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Germination, Respiration and Photosynthesis in Seeds of Dwarf Mistletoe (*Arceuthobium*)

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Germination, respiration, and photosynthesis in seeds of dwarf mistletoe (Arceuthobium) were studied. The effects of 1 hour soakings of seeds in aqueous solutions of 1, 2, or 3% H_2O_2 or 1, 2, 3, 4, or 5% Chlorox on germination of seeds were tested. Germination rates for seeds from three consecutive years (1975-1977) were obtained. Germination rates varied widely. This variance transcended subtle changes in treatment. Pretreatment of seeds with Chlorox resulted in signifi-

cantly lower germination rates than those of the control. Chlorophyll concentrations were determined for seeds and aerial shoot tissue of four species. Seeds of A. douglasii had the highest chlorophyll concentration (0.39 mg/g fresh weight) while seeds of A. tsugense had the lowest concentration (0.25 mg/g fresh weight). Net O_2 uptake by seeds of A. campylopodum in dark and in light was measured by manometric and polarographic methods. The mean values were 353 µl O_2 $g^{-1}h^{-1}$ in the dark and 201 µl O_2 $g^{-1}h^{-1}$ in the light. The difference between these rates is apparently due to O_2 evolution during photosynthesis. In light the seeds can fix 43% of the CO_2 produced by respiration. Experiments in which seeds were exposed to $^{14}CO_2$ in light confirmed that the seeds are able to fix CO_2. Extraction of seeds with ethanol showed that 97-99% of the incorporated ^{14}C was ethanol soluble. Ten to sixteen percent of the ethanol fraction was chloroform soluble while the rest was H_2O soluble. Ion exchange separation of the H_2O phase showed that 11-25% of ^{14}C activity was cationic, 15-29% anionic, and 53-67% neutral.

GERMINATION, RESPIRATION AND PHOTOSYNTHESIS IN SEEDS OF DWARF MISTLETOE (ARCEUTHOBIUM)

by

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INTRODUCTION

Dwarf mistletoes (Arceuthobium sp.) are parasitic angiosperms which grow upon members of the Pinaceae in North America. They adversly affect their hosts in a variety of ways: by reducing tree growth, by distorting normal patterns of growth, by reducing seed yield, by reducing resistance to other diseases and insects, and in severe cases by causing death (Beckman, 1964; Hull and Leonard, 1964). Childs and Shea (1967) estimate the annual loss of wood in Oregon and Washington due to dwarf mistletoe to be 147 X 10^6 cubic feet. The infection process begins when the radicle of a germinating seed forms a holdfast. The holdfast develops a wedge-like piece of tissue which then penetrates the host (Hawksworth and Wiens, 1972). Knowledge of dwarf mistletoe seed physiology is important to the establishment of effective methods of control of the parasite.

While the primary purpose of this investigation was to examine the photosynthetic capacity of dwarf mistletoe seeds, other physiological characteristics of the seeds were studied. Since it was necessary to germinate substantial quantities of seeds, germination data was accumulated. Owing to fungal contamination of germinating seeds several experiments were designed to test the effectiveness of various delutions of H_2O_2 and Chlorox as anti-fungal pretreatment solutions. Chlorophyll concentrations were determined for seeds and aerial shoots of four species of Arceuthobium. Net O_2 incorporation by seeds of A. campylopodum in light and dark was measured by manometric and polaro-

graphic methods. Experiments were carried out to determine the extent of assimilation of $^{14}CO_2$ in the light and in the dark.

LITERATURE REVIEW

GERMINATION

Beckman and Roth (1968) and Knutson (1974) have defined germination as the stage when the radicles have emerged from the endocarps of the seeds. In vitro germination of dwarf mistletoe seeds is dependent on various factors including a dormancy or an after ripening period, storage conditions, length of storage, and environmental conditions such as presence or absence of light, light intensity, moisture, presence of pathogens (fungi and bacteria), and temperature (Beckman, 1964; Scharpf, 1970; Knutson, 1974; and Wicker, 1974). Water is the most important requirement for germination (Kuijt, 1969). While light is not presently considered to be an absolute requirement for germination, both Scharpf and Parmeter (1962) and Beckman (1964) reported that light significantly increased the percentage of seeds which germinated. Beckman (1964) reported germination rates over 70% for seeds exposed to light at 680 foot candles as compared with rates of 35% for seeds germinated in the dark.

Various germination rates for different species under light have been reported. Bonga (1965) reported 75% germination in seeds of A. pusillum which had been surface sterilized with 3% H_2O_2. He found that NaOCl, $MgCl_2$, and ethanol were unsatisfactory as surface sterilizers. Beckman and Roth (1968) examined the effect of various constant temperatures during storage upon germination of A. campylo-

podum. They appeared to have a short dormancy as only 2% of seeds stored for 30 days at 1.5°C germinated while 80% of seeds stored for 180 days at 1.5°C germinated. Knutson (1974) reported an apparent dormancy period for A. campylopodum in the germination rates were low (10-20%) immediately after harvest but increased to a maximum of 70-80% in seeds stored for 80-100 days. Germination dropped to 10-20% after 450 days. Wicker (1974) reported germination rates resulting from several different treatments of several species. In seeds stored 30-165 days at 5°C he reported the following rates: A. abietenum, 77%; A. americanum, 78%; A. campylopodum, 85%; A. douglasii, 56%; and A. tsugense, 68%. He also tested for dormancy and reported that germination percentages for four species, A. americanum, A. campylopodum, A. laricis, and A. douglasii, increased from 0% at dispersal time to 55-85% at two weeks post-dispersal. The rates leveled off during the next three weeks to yield maximum rates of 80-90% except for A. laricis, which had maximum rates between 50 and 60%. This one to two week dormancy is much shorter than those reported earlier (Beckman and Roth, 1968, and Knutson, 1974) of 30-60 days for seeds of A. campylopodum.

PHOTOSYNTHESIS

Most of the previous investigations concerning photosynthesis in dwarf mistletoe have been on the aerial shoots' ability to assimilate CO_2. Rediske and Shea (1961), studying photosynthesis in A. americanum growing on Pinus contorta, found that the aerial shoot assimilated CO_2 and also accumulated photosynthate, mainly sucrose, from the

host. They proposed that the dwarf mistletoe acted as a physiological girdle, intercepting all photosynthate produced distal to the infection site, blocking its basipetal translocation. Hull and Leonard (1964a) reported that A. campylopodum growing on a variety of hosts also assimilated CO_2. They also reported that dwarf mistletoe accumulated photosynthate, principally sucrose from the host throughout the year, and found that basipetal translocation of photosynthate in the host's branches was not blocked by dwarf mistletoe infections. Hull and Leonard (1964b) found the parasite photosynthate was never translocated to the endophytic system or to the host. They reported chlorophyll concentrations in dwarf mistletoes from 0.24 to 0.48 mg/g dry weight, 10 to 20% of that found in the hosts. Miller and Tocher (1975) found that aerial shoots of A. tsugensi fixed 10-15% of available $^{14}CO_2$ in one hour. Their IRGA studies showed an apparent CO_2 fixation rate of 80-90 µl CO_2 $g^{-1}h^{-1}$, 20-30% of the amount of CO_2 produced during respiration.

Tainter (1971) reported that chloroplasts in A. pusillum contained no distinct grana, but a variable number of parallel lamellae. The chloroplasts accumulated large amounts of starch and were functional. Chloroplasts were found in the aerial shoots, the endophytic system, and in the endosperm of seeds. However, Dodge and Lawes (1974) describe the chloroplasts of A. oxycedri to be large, consisting of numerous grana made up of varied number of thylakoids and extensive lamellae. The stroma contains DNA, occasional starch grains and ribosomes. These chloroplasts are typical angiosperm chloroplasts. It appears that the chloroplasts show variation between

species and more investigation is needed.

There have been several observations that dwarf mistletoe seeds contain chlorophyll and this has resulted in speculation that the seeds have photosynthetic capability. Cohen (1963), in a description of the anatomy of the seedling, described the stomates, attributing them with the function of gas exchange during "probable photosynthesis in the green, long-lived seedlings." Kuijt (1969) referred to the chlorophyllous endosperms of the Viscaceae as "seats of great photosynthetic activity." Scharpf (1970) commented on the possibility that photosynthesis occurs in the chlorophyllous seeds, supplying energy necessary for establishment on the host. Muir (1975) did two experiments in which he exposed ten germinated seeds each to $^{14}CO_2$ in light and in dark. He reported that the seeds exposed for 48 hours in light averaged 2,440 cpm and in dark averaged 310 cpm. He concluded that although the amount of CO_2 assimilated was small, it may play a significant role in the supply of energy to the seeds.

MATERIALS AND METHODS

COLLECTION AND STORAGE OF SEEDS

Seeds were collected during September and October of 1975, 1976, and 1977 (see Table I for dates and sites). Seeds were collected by placing a brown kraft bag over aerial shoots with ripe fruits and shaking vigorously. This shaking induced the seeds to fire and stick to the bag. In some cases aerial shoots were picked from the host tree, placed in a bag and shaken vigorously with the seeds firing and sticking to the bag. Once a bag was full, it was placed in an ice chest until arrival at the laboratory. In the laboratory seeds were removed from the bags by soaking them in warm water thereby hydrating the viscin permitting the seeds to slide free. They were then rinsed several times to remove dirt and debris. When the seeds appeared to be reasonably clean, they were spread onto 9 cm filter papers and allowed to dry. The storage proceedure was slightly modified from Knutson (1974) in that the filter paper discs were strung on a string with approximately 0.5 cm between each disc, instead of being placed on a glass rod. The string was affixed to a lid of a gallon jar and suspended in the jar over a saturated NaCl solution. The lids were sealed and the jars were stored in a refrigerator at approximately 4°C. This method results in a relative humidity of 75% inside the storage jar (Knutson, 1971).

TABLE I

COLLECTION SITES

SPECIES	MONTH AND YEAR	SITES
A. campylopodum	September 1975 October 1976 September 1977	#1. collected on Pinus ponderosa growing along F. S. road 139 near Camp Sherman, Oregon, Deshutes National Forest T.13S R.9E, Section 11.
A. tsugense	October 1975 October 1976 October 1977	#2. collected on Tsunga heterophylla growing along logging roads; S47c, S47d, S47e, and near Goat Mtn.,. Estacada Ranger District, Mt. Hood National Forest T.5S R.4E, Section 11, 12, 14, 15.
A. americanum	September 1975 October 1976 September 1977	#3. collected on Pinus contorta growing along F. S. roads 2016 and 104 near Pringle Falls, Deschutes National Forest Tx21S R.9E and R.10F.
A. douglasii	September 1976 October 1977	#4. collected on Pseudotsuga menziesii growing along Hwy 216 within 1 mi. each side of Bear Spring Campground. T.5S R.10E, Sections 22 and 23. Bear Springs Ranger District Mt. Hood National Forest.
	October 1977	#5. collected on P. menziesii growing along F. S. road S483 going to Keeps Mill Bear Springs Ranger District Mt. Hood National Forest T.5S R.10E, Section 11.

GERMINATION OF SEEDS

Seeds of Arceuthobium campylopodum were used in the following germination studies. The general method used for germinating the seeds in most of the physiological experiments in this study was as follows. The seeds were first removed from filter papers by soaking in water for an hour, then they were soaked in 2% H_2O_2 for one hour to surface sterilize. Then they were rinsed three times with sterile distilled H_2O and transferred to a clean beaker and covered with sterile distilled H_2O. The water was changed daily to further discourage growth of microorganisms. When seeds germinated (the radicles ruptured the endocarp crests) they were separated from nongerminated seeds and placed in petri dishes. These seeds were watered once a day and any excess water was poured off. The seeds were kept in either a light box in a refrigerator or in an environmental growth chamber. Temperature ranged from 4 to 15°C. Light intensity was measured in the growth box at $4.3 \times 10^3 - 5.4 \times 10^3$ lux and in the growth chamber between $1.08 \times 10^4 - 1.40 \times 10^4$ lux.

The effects of different concentrations of H_2O_2 on seed germination and inhibition of fungal growth on seeds were tested using a series of solutions of 1, 2, and 3% H_2O_2. Seeds were placed in petri dishes at the rate of 100 per dish. They were then soaked in one of the solutions (four dishes/solution) for one hour, then rinsed three times with sterile distilled H_2O, and finally covered with sterile H_2O. Water was changed daily to further prevent contamination by microorganisms. Records of number of seeds germinated per plate were

kept. This experiment was run each year, 1975, 1976, and 1977, to see if germination rates showed annual variations. The second and third year five instead of four samples were used for each treatment.

During December 1976 a similar experiment was made, testing the effects of pretreatment of various strength (one to five percent) solutions of Chlorox on seed germination and fungal contamination. Fifteen samples of fifty seeds each were used, three samples per treatment. The seeds were placed in petri dishes and soaked in either 1, 2, 3, 4, or 5% solutions of Chlorox for one hour. Then the seeds were rinsed three times with sterile distilled H_2O and then were covered with sterile distilled water, which was changed daily. Percentages of germination were recorded.

In January 1978 an experiment was carried out to determine if a short exposure (five minutes) to red light enhanced germination of A. campylopodum seeds. Two 100 seed samples in petri dishes (uncovered) were placed in a box with a red light filter (650 nm) taped over the top. Light was provided by a 150 watt light in a goose neck lamp. Light intensity was measured at 3.2×10^3 lux using a General Electric type X23 light meter. The box was placed in a refrigerator during the five minute exposure time to avoid excessive heating. A control of 130 seeds was used. After the exposure seeds were soaked for one hour in 2% H_2O_2, rinsed with sterile distilled H_2O, and were covered with sterile distilled H_2O. The control was similarly treated with 2% H_2O and soaked in sterile water. The water was changed daily. Germination records were kept for a thirty day period.

DETERMINATION OF CHLOROPHYLL CONCENTRATIONS IN SEEDS AND AERIAL SHOOTS OF FOUR SPECIES OF DWARF MISTLETOE

Chlorophyll concentrations were determined for seeds and aerial shoots of four species of *Arceuthobium*: *A. campylopodum*, *A. tsugense*, *A. americanum*, and *A. douglasii*.

A preweighed amount (0.500 g) of each species of seeds was used for each determination. A Sorval Micromixer was used to grind the seeds. Since the volume of the grinder container was limited, it took two or three grinding periods to grind 0.500 g of seeds. Part of the seeds were placed in the grinding container with a small volume (approximately 10.0 ml) 80% acetone. The container was attached to the mixer and the seeds were ground for fifteen minutes. The container was partially submerged in a cold water bath during grinding to prevent excessive heating. The extract was filtered three times through Whatman #1 filter discs on a Buchner funnel into a foil wrapped side-arm flask. The extract was diluted to known volume. A known volume of extract was placed in a cuvette and the absorbance was measured in a Coleman 124 Spectrophotometer. This sample was concentrated under N_2 or diluted with additional 80% acetone to give an absorbance reading between 0.60 and 1.00 at 663 nm. Then a spectrum was obtained between 700 nm and 400 nm.

The formulas that were used to estimate the chlorophyll content are those reported in Holden (1965):

$$\text{Chlorophyll a} = \frac{0.0127(A663)-0.00269(A645) \times \text{vol. of extract (ml)}}{\text{fresh weight material (g)}}$$

$$\text{Chlorophyll b} = \frac{00.0229(A645)-0.00468(A663) \times \text{vol. of extract(ml)}}{\text{fresh weight material (g)}}$$

The method used for determining aerial shoot chlorophyll concentrations was basically the same as the above proceedure with the following exceptions. Freeze-dried aerial shoot tissue (5.0 g) was homogenized with 75-100 ml 80% acetone in a Sorval Omnimixer without the micromixer attachment. Chlorophyll determinations for seeds and shoots were repeated three times for each species.

MANOMETRIC DETERMINATIONS OF O_2 UPTAKE

Rates of O_2 uptake in light and dark by dwarf mistletoe seeds were measured by manometric methods. A Gilson Medical Electronics Warburg Constant Volume Respirometer was used in these experiments in conjunction with a Neslab Constant Temperature Circulation Bath, which holds a constant temperature (25°C) even during periods of illumination. Light intensity was approximately 8.6×10^4 lux. Double sidearm Warburg flasks (21.0 ml) were used as reaction vessels. The seeds (ten, preweighed) were placed in the main compartment. Pardee buffer (0.9 ml) was placed in one of the sidearms along with a folded filter paper wick to maintain the CO_2 concentration inside the flask at one percent (Umbreit, et al. 1959). Sterile distilled water (1.1 ml) was put into the other sidearm to maintain a humid environment. Flasks used to measure dark O_2 uptake were wrapped in aluminum foil. All glass to glass connections were sealed with Lubriseal. The flasks were then connected to the manometers and placed in the lighted 25°C water bath. After a fifteen minute equilibration period the

manometer stop cocks were closed and the experiment started. Two thermobarometers were used with each experiment. Each contained 0.9 ml Pardee buffer in one sidearm and 1.1 ml sterile distilled H_2O in the other sidearm. Manometer readings were taken and recorded every fifteen minutes for the four hour duration. Flask constants (K_{O_2}) were computed for the flasks with two ml fluid volume and for 25°C using the formula from Umbreit, et al. (1959). The amount of gas exchanged equaled the change in manometer reading (h) times the flask constant (K_{O_2}).

POLAROGRAPHIC DETERMINATION OF O_2 UPTAKE

A Yellow Springs Instrument Model 53 Biological Oxygen Monitor and a Sargent Recorder were used to measure and record O_2 uptake in light and dark by A. campylopodum seeds. Temperature was kept constant by using the Neslab Constant Temperature Circulation Water Bath. Illumination was provided by a 150 watt soft white bulb in a goose neck lamp. Light intensity was 1.29×10^4 lux. During dark runs the reaction chamber was covered with a dark blue cloth and the light source turned off. The machine was calibrated as described in the instruction manual (Y.S.I. Co. 1969) using O_2 saturated distilled H_2O at 25°C. A probe test was run daily and the membrane was changed whenever necessary to insure reliability. Special stirring discs with flat surfaces were used because the regular type stirrer discs tended to mascerate the seeds.

Five seeds (preweighed) were put into the reaction chamber containing 3.0 ml O_2 saturated water. The probe was inserted and the

stirrer was turned on. After a five minute equilibration period was over, the light source was turned off and the reaction vessel was covered with the dark cloth. O_2 uptake measurements were taken during alternating fifteen minute dark and light periods.

INCORPORATION OF $^{14}CO_2$

Three samples of seeds (70-100, preweighed) in three reaction flasks were exposed to $^{14}CO_2$ for twenty-four hours (see Figure 1). Each reaction flask consisted of two parts: a glass vial and a 25 ml suction flask. A piece of rubber tubing (approximately 2.0 cm long) was fitted on the sidearm of each suction flask to provide a seal to which the vial was attached. The seeds were placed in the vials and then the vials were fitted on the rubber covered sidearms. The rubber to glass joints were sealed with silicone grease. The tops of the suction flasks were fitted with serum stoppers. A Kontes cup, a small plastic cup (volume 0.4 ml) on a plastic stem (6.0 cm), was suspended from a serum stopper (through which a hole was drilled to hold the stem of the plastic cup) into each reaction flask. Each plastic Kontes cup contained 0.15 ml of 0.2N $NaH^{14}CO_3$, equivalent to 1.5×10^6 dpm per flask. $^{14}CO_2$ was liberated in each flask by injection 0.15 ml of 2N H_2SO_4 to the $NaH^{14}CO_3$. One flask was foil wrapped to determine if CO_2 assimilation occured in the dark. The flasks were then suspended in the Warburg water bath. Temperature was kept constant at 25°C using the Neslab portable bath cooler. Light intensity was 8.6×10^4 lux.

After twenty-four hours, 5.0 ml of ten percent KOH was injected into the bottom of each reaction flask. The flask was then shaken for two hours. The sidearm vial containing the seeds was then removed, filled with hot 95% ethanol and capped. Aliquots of KOH were counted for ^{14}C activity using a Nuclear Chicago Unilux II Liquid Scintillation Counter to determine the amount of $^{14}CO_2$ remaining in the reaction flask. Each aliquot was added to a counting vial containing ten ml of scintillation fluid (12 g PPO, 330 mg POPOP, in a toluene-absolute ethanol solution 2:1 v/v). Efficiency was determined by Channels Ratio method (Wang and Willis, 1965). The seeds in hot ethanol were transferred to a blender container, connected to a Sorval Omnimixer, and ground for fifteen minutes. The extract and residue were filtered twice using preweighed filter papers on a Buchner funnel and the residue dried. The volume of the ethanol soluble fraction was measured, and aliquots counted for ^{14}C activity. The dried residues were weighed and samples were combusted in Schoninger Oxygen Flasks with 2.0 ml ten percent KOH in the bottom. Aliquots of the KOH were assayed for ^{14}C activity.

Next, the ethanol soluble fraction was evaporated under partial vacuum at 35°C to dryness in a round bottom flask. The concentrate was partitioned between water and chloroform and then transferred to a separatory funnel where the phases were separated. The volume of each phase was measured. The ^{14}C activity was assayed to determine the percentage of photosynthate incorporated in chloroform and water soluble fractions.

Ion exchange chromatography was used to separate the water

soluble fractions, using columns prepared according to instructions in Wharton and McCarty (1972). The fraction was first passed through a cation exchange column (Amberlite IRC-50, Na^+ form) and then through an anion exchange column (Dowex 2-X8, OH^- form). Aliquots of the effluents were assayed for ^{14}C activity to determine the percentage of activity remaining on the columns. The columns were eluted with 2N NaOH. Aliquots of the elution were also counted.

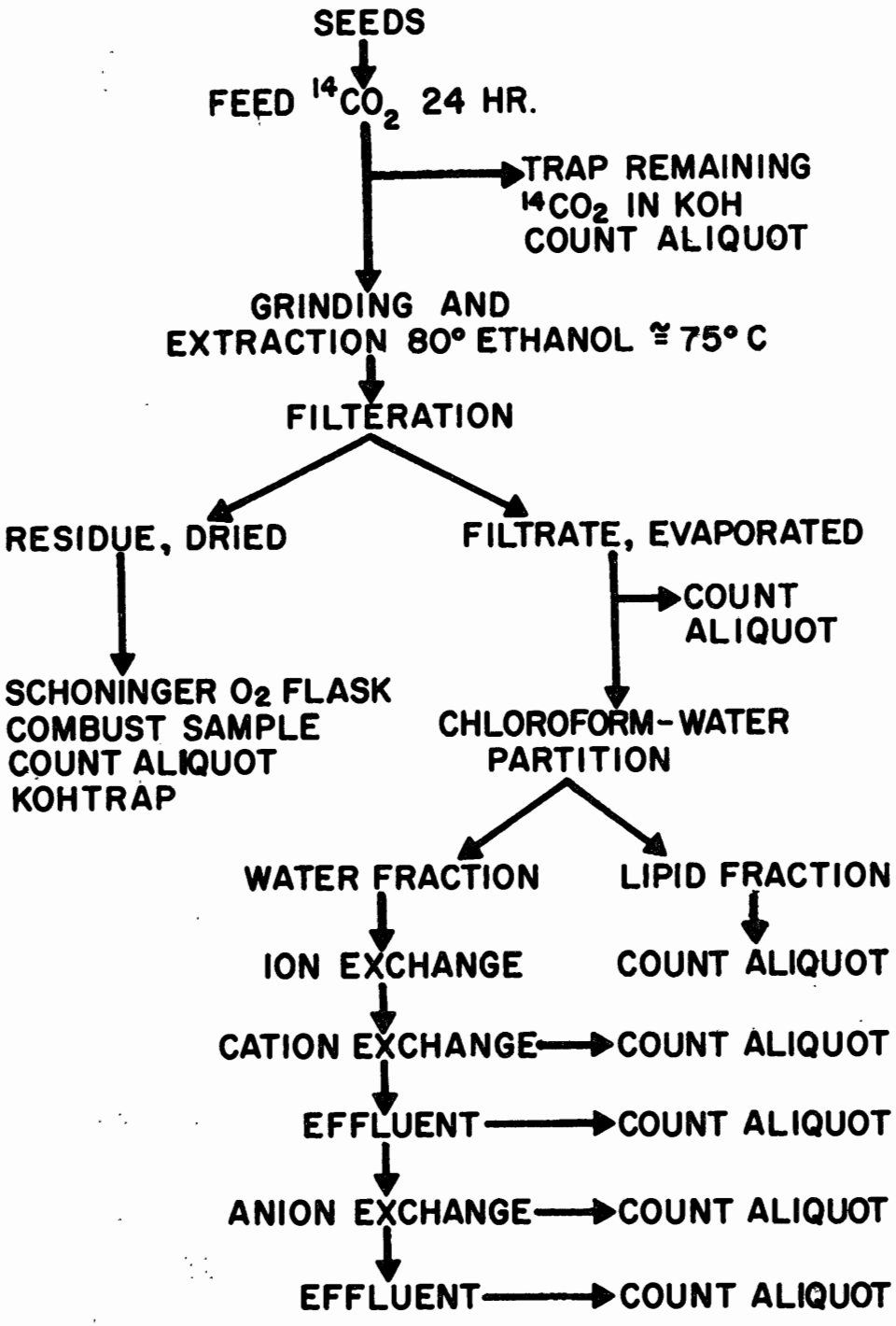


Figure 1. Flow chart for ^{14}C Label Procedure and analysis

RESULTS AND DISCUSSION

GERMINATION

Germination percentages from the experiments on the effect of H_2O_2 on seed germination are compiled in Table II and in Figure 2. Statistical treatment included one way analysis of variance (ANOVA) between treatments in a fiven year and for similar treatments between years. If the ANOVA's showed significant differences, the Student-Newman-Keuls (SNK) test was utilized to determine where specific differences between means existed. The results of the statistical analysis are summarized in Table III. In all three years there was no significant difference between the two percent and three percent H_2O_2 treatments for the respective year. In 1975 the one percent H_2O_2 treated seeds had significantly lower germination percentages than did the two percent and three percent treated seeds, but in 1976 and in 1977 the three pretreatments resulted in no significant difference in terms of percentage of seeds germinated. Comparison between the years showed that 1976 seeds had higher rates of germination than 1975 and 1977 seeds similarly treated. The 1975 seeds in the two percent and three percent treatments had significantly higher germinations than those of 1977, while the one percent treatments resulted in similar germination rates.

Fungal contamination of incubating seeds was a continous problem. Seeds treated with only sterile distilled H_2O were almost certain to

TABLE II

GERMINATION PERCENTAGES FOR SEEDS OF A. CAMPYLOPODUM TREATED WITH H_2O

$[H_2O_2]$ used in pretreatment		1%	2%	3%
1975	sample #	% seeds germinated		
	1	14	28	17
	2	13	24	20
	3	12	35	34
	4	15	33	28
	mean	13.5	30.0	25.3
	std. dev.	1.3	5.0	7.0

$[H_2O_2]$ used in pretreatment		1%	2%	3%
1976	sample #	% seeds germinated		
	1	39	44	48
	2	55	57	53
	3	20	42	60
	4	30	42	39
	5	37	66	67
	mean	36.2	50.2	53.4
	std. dev.	12.8	10.8	10.8

$[H_2O_2]$ used in pretreatment		1%	2%	3%
1977	sample #	% seeds germinated		
	1	8	9	9
	2	5	12	12
	3	12	15	10
	4	9	9	17
	5	9	10	15
	mean	8.6	11.0	12.6
	std. dev.	2.5	2.5	3.4

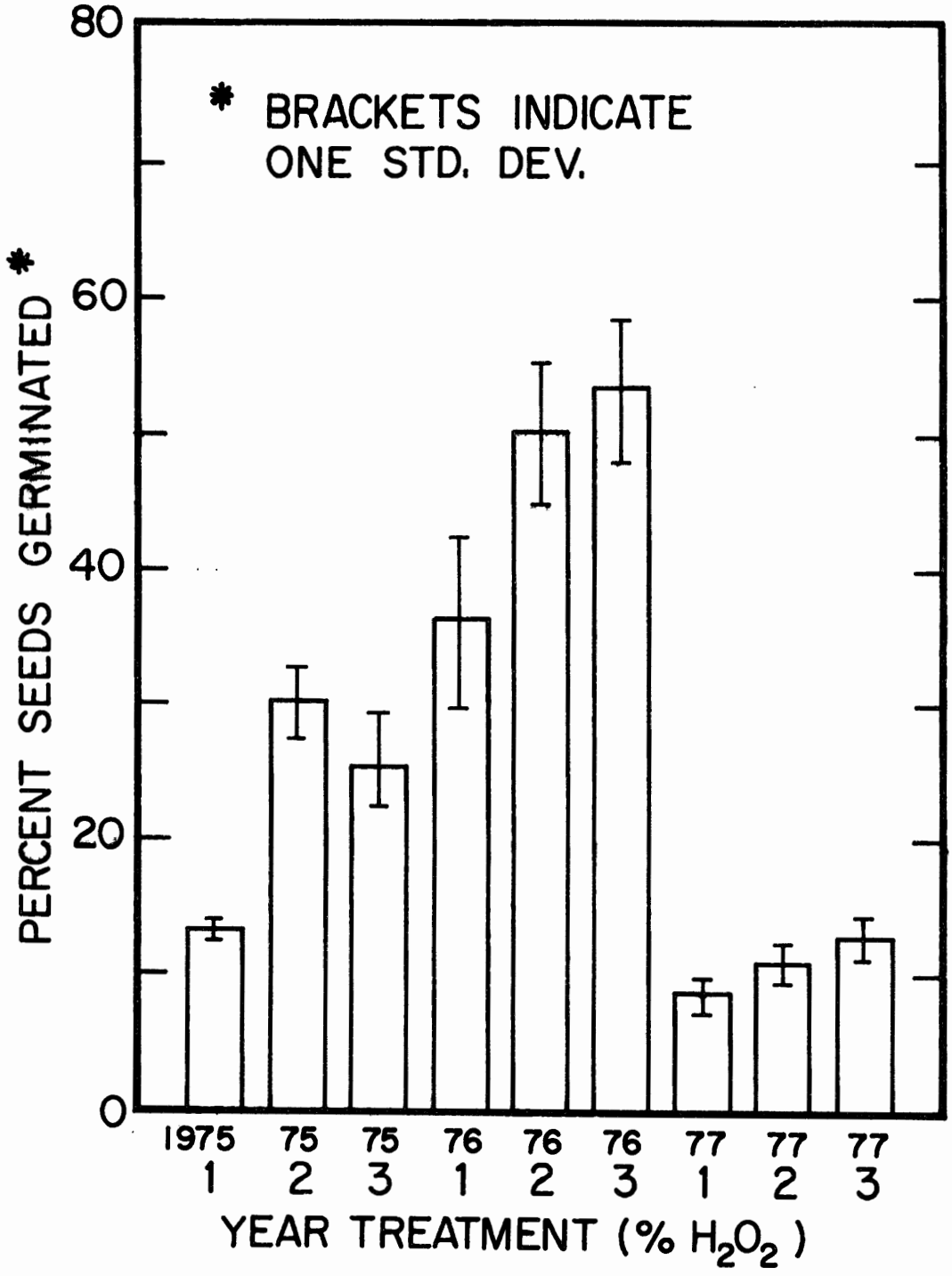


Figure 2. Annual mean germination percentages for *A. campylopodum* seeds pretreated with various strength solutions of H_2O_2

TABLE III

SUMMARY OF STATISTICAL ANALYSIS* OF GERMINATION RATES FOR SEEDS OF A. CAMPYLOPODUM

S = significant difference between two values at the .05 level of significance

NS= no significant difference between two values at the .05 level of significance

X = no statistical analysis made between two values

* Student-Newman-Keuls test

TREATMENT and Year	3% H_2O_2 1977	2% H_2O_2 1977	1% H_2O_2 1977	3% H_2O_2 1976	2% H_2O_2 1976	1% H_2O_2 1976	3% H_2O_2 1975	2% H_2O_2 1975
1% H_2O_2 1975	X	X	NS	NS	X	S	S	S
2% H_2O_2 1975	X	S	X	X	S	X	NS	
3% H_2O_2 1975	S	X	X	S	X	X		
1% H_2O_2 1976	X	X	S	NS	NS			
2% H_2O_2 1976	X	S	X	NS				
3% H_2O_2 1976	S	X	X					
1% H_2O_2 1977	NS	NS						
2% H_2O_2 1977	NS							

be overcome with fungi. It was most severe in the 1975 seeds. This is one of the possible explanations for the difference in germination rates between 1975 seeds and 1976 seeds. However, even in the absence of fungal contamination, and with similar treatment, seeds which appeared normal (green and non-shrivelled, Wicker, 1974) often did not germinate. This tendency was most apparent in the 1977 seeds where germination rates were quite low. Many of these seeds appeared to be normal but within a week after imbibition turned yellow. These seeds were separated from the green, healthy seeds but soon became heavily contaminated by fungi. It should be noted that 1977 was a year of severe drought in Oregon and, although any connection would be speculation at this point, a study of the effect of drought on fruit development in Arceuthobium would be worth while.

The maximum germination rate of 67% for A. campylopodum obtained in this study is roughly comparable to those of Knutson (1974) but somewhat smaller than those reported by Beckman (1964) and Wicker (1974). Wicker (1974) preselected against abnormal seeds in collections, and this fact may explain the 85-90% germination rates he reported. However, it can easily be seen from my data that germination rates are quite variable within the species from year to year and quite likely, from site to site.

The data from tests using dilutions of Chlorox as anti-fungal agents is in Table V. Pretreatment with Chlorox dilutions yielded low germination rates, six to twenty percent (means), as compared to fifty percent (mean) for a control using H_2O_2 (two percent). The treatment with Chlorox tended to cause many of the seeds to turn yellow within

TABLE IV

GERMINATION OF SEEDS OF A. CAMPYLOPODUM PRETREATED WITH CHLOROX

Pretreatment Solution [Chlorox]	1%	2%	3%	4%	5%
1976 sample #	Percentage Seeds Germinated				
1	36	16	16	6	9
2	18	10	13	10	6
3	6	20	6	9	5
mean	20	15	11.7	8.3	6.7
std. dev.	15	5.0	5.1	2.0	2.0

Control pretreated with 3% H_2O_2

sample #	% seeds germinated
1	50
2	60
3	46
mean	52
std. dev.	7.2

five days after treatment and this was often followed by fungal infection. Analysis of variance showed no significant difference between the resultant germination rates for the five different treatments. It appears that Chlorox is toxic to many seeds even at the one percent concentration.

Attempts to germinate seeds within thirty days after dispersal proved futile, but by forty-five to sixty days post dispersal, average germination rates were obtainable for seeds of A. campylopodum. This corresponds to apparent dormancy periods reported by Beckman and Roth (1968) and Knutson (1974). However, Wicker (1974) reported maximum germination rates were obtained within two weeks after dispersal. Thus there is controversy over the length of the dormancy expressed in seeds of A. campylopodum.

Presently it is thought that under natural conditions germination takes place in early to late spring (Hawksworth and Weins, 1972). Beckman (1968) reported that some seeds stored in the field germinated in February. He proposed that a prolonged cold period is required which caused the degradation of a hypothetical chemical inhibitor in the seed allowing germination. He also reported germination over wide ranges of temperature (5-30°C). Light, temperature, and moisture are requirements for germination but individually do not appear to be controlling when germination occurs. Beckman (1974) also investigated the effects of red light and far red light on seed germination and found that germination was slightly enhanced by both, red light being slightly more effective than far red light.

My preliminary experiments showed no significant difference

between a dark control of 130 seeds and two 100 seed samples exposed to red light at 650 nm for five minutes. It is apparent that our knowledge of factors controlling germination is still inadequate and that further investigation of a germination inhibitor and of other possible controlling factors is needed.

CHLOROPHYLL CONCENTRATIONS

Chlorophyll concentrations of seeds and aerial shoots are presented in Tables V and VI. One way analysis of variance was used to analyze total chlorophyll concentration. SNK tests were used to determine significant differences between the mean values for seeds and aerial shoots of the four species. Results of these analyses are summarized in Table VII. Seeds of A. douglasii had the highest chlorophyll concentration of 0.39 mg/g fresh weight while seeds of A. tsugense had the lowest concentration of 0.25 mg/g fresh weight. Statistical comparison showed there is no significant difference between chlorophyll concentrations in seeds and aerial shoots of A. douglasii and A. campylopodum while there is significant difference between the total chlorophyll content of seeds and aerial shoots of A. americanum and A. tsugense. The ratio of chlorophyll a to chlorophyll b was generally greater in the aerial shoots than in the seeds. These ratios varied from a low of 1.30:1 in seeds of A. tsugense to 2.72:1 in aerial shoots of A. douglasii.

Hull and Leonard (1964 b) reported substantial variances in aerial shoot chlorophyll content in two species of dwarf mistletoe ranging from 0.24 to 0.48 mg/g tissue. They reported chlorophyll a to

TABLE V

SEED CHLOROPHYLL CONCENTRATIONS

Species	[chl a] mg/g fr.wt	[chl b] mg/g fr.wt	[chl a+chl b] mg/g fr.wt	Ratio chl a = chl b
A. campylopodum	0.21	0.12	0.33	1.75:1
	0.19	0.15	0.34	1.27:1
	0.21	0.14	0.35	1.50:1
	0.20	0.15	0.35	1.33:1
mean	0.20	0.14	0.34	1.43:1
std. dev.	0.0095	0.0141	0.0095	
A. douglasii	0.23	0.16	0.39	1.44:1
	0.23	0.17	0.40	1.35:1
	0.22	0.17	0.39	1.29:1
mean	0.23	0.17	0.39	1.35:1
std. dev.	0.0058	0.0058	0.0058	
A. americanum	0.19	0.14	0.33	1.36:1
	0.17	0.12	0.29	1.42:1
	0.19	0.13	0.32	1.46:1
mean	0.18	0.13	0.31	1.38:1
std. dev.	0.0115	0.0100	0.0208	
A. tsugense	0.14	0.11	0.25	1.27:1
	0.14	0.11	0.25	1.27:1
	0.15	0.11	0.26	1.36:1
mean	0.14	0.11	0.25	1.27:1
std. dev.	0.0058	0.0000	0.0058	

TABLE VI

AERIAL SHOOT CHLOROPHYLL CONCENTRATIONS

Species	[chl a] mg/g fr.wt	[chl b] mg/g fr.wt	[chl a+chl b] mg/g fr.wt	Ratio chl a = chl b
A. campylopodum	0.21	0.15	0.37	1.40:1
	0.21	0.11	0.32	1.91:1
	0.22	0.16	0.37	1.38:1
mean	0.21	0.14	0.35	1.50:1
std. dev.	0.0057	0.0265	0.02887	
A. douglasii	0.28	0.12	0.40	2.33:1
	0.27	0.08	0.35	3.38:1
	0.28	0.10	0.38	2.80:1
mean	0.28	0.10	0.38	2.80:1
std. dev.	0.0058	0.0200	0.0252	
A. americanum	0.22	0.12	0.34	1.83:1
	0.23	0.13	0.36	1.77:1
	0.23	0.11	0.35	2.09:1
mean	0.23	0.12	0.35	1.92:1
std. dev.	0.0058	0.0100	0.0100	
A. tsugense	0.20	0.13	0.33	1.54:1
	0.20	0.09	0.29	2.22:1
	0.19	0.10	0.30	1.90:1
mean	0.20	0.11	0.31	1.82:1
std. dev.	0.0058	0.0208	0.0208	

TABLE VII

SUMMARY OF STATISTICAL ANALYSIS* OF CHLOROPHYLL CONCENTRATIONS FOR SEEDS AND AERIAL SHOOT TISSUE OF 4 SPECIES OF ARCEUTHOBIUM

S = significant difference between 2 values at the 0.05 level of significance

NS= no significant difference between 2 values at the 0.05 level of significance

* Student-Newman-Keuls tests

A. a. = Arceuthobium americanum

A. c. = Arceuthobium campylopodum

A. d. = Arceuthobium douglasii

A. t. = Arceuthobium tsugense

TISSUE [CHLOROPHYLL] mg/g fr. wt	A.a. shoots 0.35	A.d. shoots 0.38	A.t. shoots 0.31	A.c. shoots 0.36	A.t. seeds 0.25	A.a. seeds 0.31	A.d. seeds 0.39
A.c. seeds 0.34	NS	NS	S	NS	S	S	S
A.d. seeds 0.39	S	NS	S	NS	S	S	
A.a. seeds 0.31	S	S	NS	S	S		
A.t. seeds 0.25	S	S	S	S			
A.c. shoots 0.36	NS	NS	S				
A.t. shoots 0.31	S	S					
A.d. shoots 0.38	NS						

chlorophyll b ratios in A. campylopodum aerial shoots growing on five different hosts, ranging from 1.9:1 to 3.4:1. However, one should realize they were using Gill's system of classification which previously lumped several presently recognized species into one large species, A. campylopodum. Therefore this seemingly high amount of intraspecific variation of chlorophyll content and ratios of chlorophyll a to b is in fact interspecific variation.

The biological implication of these findings comes from comparing chlorophyll concentration with photosynthetic capacity. Hull and Leonard (1964 b) reported chlorophyll concentrations for host foliage, four species of Phoradendron, and six species (present taxonomic designation) of dwarf mistletoes. Total chlorophyll concentration for dwarf mistletoes was twelve percent to twenty-four percent that of the hosts and fifteen percent to fifty-two percent that of Phoradendron species. Phoradendron was found to fix quantities of CO_2 comparable to host foliage while dwarf mistletoes fixed only five to ten percent of the amount of CO_2 fixed by the host (Hull and Leonard, 1964 b). The photosynthetic capacity corresponds to the amount of chlorophyll present in the mistletoe. It is reasonable on the basis of similar low chlorophyll concentrations in seeds and aerial shoots of dwarf mistletoes, to assume that the seeds will have similar low photosynthetic rates as compared to the aerial shoots.

O_2 CONSUMPTION IN LIGHT AND IN DARK

O_2 consumption in light and in dark by seeds of A. campylopodum was measured by manometric and polarographic methods. These seeds

were imbibed ten days before and had germinated three days prior to use. Data from manometric measurements is compiled in Table VIII. The data were analyzed using one way analysis of variance. The mean value for O_2 consumption in the dark was 352 µl O_2 $g^{-1}h^{-1}$ and in the light was 201 µl O_2 $g^{-1}h^{-1}$. The statistical test showed a significant difference between the resultant means of the two treatments. Figure 3 displays the results of the experiments on March 23, 1978, in graphical form. Rates were linear during the experiment.

The results from the polarographic method are in agreement with those above. The mean O_2 consumption in the dark is 353 µl O_2 $g^{-1}h^{-1}$ and in the light, 214 µl O_2 $g^{-1}h^{-1}$. The data is reported in Table IX. These two values are significantly different at the 0.05 level of significance.

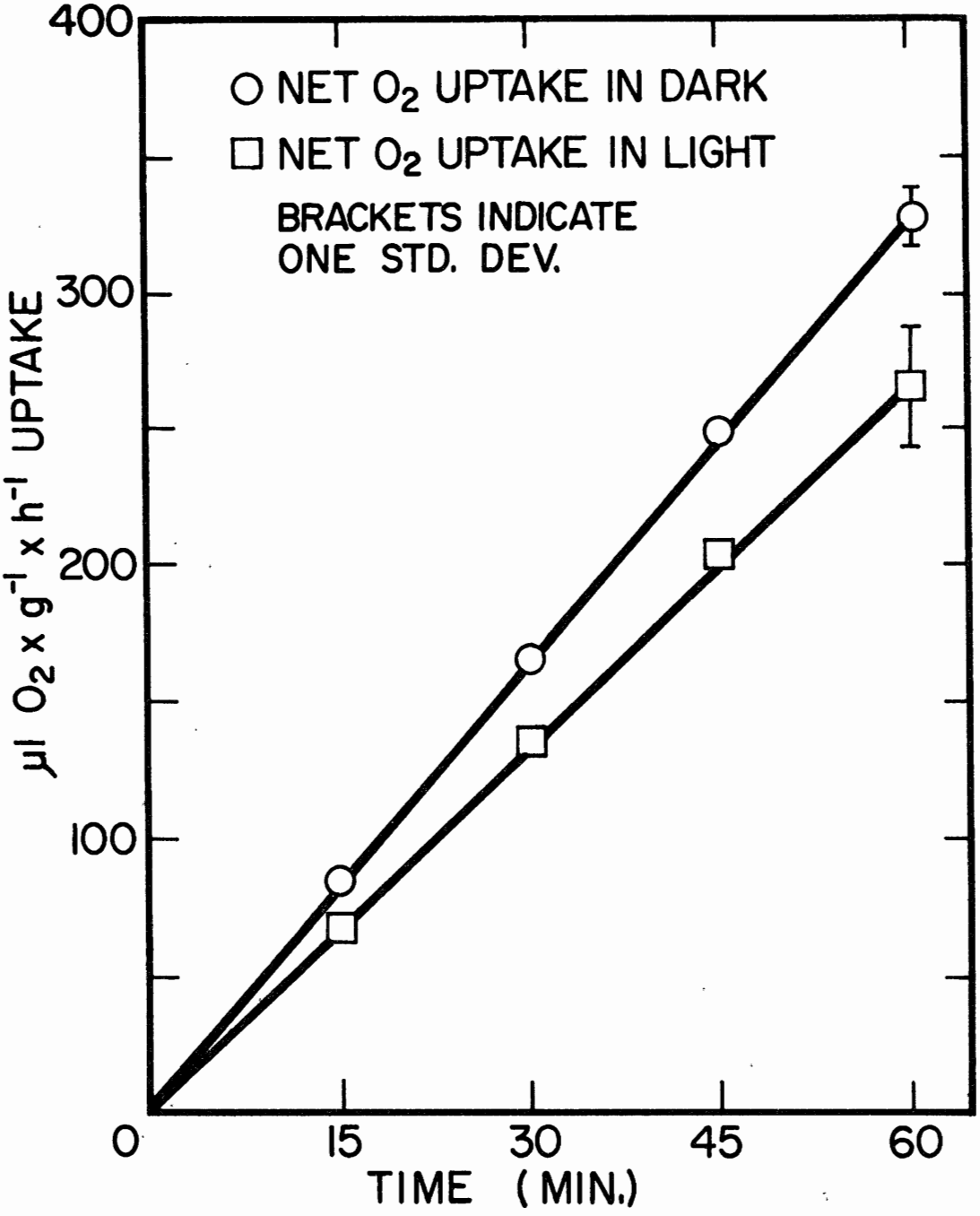
One explanation for the difference in O_2 consumption in light and dark is that the difference is due to O_2 evolved through photosynthesis. If this is true, the difference would equal the apparent photosynthetic rate. Subtracting the mean light O_2 consumption rate from the mean dark O_2 consumption rate gives the mean apparent photosynthetic rate of 151 µl O_2 $g^{-1}h^{-1}$, which is equivalent to 42.9% of the O_2 $g^{-1}h^{-1}$ consumed during respiration. Miller (1975) used similar reasoning, attributing the difference between light and dark rates of CO_2 evolution by aerial shoot tissue to photosynthesis. He found that the aerial shoots evolved 301 µl CO_2 $g^{-1}h^{-1}$ in the dark and 213 µl CO_2 $g^{-1}h^{-1}$ in the light. The difference of 88 µl CO_2 $g^{-1}h^{-1}$ being the apparent photosynthetic rate, which is less than the rate, 151 µl O_2 $g^{-1}h^{-1}$, for the seeds. However, A. campylopodum seeds have twelve

TABLE VIII

O_2 UPTAKE BY SEEDS OF A. CAMPYLOPODUM IN LIGHT AND DARK MEASURED BY MANOMETRIC METHODS

Date	Flask #	O_2 Uptake in the dark $\mu l\ O_2\ g^{-1} h^{-1}$
1/19/77	1	315
	2	373
	3	340
mean		343
std. dev.		29
2/3/77	1	319
	2	377
	3	360
	4	335
	5	380
	6	439
mean		369
std. dev.		42
1/19/78	1	387
	2	330
	3	353
mean		357
std. dev.		40
3/23/78	1	353
	2	334
	3	320
	4	315
mean		330
std. dev.		17
mean (all flasks)		352
std. dev.		33

Date	Flask #	O_2 Uptake in the light $\mu l\ O_2\ g^{-1} h^{-1}$
2/3/77	7	246
	8	234
	9	241
mean		240
std. dev.		6
2/25/77	1	251
	2	167
	3	148
	4	178
mean		186
std. dev.		45
11/1/77	1	209
	2	196
	3	153
mean		186
std. dev.		29
1/19/78	4	122
	5	96
	6	107
mean		108
std. dev.		13
3/23/78	5	268
	6	293
	7	201
	8	304
mean		266
std. dev.		46
mean (all flasks)		201
std. dev.		63



EACH POINT ON THE GRAPH REPRESENTS THE MEAN OF FOUR FLASKS CONTAINING TEN SEEDS. 3/23/78

Figure 3. O_2 uptake in light and in dark by seeds of *A. campylopodum*.

TABLE IX

O_2 UPTAKE BY SEEDS OF *A. CAMPYLOPODUM* IN LIGHT AND IN DARK MEASURED BY POLAROGRAPHIC METHODS

O_2 UPTAKE IN THE DARK

SAMPLE*	O_2** UPTAKE ($\mu l\ O_2\ g^{-1}h^{-1}$)
1	343
2	376
3	352
4	342
mean	353.3
std. dev.	15.8

O_2 UPTAKE IN THE LIGHT

SAMPLE*	O_2** UPTAKE ($\mu l\ O_2\ g^{-1}h^{-1}$)
1	229
2	235
3	220
4	171
mean	213.8
std. dev.	29.2

* Each sample consisted of 5 seeds

** Each value reported is calculated from the average of two 15 min. measurements

percent more chlorophyll than A. tsugense.

The respiration rate of 350 µl O_2 $g^{-1}h^{-1}$ is equivalent to 0.688 mg CO_2 $g^{-1}h^{-1}$ if one assumes a RQ of 1.0. This value can be converted to a per seed basis by multiplying it times 0.003 g/seed (empirically derived) which equals 0.002 mg CO_2 $seed^{-1}h^{-1}$. Thus if one assumes a constant respiration rate during twenty-four hours the seed would lose .048 mg CO_2 or 1.6% of its weight. If one assumes twelve hours of light, the seed would be able to fix 0.010 mg of CO_2 (at 0.0084 mg CO_2 $seed^{-1}h^{-1}$) or twenty-one percent of the CO_2 expelled during respiration. Photosynthetic carbon fixation increases the efficiency of the seeds' nutrition and may provide the fine difference between life and death.

$^{14}CO_2$ INCORPORATION BY SEEDS OF A. CAMPYLOPODUM

The results from $^{14}CO_2$ labelling of seeds in light and in dark and subsequent analysis of location of ^{14}C are reported in Table X and XI. The results show that seeds of A. campylopodum assimilated CO_2 in light. CO_2 assimilation in the light was approximately 350 times CO_2 assimilation in the dark. The seeds in light incorporated twenty-five percent of the original $^{14}CO_2$ present in the reaction flask during twenty-four hours. However, it should be remembered that the seeds are respiring at a greater rate than they are photosynthesizing thus in the course of twenty-four hours substantial dilution of the labelled $^{14}CO_2$ took place. Therefore, quantification of photosynthetic rates of the seeds by this method is not reliable.

Extraction of seeds with ethanol showed that 97-99% of the

TABLE X

DISTRIBUTION OF ^{14}C IN SEEDS OF A. CAMPYLOPODUM

SAMPLE	DARK EXPOSURE 1	2	LIGHT EXPOSURE 3	4	5	7
Activity of solid residue (dpm)	1121	0	6,538	7,024	4,451	8,047
Activity of ethanol extract on a per gram basis	0	0	351,311	330,028	323,464	339,450
Total activity incorporated by seeds	1121	0	357,894	337,052	324,915	347,497
Percent of original activity in reaction vessel incorporated by seeds*	0.07	0.00	24.90	23.40	23.40	25.10
Activity of $CHCl_3$ soluble fraction (dpm and % of recovered activity from ethanol extract)	----	----	39,455 (15.6)	45,454 (16.4)	13,335 (6.5)	13,350 (5.8)
Activity of H_2O soluble fraction (dpm and % of recovered activity from ethanol extract)	----	----	214,021 (84.4)	232,112 (83.6)	191,819 (93.5)	216,834 (94.2)

*Only approx. 40% of original activity was accounted for at the end of 24 hr. The missing 60% was probably leaked to the air. Photosynthetic rates can not be derived from these results.

TABLE XI

SEPARATION OF WATER-SOLUBLE ^{14}C COMPONENTS BY ION EXCHANGE CHROMATOGRAPHY

SAMPLE	Activity Retained by Cation Column (% of H_2O phase activity)	Activity Retained by Anion Column (% of H_2O phase activity)	Activity of Neutral Effluent (% of H_2O phase activity)
3	10.8	29.2	60.0
4	25.1	22.0	52.9
5	23.7	21.0	55.4
6	17.3	15.4	67.3

incorporated ^{14}C was ethanol soluble, of this, 83-90% of the activity in the ethanol extracts concentrate was H_2O soluble. The H_2O soluble phase was separated into cationic, anionic, and neutral fractions by ion exchange chromatography. These results are also reported in Table XI. The majority (50-67%) of the label remained in the neutral fraction, while 11 to 25% of the label was in the cationic fraction, and 15to 29% of the label was in the anionic fraction.

Miller and Tocher reported the following values from ion exchange chromatography of water soluble ^{14}C from aerial shoots: cationic fraction, 12-26%; anionic fraction, 20-25%; and neutral fraction, 51-68%. Hull and Leonard (1964 b) reported a smaller percentage (one percent) of label in the cationic fraction, and a larger percentage (36-70%) in the anionic fraction. They suggested the presence of Crassulacean acid metabolism (CAM) or of enzymes active in CAM in aerial shoots based on the large amounts of ^{14}C label incorporated into malic acid. The nonexistant to very minute assimilation of ^{14}C by the seeds of A. campylopodum in the dark suggests the absence of CAM.

Tainter suggests that chloroplasts of A. pusillum resembled chloroplasts associated with C4 carbon assimilation systems. The C4 pathway is usually associated with a certain type of cell anatomy within leaves. Mesophyll cells have chloroplasts which fix CO_2 by PEP carboxylase to form malate. The malate is either directly transferred or is aminated to form aspartate and then transferred to the bundle sheath cells. In the bundle sheath cells the CO_2 carrier is decarboxylated. The CO_2 is then fixed in the chloroplast via the Calvin cycle

(Bonner and Varner, 1976). This elaborate anatomy has not been reported to occur in dwarf mistletoes, however, that does not exclude the possibility of the presence of C4 system enzymes. It is apparent that further investigation of the pathway of carbon fixation in the seed is needed.

CONCLUSION

Seeds of A. campylopodum were quite vulnerable to fungal attack during incubation in the laboratory. Presoaking of seeds with two and three percent H_2O_2 resulted in less fungal contamination and in relatively high germination percentages as compared to results obtained using Chlorox pretreatments. The seeds were found to contain chlorophyll in concentrations (0.25 to 0.40 mg/g fresh weight) comparable to those in aerial shoots which are ten to twenty percent the amounts found in host leaves (Hull and Leonard, 1964 b). The variance in chlorophyll concentrations in seeds and aerial shoots of different species supports the hypothesis of Miller and Tocher (1975) that different species of Arceuthobium vary in their dependence upon the host.

O_2 consumption by seeds in light and in dark was measured by manometric and polarographic methods. The resulting data showed good agreement. The difference between the light and dark rates was attributed to O_2 evolved during photosynthesis. This mean difference equaled 152 $\mu l\ O_2\ g^{-1}h^{-1}$. This figure equaled 42% of the dark respiration rate of 352 $\mu l\ O_2\ g^{-1}h^{-1}$. The ^{14}C labelling experiments proved that the seeds fix CO_2 in the light. This CO_2 fixation in light supports the previous reasoning that the difference between O_2 uptake in the light and in the dark was attributed to O_2 evolved in the light reactions of photosyntheses. Further investigation of the pathway of CO_2 fixation in seeds and shoots of dwarf mistletoe is warranted.

This study supports the earlier hypothesis of Cohen (1964), Kuijt

(1969), and Scharpf (1970), that the chlorophyll present in dwarf mistletoe seeds is functional. However Kuijt (1969) appears to have been overly enthusiastic in his description of Viscacean seeds as "seats of great photosynthetic activity."

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APPENDIX

TABLE XII

COMPARISON OF GERMINATION RATES BETWEEN YEARS BY PRETREATMENTS

ANOVA TABLE 1% H_2O_2 PRETREATMENT

SOURCE	DF	SS	MS	F	F(.05)
TREATMENT	2	2130.7143	1065.3571	16.90	3.98
ERROR	11	693.0000	63.0000		
TOTAL	13	2823.7143			

ANOVA TABLE 2% H_2O_2 PRETREATMENT

SOURCE	DF	SS	MS	F	F(.05)
TREATMENT	2	3842.6286	1921.3143	37.15	3.98
ERROR	11	568.8000	51.7091		
TOTAL	13	4411.4286			

ANOVA TABLE 3% H_2O_2 PRETREATMENT

SOURCE	DF	SS	MS	F	F(.05)
TREATMENT	2	4333.2071	2166.6036	36.04	3.98
ERROR	11	661.1500	60.1045		
TOTAL	13	4994.3571			

RANKED TREATMENT MEANS*

TREATMENT	MEANS	TREATMENT	MEANS	TREATMENT	MEANS
1976 - 1%	36.2000	1976 - 2%	50.2000	1976 - 3%	53.4000
1975 - 1%	13.5000	1975 - 2%	30.0000	1975 - 3%	25.2500
1977 - 1%	8.6000	1977 - 2%	11.000	1977 - 3%	12.6000

*Analysis using SNK tests showed no significant difference at the 0.05 level of significance between values connected by vertical lines.

DF = degrees of freedom

SS = sum squares

MS = mean squares

F = F test statistic

F(.05) = critical value of F at the .05 level of significance

TABLE XIII

COMPARISON OF GERMINATION RATES FROM PRETREATMENTS OF SEEDS WITH 3 SOLUTIONS (1%, 2%, 3%) OF H_2O_2 BY YEAR

ANOVA TABLE 1975

SOURCE	DF	SS	MS	F	F(.05)
TREATMENT	2	568.5000	284.2500	9.925	4.256
ERROR	9	257.7500	28.6389		
TOTAL	11	826.2500			

ANOVA TABLE 1976

SOURCE	DF	SS	MS	F	F(.05)
TREATMENT	2	836.8000	418.4000	3.144	3.890
ERROR	12	1596.8000	133.0667		
TOTAL	14	2433.6000			

ANOVA TABLE 1977

SOURCE	DF	SS	MS	F	F(.05)
TREATMENT	2	40.5333	20.2667	2.520	3.890
ERROR	12	96.4000	8.0333		
TOTAL	14	136.9333			

RANKED TREATMENT MEANS*

TREATMENT	MEANS	TREATMENT	MEANS	TREATMENT	MEANS
1975 - 2%	30.0000	1976 - 3%	53.4000	1977 - 3%	12.6000
1975 - 3%	24.7500	1976 - 2%	50.2000	1977 - 2%	11.0000
1975 - 1%	13.5000	1976 - 1%	36.2000	1977 - 1%	8.6000

*Analysis using SNK tests showed no significant difference at the 0.05 level of significance between values connected by vertical lines.

TABLE XIV

GERMINATION RATES OF SEEDS OF
A. CAMPYLOPODUM PRETREATED
WITH VARIOUS SOLUTIONS
OF CHLOROX

ANOVA TABLE

SOURCE	DF	SS	MS	F	F(.05)
TREATMENT	4	348.9333	87.2333	1.51	3.48
ERROR	10	576.6667	57.6667		
TOTAL	14	925.6000			

RANK TREATMENT MEANS

TREATMENT	MEANS*
1976 - 1%	20.0000
1976 - 2%	15.3333
1976 - 3%	11.6667
1976 - 4%	8.3333
1976 - 5%	6.6667

*Analysis using SNK tests showed no significant difference at the 0.05 level of significance between values connected by vertical lines.

TABLE XV

ANOVA TABLE CHLOROPHYLL CONCENTRATIONS IN AERIAL SHOOTS AND SEEDS OF 4 SPECIES OF DWARF MISTLETOE

SOURCE	DF	SS	MS	F	F(.05)
TREATMENT	7	0.0402	0.0057	19.0	3.61
ERROR	17	0.0050	0.0003		
TOTAL	24	0.0452			

RANKED TREATMENT MEANS*

TREATMENT	MEANS
A. d. seeds	0.39
A. d. shoots	0.38
A. c. shoots	0.36
A. d. shoots	0.35
A. c. seeds	0.34
A. d. seeds	0.31
A. t. shoots	0.31
A. t. seeds	0.25

*Analysis using SNK test showed no significant difference at the 0.05 level of significance between values connected by vertical lines.

TABLE XVI

ANOVA TABLE O_2 UPTAKE BY SEEDS OF *A. CAMPYLOPODUM* MEASURED BY MANOMETRIC METHODS

SOURCE	DF	SS	MS	F	F(.05)
TREATMENT	1	188828.8471	188828.8471	72.58	4.16
ERROR	31	80646.2481	2601.4919		
TOTAL	32	269475.0952			

RANK TREATMENT MEANS

TREATMENT	MEAN
O_2 uptake in dark	352.1937
O_2 uptake in light	200.8353

TABLE XVII

ANOVA TABLE O_2 UPTAKE BY SEEDS OF *A. CAMPYLOPODUM* MEASURED BY POLAROGRAPHIC METHODS

SOURCE	DF	SS	MS	F	F(.05)
TREATMENT	1	39200.0	39200.00	83.99	5.99
ERROR	6	2800.0	466.67		
TOTAL	7	42000.0			

RANK TREATMENT MEANS

TREATMENT	MEAN
O_2 uptake in dark	352.19
O_2 uptake in light	200.84