♂*Arceuthobium douglasii*

ECOLOGY OF DWARF MISTLETOE SEED

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ABSTRACT

Information on seed viability, germinability, dormancy, and longevity for several species of Arceuthobium is presented and methods for testing these qualities during storage are described. Data on the effects of temperature, relative humidity, and light on seed vitality are reported. Some mold fungi capable of reducing the vitality of dwarf mistletoe seed are identified.

INTRODUCTION

Experimental investigations of dwarf mistletoes (*Arceuthobium* spp.) that involve mass artificial inoculations require large amounts of seed. Fruit of the species discussed in this paper mature in September-October and seed are cast by an explosive fruit mechanism. Thus, it is most convenient and practical to collect the seed at this time (Wicker 1967a).

Unless seed are to be used immediately after collection, maximum germinative capacity must be maintained in storage. Consequently, storage practices can be evaluated only in terms of net effect upon the physiological condition of seed as represented by seed viability, germinability, longevity, and dormancy. These attributes are influenced by such environmental factors as seed moisture content, oxygen supply, temperature, light, relative humidity, and time, as these relate to the quality and rate of respiration.

When my studies were initiated, investigation of many aspects of the biology of dwarf mistletoes in controlled experiments was difficult, if not impossible, because of insufficient knowledge of seed ecology. Successful artificial inoculations were possible only during September-October when seed were being cast, and then only within natural environments. Even so, success was low, seldom exceeding 5%. Most available information relating to seed physiology and ecology was fragmented, general, and largely empirical (Peirce 1905; Heinricher 1915a, 1915b, 1917; Palhinha 1942; Weir 1918). Reliable methods of seed storage and of testing viability or germinability were not available; consequently, little was known about longevity and dormancy.

Previously, I reported practical methods for collecting and storing dwarf mistletoe seed (Wicker 1967a). In the current report, I present information on viability, germinability, dormancy, and longevity of seed of several *Arceuthobium* taxa. If this information is utilized, germinable seed can be available throughout the year for cultural studies.

Coincidental with my studies, Scharpf and Parmeter (1962) and Beckman and Roth (1968) were conducting similar investigations with *Arceuthobium campylopodum* Engelm.

SEED VIABILITY

Materials and Methods

A total of 71 naturally dispersed seed of *A. campylopodum* were collected from the foliage and branches of *Pinus ponderosa* D. Don near Spangle, Wash., in November. Only seed that appeared normal were collected; molded, shriveled, or discolored seed were discarded. In the laboratory, seed were presoaked in distilled water at 22°C for 24 h and then were used for the initial evaluation of 2,3,5-triphenyl tetrazolium chloride (TTC) as a viability test (Flemion and Poole 1948; Lakon 1949, 1954; Smith 1951; On 1952; Parker 1953; Grano 1958; Scharpf and Parmeter 1962). The test solution was prepared by dissolving 100 mg of TTC in 100 ml of de-ionized water.

The presoaked seed were placed in small glass vials containing the TTC test solution. The vials were capped and kept at room temperatures (22-23°C), dark for 3 days. At that time seed were removed from the test solution, excised longitudinally, and the embryo examined for red coloration indicating viability. Any red staining of the embryo was read as a positive (viable) test.

Following this initial test with naturally dispersed seed, the same procedure was used to test laboratory stored seed. Sample lots of 100 seed from each of six *Arceuthobium* taxa that had been in dry, cold, laboratory storage (5°C, 35-45% RH, dark; Wicker 1967a) for 60 days and comparable sample lots of seed stored at room air temperature and room light for 60-67 days were tested. All laboratory stored seed were collected in September and October at the locations reported by Wicker (1965).

Seed of *A. laricis* (Piper) St. John were used in a test to compare the efficacy of 0.1% ethanolic TTC with the 0.1% aqueous TTC. Six lots of 50 seed each were removed from dry, cold, laboratory storage (76 days); three lots were treated with aqueous TTC and three lots with ethanolic TTC. This test was repeated with two lots of 50 seed each of *A. campylopodum* and *A. laricis* following 86 days of dry, cold storage.

Results

Data from the preliminary test using naturally dispersed seed of *A. campylopodum* were very encouraging. Sixty-three of 71 seed (88.7%) showed a positive reaction to TTC.

Results of the test using seed of six taxa of dwarf mistletoe that had been in dry, cold, laboratory storage for 60 days are shown in table 1. These results are very favorable for the viability test and for this particular laboratory storage method.

The results with seed that had been stored at room temperature for 60 days were all negative; no red coloring of the embryo. A second test conducted 7 days later also yielded negative results.

The next test employed *A. laricis* seed that had been in dry, cold, laboratory storage for 76 days. The three lots of 50 seed each treated with the aqueous test solution showed 94%, 88%, and 92% viability; results of the ethanol treatment were all negative.

The results of tests after 86 days of dry, cold, laboratory storage verified the previous test. The seed of *A. laricis* treated with the aqueous test solution showed 94% viability, while the results of the ethanol test solution were again negative. The seed of *A. campylopodum* showed 92% viability when the aqueous test solution was used and zero percentage when the ethanol test solution was used.

Table 1.--*Seed viability as determined by 2,3,5-triphenyl tetrazolium chloride after 60 days of dry, cold, laboratory storage*

Dwarf mistletoe (*Arceuthobium*)	Viability[1]
	%
A. abietinum Engelm. ex Munz	90
A. americanum	76
A. campylopodum	92
A. douglasii	91
A. laricis	94
A. tsugense	87

[1]Based on 100 seed per test.

SEED GERMINATION

Materials and Methods

Petri plates (15 by 90 mm) containing three discs of filter paper (9 cm) were normally employed as germinators. The germinators were sterilized by dry heat for 4 h at 185°C. The filter paper in some germinators was saturated with sterile distilled water; in others it was dry except for moisture in the saturated external viscin on the seed. These germinators are referred to hereafter as "wet" and "dry."

All seed had been in dry, cold, laboratory storage for 100-150 days prior to the germination tests. Unless otherwise stated, (1) all seed were presoaked 18-24 h in sterilized distilled water prior to other treatment and prior to being placed in germinators, and (2) all series of tests were run in duplicate--one in wet germinators, the other in dry germinators. Wet germinators were checked periodically; when seed and filter paper appeared to be dry, sterile distilled water was added until free water was evident in the plates. Tests were run at 5°, 10°, 15°, 20°, and 25°C, at room temperature (22°-23°C), and at "greenhouse temperatures" (ambient). Tests were conducted in the light and in the dark. Complete darkness was not insured, because other workers used the incubators and seed were exposed to room light when incubator doors were open. Percentage germination was determined after 40-111 days.

Some seed received additional pretreatments with various chemicals for surface sterilization or with enzymes to decompose the external viscin. All pretreatments itemized below were performed at room temperatures in the laboratory:

1. Five minutes in 95% ethyl alcohol followed by two 3-min rinses in sterile distilled water.

2. Five minutes in 6% NaClO (full strength Chlorox) followed by two 3-min rinses in sterile distilled water.

3. Two hours in 6% HCl solution (84 ml water plus 16 ml. of 37% HCl, reagent grade) followed by a 10-min rinse in sterile distilled water.

4. Two hours in a 16% H_2SO_4 solution (84 ml water plus 16 ml of 96% H_2SO_4, reagent grade) followed by a 10-min rinse in sterile distilled water.

5. Three minutes in 95% ethyl alcohol, 3 min in 6% NaClO, then 5 min in sterile distilled water.

6. Thirty-six and 72 h in a pectinase solution (500 mg pectinase/10 ml water) followed by a 5-min rinse in sterile distilled water.

7. Thirty-six and 72 h in a hemicellulase solution (500 mg hemicellulase/ 10 ml water) followed by a 5-min rinse in sterile distilled water.

Other chemicals and organic materials were employed in attempts to stimulate seed germination. Some seed were soaked for 5 days in an aqueous extract from Douglas-fir bark and others for 5 days in a horse dung infusion. The seed were incubated in the laboratory (10°C, 38-46% RH, dark) and in the greenhouse at ambient temperatures.

Paired seed lots were treated with dimethyl sulfoxide (DMSO) for various periods of time ranging from 30 min to 4 h. One seed lot received a posttreatment rinse in water for 30 min; the other lot for 1 h. The seed were then placed in dry petri plate germinators and stored in a growth chamber (10°C, 35-55% RH, dark) for the germination period.

Seed were treated for 5 h in 12.5% to 100% solutions of ethylene chlorohydrin, a bud-forcing chemical (Denny 1926a, 1926b; Kotowski 1926; Vacha and Harvey 1927; Deuber 1932). Upon removal from the solutions, seed were placed into dry petri plate germinators and stored in an incubator at 10°C, 35-45% RH, dark. An untreated control was similarly incubated.

The effect of hydrogen peroxide (H_2O_2) (Miége 1908; Leggatt 1929; Parker and Hill 1955; Ching and Parker 1958; Ching 1959) on dwarf mistletoe seed germination was evaluated. *Arceuthobium laricis* seed were removed from dry, cold, laboratory storage (150 days), pretreated 12-24 h with sterile distilled water, then placed in small vials containing aqueous H_2O_2, and sealed. Seed remained in the solutions for the germination period (10 days). Five different concentrations of H_2O_2 were tested at room temperatures under both light and dark conditions.

Following the initial test of the effects of H_2O_2 on *A. laricis* seed germination, the germinative capacities of seed of eight dwarf mistletoe taxa were subsequently tested. Seed were removed from dry, cold, laboratory storage (30-165 days), pretreated with sterile distilled water (18-24 h), and treated in 3% H_2O_2 at room temperature under typical laboratory light conditions.

Additional dwarf mistletoe seed were then tested with 3% H_2O_2 to determine whether or not the distilled water pretreatment was influencing the stimulatory action of H_2O_2.

The H_2O_2 (3%) germination test was compared with the aqueous TTC viability test on *A. americanum*, *A. campylopodum*, and *A. laricis* seed. Tests were run in triplicate following the same procedures outlined for the initial tests with *A. laricis* seed.

Hydrogen peroxide (3%) was used to investigate the effect of aqueous TTC on dwarf mistletoe seed. Seed were removed from dry, cold (5°C, 35-45% RH, dark) storage and soaked for 20 h in distilled water. Fifty seed each of *A. campylopodum* and *A. laricis* were put in the 0.1% TTC solution and 50 seed (controls) of each taxon were placed in distilled water. All seed were kept in the dark at room temperature. After 3 days, the seed were removed from these solutions, placed in 3% H_2O_2, and kept in the dark at room temperature for 10 days, at which time they were observed for germination.

Growth substances were also used in germination tests (Crocker 1948; Crocker and Barton 1957). Samples of 100 seed each were placed in thiamine chloride, glutathione

(1 mg/100 ml water), and ripe coconut milk and left (5°C, dark) in these solutions for 70 days.

Another technique involved submerging seed in water for the entire germination period. Three hundred and seven seed of *A. laricis* that had been in dry, cold, laboratory storage for ca. 145 days were put into a quart jar fitted with a copper screen cap. The jar was placed under running tap water in a laboratory sink for 3 days. After that, the water was run long enough every 2-3 days to effect a change of water on the seed. The seed remained in the water for 43 days.

Germination was also attempted on agar plates. Seed were soaked in tap water for 2-3 days and the viscous covering of each was removed to expose the endosperm. The seed were then treated for 2 min with 6% NaClO followed by a 5-min sterile distilled water rinse. Seed were sown individually on dwarf mistletoe infusion agar plates (50 g of dwarf mistletoe plants were chopped in an electric blender, steeped in distilled water (ca. 10 min), and 20 g of agar were added per 1,000 ml filtrate). Twenty-one seed of *A. campylopodum* that had been in dry, cold, laboratory storage for ca. 105 days and 11 seed of *A. tsugense* (Rosendahl) G. N. Jones that were collected from host twigs were so treated. The plates were stored in the dark at 10°C.

A "rag-doll" germinator was employed in one test. Four paper towels were moistened with distilled water and folded lengthwise to a width of 2 inches. The ends were folded over, the folded towels wrapped with heavy wax paper, and fastened with paper clips prior to being sterilized in an autoclave. Two hundred seed each of *A. laricis* and *A. campylopodum* were taken from dry, cold, storage, placed on the sterilized paper towels inside the "rag-doll," and tested for germination at each of three temperatures. One hundred seed of each species of dwarf mistletoe received further pretreatment for 5 min in 95% ethyl alcohol followed by 15 min in sterile distilled water. The second 100 seed of each species (controls) received no further treatment. The four lots were placed in separate sterilized "rag-dolls." Tests were run at 5°, 10°, and 15°C for 40 days.

Results

All seed tested for germination in the wet and dry germinators failed to germinate, regardless of pretreatment, temperature, moisture, or light conditions used. All seed pretreated with water only and tested in wet germinators molded; seed in dry germinators shriveled, but showed only slight amounts of mold development. Additional pretreatments with alcohol, sodium hypochlorite, hydrochloric acid, and sulfuric acid reduced the amount of mold development, but did not prevent it.

No molds developed on seed treated with pectinase or hemicellulase, which decomposed the external mucilaginous material of the seed. The 72-h pretreatment apparently was too long at the concentrations used; all seed so treated turned white and failed to germinate. Seed were not tested by the TTC or H_2O_2 method at the end of the germination period (91 days). Seed treated with the two enzymes for 36 h also failed to germinate in the dry germinators, but did not turn white. Decomposition of the mucilaginous material appeared to be less than in the 72-h treatment. Seed of *A. laricis* treated for 36 h subsequently were tested by the H_2O_2 method after 85 days in the dry germinators at 10°C. Percentage germination for pectinase treated seed was 22; for the hemicellulase treated seed, 16.

The horse-dung infusion and Douglas-fir bark extract failed to stimulate seed germination after 111 days in the germinators. Seed in the wet germinators molded and those in dry germinators shriveled. Posttreatment with H_2O_2 was not attempted because the treatment had not yet been developed.

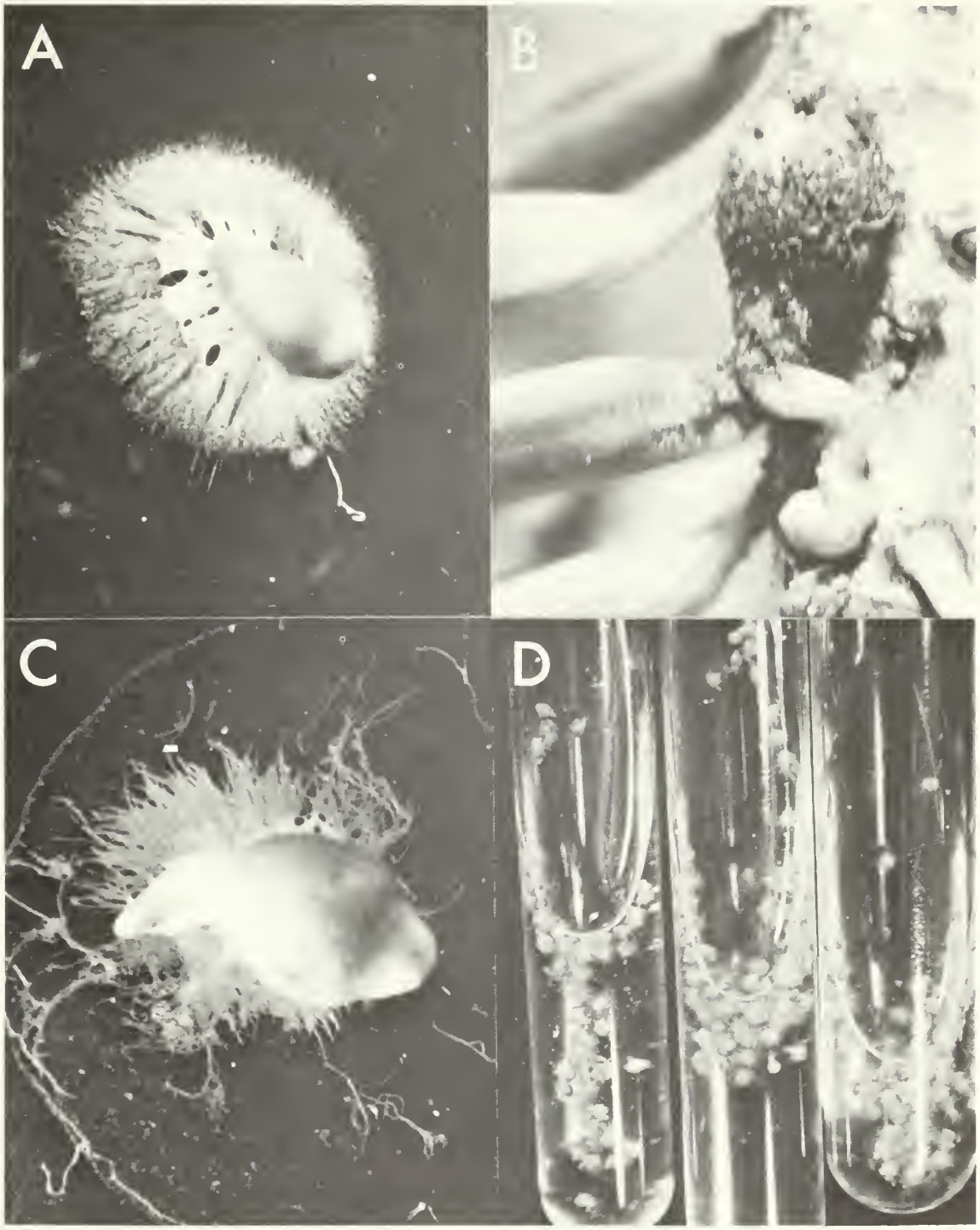

Figure 1.--Dwarf mistletoe seed. (A) Recently expelled seed adhering to a glass surface. Seed was wetted with water to allow the filamentous mucilage cells to fully expand. Upon drying, the mucilage cemented the filaments to the glass surface. X14.4. (B) Seed germinating upon a host's twig in a natural environment. An irregular mound of tissue (holdfast) has developed at the tip of the radicle and is attached to the bark by groups of surface initials. X20. (C) Seed germinated in 3% H_2O_2 solution and placed on a glass surface. Mucilage and mucilage cell have been destroyed to the point the seed will not adhere to the glass surface. X20. (D) Seed germinating in H_2O_2 solution. X1.44.

The DMSO-treated seed showed no germination after 81 days, at which time a malfunction in the growth chamber controls resulted in temperatures exceeding 200°F, thus terminating the experiment. No molds developed on these seed.

After 88 days in the dry petri plate germinators, ethylene chlorohydrin-treated seed and the control had failed to germinate. The seed were placed at this time in H_2O_2 and the germinative capacity of all treated seed was zero, but the germinative capacity of the control sample (18-24 h water pretreatment only) was 60%. Although some seed in the control were molded, treated samples showed no mold development.

The germination tests employing hydrogen peroxide were very encouraging (Wicker 1962). Germination of laboratory stored seed was obvious after 4 days (fig. 1). Treatment was continued for 10 days with hope that all viable seed would germinate. Results of the initial test with *A. laricis* seed, conducted at room temperature under dark conditions, are given in table 2.

Hydrogen peroxide (3%) was equally effective for determining the germinative capacities of dwarf mistletoe seed for the eight taxa tested (table 3). The stimulatory effect of H_2O_2 was enhanced by pretreating the seed with distilled water for 18-24 h (table 4).

Comparisons of the H_2O_2 (3%) germination test with the 0.1% aqueous TTC viability test showed that percentage germination in H_2O_2 was always less than viability as indicated by the TTC test for the three taxa tested. The data (table 5) represent averages for the three samples of 50 seed each.

The dwarf mistletoe seed treated with 0.1% aqueous TTC for 3 days and subsequently treated for 10 days with 3% H_2O_2 failed to germinate. The controls, however, showed 74% germination for *A. campylopodum* and 66% for *A. laricis*.

None of the dwarf mistletoe seed treated with thiamine chloride, glutathione, or raw coconut milk germinated. Upon termination of these tests, seed were treated with 3% H_2O_2. Germination was uniformly low, ranging from 12% to 22% (table 6).

Seed of *A. laricis*, which were continuously submerged in water, showed a germination percentage of 27. The nongerminating seed were not tested for viability (TTC) nor treated with H_2O_2.

Molds were again a major problem in germination tests using agar plates. Only three *A. campylopodum* seed and one *A. tsugense* seed germinated. The rest were destroyed by molds. All the seeded plates were placed in the incubator in an inverted position to reduce the chance of contamination from external sources. The four seed that germinated showed a tropic response; radicles grew down away from the agar surface.

Fungi that developed on agar plates seeded with *A. campylopodum* were predominantly *Penicillium* spp. and *Aspergillus* spp., whereas the only fungus found on plates seeded with *A. tsugense* was *Pestalotia maculiformans* Guba & Zeller.

All seed tested in "rag-doll" germinators failed to germinate. The controls (water pretreatments) were severely molded with *Penicillium* spp. Alcohol-treated seed had molded also, but not as much as the controls. The alcohol pretreatment was later found to be toxic to seed. Seed so treated and then placed in dry germinators for 150 days at 0°, 5°, and 10°C did not germinate. But, when seed were subsequently treated with H_2O_2, with appropriate checks, the alcohol pretreatment reduced germination by 16%-60%.

Table 2.--*Percentage germination (50 seed/sample)*[1] *and length of radicles for seed of* Arceuthobium laricis *treated for 10 days in* H_2O_2

H_2O_2 (%)	Germination	Average length of radicle
	%	*mm*
0.0 (water)	0 (84)[2]	
1.0	84	0.35
2.0	78	.49
3.0	76	.54
4.0	82	.54
5.0	76	.69
Dry	0 (82)[2]	0

[1]Seed had been stored 150 days, 5°C, 35-45% RH, dark. Pretreated 18-24 h with sterile distilled water following removal from storage and prior to H_2O_2 treatment.
[2]Subsequent treatment for 10 days with 3% H_2O_2 gave this percentage germination.

Table 3.--*Germination of 100-seed samples*[1] *treated with 3%* H_2O_2 *at room temperature and light for 10 days*

Dwarf mistletoe *(Arceuthobium)*	Germination
	%
A. abietinum	77
A. americanum	78
A. campylopodum	85
A. cyanocarpum Coulter and Nelson[2]	57
A. douglasii	56
A. laricis	84
A. pusillum Peck[3]	48
A. tsugense	68

[1]Seed had been stored 30-165 days, 5°C, 35-45% RH, dark. Pretreated 18-24 h with sterile distilled water prior to H_2O_2 treatment.
[2]205 seed.
[3]257 seed.

Table 4.--*Effect of pretreatment in distilled water on seed germination of 50-seed samples*[1] *treated with 3%* H_2O_2 *at room temperature for 10 days*

Dwarf mistletoe (*Arceuthobium*)	Water pretreatment	Germination	Average length of radicle
		%	*mm*
A. americanum	Yes	78	0.59
A. americanum	No	68	.52
A. laricis	Yes	76	.31
A. laricis	No	70	.26

[1]Seed had been stored 160 days, 5°C, 35-45% RH, dark.

Table 5.--*Comparison of H_2O_2 germination and TTC viability tests*

Dwarf mistletoe *(Arceuthobium)*	Viability (0.1% aqueous TTC)	Germination (3% H_2O_2; 22°C)
	%	%
A. americanum	84	77
A. campylopodum	90	78
A. laricis	79	71

Table 6.--*Germination of seed placed in H_2O_2 after being treated with growth substances*

Treatment	Dwarf mistletoe *(Arceuthobium)*	Germination[1]
		%
Thiamine chloride	*A. campylopodum*	21
	A. laricis	17
Glutathione	*A. campylopodum*	13
	A. laricis	18
Coconut milk	*A. campylopodum*	12
	A. laricis	22

[1]3% H_2O_2 solution, room temperature, 10 days.

SEED DORMANCY

Materials and Methods

Several hundred seed of *A. americanum* Nuttall ex Engelm., *A. campylopodum*, *A. laricis*, and *A. douglasii* Engelm. were collected during a 2-day period in mid-September 1962. At the time of collection, sample lots of 100 seed of each taxon were soaked in water for 12-15 h, then placed in small vials containing 20 ml of 3% H_2O_2 solution, and sealed. In the interim, the remaining seed were held in dry, cold, laboratory storage. Each week thereafter, lots of 100 seed were removed from storage and placed in H_2O_2 until maximum germination was attained. Each weekly series of tests was of 10 days duration at room temperature in diurnal light.

Results

This experiment was terminated after 5 weeks. Maximum germination apparently was reached prior to its termination (fig. 2). The seed of *A. laricis* were of poor quality as is verified later, but the delay in germination is demonstrable.

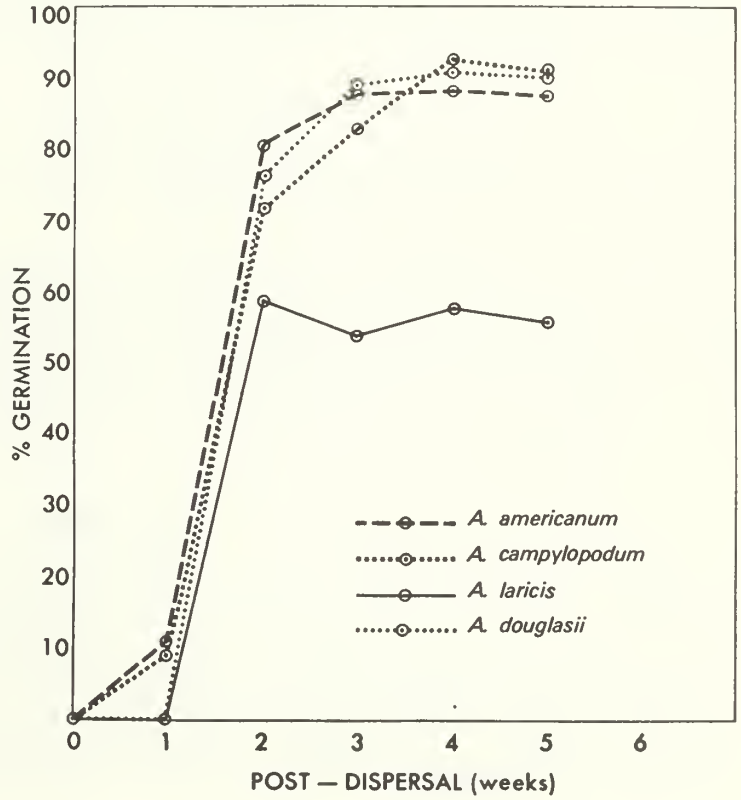

Figure 2.--Percentage germination of dwarf mistletoe (Arceuthobium) *seed during the first 5 weeks of storage following dissemination.*

TEMPERATURE

Materials and Methods

All seed used to study temperature effects had been in dry, cold, laboratory storage.

Eight hundred *A. laricis* seed were removed from storage after 60 days and counted into lots of 50 seed each. Germinative capacity (3% H_2O_2) was 84%. Duplicate lots were tested at each temperature employed. One seed lot received a 24-h pretreatment in sterile distilled water. The second lot received a 24-h soak in tap water, 15 min in 95% ethyl alcohol, and a 15-min rinse in sterile distilled water. Seed lots were scattered in open petri plates and stored in incubators at 0°, 5°, 10°, 15°, 20°, and 25°C. Identical samples were frozen at -3°C, for 32 days and then placed at 10°C for 145 days.

There was little to no control of the relative humidity in these incubators because they were being used by other workers. The incubators were kept dark, except when the doors were open. Germination tests (3% H_2O_2) were run on all seed samples after 177 days.

In another experiment, seed of three dwarf mistletoes that had been in dry, cold, laboratory storage for 30 days were counted into lots of 50 seed each, scattered in open, sterilized petri plates, and placed in dark incubators set at desired temperatures. Seed received no pretreatment. Sample lots were tested for germinability (3% H_2O_2) after 1, 3, 6, 9, and 12 mo. Germinative capacity was 95% for *A. campylopodum*, 89% for *A. douglasii*, and 53% for *A. laricis* when removed from storage.

Results

Data (table 7) show that storage temperatures affect seed viability and indicate the rather pronounced detrimental effects of the ethyl alcohol pretreatment on *A. laricis* seed. Additional data on the time-temperature-viability relationships for seed of three species of *Arceuthobium* during storage are shown in figures 3, 4, and 5. In all three species, optimum storage temperature is near 0°C. Seed source was a confounding factor in these tests. *Arceuthobium campylopodum* seed were from a single source. *Arceuthobium douglasii* and *A. laricis* seed were from mixed sources and, hence, may not have been uniform.

Table 7.--*Effect of storage temperatures on viability of seed of* Arceuthobium laricis

Temperature (°C)	Water	Ethyl alcohol, 95%	Water	Germination[1] (3% H_2O_2)
	h	*min*	*min*	%
-3[2]	24	15	15	28
-3[2]	24	0	0	42
0	24	15	15	12
0	24	0	0	74
5	24	15	15	14
5	24	0	0	60
10	24	15	15	12
10	24	0	0	52
15	24	15	15	0
15	24	0	0	0
20	24	15	15	0
20	24	0	0	0
25	24	15	15	0
25	24	0	0	0

[1]Based on 50 seed/sample after 177 days' storage.
[2]32 days at -3°C; 145 days at 10°C.

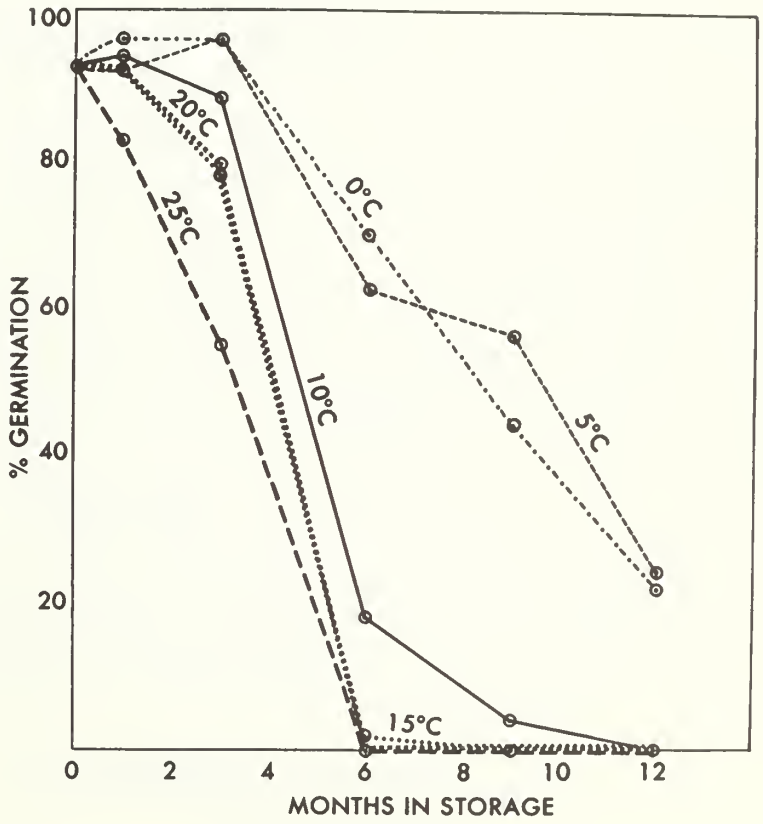

Figure 3.--Effect of storage temperatures on germinability of seed of Arceuthobium campylopodum.

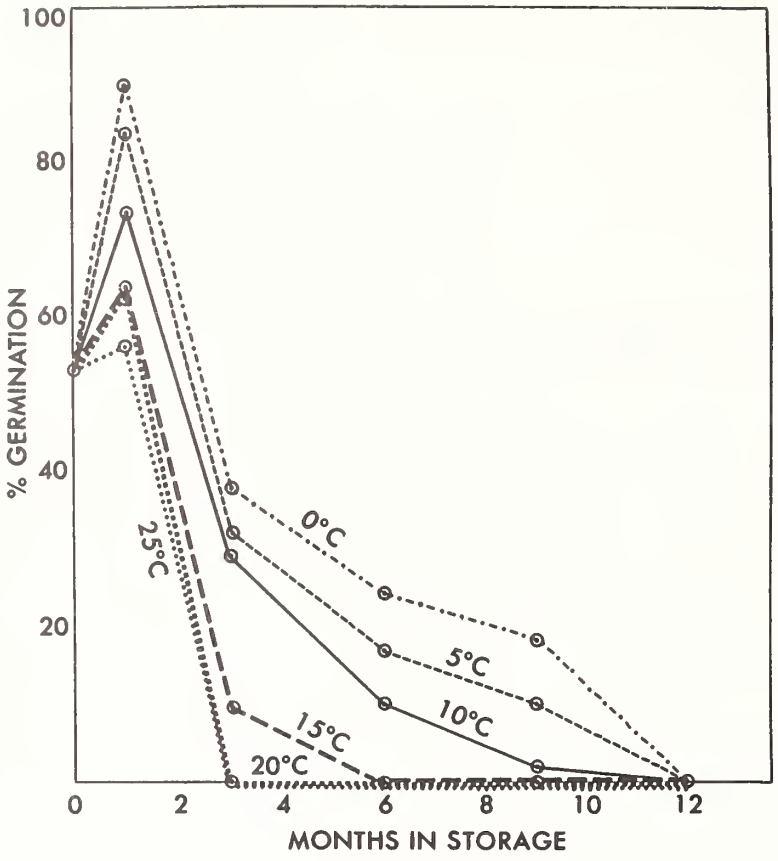

Figure 4.--Effect of storage temperatures on germinability of seed of Arceuthobium laricis.

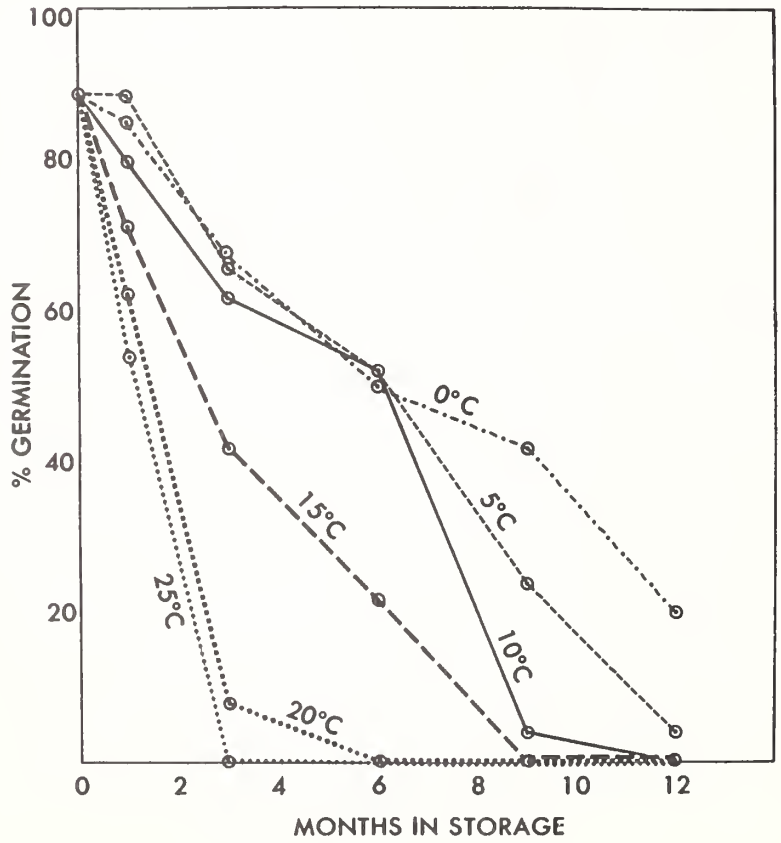

Figure 5.--Effect of storage temperatures on germinability of seed of Arceuthobium douglasii.

RELATIVE HUMIDITY

Materials and Methods

Seed of *A. campylopodum* and *A. laricis* were removed from dry, cold, laboratory storage (ca. 120 days), soaked in tap water for 12-14 h, and counted into lots of 50 seed each. Each lot was placed on a 4-in square of cheesecloth. Corners of the cheesecloth were brought together and fastened with wire, thus enclosing the seed in a cloth sack. Seed were soaked for 15 min in a 0.1% mercuric chloride solution, washed for 5 min in each of three successive changes of sterile distilled water (Scharpf and Parmeter 1962), removed from the cheesecloth, and scattered in sterile glass vials (7x2 cm). The vials were then placed in 1-qt widemouthed jars containing saturated salt solutions, prepared according to the method described by Winston and Bates (1960). Three lots of 50 seed each were placed into each container, which was then sealed. Four different relative humidities were tested at each of two temperatures (5° and 20°C). Seed samples were removed after 30, 60, and 90 days. Percentage germination was determined by the hydrogen peroxide method (3%, 10 days, room light).

Results

At the beginning of the test (i.e., after 120 days dry, cold, laboratory storage), germination was 86% for *A. campylopodum* seed and 35% for *A. laricis* seed. The initial germinative capacity of *A. laricis* seed was too low to indicate the effects of relative humidity or of temperature. The effect of temperature on *A. campylopodum* seed was pronounced (table 8). The data further suggest that high relative humidities may adversely affect the viability of stored seed.

Table 8.--*Germination of dwarf mistletoe* (Arceuthobium) *seed after 30-90 days storage at 5° and 20°C at four different relative humidities*

Dwarf mistletoe	Temperature	Relative humidity	Germination[1]		
			30 days	60 days	90 days
	°C	%	%	%	%
A. campylopodum	5	14.0	74	80	70
	5	40.0	72	78	68
	5	75.0	64	68	34
	5	98.5	66	66	52
	20	12.5	8	14	0
	20	38.0	10	8	0
	20	75.0	4	10	0
	20	98.0	[2]0	[2]0	[2]0
A. laricis	5	14.0	2	4	0
	5	40.0	10	6	0
	5	75.0	2	6	0
	5	98.5	8	4	0
	20	12.5	2	0	0
	20	38.0	0	0	0
	20	75.0	2	0	0
	20	98.0	0	0	0

[1]Germination after 10 days in 3% H_2O_2, 22-23°C.
[2]Contaminated by *Penicillium* spp.

LIGHT

Materials and Methods

Seed of *A. campylopodum* that had been in dry, cold, laboratory storage for ca. 65 days were counted into lots of 100 seed each and scattered in open petri plates. Seed were then stored at 5°C, 35-45% RH, and ca. 700 fc of light from white fluorescent bulbs for 8 h each day for 6 mo. Samples were tested for viability at 60-day intervals by the TTC method. Seed lots left in dry, cold, laboratory storage (dark) were used as checks.

In a second experiment, light intensity was increased to ca. 3,000 fc for 8 h each day. Seed of *A. campylopodum* and *A. laricis* that had been in dry, cold, laboratory storage for 30 days were soaked in tap water for 18-24 h, then counted into lots of 100 seed each and scattered in open petri plates. Temperature and relative humidity were the same as before. Duplicate samples (controls) were stored in the dark. Samples were tested for germinative capacity every 30 days by the hydrogen peroxide method.

Results

Based on two 100-seed samples and determined by the aqueous TTC method, viability of *A. campylopodum* seed was 90.5% at the beginning of the first light treatment. Light had no effect on seed viability under the conditions of the experiment (table 9).

Table 9.--*Viability of* Arceuthobium campylopodum *seed stored under illumination and in the dark*

Treatment time (days)	Viability[1]	
	Light	Dark
	%	%
60	84	81
120	68	71
180	54	56

[1]Determined by 0.1% TTC (in water).

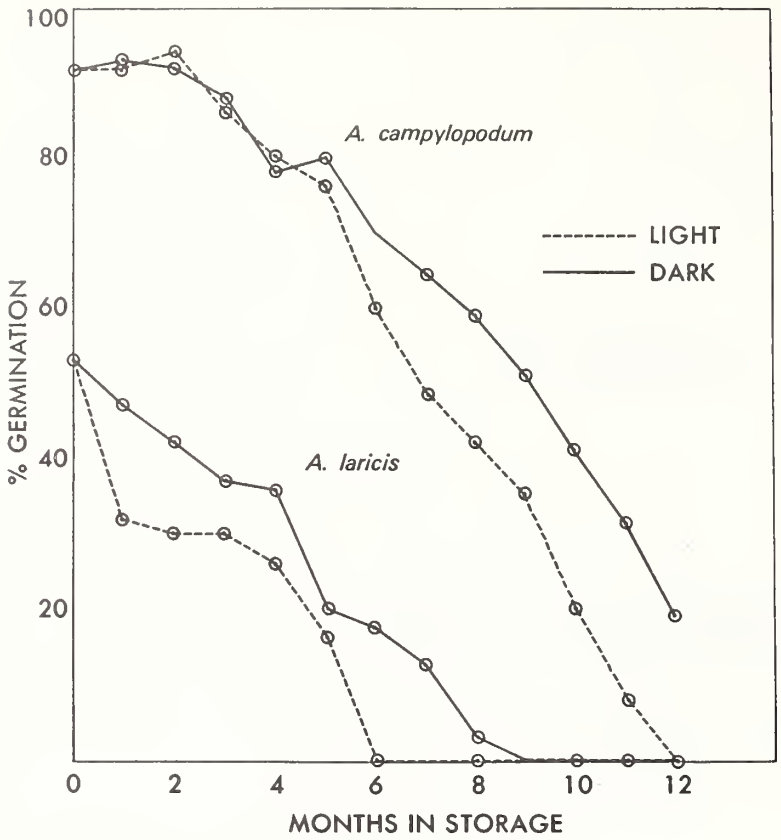

Figure 6.--Effect of ca. 3,000 foot-candles of of light (8 hours/day) during storage (5°C, 35-45% RH) on the germinability of dwarf mistletoe seed.

Results of the second experiment are illustrated in figure 6. The seed of *A. campylopodum* were all from the same source and were of high quality (93% viability) at the beginning of the tests. Differences between storage under illumination and dark were not marked until after 6 mo of treatment. This suggests that the effect at this lighting (3,000 fc, 8 h/day) was one of temperature rather than light.

All *A. laricis* seed were also from a single source but the viability of these seed was relatively low (53%) at the start of the tests. Differences between light and dark were rather sharp after 30 days of treatment.

MOLDS AND BACTERIA

Materials and Methods

Fungi and bacteria from molded seed were grown on 2.5% potato dextrose agar (PDA) plates. Each plate was inoculated with a single, molded seed and incubated in the dark at 20°C for a week. Successive transfers of all colonies were made until pure cultures were obtained. Seed used were from three sources: (1) *A. laricis* collected at Priest River Experimental Forest (PREF), Priest River, Idaho, that molded during stratification in moist, sterile sand (Wicker 1965, 1967a); (2) 20 seed each of (a) *A. laricis* collected at Quartz Creek and PREF, Bonner County, Idaho; (b) *A. laricis* from Sherman Creek, Ferry County, Wash.; and (c) *A. campylopodum* collected 4 miles north of Spangle, Spokane County, Wash., that had molded during moist laboratory storage at 5°C (Wicker 1965, 1967a); and (3) thirty-eight seed of *A. laricis* collected at PREF and 42 seeds of *A. douglasii* collected 3 miles east of Viola, Latah County, Idaho, that molded during the winter in the field on their coniferous hosts at an artificial inoculation plot, PREF.

Results

The fungal isolates from seeds of Source 1 were tentatively identified to species. Fungi isolated from 78 seeded PDA plates and their frequency of occurrence were as follows: *Alternaria tenuis* Nees, 79.5%; *Mucor sphaerosporus* Hagem, 70.5%; *Trichoderma lignorum* (Tode) Hatz, 60.3%; *Rhisopus microsporus* van Tiegh. and *Rhizopus stolonifer* (Ehr. ex Fr.) Vuill., 53.8%; *Stemphylium botyrosum* Wallroth, 39.7%; *Penicillium decumbens* Thom, 29.5%; *Fusarium* spp., 20.5%; miscellaneous fungi, 6.4% bacteria, 67.9%.

Many fungi were isolated from seeds of Source 2 (table 10). None of these isolates have been identified to species. Some isolates could not be identified even at the generic level; these are shown in table 10 as "miscellaneous fungi." Source 3 seed of *A. laricis* and *A. douglasii* produced even a greater array of fungi (table 11). Again, some rarely occurring, nonsporulating isolates were not identified and are listed as "miscellaneous fungi" in the table.

Table 10.--*Fungi isolated from dwarf mistletoe* (Arceuthobium) *seed which molded during moist, cold storage*

Genera	Frequency	
	A. campylopodum	*A. laricis*
	%	%
Cephalosporium	--	55.0
Alternaria	55.0	45.0
Aureobasidium	35.0	40.0
Candida	40.0	10.0
Paecilomyces	40.0	20.0
Penicillium	--	35.0
Hormiscium	--	25.0
Kloeckera	--	25.0
Stemphylium	25.0	--
Streptomyces	20.0	--
Trichoderma	15.0	15.0
Botrytis	--	15.0
Pestalotia	10.0	--
Rhizoctonia	--	5.0
Cladosporium	--	5.0
Miscellaneous fungi	15.0	35.0

Table 11.--*Fungi isolated from dwarf mistletoe* (Arceuthobium) *seed which molded on the host trees in the field*

Genera	Frequency	
	A. laricis	*A. douglasii*
	%	%
Epicoccum	73.7	52.4
Stemphylium	13.2	23.8
Hormiscium	7.9	21.4
Phyllosticta	13.2	9.5
Coniothyrium	--	11.9
Aureobasidium	7.9	9.5
Alternaria	7.9	7.1
Candida	7.9	4.8
Rhizoctonia	5.3	7.1
Streptomyces	5.3	--
Fusarium	5.3	--
Monilia	2.6	--
Pestalotia	2.6	--
Mucor	2.6	--
Cephalosporium	2.6	--
Helminthosporium	2.6	--
Penicillium	--	2.4
Paecilomyces	--	2.4
Miscellaneous fungi	18.4	31.0
Yeast	15.8	2.4
Bacteria	23.7	16.7

DISCUSSION

Various physical, biochemical, and cultural factors, as well as direct germination, have been employed to measure seed quality (viability and germinability). In some of the literature (U.S. Dep. Agric. 1948; Ching and Parker 1958; Kramer and Kozlowski 1960; Mayer and Poljakoff-Mayber 1963), viability and germinability are not clearly differentiated. In reporting these investigations, the two terms are used as defined by Amen (1963) and Webster's Third International Dictionary. Viability--the ability to maintain life--may or may not be a measure of germinability. The fact that a seed is living does not imply that it will germinate. Rarely is germinability 100% of viability. Viability tends to decrease with time; nevertheless, the germinability of those seed that are still viable may increase with age (Amen 1963). Germinability--the ability of an embryo to resume growth--is a measure of viability, but the reverse is not necessarily true.

Viability of dwarf mistletoe seed (table 1) is readily and rather accurately determined by the 0.1% aqueous 2,3,5-triphenyl tetrazolium chloride (TTC) viability test. The test is run at room temperature (22°C) for 3 days in the dark. The ethanol TTC test was unsatisfactory for *Arceuthobium* seed under the conditions of the experiments. The ethanol insolubility of the viscin, which coats the seed, perhaps was a limiting factor in these tests. Also, 95% ethanol is toxic to seed (table 7), probably because it rapidly reaches the embryo in the area not covered by the viscin. Viability determined by the aqueous TTC test was 7-12% higher than germinability determined by the H_2O_2 method (table 5).

The most important environmental requirements for seed germination are water, oxygen, and favorable temperature. Light is required by some seed, but is not essential for germination of *Arceuthobium* spp. Specific requirements vary with plant species. The important processes required for seed germination are water absorption, digestion, respiration, and assimilation (Kramer and Kozlowski 1960; Mayer and Poljakoff-Mayber 1963). According to Toole and Hendricks (1956), seed germination reflects a balance between germination promoters and inhibitors; the ratio is specific for each species.

All conditions defined for seed germination were met individually or in combinations by these investigations. However, mature, naturally discharged dwarf mistletoe seed failed to germinate during laboratory storage. Most factors tested, e.g., stimulants, sterilants, enzymes, temperature, relative humidities, light, etc., failed to stimulate germination.

Stored seed placed on agar plates germinated if they remained free of mold fungi. This is a laborious and impractical method when working with large numbers of seed. Some seed germinated when submerged in water for 43 days. Preparation time and the low percentage germination restrict the usefulness of this method. Nongerminating seed were not subsequently treated with H_2O_2; but, it is doubtful that the short storage in water is detrimental because some seed germinated. Both of these methods require 35-50 days for germination of stored seed; however, results suggest that free water is necessary for germination. Bonga (1972) has recently reported evidence which also supports this conclusion. The low germinative capacity of dwarf mistletoe seed submerged in water suggests that oxygen was a limiting factor. Later investigations showed that temperature was also a limiting factor.

Hydrogen peroxide stimulates germination of stored dwarf mistletoe seed; germination is visible macroscopically within 4 days. This treatment has proved to be an invaluable tool in investigations of seed storage conditions, dormancy, and ecology and in artificial inoculation studies. Problems created by molds and bacteria are eliminated. Tests with H_2O_2 have been run under light and dark conditions and at 10°C and 22°C without significant variation. Germination is so rapid in H_2O_2 that the effects of temperature are nullified. Thus, asepsis, rapidity, and simplicity are major attributes of the H_2O_2 method. Rate of germination, as measured by radicle length, increased as concentrations of the test solution increased up to 5% H_2O_2 solution.

The H_2O_2 germination method was effective for seed of the eight taxa tested, including *A. pusillum*. Bonga (1965) cited my report (Wicker 1962) that H_2O_2 stimulated seed germination in several dwarf mistletoes, but concluded from his own data that "such a reduction in germination time,...was not noted for *A. pusillum*." He might have corroborated my findings if he had followed the germination testing method that I reported. The stimulatory effect is enhanced by pretreating the seed for 18-24 h in distilled water. Percentage viability was lower when determined by this method than by the TTC method. However, the H_2O_2 method is a germinability test as well as a viability test. It is more reliable and more useful than chemical staining tests for viability because such tests check on only one definite reaction which may be correlated with germinability. Furthermore, seed treated with TTC for 3 days and subsequently placed in H_2O_2 for 10 days did not germinate. Thus, although TTC is useful for a destructive viability test, it is lethal to seed of *Arceuthobium* spp. The TTC staining test results in formation of color-visible products in the presence of active oxidizing enzymes and may inactivate the enzymes.

The mode of stimulation by H_2O_2 in germination is not known. Ching (1959) discussed four possible stimulatory mechanisms of H_2O_2. The H_2O_2 treatment supplies seed with both water and oxygen but since H_2O does not show the stimulating effect of H_2O_2, the additional oxygen must be the key factor. Based on this assumption, the first approach to an understanding of the mode of activating germination would be to determine the fate of the oxygen. Perhaps radioactive tracer techniques employing $H_2O_2{}^{14}$ or $H_2O_2{}^{15}$ could be applied here.

Data illustrated in figure 2 indicate that a short period of seed dormancy may exist for the four taxa investigated. The concept of seed dormancy was recently reviewed by Amen (1963). He lists five major causes of seed dormancy: (1) rudimentary embryos; (2) physiologically immature embryos (inactive enzyme systems); (3) mechanically resistant seed coats; (4) impermeable seed coats; and (5) presence of germination

inhibitors. In general, seed dormancy is classified as either seed coat or embryo dormancy. The duration of seed dormancy ranges widely among species from a few days to several years. Seed dormancy is specific for the species, but not all seed of a given species are necessarily dormant.

Arceuthobium does not have a true seed coat (Johnson 1888; Thoday and Johnson 1930; Gill 1935; Cohen 1962, 1963). The seed consists essentially of a naked embryo and endosperm encased by the crushed tissues of the ovarian mound or the pericarp of the fruit, or perhaps both (Gill and Hawksworth 1961). Therefore, seed coat dormancy cannot be a factor.

Scharpf and Parmeter (1962) state that *A. campylopodum* seed require no after-ripening period. However, I believe their data on seed germination clearly indicate an after-ripening period because some seed germinated within 30 days but maximum germination was not achieved until after 60-90 days. Furthermore, if the enzymatic and chemical changes necessary for germination occur in less than 30 days, this period of embryo dormancy could not be defined in their data because 30 was the minimum days of germination used. Beckman and Roth (1968) speak of "a dormancy factor of unknown nature....The presumed inhibitor, probably of a chemical nature and associated with the seed covering,...." Cohen (1963) reported that the embryo of dwarf mistletoe seed develops directly into the seedling without cessation of growth, a condition which he calls "vivipary." Hawksworth (1961) stated that seed of *A. vaginatum* subsp. *cryptopodum* (Engelm.) Hawksworth and Wiens germinate within 1 month following dissemination in late July and early August.

If dwarf mistletoe seed can be assumed to be mature at the time of natural dissemination, then the data (fig. 2) would indicate a dormant period of ca. 24 days for the four species tested. The validity of the assumption is questionable, however, because physiological maturity at the time of seed dissemination is not completely understood.

Temperature and relative humidity are the critical factors in respect to viability and longevity during seed storage. Higher temperatures most likely increase cell metabolism and enzyme activity. High relative humidities or free water at low temperatures not only provide conditions favorable for development of molds but probably cause a deficient oxygen supply. High moisture content lowers seed tolerance to heat (Stanley and Butler 1961).

The effect of relative humidity is not as striking as that of temperature (table 8). Nevertheless, both trends are there for seed of *A. campylopodum*. Data on *A. laricis* are not reliable because seed were of poor quality (35% viability). The effects of both increasing temperature and relative humidity (>75%) decreased germination.

Temperature affects both viability and longevity of stored seed. A valid comparison cannot be made with the data in table 8 between *A. campylopodum* and *A. laricis*, because viability was not comparable at the beginning of the experiment. The data for *A. laricis* (table 7) indicate the temperature effect. The alcohol pretreatment markedly reduces germination as does storage at constant freezing temperatures (-3°C). The maximum temperature for storage of *A. campylopodum* (fig. 3), *A. laricis* (fig. 4), and *A. douglasii* (fig. 5) seed for 10 months or more is between 5° and 10°C. Under the conditions of the experiment, the optimum temperature for all three taxa is the lowest temperature used, 0°C. These storage temperatures are in agreement with those reported by Scharpf and Parmeter (1962) for *A. campylopodum*.

An effect of light on seed viability was not detected under the conditions of these experiments. Light did not affect viability of *A. campylopodum* seed after 180 days of storage (table 9). Results presented in figure 6 are considered to be due to temperature rather than light *per se*.

Molds were a very disturbing problem throughout the investigations of seed storage, viability, and germination. The only reference to molds in the published literature on dwarf mistletoes was by Scharpf and Parmeter (1962), although J. R. Weir mentions the problem in unpublished field notes. Apparently, Beckman and Roth (1968) encountered this problem, but not Knutson (1971). Most of the literature on molds of seed deal with stored grains and forest tree seed (Christensen 1957; Gibson 1957; Lawrence and Rediske 1962; Salisbury 1953; Shea 1960; Timonin 1964; Noble and others 1958; Tuite and Christensen 1955, 1957).

The fungi listed do not represent the range of seed coat microflora for dwarf mistletoes because conditions of experimentation (media, temperature, pH, light, etc.) were selective in respect to the growth of fungi; therefore, the tables present a restricted sample of the seed microflora for a particular set of conditions. The fungi (tables 10-11) isolated also reflect the different localities of seed collection. *Arceuthobium campylopodum* came from a single location near Spangle, Wash., whereas *A. laricis* came from Sherman Creek, Ferry County, Wash., and Priest River Experimental Forest in northern Idaho. *Arceuthobium laricis* seed (table 11) were collected at Priest River Experimental Forest and *A. douglasii* at Sherman Creek, Ferry County, Wash., and 3 miles east of Viola, Latah County, Idaho. The majority of the fungi are common airborne contaminants, but their effect on seed viability and decay, particularly under conditions of high relative humidity, certainly deserve recognition. The mucilaginous coating of dwarf mistletoe seed, when moist, is a suitable substrate for these fungi. Seed may be destroyed by these fungi in the field during the overwintering period as well as during laboratory storage (Wicker 1967b). In this respect, they can be considered a facet of biological control.

CONCLUSIONS

Viability of dwarf mistletoe seed is accurately measured by the aqueous TTC test. Hydrogen peroxide rapidly stimulates germination. There is a brief period (ca. 24 days) of physiological dormancy indicated for the four taxa tested.

Temperature and relative humidity affect vitality and longevity of dwarf mistletoe seed. An effect of light was not detected. Free moisture for prolonged periods is conducive to mold development and deficient oxygen for metabolic processes. The mucilage covering the seed is a suitable substrate, when moist, for numerous fungi which may destroy the seed. I concur with Kuijt (1960) in that "no particularly unusual conditions" are required for germination.

It is established that many factors of the environment affect vitality, longevity, and germinability of dwarf mistletoe seed. It is well to remember that these factors never function individually or constantly in their effect upon the organism. Therefore, the effects of environmental factors upon the vital processes of dwarf mistletoe seed are meaningful only in terms of their composite actions and interactions.

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Information on seed viability, germinability, dormancy, and longevity for several species of Arceuthobium is presented and methods for testing these qualities during storage are described. Data on the effects of temperature, relative humidity, and light on seed vitality are reported. Some mold fungi capable of reducing the vitality of dwarf mistletoe seed are identified.

KEYWORDS: hydrogen peroxide, 2,3,5-triphenyl-tetrazolium chloride, Arceuthobium abietinum, Arceuthobium americanum, Arceuthobium campylopodum, Arceuthobium douglasii, Arceuthobium laricis, Arceuthobium tsugense, Arceuthobium cyanocarpum, Arceuthobium pusillum.

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Headquarters for the Intermountain Forest and Range Experiment Station are in Ogden, Utah. Field Research Work Units are maintained in:

Boise, Idaho
Bozeman, Montana (in cooperation with Montana State University)
Logan, Utah, (in cooperation with Utah State University)
Missoula, Montana (in cooperation with University of Montana)
Moscow, Idaho (in cooperation with the University of Idaho
Provo, Utah (in cooperation with Brigham Young University)
Reno, Nevada (in cooperation with University of Nevada)