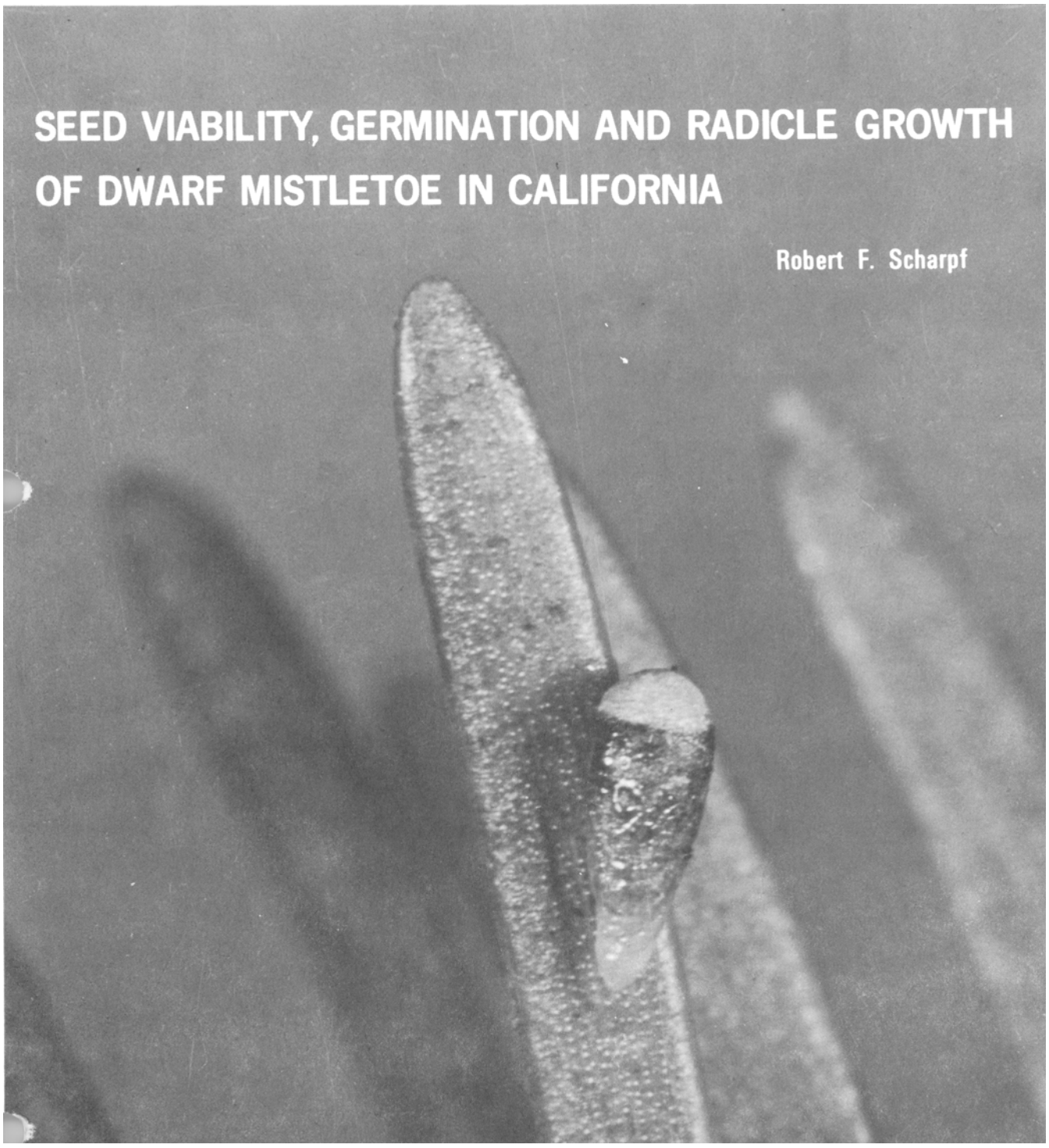
PACIFIC SOUTHWEST
Forest and Range
Experiment Station

FOREST SERVICE
U. S. DEPARTMENT OF AGRICULTURE
P.O. BOX 245, BERKELEY, CALIFORNIA 94701

SEED VIABILITY, GERMINATION AND RADICLE GROWTH OF DWARF MISTLETOE IN CALIFORNIA

Robert F. Scharpf



U.S.D.A. FOREST SERVICE RESEARCH PAPER PSW- 59 /1970

Scharpf, Robert F.

1970. **Seed viability, germination, and radicle growth of dwarf mistletoe in California.** Berkeley, Calif. Pacific SW. Forest & Range Exp. Sta., 18 p., illus. (U.S.D.A. Forest Serv. Res. Paper PSW-59)

Two species of dwarf mistletoe were studied: *Arceuthobium abietinum* (Engelm.) Hawksworth and Wiens and *A. occidentale* Engelm. Viability of fresh seeds was high and not significantly influenced by year of collection, where collected, or plant from which collected. Temperature affected viability most noticeably. It also significantly affected rate and percentage of seed germination. Longevity of radicles of germinated seeds was inversely proportional to temperatures above 5°C.

Oxford: 176.1 *Arceuthobium* spp. (79):181.524/525-111.5.
Retrieval Terms: Arceuthobium abietinum; Arceuthobium occidentale; seed viability; germination; temperature.

Scharpf, Robert F.

1970. **Seed viability, germination, and radicle growth of dwarf mistletoe in California.** Berkeley, Calif. Pacific SW. Forest & Range Exp. Sta., 18 p., illus. (U.S.D.A. Forest Serv. Res. Paper PSW-59)

Two species of dwarf mistletoe were studied: *Arceuthobium abietinum* (Engelm.) Hawksworth and Wiens and *A. occidentale* Engelm. Viability of fresh seeds was high and not significantly influenced by year of collection, where collected, or plant from which collected. Temperature affected viability most noticeably. It also significantly affected rate and percentage of seed germination. Longevity of radicles of germinated seeds was inversely proportional to temperatures above 5°C.

Oxford: 176.1 *Arceuthobium* spp. (79):181.524/525-111.5.
Retrieval Terms: Arceuthobium abietinum; Arceuthobium occidentale; seed viability; germination; temperature.

CONTENTS

The Author

ROBERT F. SCHARPF, a plant pathologist, is studying problems in forest diseases and their control. Native of St. Louis, Mo., he earned a forestry degree (1954) at the University of Missouri. He also holds a master's degree in forestry (1957) and a doctorate in plant pathology (1963) from the University of California, Berkeley. He joined the Forest Service in 1960, and has been with the Station's Berkeley research staff since then.

Influences on viability and germination of seeds have been studied intensively for many years, particularly for the numerous commercially important agricultural, ornamental, and timber species. Consequently, a voluminous literature exists on the physiology of seeds (Barton 1967).

Some attention has also been given to the physiology of seeds of the mistletoes, family Loranthaceae, because the mistletoes are damaging parasites of other plants and also because they have certain unusual seed features. Among these features are: (a) the seeds of mistletoes must germinate, grow, and establish a parasitic relationship with a host plant or they will die; and (b) the seeds of mistletoes are not exposed to a subterranean or soil surface environment, but on the contrary, are found on the surface of branches or twigs above the ground and therefore are exposed to climatic or environmental conditions that differ from those of seeds in the soil.

The seeds of dwarf mistletoes, genus *Arceuthobium* in particular, are quite different in several ways from seeds of most plants. For one, the seed is borne in an elastic fruit, usually one per fruit, that when ripe bursts and shoots it for a considerable distance like a miniature rifle projectile. This means of dispersal is accomplished by the buildup of internal water pressure.

The seeds do not have a seed coat, but are surrounded by an endocarp to which are attached long filamentous cells, commonly called "viscin cells." These cells rupture at seed discharge, and release a sticky mucilage which enables the seeds to stick to objects they strike. The mucilage, when wet by rains, becomes slippery however, and enables the seed to slide along a needle to a branch or move firmly into position on a branch where germination and penetration can occur. With prolonged rain the viscin washes from the seed. The viscin cells firmly attach the seed to the host upon drying. These dried cells also function as a seed coat by providing a protective shell around the endosperm and embryo, thus reducing water loss.

The presence of chlorophyll in the endosperm is another unusual feature of dwarf mistletoe seeds. Except in some mistletoes, chlorophyll does not occur in the endosperm of flowering plants (Kuijt 1960). It is possible that photosynthesis does take place in the seed and supplies the embryo with food and energy necessary for germination and infection of the host.

Some studies have been made of the physiology of seed of dwarf mistletoes, but much information is lacking. Therefore, because dwarf mistletoes are extremely damaging parasites of conifers in the Western United States, any information that may aid in their control is useful.

This paper reports results of studies undertaken to discover more about the influences on seed viability, germination, and radicle growth of two species of dwarf mistletoe: *A. abietinum* (Engelm.) Hawksworth and Wiens and *A. occidentale* Engelm.[1] so that better understanding might lead to better control.[2] These two species were selected for study because I believe they represent dwarf mistletoes that grow under markedly different environmental regimes in the Western United States. *A. abietinum* grows on red fir *(Abies magnifica* A. Murr.*)* and white fir *(A. concolor* [Gord. and Glend.] Lindl.*)* in the higher elevations of the Sierra Nevada; whereas *A. occidentale* commonly occurs on pines *(Pinus sabiniana* Dougl. and *P. radiata* D. Don*)* in the foothills of the Sierra Nevada, in the coastal mountains, and at near sea level along the California coast.

---

[1]Recent studies by Frank G. Hawksworth at the Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado and Delbert Wiens, Department of Biology, University of Utah, Salt Lake City, Utah have shown that *A. abietinum* (previously *A. campylopodum* f. *abietinum)* and *occidentale* (previously *A. campylopodum* f. *campylopodum*) are no longer forms of *A. campylopodum* but distinct species.

[2]The information reported is based on a dissertation submitted to the Graduate School, University of California, Berkeley, in partial fulfillment of the requirement for the Ph.D. degree.

VIABILITY

The term "viability" has been variously used for seeds and has led to confusion. To some writers, viable seeds are those that have the capacity to germinate. To others, any living seeds are viable. Chemical tests have also been used to determine viability. These tests detect chemical reactions that usually but not always occur in living systems. Therefore, in my review of the literature on seed physiology, it must be kept in mind that viability is not a precise term.

For the most part the environmental conditions that influence viability are not well understood. Weir (1918) suggested that *Arceuthobium* sp. seeds stored over the winter under cool, moist conditions could be used for inoculation in the spring under warm, dry conditions. However, seeds lost viability rapidly, presumably as a result of evaporation of moisture from the endosperm. Gill (1954) and Hawksworth (1961), studying *A. vaginatum* (Willd.) Presl, reported that seeds which remained dormant on hosts for a period of 2 months failed to germinate. Scharpf and Parmeter (1962) found that dry stored seed of *A. occidentale* retained viability longer at near-freezing temperatures (2°C.) than at temperatures much above or below freezing. Wicker (1967) also noted a high percent viability of seeds of three species and four subspecies of dwarf mistletoe stored dry at 5°C. for 60 days. Seeds stratified in sand or stored under moist cold conditions lost viability rapidly because of deterioration by mold fungi. According to Beckman and Roth (1968), viability of dry stored seeds of *A. campylopodum* f. *campylopodum* was highest at 1.5°C. and decreased as temperature increased.

Heinricher (1915) suggested that light prolonged the viability of seeds of *A. oxycedri (DC.)* M. Bieb. He reported that seeds kept in the dark suffered "injury and destruction" of the embryo. After 3 months' storage in the dark only 7 percent of the seeds germinated when transferred to moist filter paper.

Because information is lacking on influences on seed viability, several studies were undertaken. These include (a) the variation in viability of freshly collected seed from different dwarf mistletoe plants in different years and from different areas; and (b) specific influences such as temperature, light, moisture content of seeds, and relative humidity.

Determination of Viability

Before any intelligible discussion can take place, a definition of viability is needed. As mentioned previously, viability often means the ability of seeds to germinate. However, preliminary studies showed that dwarf mistletoe seeds are not only slow to germinate but require some rather specific conditions for germination. Therefore, a commonly used chemical test was used as the standard method to determine viability in the present study (Flemion and Poole 1948). The test is commonly called the "tetrazolium or TTC test." Scharpf and Parmeter (1962) summarized the use of this method on seeds of *A. occidentale.*

As applied in the present study, the method is essentially as follows: dwarf mistletoe seeds were first placed in water until the viscin layer became swollen. They were then placed in rows on a circular piece of filter paper 9 cm. in diameter and allowed to dry; the dried viscin layer held the seeds on the paper. Each seed was then cut in half longitudinally with a sharp razor blade. The filter paper bearing the cut seeds was then placed in a 100-mm. petri dish, covered with a 0.1 percent solution of 2,3,5-triphenyltetrazolium chloride (TTC) at pH 7-8, and kept dark for 12 to 24 hours. The cut seeds were then examined for staining. Viable seeds changed the colorless TTC into an insoluble, red pigment which was observed in the embryo and endosperm. Nonviable seeds remained unstained. Seeds showing any stain were considered viable.

The accuracy of the TTC test with dwarf mistletoe seeds was initially not known. But because it had been used successfully as a test for viability with numerous other species, I assumed it would give a quick estimate of the viability of seeds of dwarf mistletoes. Later in the study, after the conditions for germination had been worked out, viability as indicated by the TTC test was compared to actual germination at what were considered near-optimum conditions. The conditions most nearly optimum for germination are summarized elsewhere in this paper. In general, they are as follows: aseptic germination of seeds on sterile distilled water at 13°C. and 500 foot-candles of light from cool-white fluorescent bulbs. Under these conditions, the viability of seed of *A. abietinum* by both a germination and a TTC test was compared *(table 1).*

As was expected, viability decreased with length of time in storage. This was shown by both the TTC and the germination tests.

Viability, as indicated by TTC, ranged from 8 to 15 percent higher than actual germination of the seeds under near-optimum conditions. The differ-

Table 1.—*Viability of stored dwarf mistletoe seeds as determined by TTC and germination tests*

Days Storage at 2°C.	TTC test		Germination test	
	Seeds	Viability	Seeds	Viability
	Number	*Percent*	*Number*	*Percent*
5	100	96	100	85
60	100	89	779	74
180	100	56	334	48

ences, however, were not statistically significant.[3] The TTC test therefore proved to be a rapid, useful technique for determining the approximate percentage of viable seeds. Consequently, all determinations of viability reported in this section are considered valid.

Variation

Knowledge of the difference in viability among seed lots is necessary; in comparative studies of viability, tests must be standardized. Therefore, the differences in viability of dwarf mistletoe seeds collected from different areas, in different years, and from different plants were investigated.

Area of Seed Collection

Seeds of *A. abietinum* were collected in 1958 from three widely spaced points in California. Viability tests were made using 200 seeds per test selected at random from seeds from several dwarf mistletoe plants. Results were as follows:

	Viability of fresh seeds (percent)
Collection point:	
Pinecrest, Tuolumne County	97.0
Bucks Lake, Plumas County	97.5
Latour State Forest, Shasta County	97.5

Differences in viability due to area of collection were not found.

Year of Seed Collection

Variation in viability of fresh seeds collected over a period of 4 successive years was also tested, using 200 seeds per year of *A. abietinum* and 100 seeds per year of *A. occidentale.*

	Percent viability			
	1958	1959	1960	1961
Species:				
A. abietinum	97.0	97.5	97.5	97.5
A. occidentale	98	98	98	98

Any differences in viability between the two species of the parasite or among the tests over the years 1958 through 1961 were too small to be detectable.

Individual Plant Source

Differences in viability of seeds collected in 1960 from individual dwarf mistletoe plants were also tested. Each treatment group included 100 seeds from four plants. Results are shown below:

	Days in 2° C. Storage	Range in percent viability
Species:		
A. abietinum	37	86-90
A. abietinum	29	85-89
A. occidentale	1	96-98

The variation in viability was not significantly different for lots of seeds from the same plants or among several plants of the same species.

In general, therefore, I concluded that viability of freshly collected seeds of dwarf mistletoe was consistently high and not significantly influenced by year of collection, area of collection, or individual plant source.

Influences on Viability in Stored Seeds

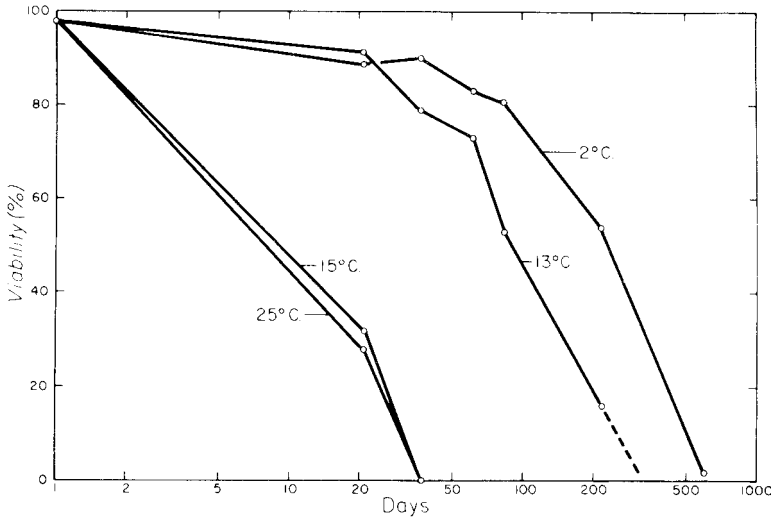
Temperature

Temperature is known to be a marked environmental influence on viability of seeds of many plants. Therefore, the effect of constant temperature on the viability of dwarf mistletoe seeds was studied. Freshly collected, air-dry seeds were placed in petri dishes and stored in the dark at temperatures of -15°C., 2°C., 13°C., and 25°C. At periodic intervals 100 seeds from each temperature group were removed and tested for viability with TTC.

The temperature at which the seeds were stored had a marked influence on viability of both species *(figs. 1,2).* The effect of time on viability was significantly nonlinear at any one temperature. For both species of dwarf mistletoe tested, seeds retained viability longest at 2°C., while 13°C. was significantly less favorable for survival of stored seeds. Viability declined rapidly in storage at -15°C. and at 25°C.; these were the least favorable temperatures for survival. The two species did not differ significantly in their response to constant temperatures, except at

[3]The statistical significance of the data reported in this paper is based on chi-square tests or F tests. The 5-percent level of significance is used throughout.

Figure 1–*Both time and temperture affected viability of stored seeds of A. abietinum.*



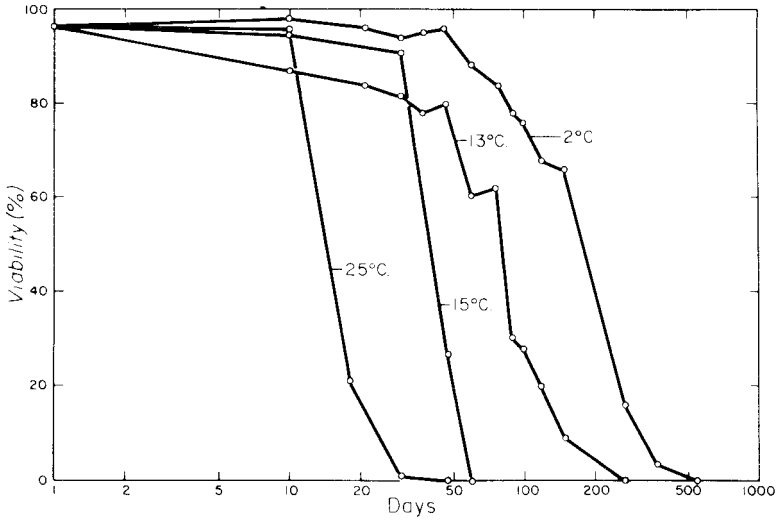


Figure 2.–*Both time and temperature affected the viability of stored seeds of A. occidentale.*

-15°C. At this temperature *A. occidentale* lost viability less rapidly than *A. abietinum.*

Light

Nothing is known about the effect of light on viability of seeds of dwarf mistletoe species of North America. Therefore, experiments were made to determine the influence of darkness, natural sunlight, and artificial light on viability of *A. abietinum* and *A. occidentale.*

Seeds were moistened and placed on circular pieces of filter paper, as described for the TTC test. Several pieces of the paper bearing seeds of each species were then placed in open petri dishes–one piece of paper per dish–and exposed to the desired light and temperature conditions. Other pieces of filter paper with the seeds were wrapped in aluminum foil to exclude light.

The following temperature and light conditions were used:

1. Natural sunlight in a greenhouse at 16°C.
2. Darkness in a greenhouse at 16°C.
3. Artificial light (500 foot-candles from cool white fluorescent bulbs) in a growth chamber at 13°C.
4. Darkness in a growth chamber at 13°C.

At periodic intervals 100 seeds of each test group were removed and tested with TTC for viability. Results of the tests on seeds of *A. occidentale* held in the dark and under natural sunlight were:

	Percent viability	
	Light	Dark
Days storage at 16°C.:		
0	98	98
15	81	86
44	62	73
63	47	58
79	34	44

Significantly lower viability was found in seeds held under conditions of natural sunlight than those in the dark. But no significant difference in viability was found between seed in continuous artificial light and in the dark for either species of dwarf mistletoe *(table 2).* In all tests the decrease in viability was essentially linear with respect to time.

Table 2.– *Viability of dwarf mistletoe seeds exposed to darkness and continuous artificial light*

Days storage 13°C.	Percent viability	
	In light	In dark
A. ABIETINUM		
0	97	97
32	90	91
60	71	74
89	53	60
120	39	44
135	32	33
A. OCCIDENTALE		
0	82	82
14	77	78
31	71	76
41	64	71
51	52	55

Relative Humidity

Because dwarf mistletoe seeds are deposited on needles and branches of conifers and are exposed to a natural air environment, the influence of relative humidity on viability was studied. Seeds of *A. abietinum* were exposed to several different regimes of relative humidity at constant storage temperatures of 15°C. and 25°C. Humidity control was obtained with saturated salt solutions (Hodgeman 1951-52; Anonymous, International Critical Tables 1926).

Small, airtight humidity chambers were made from pint mason jars. Each chamber contained a salt solution, a glass cylinder, and a piece of plastic window screen on which the seeds were placed. Seeds to be tested were surface sterilized in 0.1 percent mercuric chloride ($HgCl_2$) for 15 minutes, washed in three changes of sterile distilled water, placed on sterilized pieces of the window screen, and dried overnight in a desiccator. The screens bearing the seeds were then placed on the glass cylinder in the chambers. The chambers were kept in the dark at a constant temperature. Periodically, 100 seeds were removed and tested for viability with TTC.

At both storage temperatures, relative humidity had no significant effect on viability of *A. abietinum* seeds *(table 3).* The one influence relative humidity did have on seeds in the tests was noticed at 95- and 100-percent humidities at 15°C. At these two humidities some seeds germinated, indicating that free water or a nearly saturated atmosphere provides adequate moisture for germination.

Table 3.–*The viability of seeds of A. abietinum under varying conditions of temperature and humidity* [1]

Days storage	Percent viability at 25°C. and relative humidity of				
	0	33	62	93	100
7	32	21	36	35	33
15	4	7	8	15	6
30	0	0	0	0	0
Days storage	**Percent viability at 15°C. and relative humidity of**				
	0	35	66	95	100
30	40	41	31	[2]34	231
62	28	32	27	[2]39	233

[1]Seeds 60 percent viable at the start of the test.

[2]Percentage of seeds that germinated.

Moisture Content of Seeds

To determine whether or not differences in relative humidity affected the moisture content of the seeds, a further experiment was needed. But first, the amount of free or loosely held water present in the outer viscin layer of fresh seeds had to be determined. To obtain this, seeds were handled in three ways: (a) one lot of seeds was soaked in acetone for 10 seconds to aid in drying, air dried for 5 minutes, and then placed in a desiccator for 24 hours; (b) another lot was placed in a desiccator for 24 hours without pretreatment; and (c) the third lot was air-dried at room temperature for 24 hours only.

Each seed lot was then weighed, oven-dried at 80°C. for 24 hours, and reweighed. The difference between the percent moisture content of the air-dried seeds and the percent moisture content of each of the treated lots of seeds was considered the percent moisture present in the viscin layer.

There was essentially no free water present in the viscin layer of freshly collected seeds air-dried for 24 hours *(table 4).* Furthermore the treatments had no effect on the internal moisture content of the seeds. On the basis of their oven-dry weight, fresh seeds contained about 35 percent water.

Next, I sought to determine the effect of temperature and relative humidity on the moisture content of seeds of *A. abietinum.* Viability was also tested.

Table 4.–*Moisture content of variously treated dwarf mistletoe seeds*

Seed treatment	Fresh weight per 100 seeds	Moisture Content	Difference from control
	Mg.	*Percent*	*Percent*
Acetone treatment, desiccation	263.3	36.4	+l.4
Desiccation	255.6	34.5	-.5
Air-drying (control)	257.6	35.0	– –

Seeds placed in humidity chambers, as previously described, were exposed to a desiccated atmosphere (0-5 percent relative humidity) and a near-saturated atmosphere (87-90 percent relative humidity). One chamber at each humidity was then stored in the dark at 0°C. and at 25°C. At periodic intervals 100 seeds were taken from each chamber, soaked in acetone for 15 seconds, air-dried for 1 hour to remove surface moisture that may have been absorbed by the viscin layer or may have accumulated on seeds in the chambers. After this treatment, the seeds were weighed, oven-dried at 80°C. for 24 hours, and reweighed. At the same time 100 seeds from each chamber were tested with TTC for viability.

Both temperature and relative humidity significantly affected the moisture content of seeds *(table 5).* The effect of time was essentially linear. In all chambers, moisture content reached an equilibrium after about 5 days and changed relatively little thereafter. At both temperatures, moisture content of seeds in the near-saturated atmosphere was about two to three times that of seeds in a desiccated atmosphere. As in previous tests, temperature significantly influenced viability, whereas moisture content did not. At each temperature, viability decreased at approximately the same rate, irrespective of moisture content.

Discussion

Viability of freshly collected dwarf mistletoe seeds was found to be consistently high in these studies. Area of seed collection did not influence viability, as has been reported by Bonga and Chakraborty (1968) for *A. pusillum* Peck.

Weir (1918) claimed that under hot, dry conditions, seeds lose viability rapidly as a result of water loss from the endosperm. Results of my study agree with those of Weir in that seeds did not store well under hot, dry conditions. Relative humidity of the atmosphere or moisture content of the seeds, however, did not have a marked influence on longevity. Regardless of moisture content of the seeds, viability decreased with an increase in storage temperature above freezing. At a temperature considerably below freezing, seeds also lost viability very rapidly. Although the theory was not tested, it is possible that at subfreezing temperatures moisture content of seeds may affect viability. Sudden exposure of fresh seeds to extreme cold (-15°C.) may have caused death as a

Table 5.–*The moisture content and viability of seeds of A. abietinum under varying conditions of temperature and relative humidity*[1]

Days storage	0°C.				25°C.			
	0 pct rel. humidity		90 pct. rel. humidity		0 pct. rel. humidity		90 pct. rel. humidity	
	Moisture content	Viability	Moisture content	Viability	Moisture content	Viability	Moisture content	Viability
	Percent							
5	27	95	49	94	9	50	34	51
13	26	98	50	97	16	38	37	20
21	24	89	49	95	11	24	33	22
29	(2)	(2)	(2)	(2)	14	4	36	8
53	21	90	53	92	(2)	(2)	(2)	(2)
83	21	79	57	89	(2)	(2)	(2)	(2)

[1]Seeds contained 33 percent moisture and were 98 percent viable at the beginning of the test.
[2]No determinations made.

result of the formation of ice crystals in the tissues.

The finding that seeds of dwarf mistletoe retain viability longest at near freezing temperatures agrees with results reported by Scharpf and Parmeter (1962), Beckman and Roth (1968), and Wicker (1967).

Constant exposure to light did not prolong the viability of seeds of either *A. abietinum* or *A. occidentale* as was reported by Heinricher (1915) for *A. oxycedri.* Crocker (1936) points out that Heinricher had published a report on the favorable influence of light on the viability of seeds of the true mistletoe *Viscum album* L. As a result of Heinricher's experience with *Viscum*, it is possible that he erroneously attributed loss of viability to lack of light during storage. In his early experiments, Heinricher was not able to control the temperatures at which his seeds were stored. It is likely, therefore, that unfavorable temperature conditions during storage and not the lack of light were responsible for loss of viability.

The decrease in viability of seeds exposed to sunlight over those stored in the dark in my study was probably the result of solar radiation increasing temperatures above optimum for maximum longevity.

GERMINATION

The literature contains conflicting reports on temperatures favorable for germination and possible requirements of mistletoe seeds for a period of dormancy. Weir (1918) claimed percentage of germination increased in dwarf mistletoe seeds exposed to freezing temperatures. Beckman and Roth (1968) reported that "prolonged chilling" of *A. campylopodum* is actually necessary for germination. On the other hand, Scharpf and Parmeter (1962) found that seeds of *A. occidentale* required no period of chilling, but that adequate moisture and a favorable temperature (13.3°C.) were necessary for germination. The optimum constant temperature reported by Beckman and Roth (1968) for germination of *A. campylopodum* was between 17°C. and 19°C., but the most favorable alternating night-day temperatures for germination were 5°C. to 15°C.

In general, most investigators have found that light is either required for germination of mistletoe seeds or stimulates it to some degree. Studies by Wiesner, as reported in Crocker (1936), showed that light was required for germination of *V. album.* Heinricher (1915) reported light to be necessary for germination of *A. oxycedri.* In a subsequent study (1917), he again tested the germination of *A. oxycedri* in the dark. After 18 days he noted no germination. On the 18th day, seeds were exposed to diffused daylight. After a day in the light, 3 of 19 seeds germinated, 2 of which Heinricher felt undoubtedly must have germinated while in the dark. Scharpf and Parmeter (1962) found that at 13°C. light is not necessary for germination of *A. occidentale,* but stimulates it and slightly increases percentage germination. Kuijt (1960) reported that chlorophyll occurs in the endosperm and radicle of dwarf mistletoe seeds and that these organs undoubtedly are photosynthetically active.

Heinricher (1915) reported that dwarf mistletoe seeds required a chemical stimulus for germination, provided by either living or dead organic matter. In a subsequent study, however, Heinricher (1917) corrected his early statements on germination when he found that seeds germinated on either organic or inorganic materials as long as free water was present.

In laboratory studies with a few North American species of dwarf mistletoes, Gill and Hawksworth (1961) found that removal of the viscin layer surrounding the seeds hastened and improved germination. Beckman and Roth (1968) suggested that a chemical inhibitor associated with the seed covering prevents germination. With time the inhibitor gradually breaks down to a point at which germination will occur if light, moisture, and temperature are favorable. Wicker (1962) reported that hydrogen peroxide stimulated germination of *Arceuthobium* spp.

Standard Germination Test

For the studies of influences on germination of dwarf mistletoe seeds, a uniform method of handling a fairly large number of seeds was needed. Scharpf and Parmeter (1962) described the method for germinating seeds of *Arceuthobium.*

Surface Sterilization

Preliminary germination tests in the laboratory demonstrated that the seeds of dwarf mistletoe are rather slow to germinate, and as a result require special handling to avoid contamination from micro-organisms. For example, freshly collected as well as stored seeds which were not disinfected but placed directly on moist filter paper in petri dishes were overrun with micro-organisms before seeds could

germinate. These micro-organisms, growing profusely on the surface of the seeds, prevented germination. Therefore, several methods were used in an effort to surface sterilize seeds for aseptic germination tests *(table 6).*

"Clorox,"[4] a commonly-used laboratory disinfecting agent, and propylene oxide, a gaseous sterilizing agent, proved unsuitable for surface sterilization. Mercuric chloride ($HgCl_2$), however, did provide adequate surface sterilization. In none of the tests in which a 0.1-percent solution of $HgCl_2$ was used did contamination occur. Addition of wetting agents (Amphyl[5] or alcohol) and vacuum treatment did not improve sterilization.

Additional tests were conducted to determine if $HgCl_2$ had any detrimental effects on the seeds. Seeds were soaked for 15 minutes in a 0.l-percent solution of $HgCl_2$ washed in three changes of sterile water, air-dried, and stored in petri dishes at 13°C. Control seeds were washed in distilled water only, air-dried, and stored in dishes at 13°C. Periodically, 50 treated and 50 untreated seeds were removed from storage and tested with TTC for viability. The effect of $HgCl_2$ on the viability of dwarf mistletoe seeds was:

	Percent viability	
	$HgCl_2$-treated seeds	Untreated seeds
Days storage:		
0	94	88
13	82	84
28	74	76
47	72	76
64	68	68

Viability decreased linearly with time, and was not affected by treatment with $HgCl_2$. As a result of this information $HgCl_2$ was adopted as the standard sterilizing agent.

A standardized method of handling seeds for germination, based on the results of the previous tests, was adopted.

About 100 dwarf mistletoe seeds were enclosed in a bag made from a 15 cm. square of cheesecloth. The four corners of the cloth were gathered up and wired together to form the bag. In the bag seeds were surface sterilized in 0.1 percent $HgCl_2$ for 15 minutes, washed for 10 minutes each in three changes of sterile, distilled water, and placed in a 100-mm. petri dish. The bag was opened in the dish with sterile forceps and the seeds distributed evenly over the cloth. Sufficient water for germination was present on the cloth and seeds. The dish was then covered and sealed with drafting tape to prevent evaporation of moisture. At periodic intervals, the seeds in the dishes were examined with a dissecting microscope for germination. Seeds were considered germinated when the radicle emerged from the endocarp.

By this method, large numbers of seeds could be handled fairly easily. For the various studies undertaken, the seeds could be either germinated directly on the cheesecloth in dishes or removed from the cheesecloth after surface sterilization and placed on other substrata for testing.

Influences on Germination

Temperature

To determine the effect of constant temperature on the germination of dwarf mistletoe seeds, seeds of *A. abietinum* were surface sterilized, put in dishes as previously described, and exposed to a range of constant temperatures. At each temperature, 100 seeds were used, and all tests were made in the dark. At periodic intervals, the seeds in the dishes were observed for germination.

Temperature affected both the rate of germination and percentage germination *(fig. 3).* Rate of germination increased with temperature between 0°C. and 13°C. and decreased between 13°C. and 22°C.

Percentage germination did not progressively increase with temperatures above freezing but was

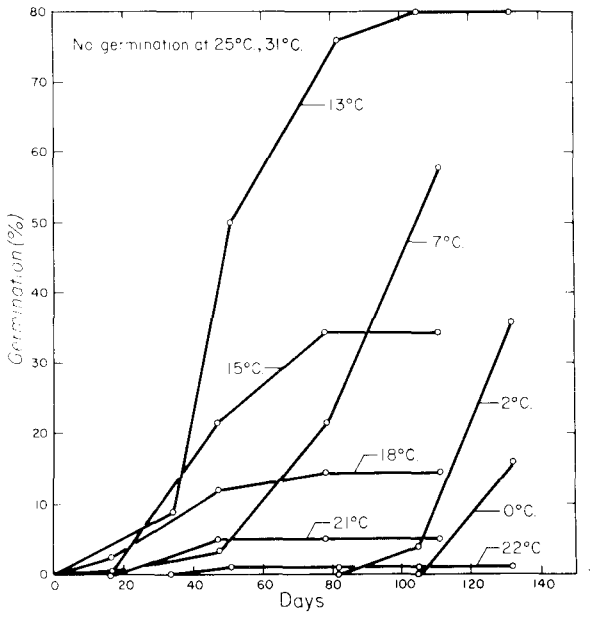


Figure 3.– *Temperature affected the germination of seeds of A. abietinum.*

[4]Trade names and commercial products or enterprises are used solely for necessary information. No endorsement by the U.S. Department of Agriculture is implied.

[5] See footnote 4.

Table 6.–*Results of surface sterilization of seeds of dwarf mistletoe for aseptic germination, by treatments*

Chemical agent	Strength of active ingredient (percent)	Treatment	Plates used[1]	Plates contaminated with microorganisms after 1 month
Sterile water	– –	Three 10-min. washes, sterile water	4	4
Clorox	1	10-min. soak; one 10-min. wash, sterile water	4	4
	1	15-min. soak; three 10-min. washes, sterile water	2	2
	2		2	2
	3		2	2
	4		2	2
	5		2	2
Clorox and amphyl	1	Ditto	2	2
$HgCl_2$	0.1	Ditto	4	0
$HgCl_2$ and amphyl	0.1	Ditto	4	0
$HgCl_2$ and alcohol	0.1	Ditto	4	0
$HgCl_2$ and vacuum	0.1	Ditto	4	0
$HgCl_2$ and amphyl and vacuum	0.1	Ditto	4	0
Propylene oxide	2 cc per 5,000 cc air	24 hours sterilization and 24 hours aeration	5	5

[1]Twenty-five seeds per plate.

highest at 13°C. and decreased above and below this temperature. No germination of seeds occurred at temperatures above 22°C. Some germination did occur at 0°C., but only after about 4 months.

pH

To ascertain the effect of pH on the germination of *A. abietinum* phosphate buffer solutions at a pH of 5, 6, 7, 8, and 9 were used. "Hydrion" buffer capsules were used to make the solutions, one capsule per 100 ml. solution. The solutions were placed in 125-ml. flasks and the flasks plugged with cotton and autoclaved. After autoclaving, the sterile buffer solutions were checked with pH paper and found to be within about ± 0.2 pH of the desired pH. Non-buffered, sterile distilled water at a pH of 6.5 was used as a control. Cheesecloth bags containing 25 seeds each were surface sterilized, washed in sterile distilled water, and then placed in the test solution for one-half hour. Two bags of seeds were used at each pH. After the half-hour treatment the seeds were distributed over the cheesecloth in petri dishes and placed in the dark at 16°C. for germination.

The range of pH from 5 to 9 had no significant effect on the germination of dwarf mistletoe seeds. Germination of seeds in the phosphate buffer solutions did not differ significantly from that of seeds in sterile distilled water. Germination at the pHs tested and in the non-buffered control ranged from about 30 to 40 percent after 3 months.

Influences on Dormancy

Seeds of dwarf mistletoe are reported to undergo a period of dormancy before germinating. Because seeds of some dwarf mistletoes overwinter on hosts and usually are rather slow to germinate in the field, tests were made to determine if they require a period of dormancy for maximum germination. Several methods commonly used to break dormancy were used. These included exposure to light, scarification, after-ripening, removal of germination inhibitors, and chemical stimulation of germination.

Information on dormancy in dwarf mistletoes sheds light on the conditions that influence germination in nature and also facilitates the handling, storage, and germination of seeds in the laboratory.

Light

Previous studies had shown that light was not necessary for germination of *A. occidentale* but suggested that light stimulates germination (Scharpf and Parmeter 1962). Additional tests were made, therefore. Because it was desirable to maintain constant temperatures during germination, the tests were carried out under artificial light in temperature controlled chambers. Temperatures near optimum for germination (13°C. and 16°C.) were used.

One hundred seeds handled as described for germination were used at each temperature and under

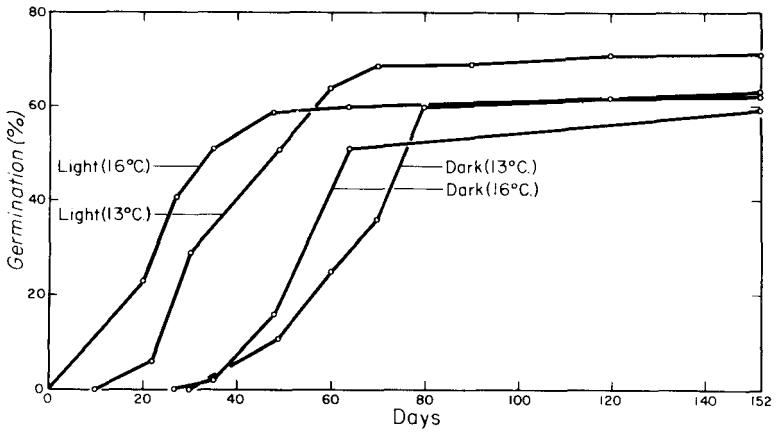


Figure 4.–*Light reduced the time needed for germination of seeds of A. occidentale.*

each light condition. The source of light was a bank of four 40-watt, cool-white fluorescent bulbs. The seeds were exposed to approximately 500 foot-candles of light. At intervals the seeds were examined for germination. At both temperatures, light significantly shortened the time of germination of seeds *(fig. 4).* Germination began after about a week to 10 days in the light, whereas between 3 to 4 weeks were required for beginning germination in the dark. Percentage germination was greater in the light than in the dark at 16°C. but not significantly different in the light and dark at 13°C.

Scarification

Both mechanical and chemical methods of scarification aimed at making the seedcoat more permeable to water have been used to break seed dormancy (Crocker and Barton 1953). In tests with dwarf mistletoe, both types of methods were used. Dwarf mistletoe seeds have no seedcoat, but I wanted to determine if the viscin layer and endocarp surrounding the seeds inhibit germination by preventing water or oxygen from entering the seeds.

Six bags, each containing 50 dwarf mistletoe seeds, were prepared for seed sterilization. One each of three bags was dipped into acetone, petroleum ether, and a 1-molar solution of ammonium hydroxide for 10 minutes. Seeds in the fourth bag were placed in concentrated sulfuric acid in a petri dish before being placed in the bag, to prevent breakdown of the cheesecloth bag by the strong acid. Before the seeds in the fifth bag were bagged, they had been mechanically scarified by carefully cutting away with a sharp razor blade a small part of the viscin layer and endocarp. The seeds in the last bag were left untreated as a control. The seeds in all bags were then surface sterilized, plated, and placed in the dark at 15°C. to germinate. The seeds were observed at intervals for germination.

Both chemical and mechanical methods of scarification failed to stimulate germination *(fig. 5).* In the

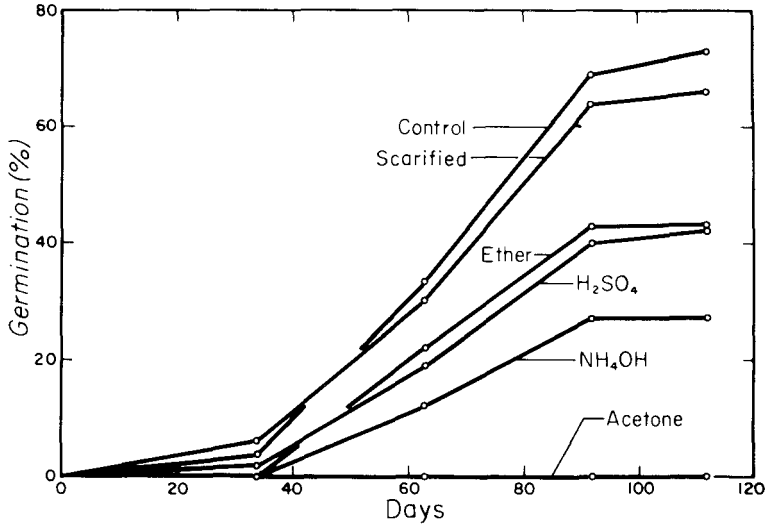


Figure 5.–*Mechanical and chemical scarification did not favorably affect germination of seeds of A. occidentale.*

tests using acetone and ammonium hydroxide, germination was inhibited significantly. In the tests using ether and sulphuric acid, the results were not significantly different from those of the control.

After-Ripening

The seeds of many plants, including a number of the coniferous hosts of dwarf mistletoe, require a period of exposure to low temperatures during which they undergo certain changes known as after-ripening (Crocker and Barton 1953; U.S. Forest Serv. 1948). After-ripening requirements are often best met by stratification (exposure to moist conditions at low temperatures). But preliminary tests in these studies and in studies reported by Wicker (1967) showed that dwarf mistletoe seeds not handled aseptically became overrun with micro-organisms during stratification and moist cold storage. Tests were made to determine the effect of stratification under aseptic conditions and low-temperature dry storage on the germination of dwarf mistletoe seeds.

Four lots of freshly collected seeds of *A. occidentale,* 100 seeds per lot, were bagged, surface sterilized, and placed on moist cheesecloth in sterile petri dishes. Two of the lots of seeds were then placed in the dark at 13°C. to germinate. The other two lots of seeds were placed at 0°C. to stratify for 2 months. An additional two lots of freshly collected seeds were placed in petri dishes and stored dry 0°C. for 2 months.

After the 2-month period, the stratified seeds, still on moist cheesecloth in petri dishes, were placed in the dark at 13°C. to germinate. At the same time, the seeds stored dry at 0°C. were bagged, surface sterilized, placed in sterile petri dishes, and put at the same temperature to germinate. At periodic intervals the seeds were observed for germination.

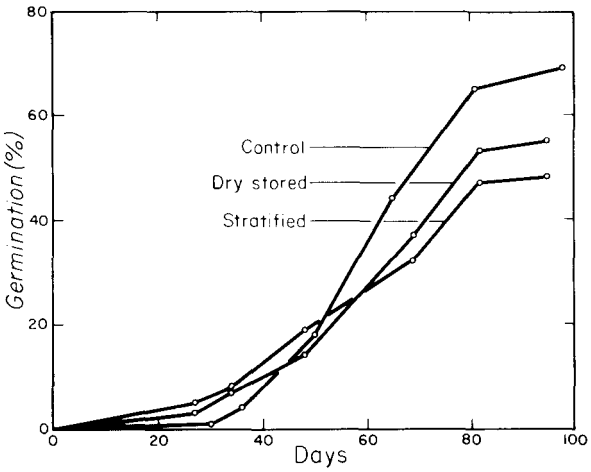


Figure 6.–*Stratification and dry cold storage did not favor germination of seeds of A. occidentale.*

Neither dry storage nor stratification favored the germination of dwarf mistletoe seeds *(fig. 6).* Seeds kept for 2 months for after-ripening under each of these conditions showed a significantly lower percentage of germination than did fresh seeds allowed to germinate immediately. Also, percentage of germination was significantly higher in seeds stored dry than in those stratified. Thus, seeds of dwarf mistletoe do not require a period of after-ripening for germination.

Germination Inhibitors

The fleshy coating of some seeds has chemicals that prevent germination (Crocker and Barton 1953; Evenari 1949). Beckman and Roth (1968) have suggested that certain chemicals might be present in the viscin layer of dwarf mistletoe seeds which prevent germination.

A preliminary investigation showed that the viscin layer surrounding the seeds of dwarf mistletoe is primarily pectic. The presence of pectin was determined by a specific test for pectin based on the reaction of hydroxamic acid with ferric ion (McCready and Reeve 1955).

A study was made to determine if the pectic portion of the viscin coating or any materials contained in the pectic matrix affected germination of *A. occidentale.* To test this, the viscin layer surrounding the seeds was removed before germination with pectic enzymes (Durbin 1956). Durbin used the enzyme pectinase to remove pectic substances surrounding the seeds of cacti. For my study, the pectic material in the viscin layer of dwarf mistletoe seeds was removed with the enzyme polygalacturonase (PG). An attempt was also made to remove germination inhibitors by soaking the seeds in distilled water.

Dwarf mistletoe seeds were placed in bags, for surface sterilization, 100 seeds per bag. Five bags in all were made. Two of the bags were placed in 125 ml. flasks, each of which contained 50 ml. of a 3-percent solution of PG; and two bags were placed in 125 ml. flasks, each of which contained 50 ml. of sterile distilled water. The four flasks were then gently agitated in a biological shaker at room temperature. The seeds in one flask containing PG and one flask containing water were removed from the solutions after 3 hours' agitation, surface sterilized, placed in sterile petri dishes, and placed in the dark at 16°C. to germinate. The remaining seeds in each flask were agitated for a total of 24 hours, removed from the solutions, handled as described above, and stored at the same temperature. As a control, untreated seeds

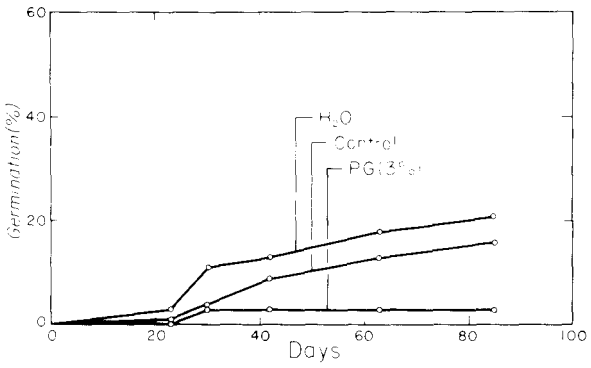


Figure 7.–*After 24-hour treatment with the pectic enzyme, polygalacturonase, seeds of A. occidentale showed a drop in level of germination.*

in one bag were surface sterilized, plated, and stored under the same conditions. The seeds in all plates were examined periodically for germination.

The 3-hour treatment with PG was sufficient to remove the viscin layer. But part of the viscin layer remained on seeds that were treated with water only, for 3 hours.

No significant differences in percent germination were found among the tests using control seeds, untreated seeds, and seeds treated with PG for 3 hours. About 20 percent germination occurred in all the tests after 3 months.

In the seeds treated with PG for 24 hours, not only was all the pectic material removed from the viscin layer but also the endocarp was partly digested from nearly all the seeds. In many seeds a considerable portion of green endosperm was exposed. The viscin coating was also missing from the seeds soaked in distilled water for 24 hours.

Seeds treated with PG for 24 hours showed a significantly lower percent germination than untreated seeds *(fig. 7).* No significant difference was noted in percent germination for the seeds soaked in water for 24 hours as compared to control seeds.

Chemical Stimulation

Chemicals stimulate the germination of seeds of many plants (Crocker and Barton 1953). The seeds of certain phanerogamic parasites require a chemical stimulus for germination provided by certain species of plants (Srinivasan and Subramanian 1960). Nash and Wilhelm (1960) have found that a plant growth regulator—gibberellic acid—helped stimulate seed germination in two species of the parasitic genus *Orobanche.*

Host Branch Tissue

To test for chemicals in host tissue that may stimulate or suppress germination, seeds of *A. occidentale* were placed on branch segments in agar in petri plates. To prepare the medium, several l- to 3-year-old branches of Digger pine were cut into segments about 1 inch long and sterilized with gaseous propylene oxide for 24 hours (Hansen and Snyder 1947). After sterilization, 25 segments were placed in sterile petri dishes, five segments per dish. Twenty ml. of 2-percent warm, sterile water agar was then poured into each plate. As the plates cooled, the segments became partially imbedded within the solidified agar. Five plates, each containing 20 ml. agar, were prepared as controls.

Seeds of *A. occidentale* were surface sterilized and placed on the agar plates so that they touched the branch segments. Four seeds were placed along each branch segment. As a control, 100 seeds–20 seeds per plate–were evenly distributed over the surface of the water agar plates. All plates were then held under artificial light at 16°C. At periodic intervals the seeds were examined for germination.

Host tissue had no significant effect on the germination of dwarf mistletoe seeds. About 30 percent of the seeds in both tests germinated after 3 months. Both rate of germination and percent germination of seeds on host tissue were the same as that for seeds on water agar.

Plant Hormones

Studies were made to determine the effect of a plant hormone indole-3-acetic acid (IAA) on the germination of dwarf mistletoe seeds. A sterile stock solution of the hormone was made by first dissolving a known amount of the compound in 5 ml. absolute ethyl alcohol and then adding this solution to 95 ml. sterile distilled water. Serial dilutions were then made from the stock solution to give sterile 100-ml. test solutions at hormone concentrations of 1, 10, and 100 p.p.m. One flask of sterile distilled water was also prepared.

Seeds of *A. occidentale* were placed in bags, 50 seeds per bag, treated by the standard germination method, and washed thoroughly in sterile distilled water. Two bags were then soaked in each of the test solutions for approximately l/2 hour. The bags were then removed, opened, and placed in sterile petri dishes. The dishes were stored in the dark at 13°C. At periodic intervals the seeds were observed for germination.

Treatment with IAA did not stimulate germination *(fig. 8).* With IAA at concentrations of 1 and 10 p.p.m. there was no significant effect on germination, whereas at 100 p.p.m. IAA rate and percentage germination were significantly reduced.

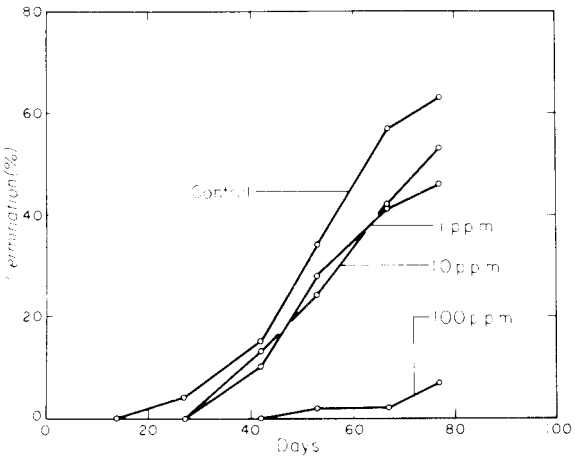


Figure 8.–*Germination of seeds of A. occidentale was not increased by varying concentrations of indole-3-acetic acid.*

Potassium Nitrate

Because seeds of *Arceuthobium* are sensitive to light, a test was made to determine if nitrate (potassium nitrate) would substitute for this response to light, as it has been reported to do for seeds of some plants (Crocker and Barton 1953). Solutions of potassium nitrate at concentrations of 1 normal, 0.1 normal, and 0.01 normal were prepared and 100 ml. of each solution placed in 125-ml. flasks and sterilized by autoclaving. One flask of distilled water was also sterilized and used as a control

Seeds of *A. occidentale* were placed in cheesecloth bags, 100 seeds per bag, surface sterilized, and then soaked in the nitrate solutions for about l/2 hour. The bags were then opened and placed in sterile petri dishes and then stored in the dark at 16°C. At periodic intervals, the seeds were examined for germination.

Potassium nitrate had a significant effect on the germination of dwarf mistletoe seeds *(fig. 9).* Seeds in 1 normal potassium nitrate failed to germinate. In the 0.l normal solution, germination was not statistically different from that of seeds of distilled water. However, a significantly higher percentage germination occurred in 0.01 normal potassium nitrate than in distilled water.

Discussion

The conflicting reports in the literature on the germination of dwarf mistletoe seeds may be due in part to the lack of knowledge about important interrelated variables, such as temperature, moisture, and light, and their influence on germination. Furthermore information about dormancy requirements of dwarf mistletoe seeds has been lacking.

In my studies attempts were made to standardize conditions for germination and also to separate out the various conditions that may influence germination or dormancy.

Temperature was an important influence on germination of dwarf mistletoe, but it did not appear to affect seed dormancy, as reported by Weir (1918) and Beckman and Roth (1968).

The temperature most nearly optimum (13°C.) for germination of *A. occidentale* in the present tests was the same as that reported by Scharpf and Parmeter (1962). This was somewhat lower than the near-optimum constant temperature (17°-19°C.) reported by Beckman and Roth for *A. campylopodum* f. *campylopodum*.. It is interesting that their night-day alternating temperature regime most favorable for germination was 5° and 15°C., both of which temperatures are below the optimum constant temperature for germination.

The requirement of light for germination of dwarf mistletoe, reported by Heinricher (1915), was not confirmed in the present studies. *Arceuthobium* is different in this respect from most other genera of mistletoes (Crocker 1936; Gill and Hawksworth 1961). However, light did increase both the rate and percentage germination of seeds, as previously reported by Scharpf and Parmeter (1962). As mentioned earlier, Kuijt (1960) noted that chlorophyll occurs in the endosperm of the seed and in the radicle. Possibly in the light, seeds can carry on limited photosynthesis which stimulates and enhances germination to some degree.

Several other possible influences on dormancy of dwarf mistletoe seeds were tested. These included scarification–both mechanical and chemical; after-ripening–both dry storage and stratification; removal of germination inhibitors in the viscin layer; and

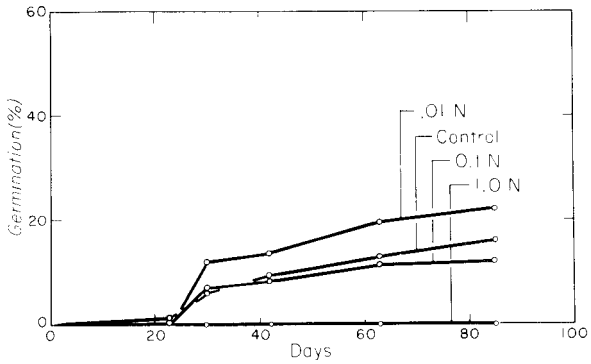


Figure 9.–*Potassium nitrate affected the germination of seeds of A. occidentale.*

chemical stimulation of germination by using host tissue, hormones, and potassium nitrate. In all but one instance–one test with potassium nitrate–germination was either not significantly affected or was inhibited. These results are in contrast with those of Beckman and Roth (1968), who reported a chemical inhibitor associated with the seed coating of *Arceuthobium.* The stimulation of germination by potassium nitrate at the proper concentration may be explained on the basis that this chemical is known to substitute for light in a number of light-sensitive seeds (Crocker and Barton 1953) and may act in the same manner with dwarf mistletoes. Stimulation of germination with hydrogen peroxide for several western dwarf mistletoes, as reported by Wicker (1962) was not tested. This method of using hydrogen peroxide for germination of *A. pusillum* proved unsatisfactory for Bonga (1965).

RADICLE GROWTH AND HOLDFAST FORMATION

The radicles of seeds of mistletoes, including *Arceuthobium,* undergo a period of independent growth between germination and infection of the host. During this period, several stages in radicle development have been reported (Kuijt 1960; Gill and Hawksworth 1961; Scharpf and Parmeter 1967). In general, the developmental stages are (a) an initial stage of linear growth following germination, (b) cessation of linear growth and formation of a "holdfast," (c) production of one or more structures from the holdfast, which penetrate the host.

For the most part only general information is available about the influences on radicle growth and holdfast formation of mistletoes including *Arceuthobium.* Early studies as reviewed by Gill and Hawksworth (1961) have shown that radicles of several mistletoes are negatively phototropic. Negative phototropism has also been reported for several species of dwarf mistletoes (Peirce 1905; Heinricher 1915; Hawksworth 1961). The presence of chlorophyll in the endosperm and radicle (Kuijt 1960) indicates that they are undoubtedly photosynthetically active.

Radicles are reported to continue linear growth until they make contact with an obstacle before initiating a "holdfast" (Hawksworth 1961; Peirce 1905; Scharpf and Parmeter 1967; Weir 1918). Apparently some pressure on the apex of the radicle is necessary before differentiation of the holdfast takes place.

Bonga (1965) reported that radicle growth of *A. pusillum* was not affected by several organic and inorganic media. Addition of host twig extracts also did not stimulate growth, nor was growth affected by changing the pH of the media or soaking the seeds in gibberellic acid.

Bonga and Chakraborty (1968) found that at 15°C., dissected embryos of *A. pusillum* grew more in the light than in the dark, but at 21°C. there was no difference. For "seed cultures," however, radicles grew more in the dark than in the light at both 15° and 21°C. And indole-3-acetic acid (IAA) was found to stimulate holdfast formation on dissected seedlings.

Influences on Growth and Holdfast Formation

To determine the influences on growth and differentiation of the radicles of dwarf mistletoe seeds, a series of tests was made in the laboratory. Specifically, the effects of temperature, light, host tissue, and pH on linear extension of radicles and on holdfast formation were studied.

Pregerminated seeds of *A. occidentale* were used for all tests. The seeds were pregerminated by the standard method described earlier at 13°C. under 500 foot-candles of light for about 1 month. After germination, the seeds were aseptically transferred to a medium on which they were to be tested *(table 7).*

At periodic intervals during the tests, the seeds were examined for linear growth and differentiation under low power (10X) of a dissecting microscope. Growth of the radicles was measured to the nearest 0.l mm. with an ocular micrometer; growth was considered to be only the linear extension of a radicle. Radicles that failed to elongate or shriveled up were considered dead, and measurements were stopped. Only radicles with measurable growth were included. The amount of linear extension was assumed to be directly related to conditions favorable for growth. Formation of holdfasts by the growing radicles was also noted.

In all tests, living radicles grew in length during the entire period of observation; no holdfasts were formed on radicles in any of the plates *(fig. 10).* In those seeds under constant illumination, a negatively phototropic response of the radicles occurred, causing a number of the radicles to penetrate below the

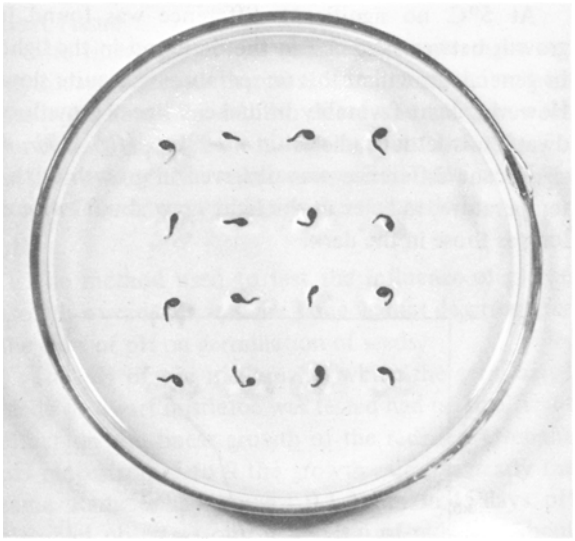


Figure 10.–*Germinated seeds of A. occidentale were grown on water agar in the light at 16°C. for about 2 months.*

surface of the medium. Also, radicles in the light took on a red to red-brown pigmentation.

No similar tropic response was observed for the radicles in total darkness, and these radicles did not become pigmented but remained more or less light green or colorless in appearance. For nearly all seeds in the dark, radicles grew along the surface of the media.

Temperature

Temperature had a noticeable effect on survival of radicles of dwarf mistletoe *(fig. 11).* In general, radicle survival was inversely proportional to increased temperature. Above 30°C., radicles failed to grow or survive. At 25°C., more than 80 percent of the radicles had died and shriveled up after 39 days. At 19° and 16°C. more than 80 percent of the radicles had died after 90 days. Best survival occurred at 10° and 5°C. Less than 50 percent of the radicles died after 90 days at these temperatures.

Unlike radicle survival, radicle growth was directly proportional to increased temperature *(fig. 12).* Radicle growth was greatest at 25° and 19°C. (there was no significant difference in growth between these two temperatures). But growth was significantly different between 16° and 25°C. No significant difference in growth was found between 10° and 5°C.; at these two temperatures, however, growth was significantly slower than at 16°C.

Light

Light did not affect the survival of radicles during the test period. Nearly the same number of radicles were alive in the light as in the dark at each temperature.

Table 7.–*Conditions of tests on radicle growth and holdfast formation of dwarf mistletoe*

Test	Test conditions	Medium[1]	Number of seeds
Temperature	Constant temperatures of 5°, 10°, 16°, 19° 25°, 30°, 37°, and 43°C. in the dark	2 pct H_2O agar	2 plates of 16 seeds per temperature
Light	5° and 15°C. in total darkness and under constant illumination (500 ft-c) from cool-white fluorescent bulbs	2 pct H_2O agar	2 plates of 16 seeds under each temperature and light condition
Host tissue	16°C. total darkness	2 pct H_2O agar; host tissue medium, 10 g. pine bark homogenate per 100 cc 2 pct H_2O agar	2 plates of 20 seeds and 1 plate of 25 seeds on each of the test media
pH	16°C. total darkness	2 pct H_2O agar buffered at pH 5, 6, 7, 8, 9 with hydrion phosphate buffer capsules; 1 capsule per 100 cc agar	2 plates of 16 seeds at each pH

[1]Media were autoclaved 15 minutes at 20 p.s.i.

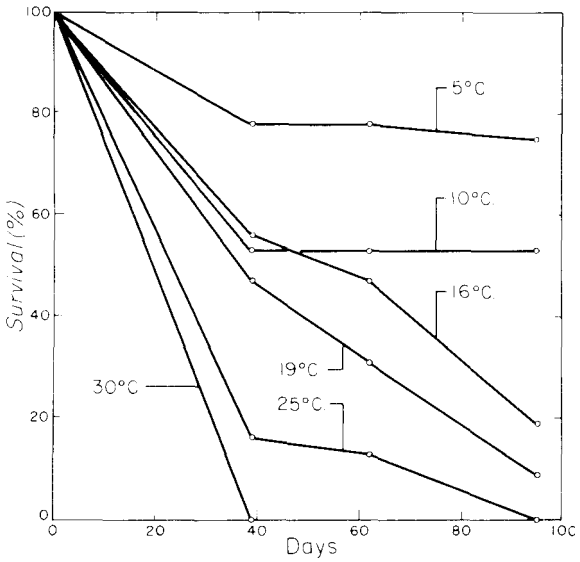


Figure 11.–*Survival of germinated seeds of A. occidentale was adversely affected by increased temperature.*

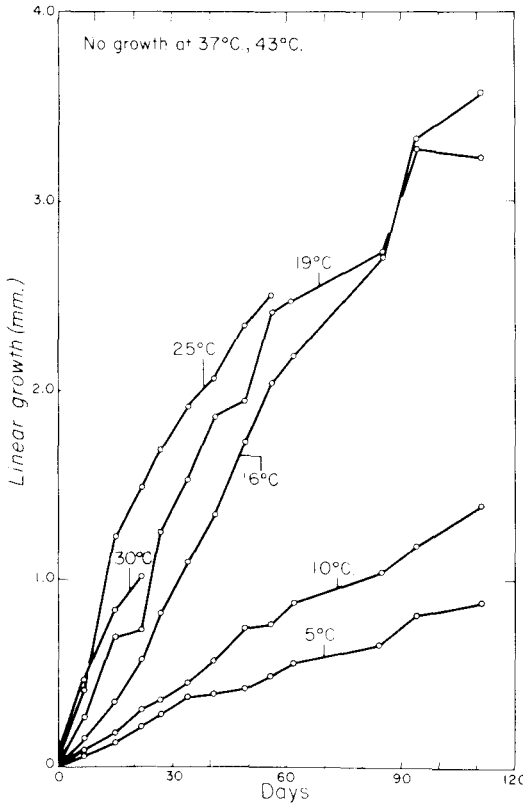


Figure 12.–*As temperature was increased, the linear growth of radicles of germinated seeds of A. occidentale increased.*

At 5°C. no significant difference was found in growth between radicles in the dark and in the light. In general, growth at this temperature was quite slow. However, light favorably influenced linear growth of dwarf mistletoe radicles at 15°C. *(fig. 13).* A significant difference was observed in growth at this temperature; radicles in the light grew about twice as long as those in the dark.

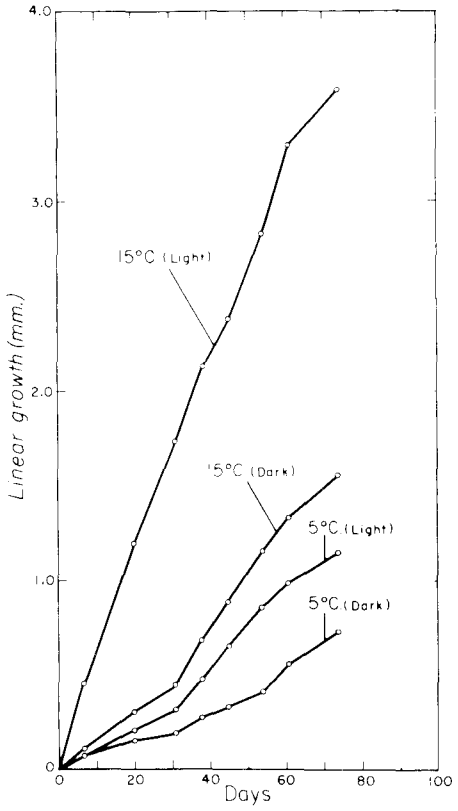


Figure 13.–*Light aided the linear growth of radicles of seeds of A. occidentale.*

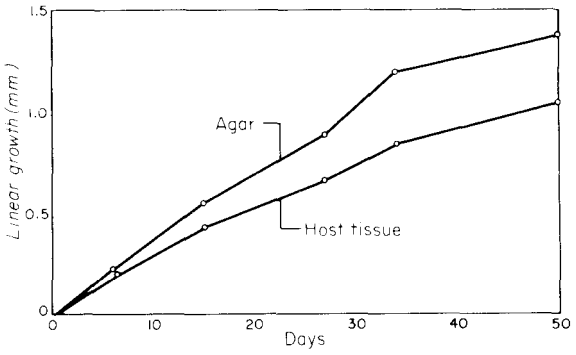


Figure 14.–*The linear growth of radicles of A. occidentale seeds was reduced on host tissue medium.*

Host Tissue

The linear growth of radicles on water agar was significantly greater than that which occurred on host tissue medium *(fig. 14).* No effect was noticed on the survival of radicles on either medium, however. On both media, most radicles remained alive and in a stage of linear growth during the test period.

pH

The method used to test the influence of pH on growth of radicles was the same as that described for the tests of pH on germination of seeds.

The pH of the medium on which the germinated seeds of dwarf mistletoe was tested had no significant effect on the linear growth of the radicles. Over the pH range from 5 to 9 the growth rate was nearly the same. Radicles had grown l.0-1.4 mm. in 45 days. pH also had no effect on the survival of radicles. About 80 percent of the radicles survived over the duration of the test period.

Discussion

In the previous tests it was found that the factors that influenced germination also influenced linear growth of radicles.

Radicle growth in general increased in direct proportion to increase in temperatures from 5° to 30°C., but survival decreased in proportion to increase in temperature. Thus, temperatures that favor most rapid growth of radicles may not necessarily be best for maximum percentage penetration and infection of hosts, mainly because of the low survival rate of radicles. Similarly, temperatures best for radicle survival also may not insure maximum percentage penetration and infection because of limited linear growth under these conditions. Probably temperatures most nearly optimum for penetration and infection of hosts by dwarf mistletoes are about midway between temperatures favoring radicle survival and those favoring maximum linear growth.

As with germination, light stimulated but was not required for growth of radicles. These findings matched Kuijt's (1960) observations that radicles are photosynthetically active, but differ from the finding by Bonga and Chakraborty (1968) that radicles of "seed cultures" grew significantly more in the dark than in the light. Strangely enough, some of their dissected embryos grew more in the light than in the dark.

One aspect of the reaction of radicles to light is particularly interesting. Radicles failed to etiolate in the absence of light; thus they reacted physiologically in the same manner as a root. On the other hand, the radicles contain chlorophyll and stomata and morphologically resemble more closely an epicotyl than a root. It appears, therefore, that the structure emerging from the seed and termed "radicle" has features characteristic of both shoot and root.

Results of my studies confirm those of Bonga (1965) in that growth of radicles was not stimulated by host extracts. In fact, in my studies, noticeably less linear growth occurred in radicles on host tissue extract medium than on water agar. Differences in pH as reported by Bonga (1965) and as shown in my studies would not account for these differences in linear growth. Possibly certain natural materials present in host tissue inhibit growth of radicles, but it appears more likely that toxic chemicals released from autoclaved host tissue may have been responsible for reduced linear growth.

Results of my studies confirmed previous reports by Weir (1918), Peirce (1905), Hawksworth (1961), and Scharpf and Parmeter (1967) in that radicles will not form holdfasts unless some obstruction to the linear growth of the radicle is present. An exception to this finding was noted by Bonga and Chakraborty (1968), who found that indole-3-acetic acid (IAA) could stimulate holdfast formation on "dissected seedlings" in pure culture.

SUMMARY

The seeds of *A. abietinum* and *A. occidentale* have a high initial viability, but are relatively short lived and do not survive from one season to the next in the field. Even under nearly optimum conditions for storage (dry at 2°C.) only about half were viable after a year. Initial viability did not vary significantly with regard to year of collection, area of collection, or plant from which collected. Temperature was the most important factor influencing viability of seeds in storage–2°C. was most nearly optimum. Other factors tested, such as light, relative humidity, and moisture content had no effect except that seeds stored moist or at high relative humidities either germinated or became overrun by mold fungi.

Proper temperature and light favored germination of dwarf mistletoe seeds although light was not required. A temperature of 13°C. was most nearly optimum for both rate of germination and maximum percentage germination of seeds. Other treatments used to promote or increase percentage germination, such as varying pH, scarification, after-ripening, removal of germination inhibitors, and chemical stimu-

lation with host tissue or plant hormones, had no appreciable effect.

Radicles of germinated seeds were significantly influenced by temperature. Survival (longevity) of radicles was inversely proportional to temperature above 5°C., whereas linear growth was directly proportional to temperatures between 5°C. and 25°C. Light did not significantly influence radicle survival, but did have a favorable effect on linear growth. The pH and the presence of host tissue had no significant influence on radicle growth and survival.

LITERATURE CITED

Anonymous
1926. **International critical tables.** l-67-68. New York: McGraw-Hill Book Co., Inc.

Barton, Sela V.
1967. **Bibliography of seeds.** 858 p. New York: Columbia Univ. Press.

Beckman, L. M., and Roth, L. F.
1968. **The influence of temperature on longevity and germination of seed of western dwarf mistletoe.** Phytopathology 58: 147-150.

Bonga, J. M.
1965. ***Arceuthobium pusillum* Peck: collection of seeds and in vitro culture of the early seedling stage.** Can. J. Bot. 43: 1307-1308.

Bonga, J. M., and Chakraborty, C.
1968. **In vitro culture of a dwarf mistletoe, *Arceuthobium pusillum*.** Can. J. Bot. 46: 161-164.

Crocker, William.
1936. **Effect of the visible spectrum upon the germination of seeds and fruits.** *In.* Biological effects of radiation. Ed. B. M. Duggar. 11: 791-827. New York: McGraw-Hill Book Co., Inc.

Crocker, William, and Barton, L. V.
1953. **Physiology of seeds.** 267 p. Waltham, Mass.: Chronica Botanica Co.

Durbin, Richard D.
1956. **A method of separating cactus seed.** Cactus & Succulent J. 28: 50.

Evenari, M.
1949. **Germination inhibitors.** Bot. Rev. 15: 153-194.

Flemion, E., and Poole, H.
1948. **Seed viability tests with 2,3,5-triphenyltetrazolium chloride.** Boyce Thompson Inst. Contrib. 15: 243-258.

Gill, Lake S.
1954. **Dwarfmistletoe of ponderosa pine in the Southwest.** U.S. Forest Serv. Rocky Mt. Forest & Range Exp. Sta. Paper 14, 9 p., illus.

Gill, Lake S., and Hawksworth, Frank G.
1961. **The mistletoes; a literature review.** U.S. Dep. Agr. Tech. Bull. 1242. 87 p.

Hansen, H. N.. and Snyder, W. C.
1947. **Gaseous sterilization of biological materials for use as culture media.** Phytopathology 37: 369-371.

Hawksworth, Frank G.
1961. **Dwarfmistletoe of ponderosa pine in the Southwest.** U.S. Dep. Apr. Tech. Bull. 1246. 112 p., illus.

Heinricher, E.
1915. **Ueber besondere Keimungsbedingungen welche die Samen der Zwergmistel, *Arceuthobium oxycedri* (D.C.) M. Bieb., beanspruchen.** Centralbl. Bakt. 42: 705-711.

Heinricher, E.
1917. **Berichtigende Mittellung uber die Keimungsbedingungen der Samen von *Arceuthobium oxycedri (D.C.)* M. Bieb.** Deut. Bot. Ges. Ber. 35: 204-212.

Hodgeman, Charles D. (Ed.)
1951-52. **Handbook of chemistry and physics.** 33rd ed. Cleveland. Ohio: Chemical Rubber Co.

Kuijt, Job.
1960. **Morphological aspects of parasitism in the dwarf mistletoes *(Arceuthobium).*** Univ. Calif. Publ. in Bot. 30: 337-436.

McCready, R. M., and Reeve. R. M.
1955. **Test for pectin based on reaction of hydroxamic acids with ferric ion.** Agr. & Food Chem. 3: 260-262.

Nash, Shirley N., and Wilhelm, Stephen.
1960. **Stimulation of broomrape seed germination.** Phytopathology 50: 772-774.

Peirce, G. J.
1905. **The dissemination and germination of *Arceuthobium occidentale* Eng.** Ann. Bot. 19: 99-113.

Scharpf, R. F., and Parmeter, J. R., Jr.
1962. **The collection, storage and germination of seeds of a dwarfmistletoe.** J. Forestry 60: 551-552.

Scharpf, R. F., and Parmeter, J. R., Jr.
1967. **The biology and pathology of dwarfmistletoe, *Arceuthobium campylopodum* f. *abietinum*, parasitizing true firs *(Abies* spp.*)* in California.** U.S. Dep. Agr. Tech. Bull. 1362, 42 p., illus.

Srinivasan, A. R., and Subramanian, C. L.
1960. **A review of literature on the phanerogamous parasites.** Indian Counc. Agr. Res., New Delhi. 96 p.

U.S. Forest Service.
1948. **Woody plant seed manual.** U.S. Dep. Agr. Misc. Publ. 654. 416 p., illus.

Weir, James R.
1918. **Experimental investigations on the genus *Razoumojskya.*** Bot. Graz. 66: l-31.

Wicker, Ed F.
1962. **Rapid germination as a viability test for seed of *Arceuthobium* spp.** Phytopathology 52: 757. Wicker, Ed F.
1967. **Seed collection and storage for *Arceuthobium* spp.** U.S. Forest Serv., Intermountain Forest & Range Exp. Sta., Res. Pap. INT-33, 13 p.

GPO 979-253

Scharpf, Robert F.

1970. **Seed viability, germination, and radicle growth of dwarf mistletoe in California.** Berkeley, Calif. Pacific SW. Forest & Range Exp. Sta., 18 p., illus. (U.S.D.A. Forest Serv. Res. Paper PSW-59)

Two species of dwarf mistletoe were studied: *Arceuthobium abietinum* (Engelm.) Hawksworth and Wiens and *A. occidentale* Engelm. Viability of fresh seeds was high and not significantly influenced by year of collection, where collected, or plant from which collected. Temperature affected viability most noticeably. It also significantly affected rate and percentage of seed germination. Longevity of radicles of germinated seeds was inversely proportional to temperatures above 5°C.

Oxford: 176.1 *Arceuthobium* spp. (79):181.524/525-111.5.
Retrieval Terms: Arceuthobium abietinum; Arceuthobium occidentale; seed viability; germination; temperature.

Scharpf, Robert F.

1970. **Seed viability, germination, and radicle growth of dwarf mistletoe in California.** Berkeley, Calif. Pacific SW. Forest & Range Exp. Sta., 18 p., illus. (U.S.D.A. Forest Serv. Res. Paper PSW-59)

Two species of dwarf mistletoe were studied: *Arceuthobium abietinum* (Engelm.) Hawksworth and Wiens and *A. occidentale* Engelm. Viability of fresh seeds was high and not significantly influenced by year of collection, where collected, or plant from which collected. Temperature affected viability most noticeably. It also significantly affected rate and percentage of seed germination. Longevity of radicles of germinated seeds was inversely proportional to temperatures above 5°C.

Oxford: 176.1 *Arceuthobium* spp. (79):181.524/525-111.5.
Retrieval Terms: Arceuthobium abietinum; Arceuthobium occidentale; seed viability; germination; temperature.