed during dormancy was analyzed for starch, sugar, lipid, and nitrogen by the procedures given in the following chart.

Statistical Analyses of Experimental Results

A randomized block design was employed for most experiments. The analysis of variance with two-way classification was used to detect differences between experimental treatments. Specific differences between treatment means were determined by the t-test of least significant difference (LSD). A comparison of all possible differences was made by arranging all sample (treatment) means in order of high to low and then substracting each value from those above (see Table 4 as an example).

The author has used transformation as discussed by Li (49, p. 447-468) and Snedecor (65, p. 318-319) as a statistical tool to reduce error in the interpretation of experimental results obtained from source material of high variability. Transformation is


Fresh material (25 gm)
samples
oven dry 24 hrs. at 105°C
calculate % M.C.
Heat to boil (95% ethanol)
Grind in Omni-mixer (2 min)
NITROGEN analysis (42, p. 806)
Filter into vol. flask (250 ml)
Repeat steps above for complete recovery
Wash (80% ethanol)
oven dry residue 48 hrs. at 65°C
STARCH analysis (42, p. 374)
Bring filtrate to 250 ml volume with 80% ethanol
Process aliquots (50 ml)
flash evaporate, add 1:1 ratio distilled water:ether, place in separatory funnel
remove ether phase and flash evaporate
calculate % LIPID
filter water phase through celite
cation column (Dowex 50W-X4 50-100 mesh)
anion column (Dowex 1-X4 20-50 mesh)
Recover sugars and other neutral substances
Concentrate and bring to volume (10 ml)
remove sample for spotting paper chromatogram (dilute with 1:1 ratio sample:70% ethanol)
Determination of sucrose and total reducing sugar by Somogyi micro method (42, p. 551)

actually a disguise for the median and in a normal population, the mean is equal to the median.

To transform, the original value is converted to the angle whose sine is the square root of the proportion or percentage; thereby called the arcsin $\sqrt{\text{percentage}}$ value, which weighs more heavily the small percentages with a small variance. Following the analysis of variance and t-test, retransformation to original units permits satisfactory interpretation of experimental results.

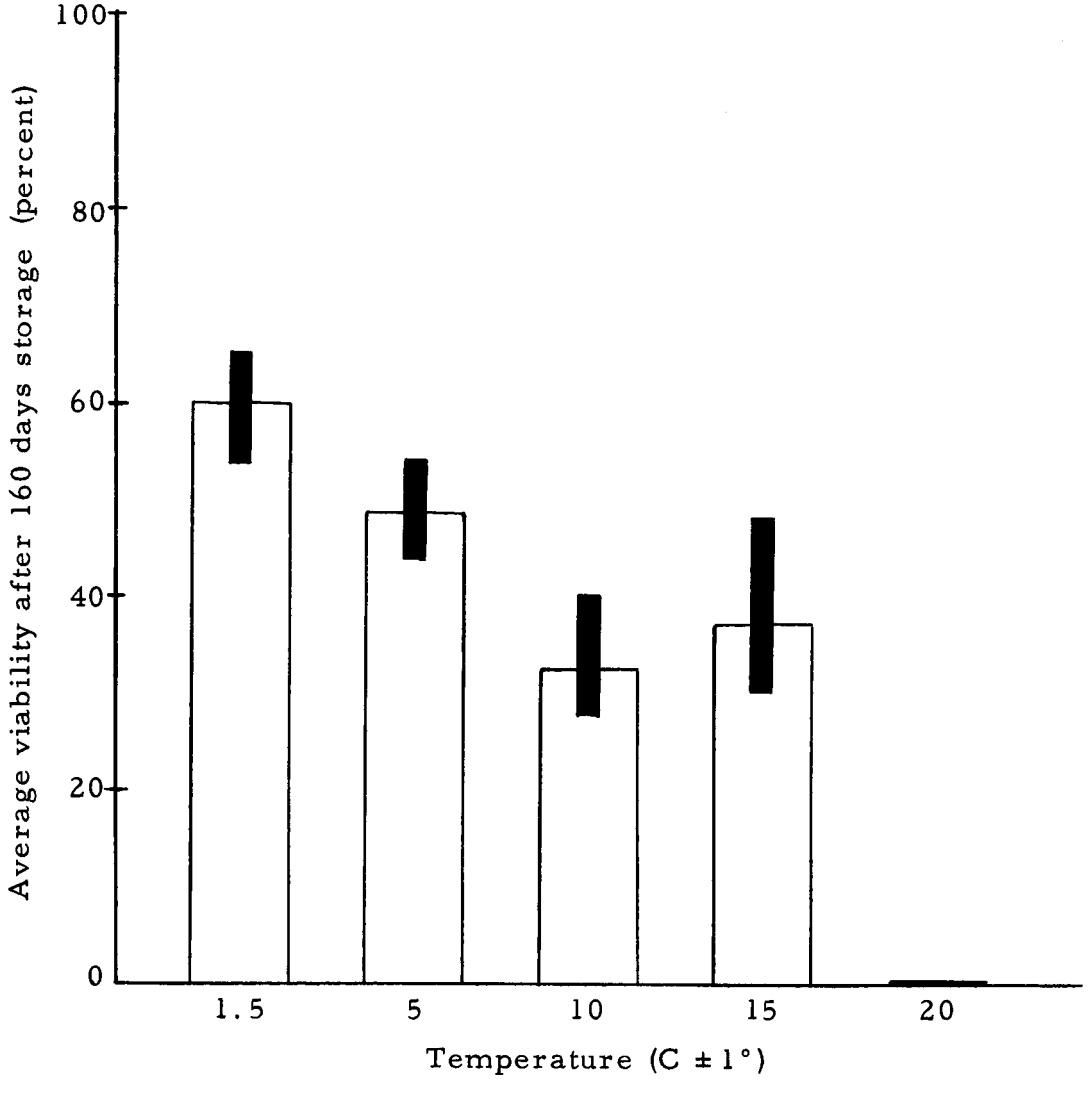
EXPERIMENTAL RESULTS

Determination of Humidities and Temperatures Limiting Deterioration of Seed During Storage

Storage of seed to be used subsequently in experimentation requires establishment of conditions insuring optimum retention of seed viability. The rate of seed deterioration during storage is inversely proportional to the suitability of ambient moisture-temperature. For example, when the temperature is increased, the tolerance of the seed toward moisture is decreased; conversely, when the temperature is decreased, the tolerance toward moisture is increased (2, p. 30).

Five samples of 250 seed per sample were used to determine the effect of temperature on viability of dwarfmistletoe seed during storage at 1.5, 5, 10, 15 and 20°C (Figure 5). Temperatures between 1.5 and 15°C caused loss of viability within 160 days. Survival of seed stored at 1.5°C was significantly better (5 percent level) than at other temperatures. Significant differences in survival also existed between seed stored at 5°C and at temperatures of 10°C and greater, while no real difference in viability existed between seed at 10 and 15°C. Thus, it appears that within the range tested, the best temperature for storage of seed of dwarfmistletoe is 1.5°C

Figure 5. The effect of storage temperature on viability of seed of western dwarfmistletoe during storage at 1.5, 5, 10, 15, and 20°C and 34-70 percent humidity. Solid bars indicate range of five samples of 250 seed per sample.



and increases of temperature of more than several degrees leads to significant reductions in viability.

Figure 6 shows the effect of atmospheric relative humidity on seed from five samples of 250 seed per sample stored at 1.5°C. Viability of seed stored at 1.5°C remained high between 45 and 70 percent relative humidity but was very poor below 25 and above 70 percent. Seed was badly overgrown by fungi and bacteria at humidities greater than 90 percent.

The moisture content of seed of dwarfmistletoe after reaching hygroscopic equilibrium over gradients of sulfuric acid shows the variations in moisture uptake under similar conditions of humidity which occurred between seed lots previously stored at different temperatures (Figure 7). As viability is lost, capacity of the seed to absorb moisture is drastically decreased. The food reserves of the seed constitute to a large part, the sites of moisture absorption within the seed. Therefore, the results of the hygroscopic equilibrium test express metabolic degradation that has occurred within the seed since discharge. For example, Figure 7 suggests that seed stored at 20°C contains fewer moisture absorption sites than seed stored at 1.5°C. This presumably occurred because the interaction of temperature and moisture, as mentioned above, resulted in higher respiration--followed by increased degradation of reserve materials.

Figure 6. The effect of atmospheric relative humidity on the viability of seed of western dwarfmistletoe during storage at 1.5°C. Averages from five samples of 250 seed per sample.

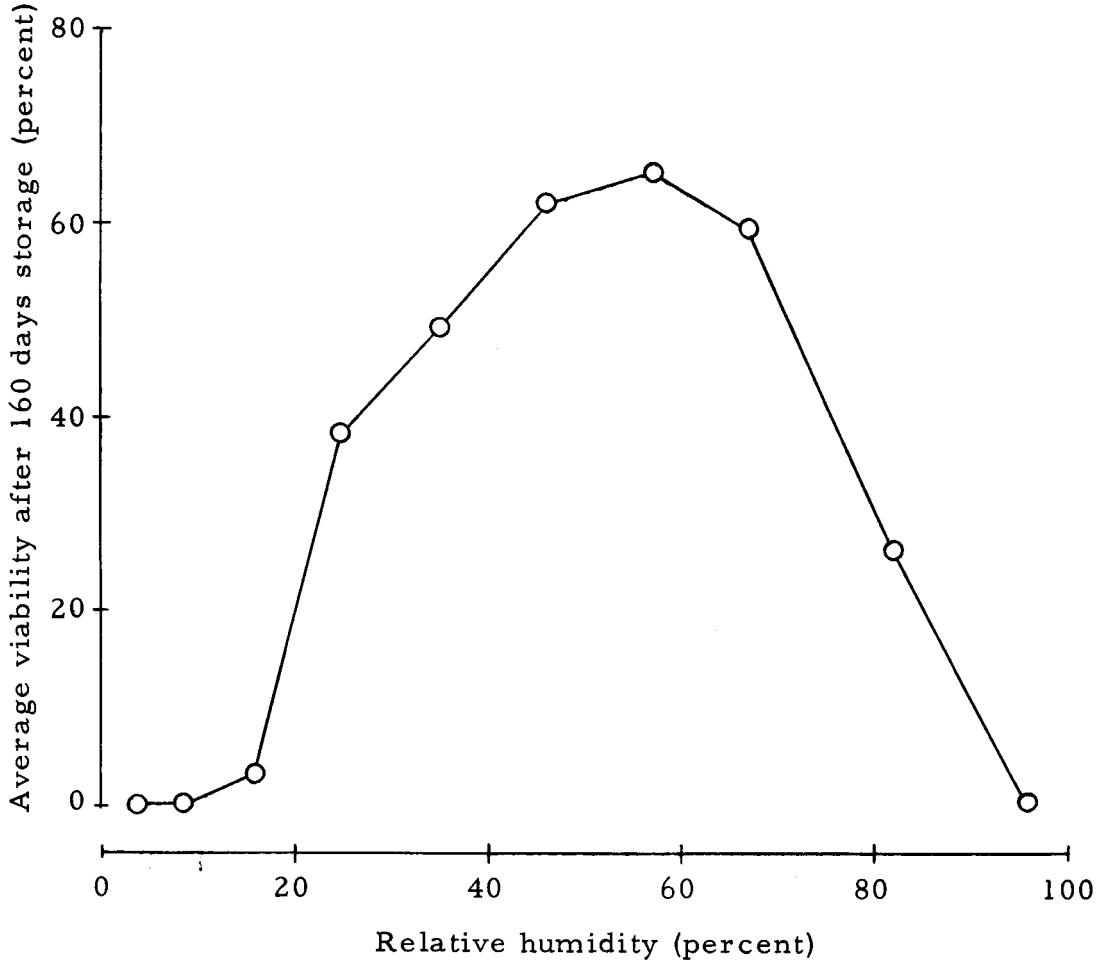
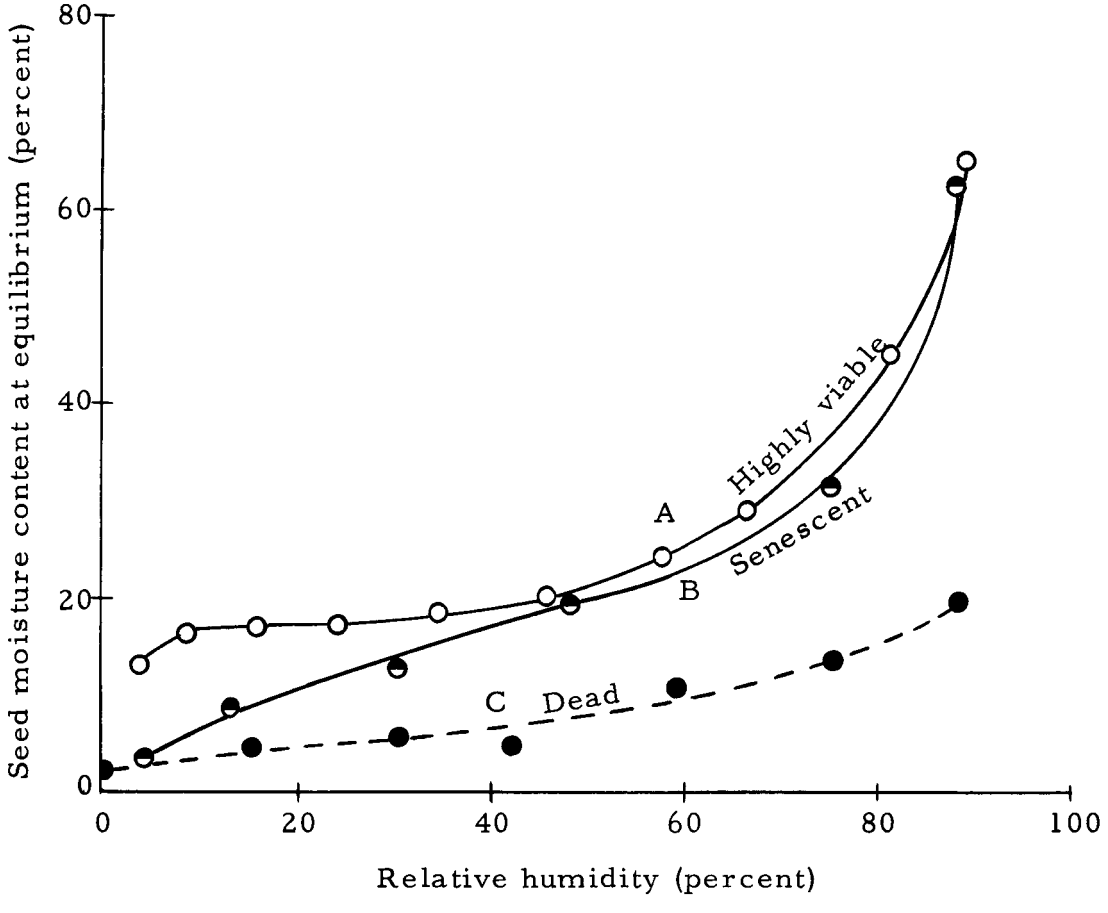


Figure 7. Moisture content of seed of western dwarfmistletoe from three conditions of storage after reaching hygroscopic equilibrium over sulfuric acid. "A" highly viable (age 30 days, viability 72 percent, stored at 1.5°C); "B" senescent (age 150 days, viability 37 percent, stored at 1.5°C); "C" dead (age 150 days, non-viable, stored at 20°C).



Physiological Mechanisms Relating to Seed Dormancy

Haber and Luippold (30) indicated from their studies of mitosis in dormant lettuce seed that: 1) the definitive characteristic of dormancy in seed is a subtle block that specifically prevents the initiation of cell enlargement, and 2) that dormancy is not a significant depression of the over-all metabolism or blockage of cellular division as the term implies. Cohen (13) reports that cellular expansion within seed of western dwarfmistletoe occurs prior to the first sign of radicle emergence. The swelling of seed prior to germination, presumably due to cellular expansions, has been observed by the author.

Dormancy *per se* is the condition in which apparently ripe seed fail to germinate when placed under conditions favoring germination (51, p. 709). In cases of after-ripening, seed must undergo natural changes following discharge before dormancy is modified and germination may occur (20, vol. 3, p. 520). Nevertheless, delayed germination appears to be the result of physiological mechanisms that maintain seed in a non-germinating state (56).

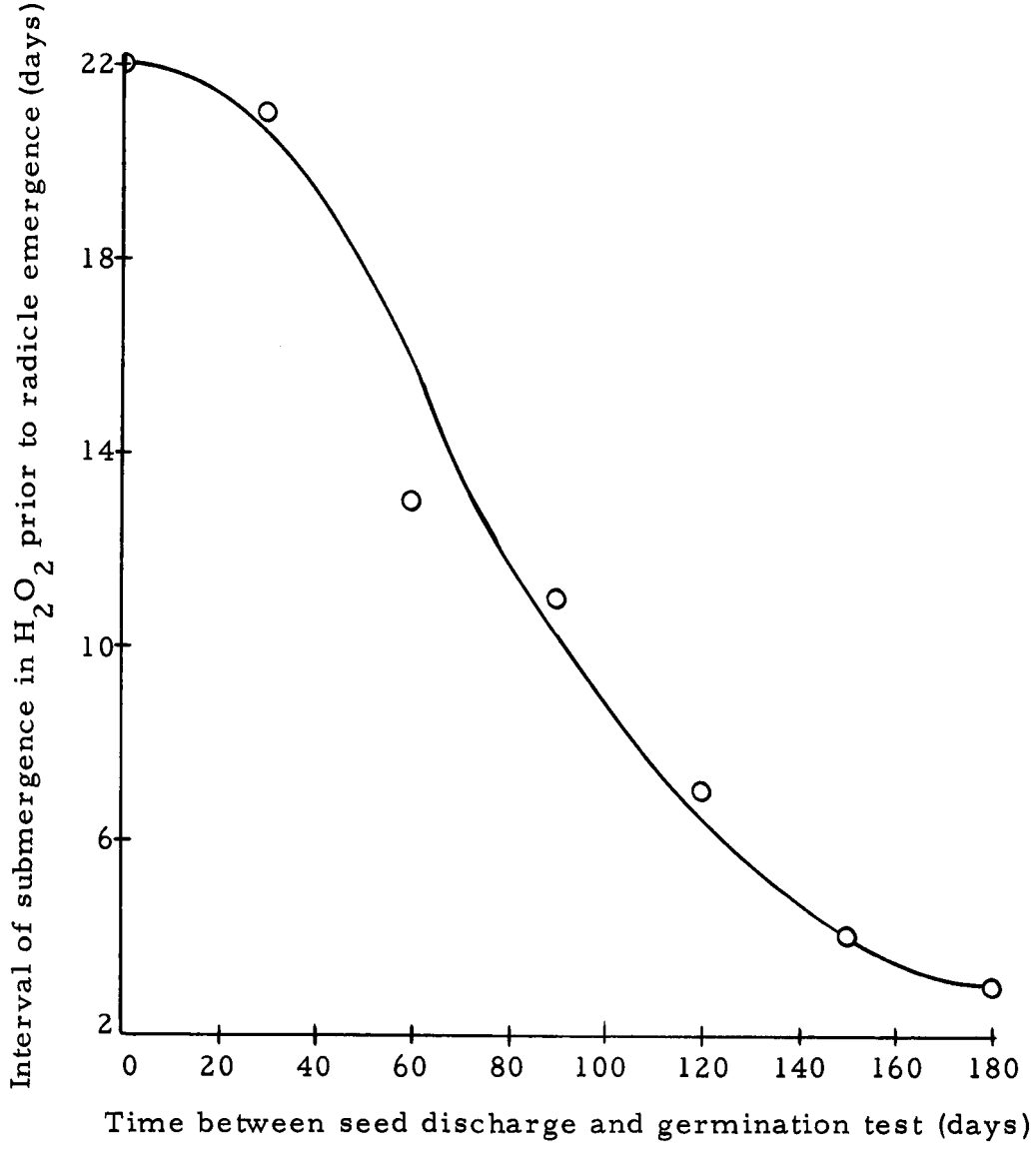
Dormancy of seed is biologically significant. It may regulate species dissemination and survival by retarding germination until prevailing environmental conditions are favorable for seedling development (68, p. 301).

Crocker (14, p. 794) reports that dormancy of seed of Viscum album is reduced to one month under optimum conditions of temperature (15-20°C), light (good) and humidity (dry air). Others (1; 26; 33; 46, p. 589) report that seed of A. vaginatum germinate upon discharge in the fall in the absence of a period of dormancy. Kuijt (46, p. 589) also reports that once germinated, the growth of the radicle is often arrested by low temperatures and only resumes when favorable conditions return. In contrast, Weir (74, p. 4) working with A. campylopodum indicated that some seed germinate immediately, but that greater germination occurred when seed was exposed to freezing temperatures. The author's observations in field and laboratory indicate that seed of A. campylopodum undergo dormancy prior to germination.

Comments by Wicker (75), pertaining to a rapid germination technique for dwarfmistletoe seed, prompted use of hydrogen peroxide to investigate dormancy of seed of dwarfmistletoe. Figure 8 shows that seed that have been stored at 1.5°C germinate more readily as the seed age. This phenomenon is called germination readiness and is expressed in days of submergence in three percent hydrogen peroxide.

The effect of temperature on seed viability during storage and dormancy was determined by the TZ test, where field storage temperatures had been between 26.5 and -10°C and laboratory storage

Figure 8. Germination readiness of seed of western dwarfmistletoe stored at a constant 1.5°C. Expressed in days of submergence in three percent hydrogen peroxide with each point representing the performance of 250 seed.



temperature was 1.5°C (Figure 9). The initial viability of seed from the three field collection and storage sites differed significantly. Retention of viability during the test also differed among seed from the three areas.

Figure 10 shows the effect of temperature on viability (as determined by TZ test) of seed within 56 days of discharge. The relationship between level of viability and temperature is almost linear. Each five degree increase in temperature results in significant losses of viability.

Attempts to break seed dormancy were made using common chemical stimulants and growth regulators. Haber and Luippold (30) indicated that both thiourea and kinetin stimulated germination in dormant lettuce seed, while gibberellic acid (GA) was ineffective. They believe that thiourea stimulates germination via initial cellular expansion even though it inhibits growth and mitosis of non-germinated seed of lettuce and potato tubers. They postulated that the inhibitory action of thiourea can be attributed solely to inhibition of cell division, while kinetin may be considered to stimulate cell division in non-germinated seed of lettuce. Toole and his associates (68, p. 308) reported that "thiourea is known to be particularly effective in promoting germination of some light-requiring or temperature-inhibited seed such as lettuce".

In these tests thiourea, kinetin and GA were effective in

Figure 9. Viability of dormant seed of western dwarfmistletoe stored at field temperatures between 26.5 and -10°C and a laboratory temperature of 1.5°C. Each point represents the average performance of 250 seed using the TZ test.

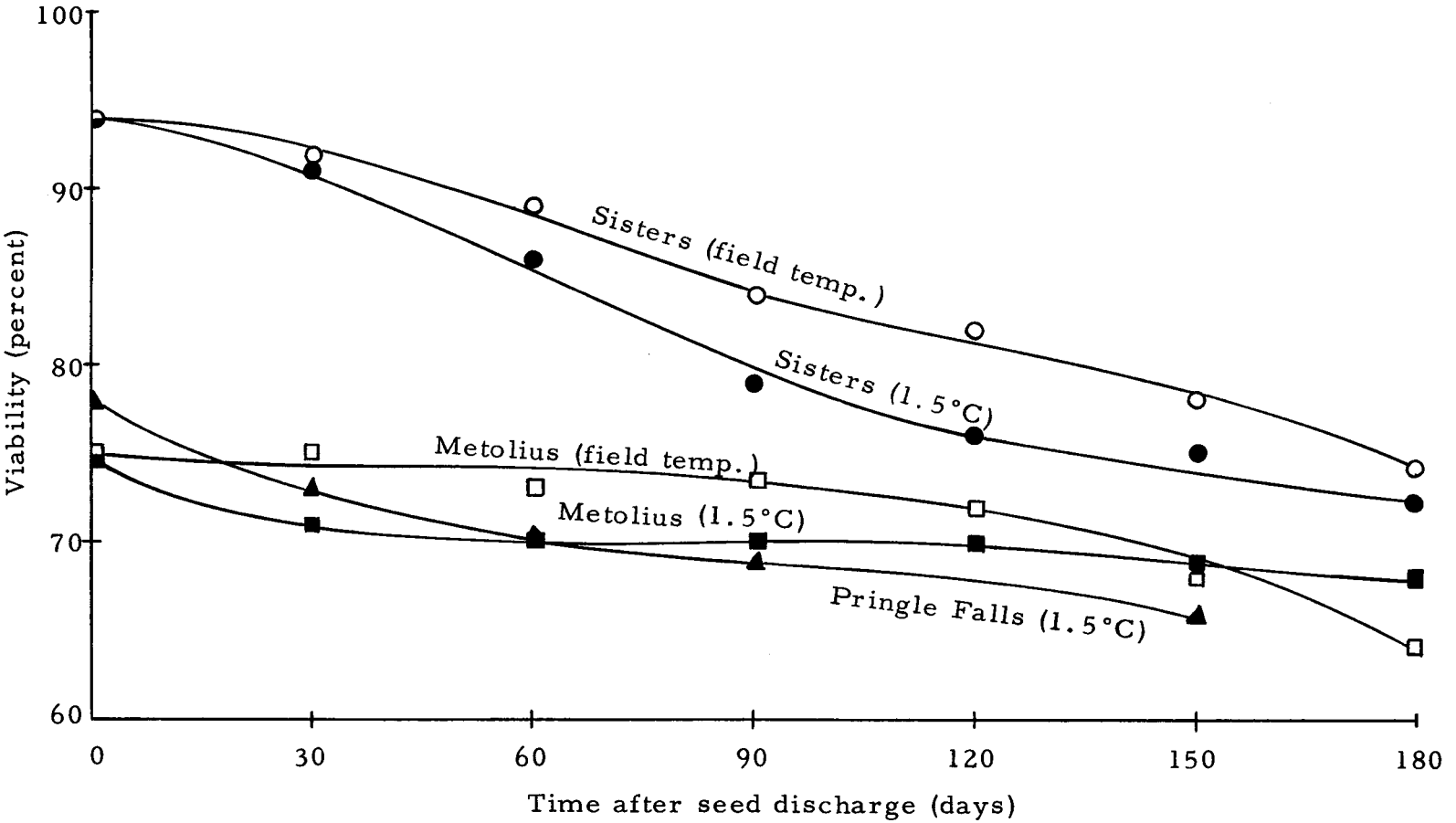
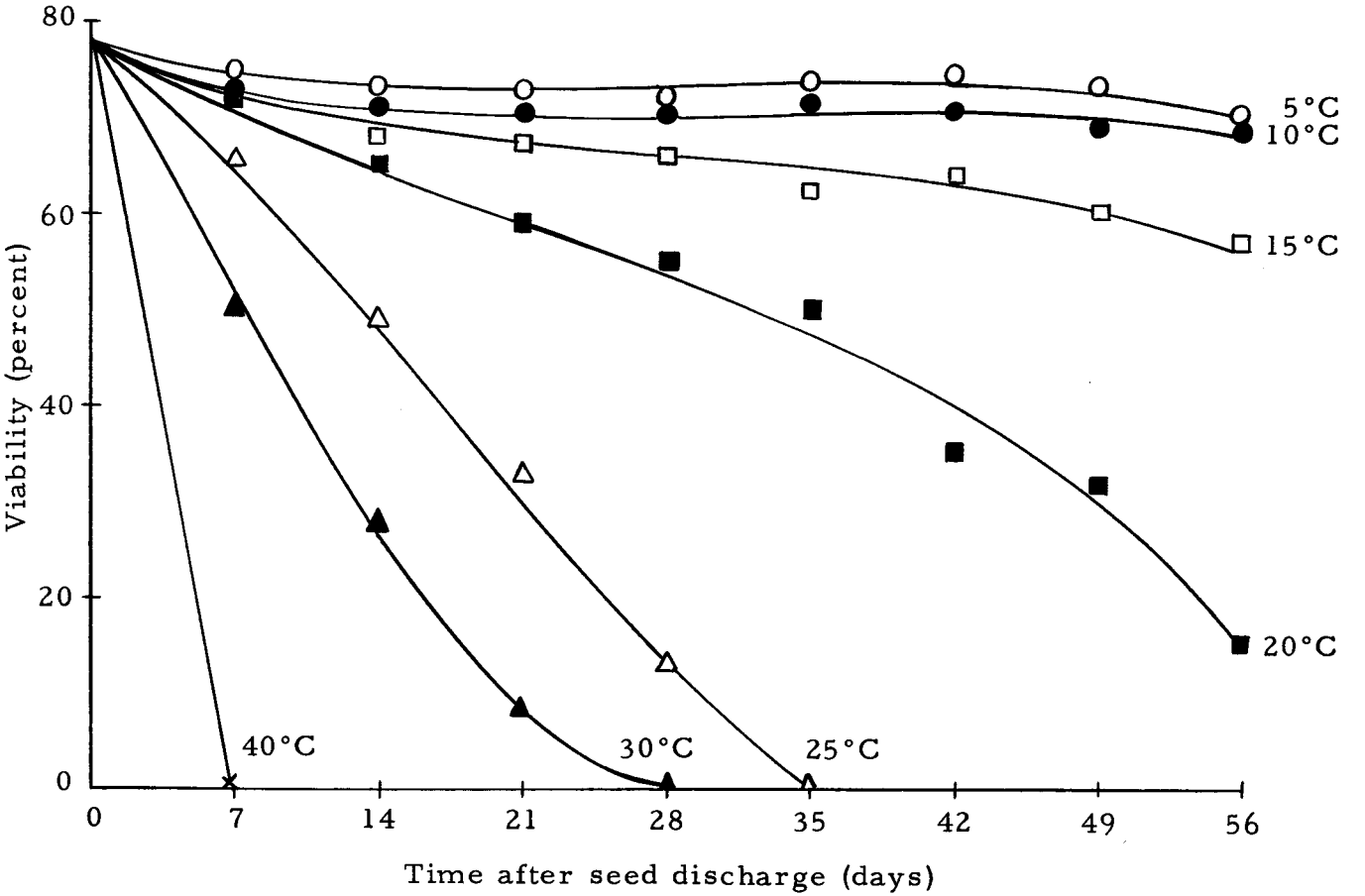


Figure 10. The effect of temperature on the viability of seed of western dwarfmistletoe within 56 days of discharge. Each point represents the average performance of 250 seed using the TZ test.



stimulating germination (breaking dormancy) when seed had aged 120 days following discharge. Table 1 indicates chemicals and growth regulators used to test breaking dormancy of dwarfmistletoe seed stored 120 days at 1.5°C. The effectiveness of thiourea is apparent. Subsequent tests using different concentrations of thiourea from 1 mg/l to 1000 mg/l indicated that concentrations below 10 mg/l failed to enhance germination while concentrations above 500 mg/l were inhibitory. Kinetin and dextrose were about 20 percent less effective than thiourea, while GA was only slightly effective.

Experiments involving separation of endosperm and embryo from the "seed coat" (see Figure 1) of freshly discharged seed suggests that an inhibitory mechanism associated with the seed coat prevents germination for about five to six months. Intact seed (see Figure 1) failed to germinate for five to six months after seed discharge, while excised endosperm and embryo germinated in two days. The inhibitor apparently breaks down at near-freezing temperatures during aging. Once degradation has progressed to a certain state, the inhibitor loses its capacity to prevent cellular expansion within the seed and germination may occur when moisture, light, and temperature are favorable.

Table 1. Summary of the effect of several chemicals on germination of seed of western dwarfmistletoe following 120 days of storage at 1.5 ± 1°C.

Supplement 1/	Accumulative mean germination rate in days 2/					Percent germination after 28 days 3/
	12	16	20	24	28	
Control (dist. H_2O)	0	0	0	3	3	1.2
Thiourea (100 mg/l)	90	124	136	160	162	64.8
Thiourea (10 mg/l)	10	87	91	110	150	60.0
Dextrose (1%)	0	19	28	73	112	44.8
IAA (10^{-6} M)	0	31	46	51	59	23.6
Gibberellin (1000 ppm)	0	11	33	36	40	16.0
Gibberellin (10 ppm)	0	0	0	15	30	12.0
Kinetin (100 ppb)	10	64	94	97	98	39.2
Kinetin (1 ppb)	0	26	67	72	75	30.0

1/ Seed soaked in 0.1% Arasan suspension for two hours then washed prior to addition of respective supplement.

2/ On basis of two replications of 250 seed per treatment.

3/ Temperature constant 20°C and photoperiod of eight hours and 120-200 foot candles intensity.

Sugar, Starch, Lipid and Nitrogen Content of Seed

Little is known of the seed reserve food metabolites of the mistletoes. Gill and Hawksworth (28, p. 21-22) present the following résumé of carbohydrates in the mistletoes:

> In the Javanese Dendrophthoe pentandra (Schoorl, 1929) glucose was essentially the only sugar and totaled about 6 percent of the fresh weight of the fruits. Glucose was also found in Viscum album (Einleger et al. 1923). Free fructose has not been reported, but sucrose was detected in Viscum album and Loranthus europaeus (Beguin 1931; Einleger et al. 1923), and 4.5 percent unidentified reducing sugars have been found in Phoradendron flavescens (Desantis and Lynn 1937). Sugars that occur in combined forms in various mistletoes include galactose, arabinose, and rhamnose.

Numerous reports of starch in the mistletoes have been made but specific reference to starch in seed of mistletoes is lacking.

Gill and Hawksworth (28, p. 23) report that leaves of Viscum album contain about six times as much nitrogen as its host, while Loranthus europaeus contains about twice as much as its host. McDowell (52, p. 26) reports the nitrogen content as one percent for both host tissue (phloem) and aerial shoots of western dwarf-mistletoe.

Einleger, et al. (19) found specific lipids in mistletoes but made no reference to lipid content of seed. The author made chemical analyses of seed constituents following standard procedures

(42). Total lipid, total sugar, reducing sugar and sucrose contents were determined on a dry weight basis, while the nitrogen content was determined on an air dry weight basis.

Table 2 shows changes in chemical composition of dormant seed stored at 20 ± 1°C. The total starch content diminished somewhat during the 150 days, while lipid content decreased almost threefold. Total sugar and sucrose content decreased sharply while reducing sugar content increased slightly. Apparently the reserve materials in the form of starch and lipids were converted and utilized at a slower rate than sugars which are basic metabolites in the respiratory process.

Figures 11, 12 and 13 are facsimiles of paper chromatographs of simple free sugars in seed of western dwarfmistletoe. Figure 11 shows the initial chromatogram of nine known saccharides (Table 3) and three concentrations of unidentified sugars from seed. Sucrose, fructose and galactose were suspected as the simple free sugars on this chromatogram. Figure 12 verified that sucrose and fructose were present as simple free sugars but raised the question of whether material B was galactose or some other saccharide. Rf calculations from Figures 11 and 12 (see Table 3), indicated that the alternative could be glucose. An extra long period of chromatogram development was used to help clarify this point, since glucose and galactose are difficult to separate over short periods of development.

Table 2. Sugar, starch, lipid, and nitrogen content of seed of western dwarfmistletoe stored at 20 ± 1°C for 150 days.

Seed age (days)	Percent viability (TZ) 1/	Moisture content (%)	Fresh weight basis (mg/gm seed)				Starch (mg/gm d. w. b.)	Percent nitrogen (air dry basis)		
			Total lipid	Total sugar	Reducing sugar	Sucrose		Endocarp + viscin	Endosperm + embryo	Entire fruit
30	72	57	24.1	121.7	33.0	84.3	345.0	3.22	6.30	6.42
120	37	53	16.2	53.6	35.2	17.5	322.0			
150	0	7	9.4	38.4	37.4	1.0	317.0			

1/ Based on average of three samples of 250 seed each.

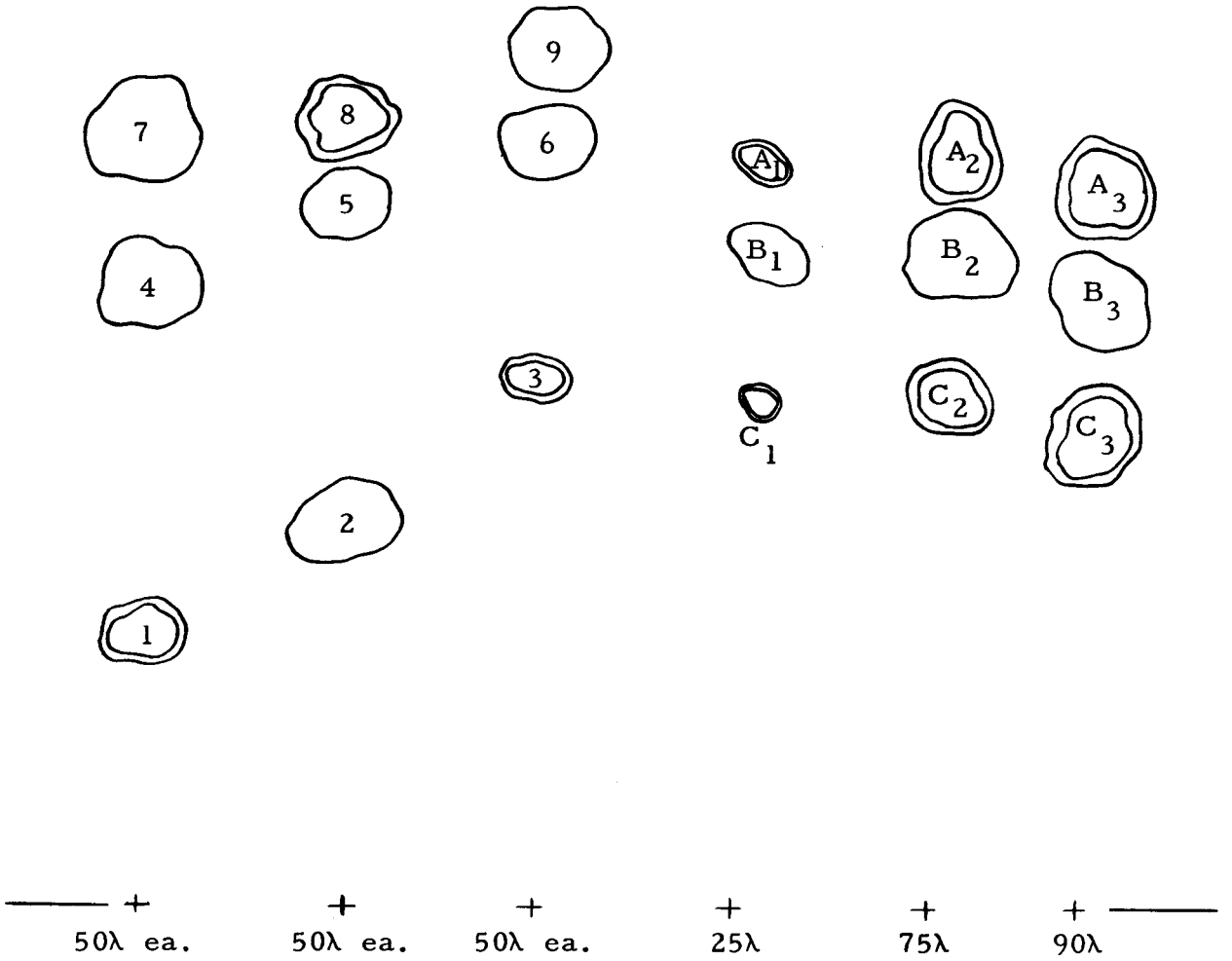


Figure 11. Chromatogram of nine known saccharides (numbers 1 - 9) and three concentrations of unidentified simple free sugars (A, B and C) from extract of seed of western dwarfmistletoe. Table 3 contains code identifications.

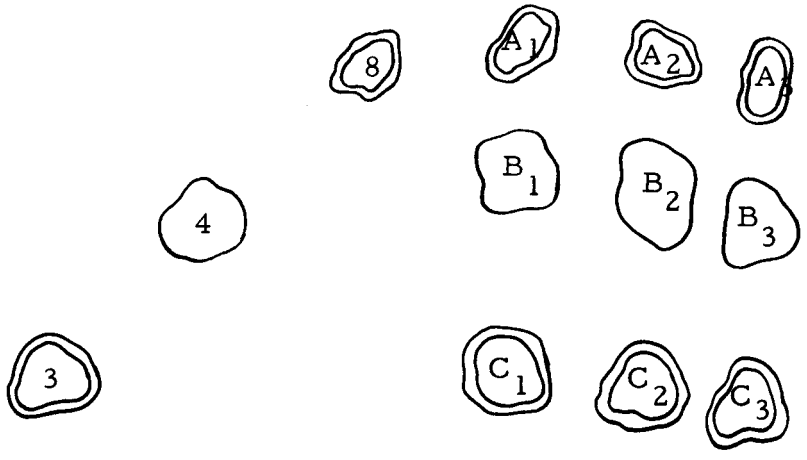
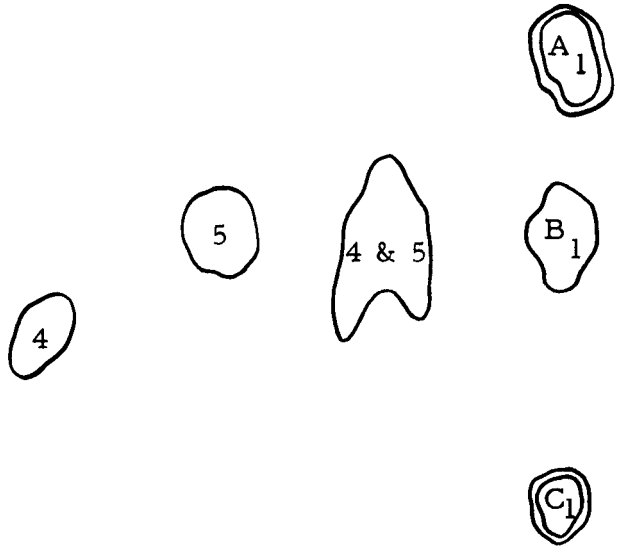


Figure 12. Chromatogram comparing sucrose (3), galactose (4) and fructose (8) with unidentified simple free sugars (A, B and C). Sugars A and C identified as fructose and sucrose on basis of Rf values (Table 3).



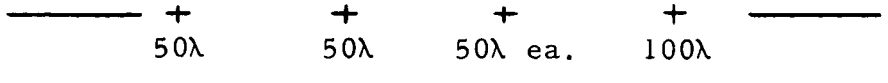


Figure 13. Chromatogram comparing galactose (4) and glucose (5) with unidentified simple free sugars (A, B and C). Sugar B identified as glucose on basis of Rf values (Table 3).

Table 3. Summary of Rf calculations and temperature effects on chromatogram color development used to identify simple free sugars found in seed of western dwarfmistletoe.

Code for known saccharides	Temperature required for color reaction 1/	Calculated Rf (Figure 11)
1 raffinose	+	0.05
2 lactose	-	0.09
3 sucrose	+	0.14
4 galactose	-	0.16
5 glucose	-	0.18
6 mannose	-	0.20
7 arabinose	-	0.21
8 fructose	+	0.23
9 xylose	-	0.28

1/ 100°C for five minutes (-) and 125°C for ten minutes (+).

Constituent 2/	Temperature required for color reaction (Figures)			Calculated Rf (Figures) 3/		
	11	12	13	11	12	13
3	+	+		0.14	0.32	
4	-	-	-	0.16	0.39	0.40
5	-		-	0.18		0.44
8	+	+		0.23	0.47	
4-5			-			0.44
A1	+	+	+	0.23	0.48	0.50
A2	+	+		0.23	0.47	
A3	+	+		0.23	0.47	
B1	-	-	-	0.17	0.42	0.44
B2	-	-		0.17	0.41	
B3	-	-		0.16	0.40	
C1	+	+	+	0.14	0.33	0.34
C2	+	+		0.14	0.32	
C3	+	+		0.14	0.32	

2/ Identification of constituents in response to temperature and relative Rf value indicate fructose (A), glucose (B), and sucrose (C).

3/ Chromatograms (Figures 11, 12 and 13) developed 24, 36, and 48 hours respectively.

Figure 13 verified the presence of glucose, sucrose and fructose but not galactose as previously indicated. The finding of free fructose in seed of dwarfmistletoe by the author complements the report by McDowell of fructose in the vegetative tissue of dwarfmistletoe.

Table 3 indicates temperature effects at 100 and 125°C on chromatogram color development and Rf values used to determine and verify the simple free sugars found in seed of western dwarfmistletoe.

Values for nitrogen found in the seed, as shown in Table 2 are three to six fold greater than nitrogen values reported by McDowell (52, p. 26) for vegetative tissue, while they are somewhat comparable to findings for Viscum album vegetative tissue as previously noted.

Influence of Temperature on Seed Germination

In general, seed germinate within a certain range of temperatures with the optimum temperature generally midway between the two extremes. It is not possible to designate the exact optimum temperature for a species unless specific criteria are employed as the index of germination (51, p. 708).

For many species the range of temperatures favorable for germination is well below the range favorable for seedling growth (67). Cieslar in 1883, was the first to demonstrate the effect of

alternating temperatures on seed germination (31, p. 295). Since then many others have demonstrated similar effects on the seed of numerous species. Toole, et al. (68, p. 311) state that "generally, an alternation from a temperature near the optimum constant value to some higher value which might be above the maximal is most effective." Yet seed of species such as brome grass, timothy, rye grass, carrot and parsley germinate almost as rapidly and completely at favorable constant temperatures (31, p. 297).

Glimcher (29) investigating the germination of Viscum cruciatum reported minimal effective temperatures of 8-10°C, but indicated that germination in the field depended on the range of temperature following dispersal. Wiesner (78, p. 415) indicated that the minimal temperature for germination of Viscum album also was 8-10°C; however, Heinricher (37, p. 595) found germination would occur when mean temperatures were 3.8°C. Hawksworth (34, p. 108) reports that the height of germination for A. vaginatum occurs between August 15 and September 15 when mean temperatures range from a maximum of 26°C to a minimum of 5°C. Scharpf and Parmeter (61) report that germination of seed of A. campylopodum parasitizing Pinus sabiniana in California may occur at temperatures of 2.5°C. Their findings also indicated that germination fails to occur at 25°C and that optimum conditions are about 13.5°C.

The author's investigations regarding temperature and

germination of seed of A. campylopodum indicate that the range of temperatures suitable for germination is higher than previously reported for any other mistletoe.

Figure 14 shows the effect of a range of constant temperatures on germination. Samples of 250 seed which had been stored for 240 days at 1.5°C were germinated at each of the following temperatures: 5, 11, 17, 19, 26, and 31°C. These seed were exposed to a 12-hour photoperiod with the light intensity between 200-500 foot candles. The temperature limitations for seed germination appear to be near 5 and 30°C with the optimum temperatures between 15-20°C. An analysis of variance indicated a significant difference in germinability within these limits. The t-test of LSD had shown that with the exception of treatment comparisons between 17 and 19°C or 5 and 26°C, significant differences in seed germination existed at all other temperature comparisons (see Table 4). When the temperature is increased from 5 to 10°C, a significant difference (five percent level) in germination occurs. When the optimum temperature range for germination is exceeded, temperature increases reduce germination at significant levels provided the temperature is greater than 26°C. Figure 15 shows the rate of germination of 250 seed each at temperatures of 5, 11, 17, 19, 26 and 31°C. Increases in temperature up to the optimum level increase the rate of germination, while further increases in temperature decrease the rate of germination.

Figure 14. The effect of constant temperature on germination of seed of western dwarfmistletoe stores at 1.5° C for 240 days. Each bar represents the performance of 250 seed.

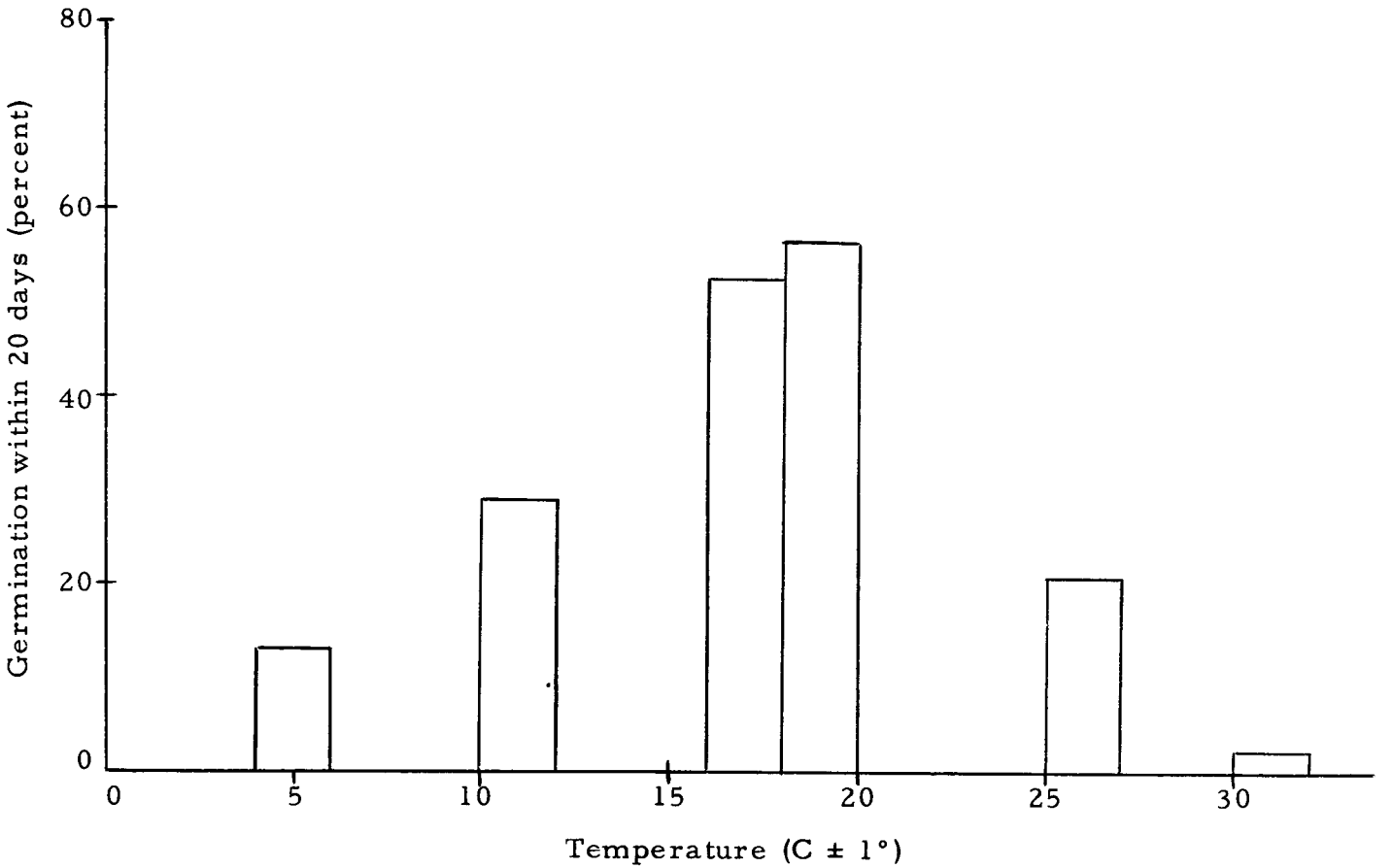


Table 4. Summary of differences in seed germination of western dwarfmistletoe in response to constant temperature (see Figure 14).

Treatment (C°)	LSD comparison of means 1/				
	31	26	19	17	11
5	SD*	NS	SD*	SD*	SD*
11	SD*	SD	SD*	SD*	
17	SD*	SD*	NS		
19	SD*	SD*			
26	SD*				

1/ Significant difference at five percent level (SD), significant difference at one percent level (SD*), and no significant difference (NS).

Figure 15. The effect of constant temperature on the rate of seed germination of western dwarfmistletoe stored at 1.5°C for 240 days. Each curve represents the performance of 250 seed.

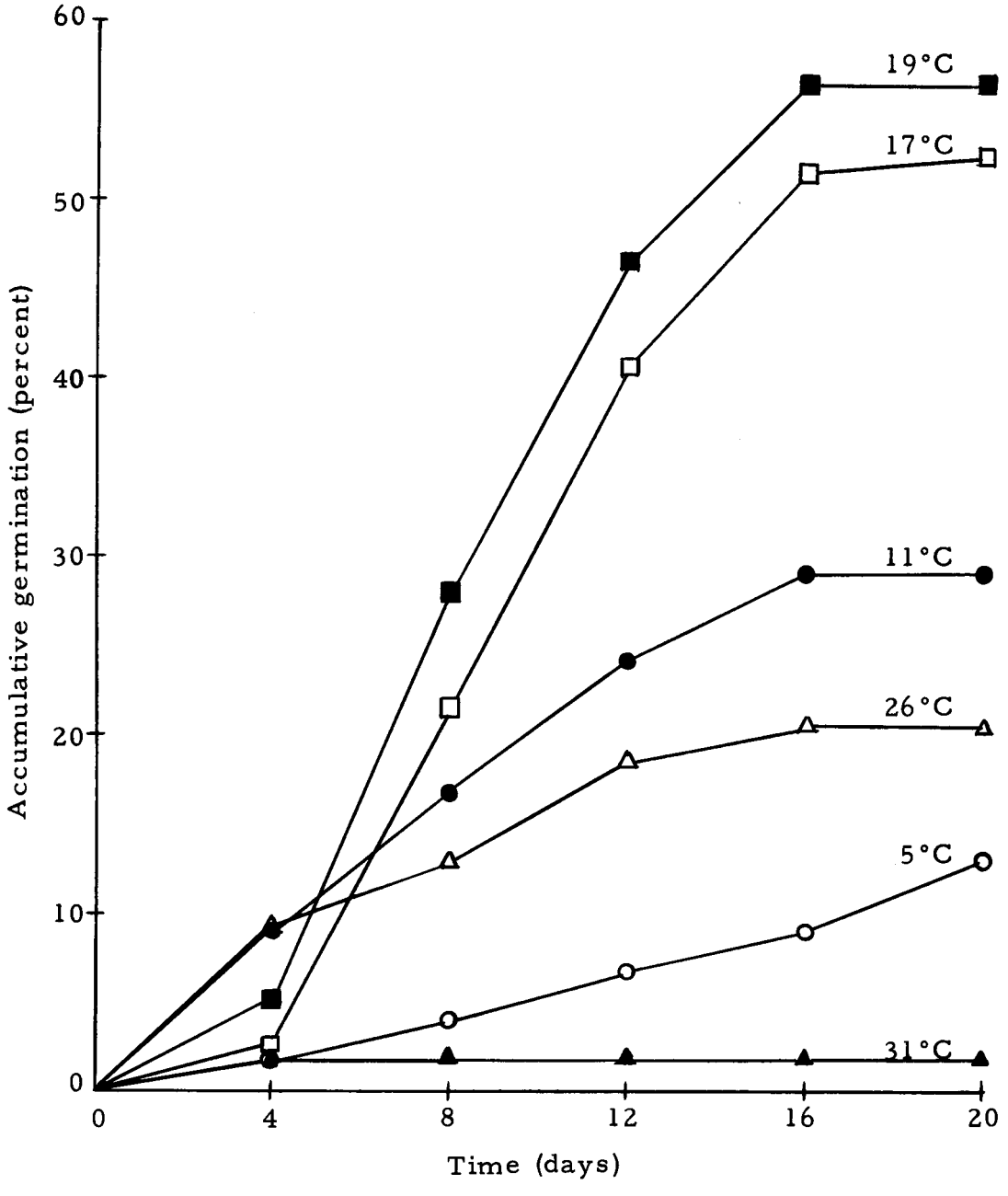


Table 5. Summary of germination percentages at different temperatures.

Night-day temperature (C°)	Seed source		Germination percentages		
	Metolius	Sisters	With light 1/	Total Darkness	Difference
10-30	x		14	6	8
		x	24	15	9
10-20	x		16	10	6
		x	47	34	13
15-20	x		17	13	4
5-20	x		26	19	5
		x	44	37	7
15-25	x		34	20	14
		x	49	35	14
20-20	x		36	20	16
		x	66	35	31
5-15	x		39	25	14
		x	56	45	11

1/ Photoperiod twelve hours at 200-600 foot candles intensity.

Figure 16. The effect of alternate night and day temperature on germination of seed of western dwarfmistletoe under two conditions of illumination. Seed stored at 1.5°C for 240 days. Germination percent represents performance of 250 seed.

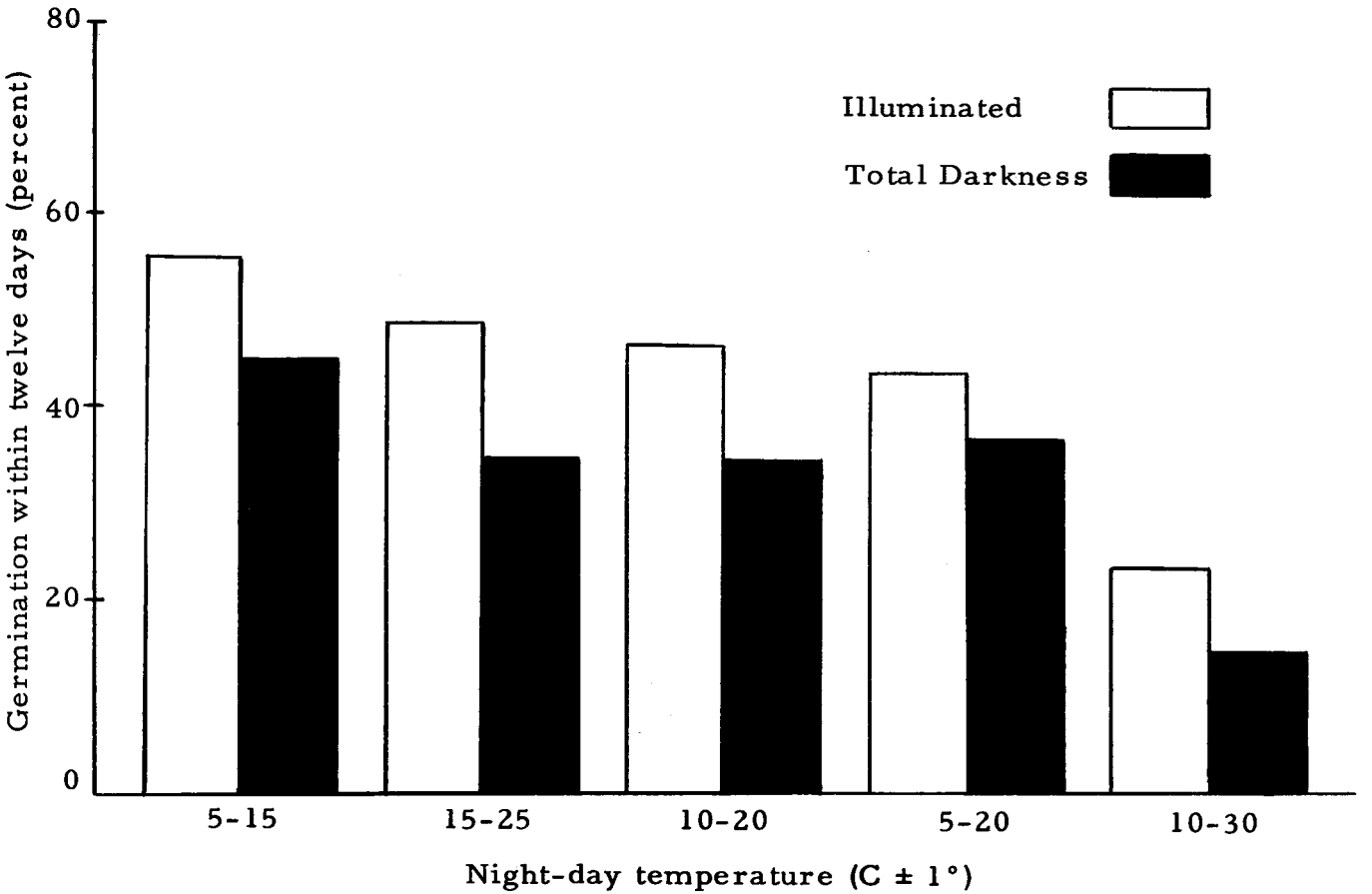


Table 6. Summary of differences in seed germination of western dwarfmistletoe under conditions of alternating temperature. Comparisons between conditions of light and total darkness (see Figure 16).

Treatment (C° and light or dark)	LSD comparison between means 1/								
	10-30d	10-301	5-20d	5-201	10-20d	10-201	15-25d	15-251	5-15d
5-151	SD*	SD*	SD*	SD	SD*	NS	SD*	NS	NS
5-15d	SD*	SD*	NS	NS	SD	NS	SD	NS	
15-251	SD*	SD*	SD	NS	SD*	NS	SD*		
15-25d	SD*	SD	NS	NS	NS	SD			
10-201	SD*	SD*	NS	NS	SD				
10-20d	SD*	SD	NS	NS					
5-201	SD*	SD*	NS						
5-20d	SD*	SD*							
10-301	NS								

1/ Significant difference at five percent level (SD), significant difference at one percent level (SD*), and no significant difference (NS).

near-freezing temperatures for 180 days germinated readily. Seed stored at near-freezing temperatures (1.5°C) for 30 days germinated at a rate of only one to two percent after 21 days exposure to optimum conditions of light, moisture and temperature.

Moisture Relations During Seed Germination

Without adequate water, cells of germinating seed are unable to carry on the vital processes of absorption, digestion, food transfer, assimilation, respiration and growth. Koller, et al. (45, p. 447) point out that germination may be affected by water relations existing prior to and during imbibition. Hawksworth (34, p. 40) reports that the average daily precipitation was 0.09 inches during the initial 15 days of optimum germination temperatures for seed of A. vaginatum. Glimcher (29) reports that Viscum cruciatum germinated in a desiccator over sulfuric acid but saturated atmospheres caused seed to rot prior to germination. Andrews (1), Gill and Hawksworth (28, p. 9), and others have pointed out that in the genus Arceuthobium, the unique viscin cells store reserve moisture required for germination in addition to binding seed against the substrate. Gill and Hawksworth also indicate that after several successive wettings and dryings, the viscin cells loose their hygroscopic quality irrespective of external moisture conditions.

It seemed logical to investigate the viscin cells, since they are

so closely associated with the moisture supply during germination. Moisture content of freshly discharged seed was 57 to 94 percent (dry weight basis), whereas after imbibition, the maximum increase in moisture content over the fresh weight was 84 percent.

Attempts to remove or dissolve the viscin cells with water were unsuccessful. In these attempts, seed were washed under continuous water pressure for 500 hours. Some deterioration occurred but for the most part the viscin cells remained functional. Tests to determine the retention of the hygroscopic quality of the viscin cells were conducted by alternate wetting and drying of seed on cheese cloth stretched across a wooden frame; seed were wet with a fine water spray then dried under a lamp. It was found that as seed viability diminished, the hygroscopic function of the viscin cells diminished.

Wiesner (78, p. 408-423) studying two European and four tropical species of mistletoe found that the former species would not germinate in water while the latter species required water. The author found that seed of western dwarfmistletoe were tolerant of reduced levels of aeration and germinated readily under water.

The capacity of seed to absorb from or relinquish moisture to the atmosphere during conditions of seed-atmospheric unbalance is altered by seed viability (Figure 7). Seed of dwarfmistletoe were unable to absorb sufficient moisture over sulfuric acid in 60 days to initiate germination, however, addition of water initiated germination

within four days. Emerging radicles were observed to grow for 120 days at 20°C without subsequent addition of water.

Influence of Light on Seed Germination

Light, temperature, and many other factors influencing seed germination act interdependently. In some cases, light regulates germination while in others it has no effect (69). In light responsive seed, germination is controlled by light duration similarly to patterns of photoperiodic control of flowering and other plant growth responses (68, p. 316).

The influences of light on germination is called photoblastism. Photoblastism may be negative or positive depending upon whether light inhibits or stimulates seed germination. Photoblastism is greatly influenced by temperature, and therefore should not alone be used to classify seed. Conditions of "positive" and "negative" photoblastism or of photo-indifference may exist in a single species or a single sample depending upon external and internal conditions. For example, Evenari (20, vol. 3, p. 534) reports that "with increasing length of after-ripening the photosensitivity increases and the photorequirement decreases until the seeds become more or less indifferent to light".

Toole et al. (67) indicate that although possibly present in all seed, the photo-reaction is not obligatory for germination of all seed.

In the past decade, findings by Borthwick and Hendricks (9) and others (7, 8) have shown that the photo-reaction controls the levels of two compounds which in turn are controlled by other reactions subject to changes of temperature. The main mechanism involved is the low energy, reversible, red/far-red mechanism, in which the pigment "phytochrome" participates. Although the "phytochrome" phenomenon operates in most light-sensitive seed, it is probably not the only mechanism responsible for regulating seed germination in photo-reactive seed (45, p. 441). In essence, light from the red portion of the spectrum enhances germination, while far-red and blue portions generally have an inhibitory effect (7; 14, p. 816; 28, p. 11).

Red and far-red wave lengths are present in normal daylight and in most artificial light (69). Piringer (55) indicates that fluorescent lamps emit a very small amount of far-red compared with red, whereas incandescent lamps emit a high proportion of far-red light. By selection of fluorescent or incandescent lamps, combined with cellophane filters, the author was able to study the "phytochrome" mechanism related to germination of seed of western dwarfmistletoe.

Previous studies involving the influence of light on germination of mistletoe seed have for the most part shown that light favors or is essential for germination. Glimcher (29) working with Viscum

cruciatum reported that germination was favored by high light intensities. He contended that once the seed receives an illumination intensity greater than five Bunsen-Roscoe units, germination continues even in total darkness. Many investigators (37; 51, p. 709; 76; 77) have indicated the essentiality of light in germination of seed of Viscum album. Wiesner (77, p. 306-315) demonstrated that germination of Viscum album seed increased with increases in light intensity, while minimum intensities required for germination were about four percent of the maximum Vienna sunlight between March 22 and April 22 (presumably 5,000 foot candles). In other investigations, Wiesner (78, p. 403-407) indicated that tropical mistletoe species would germinate in the dark while European species of mistletoe required light. Heinricher (41) found that A. oxycedri required light for germination and suggested that the less refrangible (capable of being refracted) portion of the solar spectrum is favorable, while the more refrangible portion is ineffective or injurious to seed germination.

Weir (74, p. 4), on the other hand, considered the amount of light as not critical for germination. Wagener (71) suggests that partial sunlight is more favorable in establishment of dwarfmistletoe on the host than relatively full or continuous sunlight. He contends that germination and subsequent infection can occur under comparatively minimal conditions of sunlight which, however, were not

indicated because evaluation was from estimated field intensities.

On the basis of Heinricher's (41) findings with A. oxycedri, Kuijt (47, p. 343) contends that light is essential for germination with moisture being the second major requirement. Scharpf and Parmeter (61) conclude that light, although not necessary, increases germination of A. campylopodum.

The essentiality of light for seed germination appears varied and inconclusive, particularly with regard to A. campylopodum. Therefore, studies were made of the effect of photoperiodicity, illumination intensity, and spectral quality on germination of seed of A. campylopodum Engelm. f. campylopodum.

Figure 17 shows the effect of different photoperiods (between 12 and 24 hours) on germination of seed while using artificial light to simulate a day-light intensity of 630 foot candles. Each germination percentage represents the performance of 250 seed. Temperature was maintained at 20°C and germination percentages were determined after four days' exposure to these conditions. Photo-periods > 12 hours significantly enhanced germination compared to seed exposed to total darkness. Exposures of 12 to 20 hours were not as effective in stimulating germination as were photo-periods of 22 or 24 hours (Table 7). Similar results were obtained under conditions of less illumination (460 foot candles) and longer exposure periods (12 days).

Figure 17. The effect of different photoperiods on germination of seed of western dwarfmistletoe at 20°C using light intensity of 630 foot candles. Each germination percentage represents the performance of 250 seed.

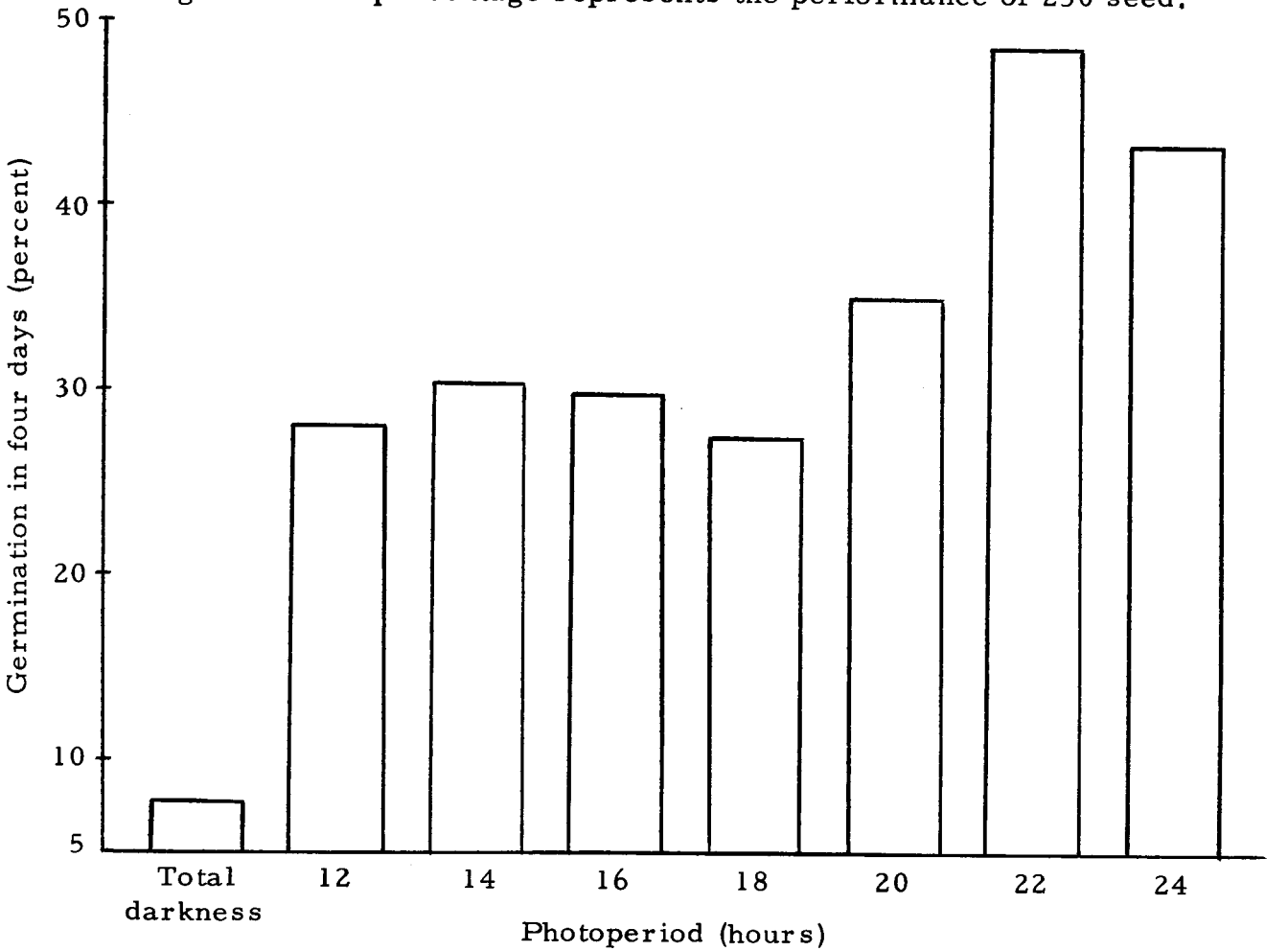


Table 7. Summary of differences in seed germination of western dwarfmistletoe resulting from different photoperiods of artificial daylight (see Figure 17).

	LSD comparison between means 1/						
	24	22	20	18	16	14	12
Total darkness	SD*	SD*	SD*	SD*	SD*	SD*	SD*
12	SD*	SD*	NS	NS	NS	NS	
14	SD*	SD*	NS	NS	NS		
16	SD*	SD*	NS	NS			
18	SD*	SD*	NS				
20	NS	SD*					
22	NS						

1/ Significant difference at five percent level (SD), significant difference at one percent level (SD*), and no significant difference (NS).

Figure 18 shows the effect of photoperiod on seed germination when using black light which is that portion of the spectrum referred to as near ultraviolet (radiation of 3100-4000 Å but mainly 3650 Å). For this study the total illumination intensity was 120 foot candles. It is of interest that photo-periods of 14 hours and greater actually inhibited seed germination, while photoperiods of 20 hours injured the seed (Table 8).

Preliminary investigations of the effect of artificial daylight intensity on seed germination indicated that intensities between 200 and 630 foot candles significantly enhanced seed germination in comparison with germination in total darkness. However, significant differences in seed germination were not apparent with different light intensities within the range of 200-630 foot candles. Light intensities between 6 and 3600 foot candles were used to determine the range of light intensity which promotes seed germination (Figure 19). Intensities < 1100 foot candles stimulate germination while intensities of 2400 foot candles and greater inhibit germination. Table 9 compares differences as to the occurrence of significance. Intensities between 200 and 1100 foot candles significantly enhanced seed germination over total darkness while the optimum intensity was about 680 foot candles where temperatures were maintained at 15-20°C for 12 days. Somewhat puzzling is the fact that an intensity of six foot candles was sufficient to promote germination significantly higher than that in

Figure 18. The effect of different photoperiods on germination of seed of western dwarfmistletoe at 20°C using black light (near ultraviolet) of 120 foot candles intensity. Each germination percentage represents the performance of 250 seed.

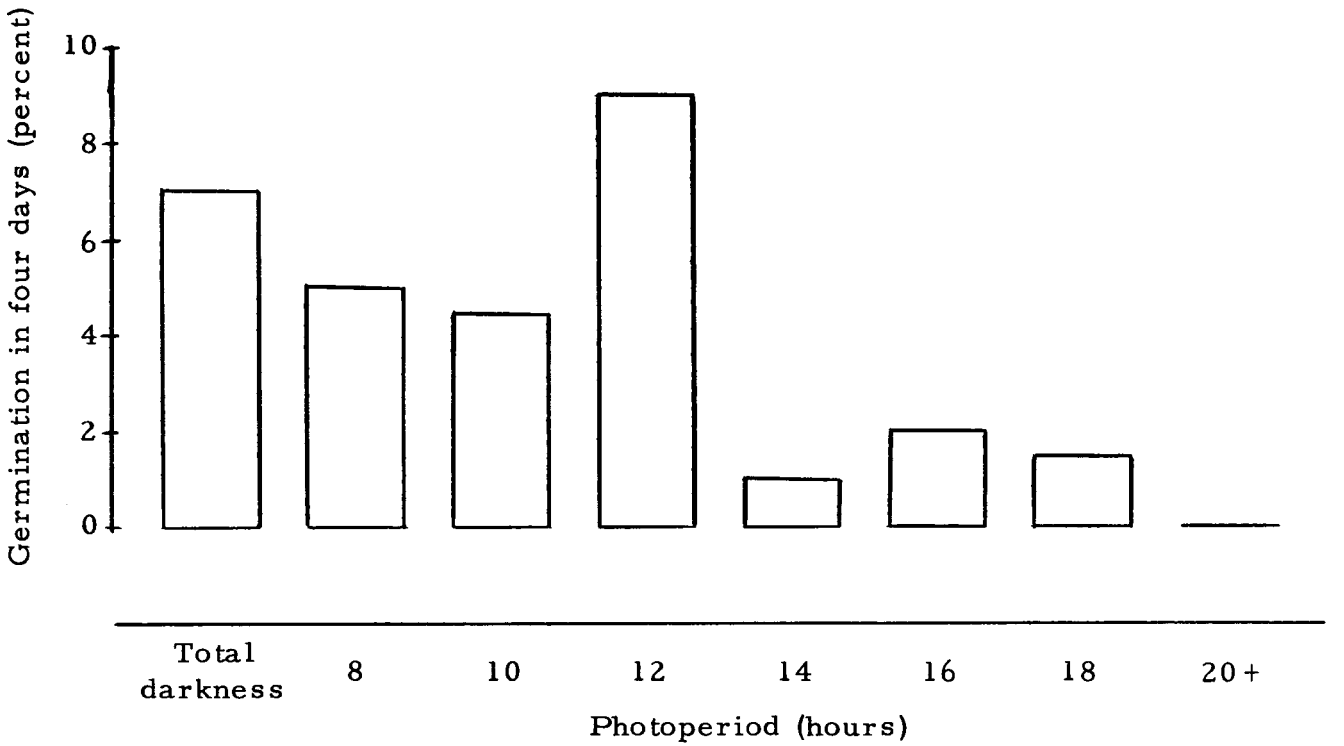


Table 8. Summary of differences in seed germination of western dwarfmistletoe resulting from different photoperiods of black light (near ultraviolet) (see Figure 18).

Photoperiod (hours)	LSD comparison between means 1/						
	20+	18	16	14	12	10	8
Total darkness	SD*	SD*	SD	SD*	NS	NS	NS
8	SD*	SD	NS	SD	NS	NS	
10	SD*	NS	NS	SD	NS		
12	SD*	SD*	SD	SD*			
14	SD*	NS	NS				
16	SD*	NS					
18	SD*						

1/ Significant difference at five percent level (SD), significant difference at one percent level (SD*), and no significant difference (NS).

Figure 19. The effect of different artificial daylight intensities on germination of seed of western dwarfmistletoe using twelve-hour photoperiod and 15-20°C. Each germination percentage represents the performance of 250 seed.

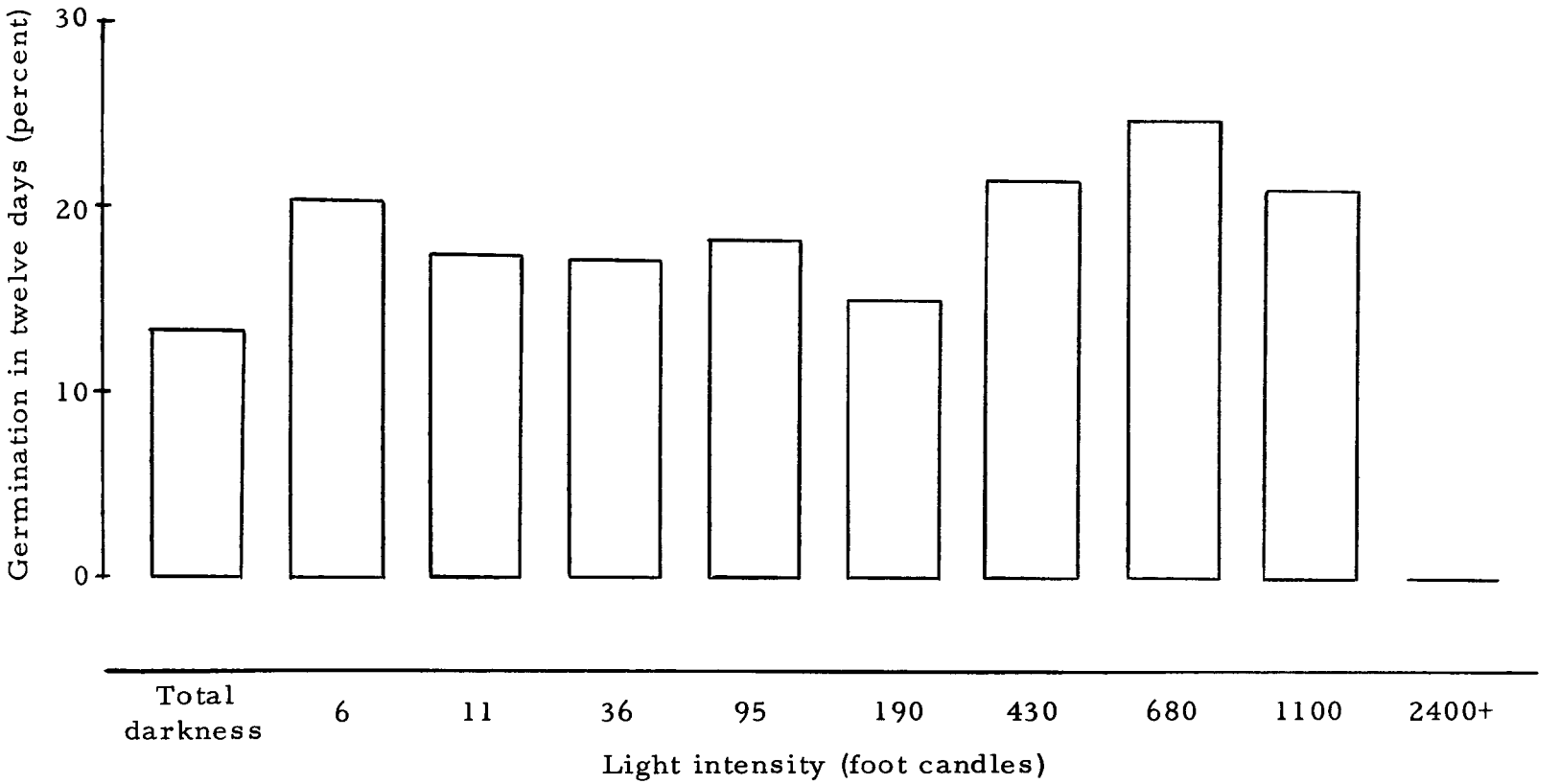


Table 9. Summary of differences in seed germination of western dwarfmistletoe resulting from different light intensities and 15-20°C (see Figure 19).

Light intensity (ft. candles)	LSD comparison between means 1/								
	2400+	1100	680	430	190	95	36	11	6
Total darkness	SD*	SD*	SD*	SD*	NS	NS	NS	NS	SD*
6	SD*	NS	SD*	NS	SD	NS	NS	NS	
11	SD*	NS	SD*	NS	NS	NS	NS		
36	SD*	NS	SD*	NS	NS	NS			
95	SD*	NS	SD*	NS	NS				
190	SD*	SD*	SD*	SD*					
430	SD*	NS	SD						
680	SD*	NS							
1100	SD*								

1/ Significant difference at five percent level (SD), significant difference at one percent level (SD*), and no significant difference (NS).

total darkness yet intensities between six and 190 foot candles did not further significantly increase germination. This behavior contrasts with Glimcher's previously cited work (29).

Figure 20 shows the effect of three different light intensities on the rate of seed germination at 20°C. Each point represents the performance of 250 seed. Table 10 shows the significance of differences in the rate of seed germination resulting from light intensities significantly accelerated seed germination over total darkness.

Special light boxes described in the "Materials and Methods" section (Figure 4) were used to investigate the effect of red and far-red light on seed germination at 20°C. Figure 21 shows the effect of red and far-red light on seed germination of western dwarfmistletoe at 20°C. Each germination percentage represents the performance of 250 seed. Although statistically significant differences in seed germination were not evident, these data suggest that red light is more effective than far-red light for seed germination.

This evidence along with other studies of photoperiod and light intensity leads to the conclusion that the "phytochrome" phenomenon is not a factor in the germination of seed of A. campylopodum Engelm. f. campylopodum.

Figure 20. The effect of three different light intensities on the rate of seed germination of western dwarfmistletoe at 20°C. Each point represents the performance of 250 seed.

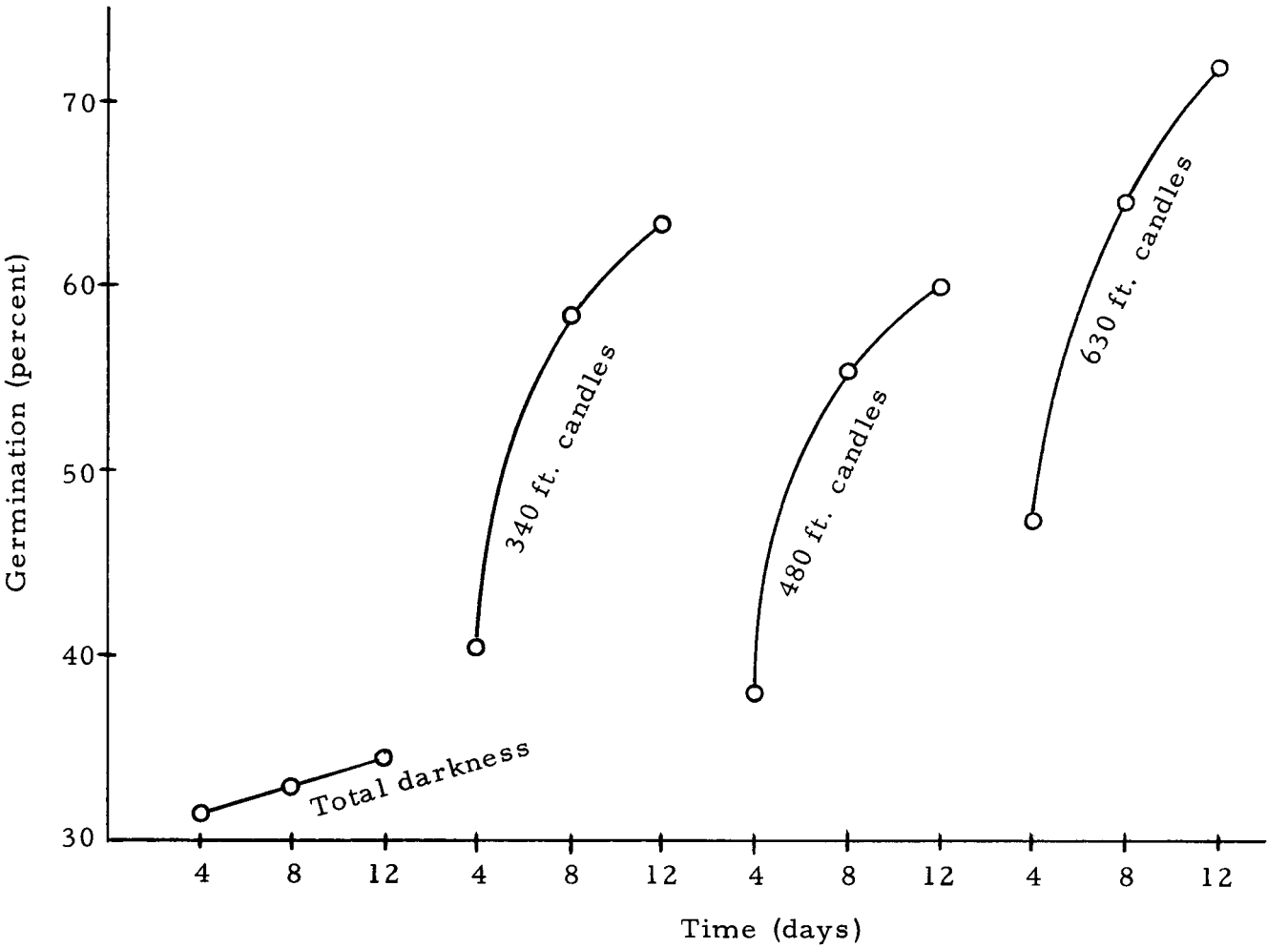
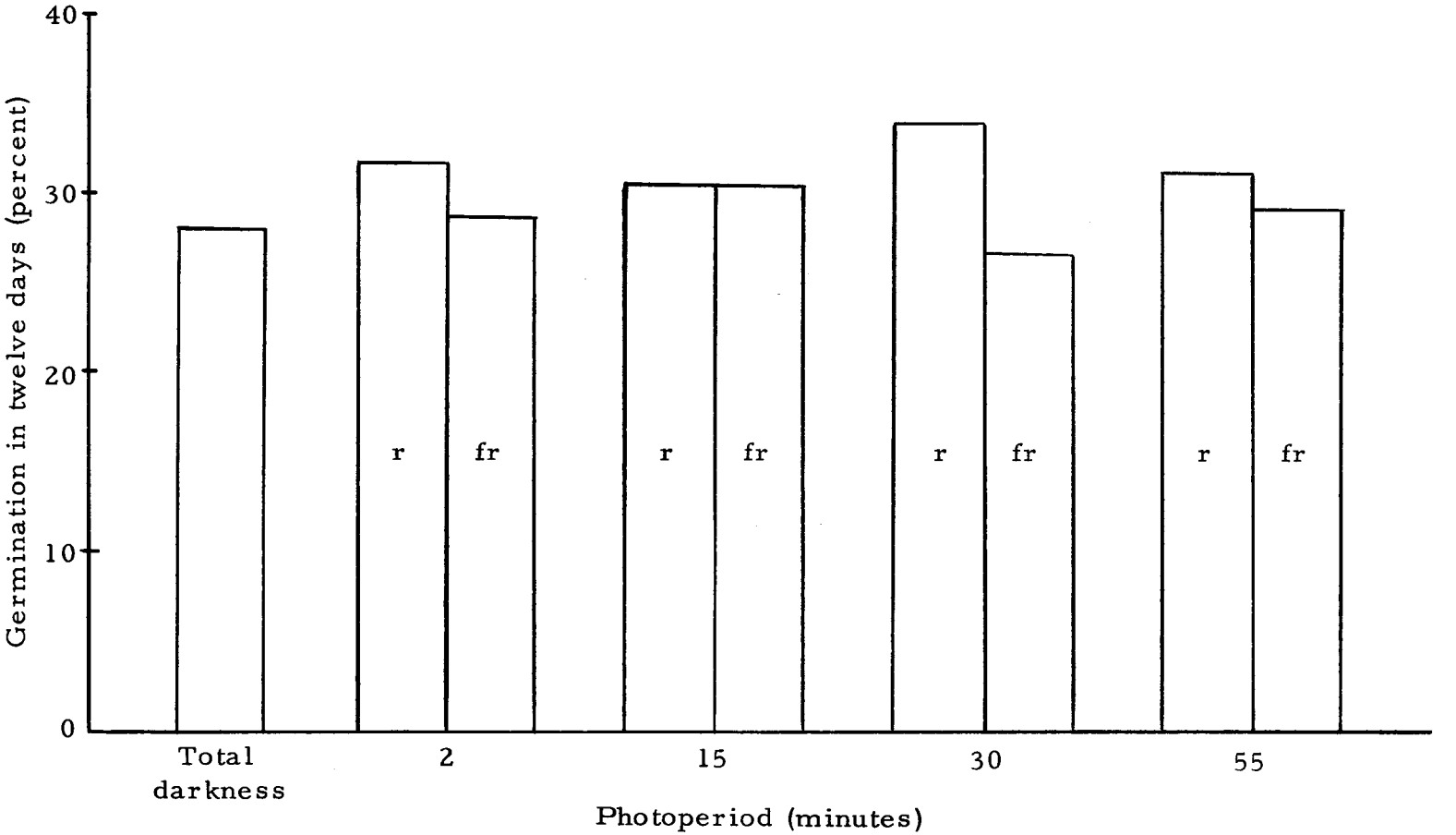


Table 10. Summary of differences in the rate of seed germination of western dwarfmistletoe resulting from three different light intensities and a temperature of 20°C (see Figure 20).

Light intensity (ft. candles)	LSD comparison between means 1/		
	630	480	340
Total darkness	SD*	SD*	SD*
340	NS	NS	
480	SD		

1/ Significant difference at five percent level (SD), significant difference at one percent level (SD*), and no significant difference (NS).

Figure 21. The effect of red and far-red light on germination of seed of western dwarf-mistletoe at 20°C. Each germination percentage represents the performance of 250 seed.



MISCELLANEOUS FIELD AND LABORATORY OBSERVATIONS

Temperature Differences Surrounding Naturally Emplaced Seed

The wide range of variable temperatures occurring from various causes, within and immediately above the vegetative cover (5, p. 327; 16, p. 164-166) are poorly represented by conventional weather records. In order to determine the effect of field temperatures on seed longevity during the 180 days following discharge, thermocouples were located adjacent to naturally emplaced seed as described under "Materials and Methods". Temperatures among the crowns of a group of pines (Figure 22) at the periphery of a severely infested, cut-over stand near Sisters, Oregon, appear in Table 11. Deviations of these temperatures from the atmospheric temperature in the open in the immediate area are also included in the table.

Deviations in temperature between atmospheric temperatures in the open and temperatures surrounding naturally emplaced seed of dwarfmistletoe on four different days within 180 days following discharge are expressed by crown classes (Figure 23). Each point represents the mean deviation of three thermocouple readings. The greatest differences occur within 30 days following seed discharge. Deviations in temperature between temperatures in the open and those surrounding seed during November, December and January were less. It is important to note that deviations in temperature

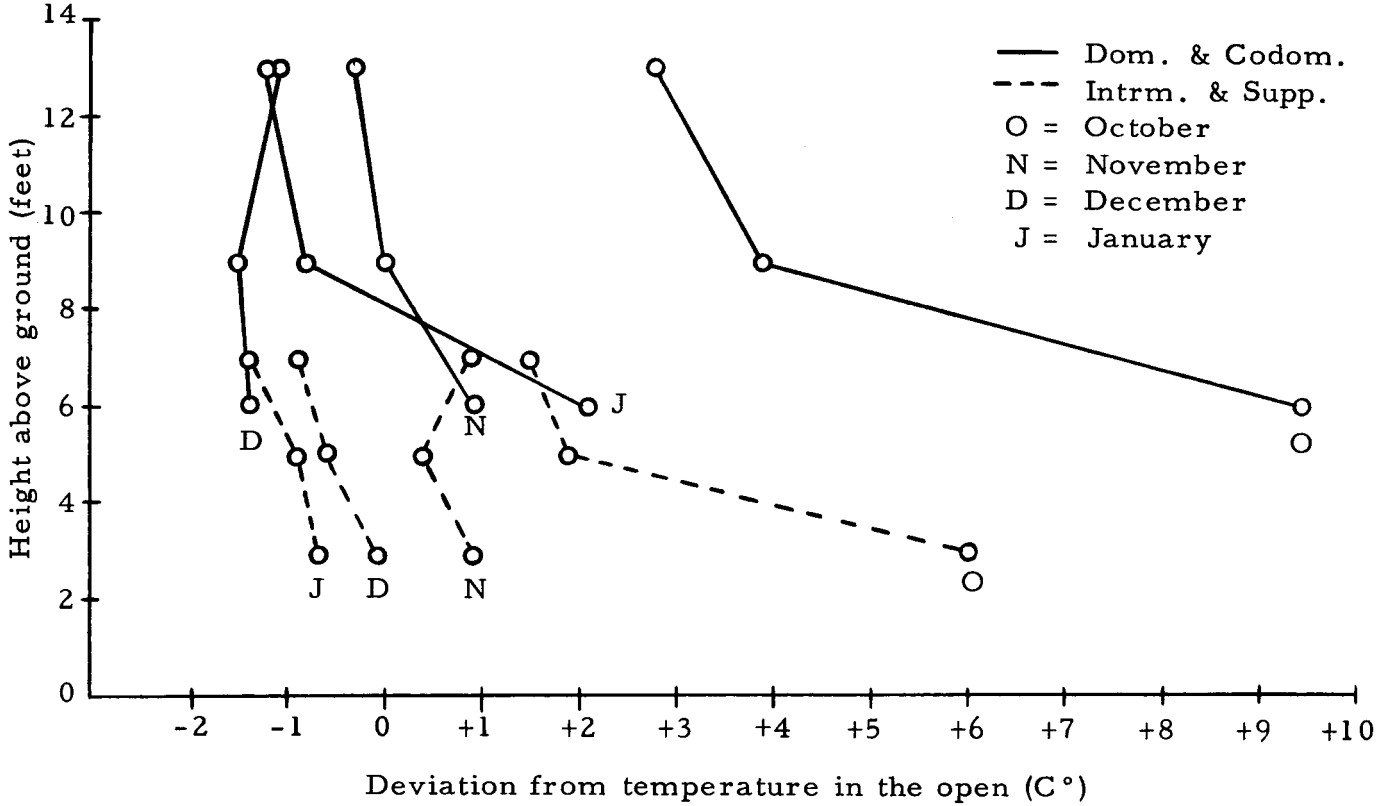
Figure 22. Differences between atmospheric temperatures in the open and temperatures surrounding naturally emplaced seed of dwarfmistletoe were recorded at nine locations in each of seven trees in the foreground.

Table 11. Differences between temperatures in the open and temperatures surrounding naturally emplaced seed of western dwarfmistletoe during 180 days following discharge. Each temperature (at seed) represents the mean reading of three thermocouples.

Dominance class	Crown exposed direct sun (%)	Region of crown 1/	Height above ground (ft.)	Temperatures on four different dates (C°)							
				Oct. 27, '62		Nov. 30, '62		Dec. 29, '62		Jan. 26, '63	
				At seed	Diff. from open	At seed	Diff. from open	At seed	Diff. from open	At seed	Diff. from open
Dominants and codominants	50-75	T	10-13	23.3	+2.8	10.8	-0.3	7.4	-1.1	-2.7	-1.2
		CL	8-9	24.4	+3.9	11.1	0	7.2	-1.5	-2.3	-0.8
		LL	5-6	29.9	+9.4	12.0	+0.9	7.1	-1.4	0.6	+2.1
Intermediates and suppressed	0-50	T	7-9	22.0	+1.5	12.0	+0.9	7.6	-0.9	-2.9	-1.4
		CL	5-6	22.4	+1.9	11.5	+0.4	7.9	-0.6	-2.4	-0.9
		LL	2-3	26.5	+6.0	12.0	+0.9	8.4	-0.1	-2.2	-0.7
Open crown	100	T	7-9	20.1	-0.4	11.4	+0.3	8.5	0	-3.5	-2.0
		CL	5-6	20.5	0	12.3	+1.2	8.4	-0.1	-2.7	-1.2
		LL	2-3	21.5	+1.0	11.6	+0.5	8.3	-0.2	-2.4	-0.9

1/ Terminal (T), central lateral (CL), and lower lateral (LL) region of tree.

Figure 23. Deviations in temperature between atmospheric temperatures in the open and temperatures surrounding naturally emplaced seed of western dwarf-mistletoe on four different dates within 180 days following discharge. Each point represents the mean deviation of three thermocouple readings (see Table 11).



within 30 days of seed discharge were sufficient for significant reduction in seed viability to occur irrespective of seed emplacement in the crown.

Variations in Field Light Intensity

Variations in field light intensity were investigated with respect to different types of vegetative cover to determine the levels of light seed are exposed to while attached to the pine host during dormancy. Measurements of field light intensities were made using a Weston illumination meter. In general, it was found that shielding by vegetative cover (such as over-story or dominant trees), reduced the light intensity beneath the cover about ten-fold. Open-grown trees or tree crowns exposed to clear skies and direct sunlight generally received from 7,000 to 10,000 foot candles illumination, while partially shaded areas in the same stand received from 500 to 1,200 foot candles illumination. Specific intensities vary with cloud cover and seasonal changes. The above variations were representative of the spring germination period.

Retention of Seed on the Host and Survival in the Field

Roth (59), while studying the natural emplacement of dwarf-mistletoe seed on ponderosa pine, reported that 55 percent of the seed intercepted by a single tree were lost prior to the spring

germination period. Of the 45 percent retained, 25 percent were attached on needles. Seed attached in this manner have little if any infection potential. In this instance, 20 percent of the seed intercepted actually were retained and in a suitable position to cause infection after germination. Hawksworth (36) working with A. vaginatum and A. americanum found that approximately 20 percent of the seed produced by these species were transferred to the twigs by mid-October. The following May germination percentages of the total seed produced was 6 and 14 percent respectively. Hawksworth (34, p. 39) also reported from earlier investigations in the southwest that less than five percent of planted seed resulted in infections. It is concluded that the infection potential of dwarfmistletoe is greatly influenced by: 1) total seed production; 2) levels of seed interception by the host; 3) initial viability of the intercepted seed; 4) temperature and moisture conditions during the first 30 days following discharge; 5) percentages of seed retention until favorable germination conditions occur; 6) position of retained seed; 7) actual percentages of seed germinating, and 8) growth rate and vigor of emerging radicles responsible for penetration into the host.

Below is listed the retention and survival of seed on the host 30 and 270 days following discharge. Forty-four percent of the retained seed germinated under field environmental conditions, while seed losses of 70 percent occurred during dormancy, resulting in an

	30 days after period of maximum seed discharge (Oct. '62)	270 days after period of maximum seed discharge (Aug. '63)
Number of random samples examined	100 1/	100
Number of samples containing seed	53	38
Total number of seed	376	106
Total number of germinated seed	0	47
Germination (%)	0	44.3
Seed retention (%)	-	29.5
Seed loss (%)	-	70.5
Level of infection potential (%)	-	12.5

1/ Each sample represents an individual branchlet with a minimum of two years growth.

estimated infection potential of 12.5 percent. The infection potential expressed as percent was determined by dividing the total number of germinated seed after 270 days by the total number of emplaced seed 30 days after discharge times 100.

Minimal Conditions Supporting Germination

First observations of seed germination were made during February 1963, while sampling seed stored under field temperatures. Temperatures in the Sisters area were mild (about 0-15°C night-day) and rain showers had resulted in wetted seed which germinated. Seed with emerged radicles were removed from the collection bags and discarded. The bags with the remaining ungerminated seed were taken back to the laboratory and placed in the refrigerator at a constant temperature of 1.5 ± 1°C. Approximately 140 days later, additional seed had germinated (19 percent) although exposed to near-freezing temperatures and atmospheric humidities of 34-74 percent. It is entirely possible that the earlier favorable climatic conditions in the field initiated resumption of meristematic processes with radicle emergence, as the expression of germination, merely being retarded by the exposure to lower temperatures. Nevertheless, meristematic activities appear to occur at 1.5°C.

Phototropic Response of Radicles

Kuijt (46, p. 589) and Hawksworth (33) report that negative phototropism is present in germinating seed of the mistletoes. The author's studies demonstrated that radicles of western dwarfmistletoes are sensitive to light and react with negative tropic responses. It may be noted that each time the light source was changed in relation to the radicles, they responded by growing away from the light (Figure 24). In Figure 24, the seed on the left appears to have exhausted its reserve food material, which may account for its failure to respond to changes of light other than swelling at the tip of the radicle. The radicle of the seed on the right continued to reverse direction in response to changes in light source.

Growth of Radicles

Table 12 indicates the average growth for 23 radicles grown under temperatures of 20 ± 5°C and with moisture restricted to the amount absorbed at initial imbibition. Some radicles grew under these conditions for more than 120 days. Of interest was the fact that as reserve food was exhausted, the radicle tip would enlarge much like a holdfast or appresorium, even when the tip was not in contact with the substratum. It is apparent that after 30 days growth (Table 12), the average radicle emplaced on the needle is

Figure 24. Negative phototropic response of radicles of two seed of A. campylopodum. Arrows indicate direction of incident light consecutively on Aug. 13, Sept. 10 and 25, and Oct. 1 and 8, 1963. A) Sept. 25, B) Oct. 8, and C) Nov. 22.

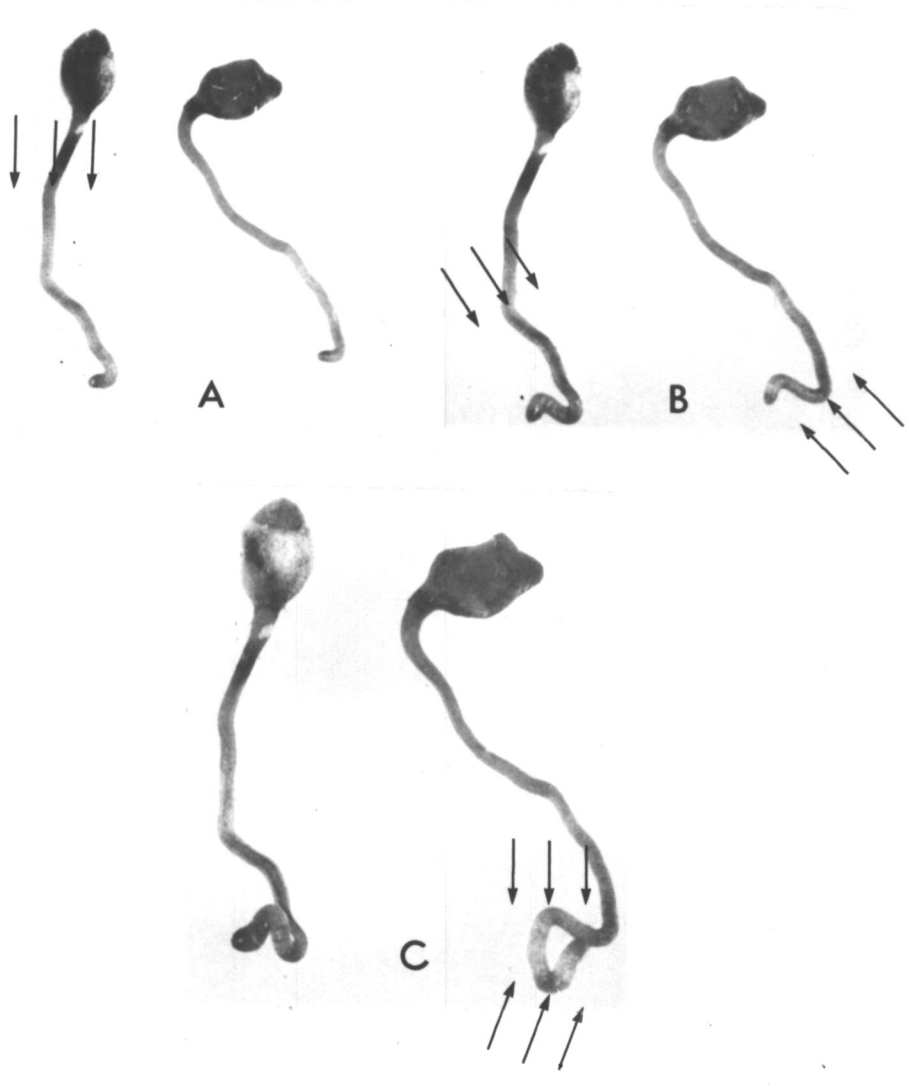


Table 12. Summary of growth of 23 radicles during 120 days at 20° C with no moisture.

Time (days)	Mean growth (mm) 1/	Mean growth per day (mm)
0-30	5.89	0.196
30-60	4.00	0.133
60-90	3.39	0.113
90-120	0.69	0.023

1/ Measurements made with a dissecting microscope.

unable to traverse from above the needle fascicle to its base (a distance of 5-11 mm in ponderosa pine) and thereby penetrate the host tissue. If this is true, seed so emplaced on needles above the fascicle may be regarded as non-infectious.

Figure 25 shows the typical germination sequences of seed of western dwarfmistletoe resulting after dormancy when exposed to optimum light, moisture, and temperature. Stages from seed discharge, imbibition, initial growth resumption after dormancy, and relative radicle growth within 30 days are shown.

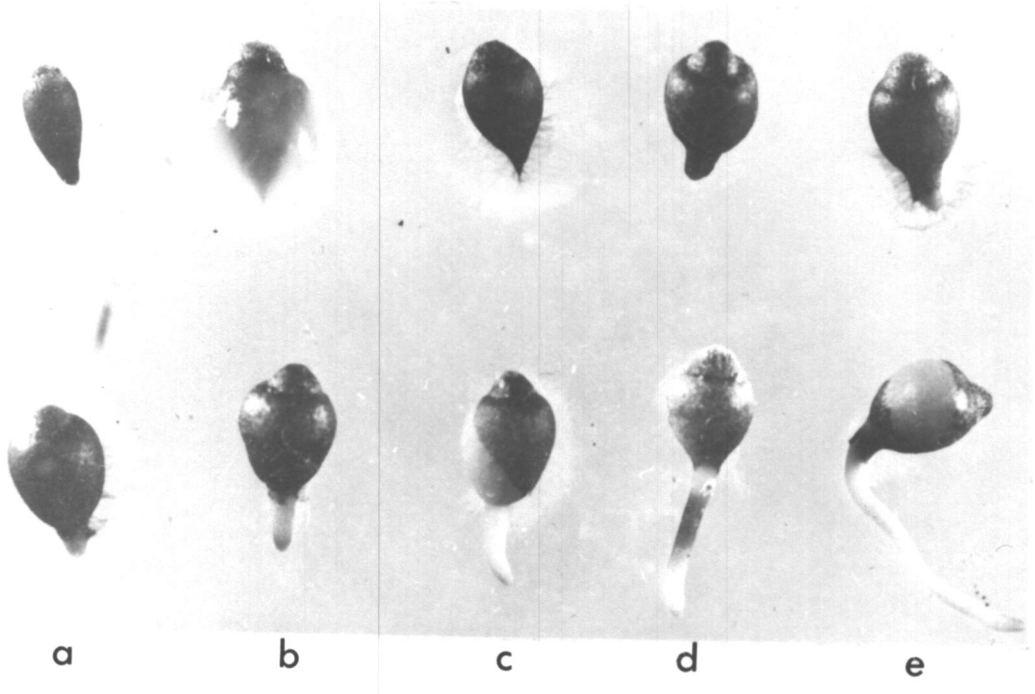


Figure 25. Typical germination sequence and relative radicle growth of western dwarfmistletoe seed. From left to right: (top) a. seed immediately after discharge; b. seed after imbibition of water; c. seed after dormancy showing initial internal swelling; d. seed showing initial radicle emerging (1-4 days); e. seed showing emerged radicle (4-8 days). (bottom) a. through e. show relative radicle growth at 4, 8, 12, 20 and 30 days respectively.

DISCUSSION AND SUMMARY

Discussion

Past investigations of Arceuthobium have been limited in scope or lacking in quantitative data. As a result, specific requirements for seed germination of Arceuthobium have not been clearly established. The essentiality of light for seed germination appears varied and inconclusive; moisture and temperature requirements favorable for seed germination have not been clarified. Therefore, studies were made of the effect of photoperiodicity, illumination intensity, and spectral quality on seed germination, as well as effects of temperature and moisture on longevity and germination of seed of western dwarfmistletoe.

Considerations of seed dormancy revealed that: 1) statistically significant differences occurred in initial viability of seed from three locations in the same forest type; 2) viability during dormancy decreased as temperature increased; statistically significant reductions in viability occurred with each five degree increase in temperature; 3) thiourea, kinetin, and gibberellic acid were effective in stimulating germination (breaking dormancy) of seed 120 days following discharge, whereas they were ineffective on younger seed; 4) excised endosperms and the contained embryos germinated in two days following discharge, whereas the intact seed failed to germinate for

five to six months (period of dormancy) after discharge; 5) deviations between atmospheric temperatures in the open and temperatures surrounding naturally emplaced seed, for 30 days following discharge, are of sufficient magnitude to promote significant reductions in seed viability, and 6) retention of seed on the host and survival during dormancy were comparable to those reported by Roth (59) and Hawksworth (36), which indicate that enormous losses occur between seed discharge in the fall and germination in the spring. Germination apparently is retarded by an inhibitor in the seed coat (endocarp plus the viscin cells of the mesocarp) which breaks down at near-freezing temperatures with aging. Inhibition persists for about six months. Use of chemical stimulants and growth regulators overcame this factor after seed had aged 120 days. This point of view contrasts with Glimcher's (29) work with V. cruciatum where the mesocarp had no inhibitory effect on germination. Seed of A. vaginatum and A. americanum, which germinate immediately after discharge, also presumably lack an inhibitor.

In these studies, seed of western dwarfmistletoe germinated on glass, wood and metal; other investigators (24, 27, 47) have found there to be no special substrate requirements for germination within the genus Arceuthobium. A. oxycedri requires a cellulose substrate to germinate and is an exception (38). It thus appears questionable

whether the character of the substratum has any influence on the germination of seed of dwarfmistletoe.

Hawksworth (35) investigated fruit and seed of several dwarfmistletoe for abnormalities. He concluded that formation of twin-seeded fruits provides increased reproductivity. I have regularly observed seed of the western dwarfmistletoe with twin embryos and endosperms within a single endocarp and found that their capacity to germinate is apparently not impared.

Attempts to germinate seed of western dwarfmistletoe over gradients of sulfuric acid were unsuccessful presumably due to the seed's inability to absorb sufficient moisture to initiate germination; however, similar attempts by Glimcher (29) with seed of V. cruciatum were successful. The hygroscopic capacity of the viscin cells appears to be correlated with seed viability in that loss of viability promotes loss of hygroscopic capacity. Attempts to remove the viscin from the seed coat with water pressure were unsuccessful presumably because of the impregnable and continuous character of the viscin cells in relation to the seed coat tissue. Western dwarfmistletoe seed are tolerant of reduced levels of aeration and readily germinate under water. Initial imbibition of moisture by the viscin cells is sufficient to supply adequate moisture for germination and growth of the radicle for 120 days at humidities between 10 and 35 percent and a constant temperature of 20°C. The assumption is that

moisture requirements for radicle growth are not dependent on the moisture conditions immediately following germination but rather at the time of initial seed imbibition prior to germination.

Gill and Hawksworth (28, p. 26) contend that light is required for germination and suggest that "this may explain the preference of mistletoes for high tree tops, open stands, or edges of dense forests." Wagener (71), on the other hand, contends that partial sunlight favors the establishment of dwarfmistletoe (A. campylopodum) more than relatively full or continuous sunlight. Heinricher (38) reported that light is essential for germination of A. oxycedri, while Scharpf and Parmeter (61) reported that germination of seed of A. campylopodum is enhanced by light, but light is not essential. In these studies seed of western dwarfmistletoe germinated in total darkness; however, light intensities between 200 and 1000 foot candles significantly enhanced germination over total darkness, while intensities greater than 2400 foot candles were injurious. Light intensities between 200 and 1000 foot candles were representative of partially shaded portions of young pine stands.

The discovery of "phytochrome" (7) elucidated the triggering mechanism of many plant growth responses to light. This pigment is believed to be a biological enzymatic catalyst which is a possible means of trapping energy from radiation for internal biochemical reactions. Phytochrome exists in two forms (P660 and P735) which

absorb light in the red and far-red portions of the spectrum with maximum absorbancies at 600 mμ and 735 mμ. The absorption at these peaks converts the one form into the other and vice versa. The active form is referred to as the P735 complex. Experimental data (7, 8, 9) indicates that different plant growth responses are influenced by the rate at which the active form (P735) is reconverted back to the inactive form (P660). Red light converts P660 to P735. Phytochrome 735 in turn stimulates seed germination while deactivation or conversion of P735 to P660 occurs under conditions of darkness or exposure to far-red irradiation. These investigations indicate that the "phytochrome" phenomenon is apparently not a regulating mechanism in seed germination of western dwarfmistletoe.

Contrary to previous reports (60, 61), seed of western dwarfmistletoe germinated under a wide range of temperatures (1.5 - 31°C). Western dwarfmistletoe seed germinated almost as rapidly and completely at favorable constant temperatures as favorable alternating temperatures. The interaction of temperature and light becomes very apparent when seed germinate under conditions of alternating temperatures. Light as an enhancing factor in seed germination is dependent on the temperature involved. For example, comparisons between treatments of light and total darkness at alternating temperatures of 5-15, 5-20, and 10-30°C revealed no significant differences in germination, whereas conditions of 10-20 and

15-25°C resulted in significant differences (Table 6). In contrast, a constant temperature of 20°C or any favorable constant temperature for germination results in significant differences in germination between treatments of light and total darkness.

Western dwarfmistletoe seed are considered carbohydrate type seed, as they contain large quantities of starch in the endosperm. Sucrose, fructose, and glucose were found to constitute the simple free sugars (see Table 2). Beevers (3, p. 1) indicates that "sucrose or its component hexoses--the major free sugars in plant--are the usual substrates for respiration in most plant cells." Recently, McDowell (52, p. 52) indicated that glucose, fructose, and sucrose are the preferential saccharides utilized during respiration by western dwarfmistletoe. Preliminary investigations in this study revealed the presence in germinating seed of several enzyme systems which suggest possible pathways by which carbohydrate substrates which were detected may be used in respiratory metabolism; hexokinase (an initial stage enzyme of glucose catabolism), amylase (an enzyme for starch reduction), and pyruvate kinase (a terminal enzyme of glycolysis). These findings lead to the conclusion that oxidative breakdown of organic substrates occurs via the Embden-Meyerhof-Parnas (EMP) pathway. As demonstrated by McDowell (52), glucose is the preferred substrate utilized during respiration by dwarfmistletoe, although fructose, sucrose, and starch may also

be used. For example, phosphorylated hexoses formed from phosphorylase action on starch may facilitate conversion of reserve starch during seed germination and subsequent growth of the radicle. The starch may also be converted to maltose by amylase action and then directly to glucose units by hydrolysis.

The pentose phosphate pathway (also called hexose monophosphate shunt), although well established in many plants, is apparently not operative in seed of western dwarfmistletoe because glucose-6-phosphate dehydrogenase or Zwischenferment (an initial enzyme of the pentose phosphate pathway) was not found. Again this suggests that catabolism of carbohydrates during seed germination occurs via the EMP pathway.

The seed contain chlorophyll as one might suspect from the green color of the endosperm and embryo; however, the occurrence and significance of photosynthesis in seed of western dwarfmistletoe has not been investigated.

Nitrogen is an essential element for all plants, and Table 2 shows that large quantities of nitrogen are present and presumably available for cellular synthesis during germination and subsequent growth of the radicle.

Gill (25) contends that biological elucidation of a parasite aids in a more effective method of control. For example, a knowledge of the effects of temperature and light on seed longevity and germination

of dwarfmistletoe may aid in more realistic evaluations of present silvicultural control practices recommended for diseased pine stands (27, 48). These practices remove vegetative screening, promote alterations in temperature and light in the stand (which may or may not be conducive to seed survival and germination), and promote larger clusters of fruit on infected hosts (27). Such conditions may lead to higher levels of disease incidence and should be investigated.

Hawksworth (33) reported the rate of spread of dwarfmistletoe in infected stands of lodgepole pine to be less than two feet per year, while Kimmey and Mielke (43) reported the maximum spread for A. campylopodum (over a 60-year period) as two feet per year. Hawksworth (32) also indicated that the spread of dwarfmistletoe in lodgepole pine stands was one and a half times greater in open stands compared to closed stands. This implies that vegetative screening (density) and light intensity factors are associated with the rate of disease spread.

Roth (58), Hawksworth (32, 33, 34) and others (43) have investigated the incidence of dwarfmistletoe in relation to vegetative screening, the distance seed are discharged, and the influence of topography (aspect or slope). Hawksworth (34) reports the most abundant period of seed germination of A. vaginatum occurs when the mean maximum temperatures are 25-28°C, yet in this study seed of A. campylopodum rapidly deteriorated at temperatures greater than

20°C (see Figure 5). It is generally accepted that comparative atmospheric temperatures are higher on southern aspects than on northern aspects, regardless of geographic location. Thus, it appears entirely feasible that the different topographic relationships reported by Roth (58)--A. campylopodum incidence is more abundant on northwesterly aspects--and Hawksworth (33)--A. vaginatum incidence is more abundant on south to southwesterly aspects--for incidence of dwarfmistletoe may be explained by the response to temperature and light conditions by these respective species. It seems apparent that physical factors of moisture, light, and temperature in relation to seed longevity and germination significantly contribute to the incidence and spread of dwarfmistletoe.

Summary

Western dwarfmistletoe seed are discharged in the fall. The seed remain dormant for six months before germinating in the spring. Dormancy is presumed to be regulated by a chemical inhibitor associated with the endocarp. Seed viability may vary from one infected stand to another, while retention of viability appears to be related to temperature and seed quality. Retention of seed viability for 10 months may be obtained by storage in the laboratory at 1.5°C. In some cases, limited viability has been observed after 48 months storage at 1.5°C.

Western dwarfmistletoe seed germinate between 1.5 and 31°C with optimum constant temperatures between 15 and 20°C and more favorable alternating night-day temperatures at 5-15°C. Absorption of liquid moisture is essential for germination to occur, and germination may occur readily at reduced levels of aeration. Germination also may occur in total darkness; however, light intensities between 200 and 1000 foot candles in conjunction with favorable temperatures significantly enhance germination. Increases in photoperiod increase germination percentages, and red light is slightly more effective in seed germination than far-red light. Black light (near ultraviolet) is injurious to seed when levels are greater than 120 foot candles and exposures exceed 12 hours. Unfavorable temperature, moisture, and light during the 30 days following seed discharge appear to be the most contributory factors toward low seed viability and low infection potentials which essentially prevent rapid spread of western dwarfmistletoe.

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