1973

Photosynthesis and Respiration of Arceuthobium Tsugense

James Roger Miller
Portland State University

AN ABSTRACT OF THE THESIS OF James Roger Miller for the Master of Science in Biology presented 23 July 1973.

Title: Photosynthesis and Respiration of Arceuthobium tsugense.

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

Richard D. Tocher, Chairman

Robert O. Tinnin

Clyde L. Calvin

Alfred S. Levinson

Dark respiration rates of the aerial shoots of Arceuthobium tsugense, obtained by manometric and IRGA techniques, show production of CO_2 to range between 155-300 µl CO_2 $g^{-1}h^{-1}$ with evidence of seasonal variation. Experiments with $^{14}CO_2$ indicate that the aerial shoots are capable of some photosynthetic CO_2 fixation, with 10-15% of the available ^{14}C incorporated by the plant tissue in one hour. The portions of the aerial shoots which are most active in CO_2 fixation are the young terminal regions. Biochemical characterization of the products of photosynthesis reveals that 80-90% of the incorporated ^{14}C is ethanol soluble. Ten

percent of the ethanol fraction is lipoidal in nature, the rest is H_2O soluble. Ion exchange separation of the H_2O soluble portion shows that 16-25% of the ^{14}C activity is cationic, about 25% anionic, with the balance neutral. Aspartic acid, glutamic acid, and valine are present in the cationic fraction, with additional free amino acids indicated. IRGA experiments indicate an apparent photosynthetic CO_2 fixation capacity of 80-90 µl CO_2 $g^{-1}h^{-1}$, or 25-30% of the amount of CO_2 produced by respiration. The significance of these findings is discussed with respect to nutrition of the parasite.

PHOTOSYNTHESIS AND RESPIRATION OF *ARCEUTHOBIUM TSUGENSE*

by

JAMES ROGER MILLER

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

in

BIOLOGY

Portland State University
1973

TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the Committee approve the thesis of James Roger Miller presented 23 July 1973.

Richard D. Tocher, Chairman

Robert O. Tinnin

Clyde W. Calvin

Alfred S. Levinson

APPROVED:

Earl Fisher, Jr., Head, Department of Biology

David T. Clark, Dean of Graduate Studies

ACKNOWLEDGEMENTS

The author wishes to express his appreciation for the assistance given by the following individuals: Thesis committee members, Doctors Richard D. Tocher, Robert O. Tinnin, Clyde L. Calvin, and Alfred S. Levinson, for their advice in the preparation of this manuscript; Dr. Earl Fisher and the Biology Department of Portland State University for financial assistance; also, Dr. Donald Knutson, for the use of research facilities at the Pacific Northwest Forest and Range Experiment Station at Corvallis, Oregon.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The dwarf mistletoes (Arceuthobium spp.) are pathogens which have a serious economic impact on coniferous forests, especially in the western portion of North America. Interest in developing effective control methods has stimulated research into the biology of these hemiparasites.

The photosynthetic capcity of dwarf mistletoes has long been a subject of speculation. The presence of chlorophyll (Freeland 1943, Hull and Leonard 1964b) provides circumstantial evidence for some capacity for photosynthetic CO_2 fixation. The light-dependent incorporation of $^{14}CO_2$ demonstrates that photosynthesis is occurring (Rediske and Shea 1961, Hull and Leonard 1964b). It is also known that $^{14}CO_2$, assimilated by the host foliage as ^{14}C-sucrose, is translocated by undefined means into dwarf mistletoe tissue (Rediske and Shea 1961, Hull and Leonard 1964a). Hull and Leonard found that $^{14}CO_2$ fixed by the aerial shoots remained there, and was not translocated, even into the endophytic system. Tainter (1971) found that the aerial shoots of A. pusillum Peck evolved less CO_2 in the light than under dark conditions; however, quantitative results cannot be calculated from his reported data since. temperature, tissue weight, light intensity, and volume of the system were all undefined.

All of the evidence supports the classification of Arceuthobium as hemiparasitic; capable of some autotrophic carbon fixation, but dependent upon its host for an unknown quantity of reduced carbon compounds. The purpose of the present investigation is to determine quantitatively the magnitude of the utilization of carbon by Arceuthobium tsugense

(Rosendahl) Jones (Hawksworth and Weins 1970), and the extent to which photosynthesis by the parasite contributes to meeting the energy requirements of the organism.

MATERIALS AND METHODS

Branches of Tsuga heterophylla (Raf.) Sarg. infected by A. tsugense were collected at Last Chance Mountain (T. 2S., R. 8E., sec. 17, Willamette Meridian) in the Mt. Hood National Forest at an elevation of 820 meters. The cut ends of infected branches were placed in water, returned to the laboratory, and stored overnight in the dark at 4°C prior to use in physiology experiments.

Dark respiration rates of aerial shoots of A. tsugense were obtained by Warburg manometric techniques. Approximately 300-600 mg of tissue were placed in foil-wrapped Warburg flasks in such a manner that the base of each shoot was immersed in 3 mls of distilled H_2O per flask. The center well of each flask contained 0.1 ml of 10% NaOH and a filter paper wick for CO_2 absorption (Umbreit, et al. 1957). Care was taken in the choice of tissue samples to use shoots which came from the same infected branch, and only those terminal 2 cm portions which were of similar size and color were used. All respiration and photosynthesis experiments were done at 25°C. After correction for thermobarometric fluctuations, the rates of O_2 consumption were calculated from flask constants. The respiratory quotient was determined using the same apparatus after the indirect method of Umbreit, et al. (1957).

Photosynthesis experiments using $^{14}CO_2$ employed Warburg flasks without the foil wrap. Aerial shoots were exposed to lights in the Warburg bath for 15 minutes prior to reaction of 2.5 μmoles (3.3×10^5 d.p.m.) of $NaH^{14}CO_3$ with 500 μl of 3N H_2SO_4 in the side arm to liberate $^{14}CO_2$. Photosynthesis proceeded for one hour after reaction, at which

time the experiment was terminated by injection of 100 µl of 10% NaOH through a rubber injection port of the second side arm which trapped all $^{14}CO_2$. Fifteen minutes after the injection, the tissue was weighed, cut into 2 mm segments, and killed in boiling 80% ethanol. The tissue was extracted 5 times with 5 ml portions of hot 80% ethanol. The ^{14}C activity of the ethanol extracts was determined by liquid scintillation counting, using a Nuclear-Chicago Unilux II. Known aliquots of A. tsugense extract were added to 5 mls of scintillation fluid (12 g PPO, 300 mg POPOP, in 3 liters toluene-absolute ethanol 2:1 v/v). Efficiency of counting was determined by the channels-ratio method (Anon. 1966).

The ^{14}C ethanol extract of A. tsugense was evaporated to near-dryness at 50°C under reduced pressure. The resultant dark brown mass was partitioned between chloroform and H_2O. The ^{14}C activity of each phase was assayed to determine the portion of photosynthate incorporated into lipid and H_2O soluble fractions. The H_2O soluble fraction was separated by ion-exchange chromatography (Cation resins Amberlite IRC-50 and Dowex 50-W, H^+ form; Anion resin Dowex 2-X, $CO_3^=$ form). The adsorbed components were eluted by washing with 1.5N NH_4OH after the method of Greenham and Leonard (1965). The cationic fraction was concentrated at 60°C under reduced pressure, and examined for free amino acids by two-dimensional paper chromatography. Solvent I= n-butanol/HAc/H_2O (120/30/50 v:v) followed by solvent II= 95% ethanol/conc. NH_4OH (180/10 v:v) or solvent III= phenol/H_2O (160g/40ml) (Ivor Smith 1960). Amino acids were detected by ninhydrin spray (0.5% ninhydrin in 95% ethanol plus 1% collidine/lutidine 1/3 v:v). Ethanol insoluble ^{14}C activity of A. tsugense tissue was determined by O_2 combustion of extracted residue in Schoniger

flasks with simultaneous trapping $^{14}CO_2$ in 2 mls 10% NaOH.

Dark respiration and apparent photosynthetic rates of aerial shoots were measured by infrared gas analysis (IRGA) at the U.S.D.A. Pacific Northwest Forest and Range Experiment Station at Corvallis, Oregon. The apparatus has a plexiglass cuvette for plant tissue, a Beckman 215 gas anlyzer, a peristaltic pump for recirculating gas in the closed system, and provisions for controlling temperature and light intensity (Krueger and Ruth 1969). Ten grams of aerial shoots were placed in the chamber with their cut bases in H_2O. Temperature was monitored using a shielded thermocouple, and varied between 20.5 and 23.5°C. Light intensity for photosynthesis experiments was $4.3x10^4$ lux. The system was calibrated with standard CO_2/air gases of 242 ppm and 378 ppm (Matheson anlysis). Instrument readouts of CO_2 concentration in microamperes were converted to ppm (0.49 ua=1 ppm). Total volume of the system is 8.1 liters.

RESULTS

Dark respiration rates of A. tsugense aerial shoots, as determined by manometric techniques, are summarized in Figures 1 and 2, and Table I. Figure 1 shows the consumption of O_2 as a function of time, and demonstrates that O_2 uptake is a linear function for the course of the experiment. The use of detached shoots creates a physiologically abnormal condition, and in time a change in the rate of O_2 consumption might occur. Talbe I shows the values obtained for the same tissue over a five hour period. It suggests that a slow loss of tissue activity may be occurring, but such changes in metabolic rates are easily avoided if data are collected within two hours after removing the aerial shoots from the host. In one experiment the cut bases of the aerial shoots were sealed with lubriseal to check the possibility that tissue wounding and release of oxidase enzymes could be responsible for O_2 consumption unrelated to normal metabolic respiration. No significant difference in O_2 uptake was observed, so the "cut surface" effect is no more than a minor factor in these experiments.

Figure 2 illustrates the seasonal variation in tissue respiration during fall, winter, and spring conditions. The highest respiratory rates (245 $\mu l\ O_2\ g^{-1}h^{-1}$) as measured by Warburg manometric techniques, occur in shoots collected during the spring. A substantial variability in tissue activity is apparent in these experiments. Standard deviations of 10-15% were observed at all times of the year, and IRGA experiments in the spring showed a variation of similar magnitude. Individual shoots which appear similar in gross morphology may show different respiratory

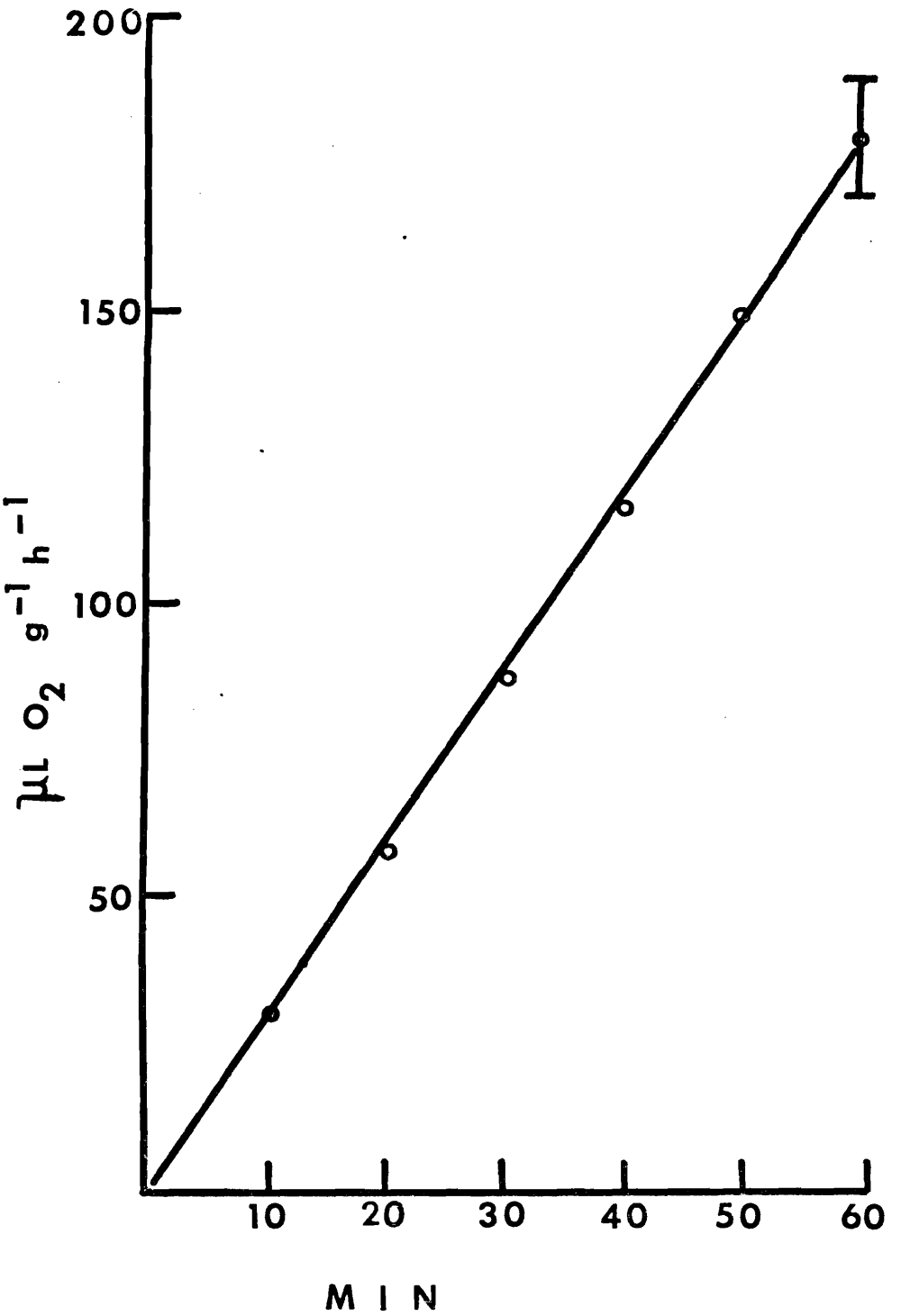

Figure 1. Dark respiration of Arceuthobium tsugense as determined by Warburg manometric techniques on 13 October 1972. Each point represents the mean of 14 flasks. Bracket indicates one standard deviation.

TABLE I

DARK RESPIRATION OF A. TSUGENSE SHOOTS, AS DETERMINED PERIODICALLY OVER A 5-HOUR TIME SPAN FROM THE SAME TISSUE

Flask #	1 Hour	3 Hours	5 Hours
1	214[(a)]	186	180
2	184	161	156
3	239	213	203
4	239	222	205
5	210	239	181
6	187	164	152
7	176	157	148
8	147	147	147
	$\bar{y}$=200 s=32	$\bar{y}$=186 s=34	$\bar{y}$=172 s=24

(a) Determined by Warburg manometric techniques on 20 October 1972 all values in µl O_2 $g^{-1}h^{-1}$

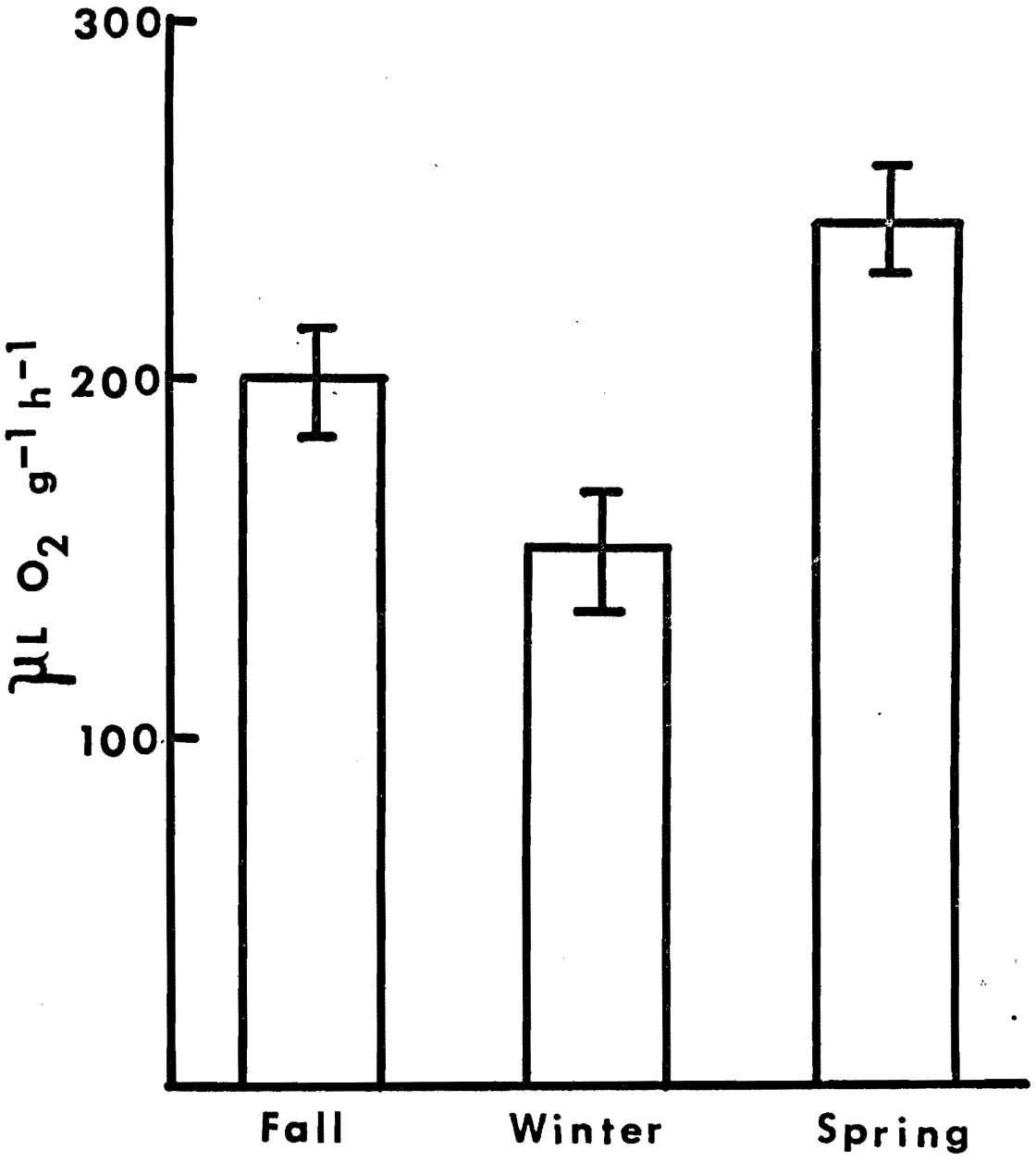

Figure 2. Seasonal variation in respiration rates of A. tsugense aerial shoots. Fall (10/20/72) Winter (1/15/73) Spring (4/473) Brackets indicate one standard deviation.

rates, possibly depending upon age or other unknown factors. No difference is apparent between male and female plants.

The respiratory quotient (µl CO_2 produced/µl O_2 consumed) measured during the fall is approximately 1.1. Apparently, despite large amounts of stored lipids in the aerial shoots of A. tsugense, fatty substances do not act as the major substrate for oxidation.

In agreement with Rediske and Shea (1961), and Hull and Leonard (1964b) for other species of Arceuthobium, I found that A. tsugense shoots can fix $^{14}CO_2$ in the light. Accurate measurements of the photosynthetic rate cannot be obtained by this method because more CO_2 is produced from respiration than is fixed by photosynthesis. Consequently, the specific activity of $^{14}CO_2$ present in the flask is diluted by relatively large quantities of unlabelled CO_2 produced during the course of a one-hour experiment. Of the 3.3×10^5 d.p.m. $^{14}CO_2$ initially present in the flasks, about 10-15% is incorporated into the plant tissue during one hour. This must be regarded as a minimum estimate of the photosynthetic capacity of A. tsugense.

In most photosynthesis experiments, the ^{14}C labelled mistletoe shoots in each flask were cut up and assayed as described in the Methods Section. In two experiments the terminal 5-8 mm portions of the shoots were analyzed separately from the older, elongated stem portions. The ^{14}C activity (dpm/mg) of the tip extracts averaged nearly twice that of the stem extracts. This indicates that more active CO_2 fixation is occurring in the young tip regions of the aerial shoots, and is suggestive that any translocation from younger to older tissues is not a rapid process.

In agreement with the findings of Hull and Leonard (1964b), 80 to 90% of the ^{14}C activity incorporated by the aerial shoots is found in the ethanol soluble fraction. The ethanol extracts averaged about 100 dpm mg^{-1} fresh tissue weight, and were used for further characterization of the products of photosynthesis.

Table II shows that about 10% of the ^{14}C-photosynthate is lipid soluble. The H_2O soluble phase was further separated into cationic, anionic, and neutral fractions by ion exchange chromatography (Table III). In general, the cationic fraction of the photosynthate was more heavily labelled in these experiments than Hull and Leonard (1964b) reported for A. campylopodum forma abietinum (Engelm.) Gill.

The free amino acids were identified by elution from the cation resin, and the use of two-dimensional paper chromatography. On the basis of R_f values in three solvent systems, and color reactions with ninhydrin, it was found that valine, aspartic acid, and glutamic acid were present, with possibly leucine and/or phenylalanine. This is in general agreement with the findings of Greenham and Leonard (1965) for other forms of A. campylopodum. Additional amino acids are definitely present in A. tsugense, but are not identified yet.

Data from infrared gas analysis experiments with aerial shoots of A. tsugense support the respiration data obtained by manometric methods. Additionally, IRGA provides quantitative measurement of CO_2 fixation in the light. Figure 3 shows CO_2 evolution of the same tissue under dark and light conditions. After the system is flushed with low CO_2 air, a short period of equilibration and gas mixing ensues. The increase of CO_2 concentration due to dark respiration is equivalent to a rate of

TABLE II

DISTRIBUTION OF ETHANOL SOLUBLE ^{14}C IN A. TSUGENSE SHOOTS

Exp.	Initial Activity of Ethanol Extract in dpm	Activity of Lipid Soluble Fraction in dpm[a]	Activity of H_2O Soluble Fraction in dpm[a]
(1)	123,000	12,500 (10%)	113,000 (90%)
(2)	133,000	9,100 (9%)	96,800 (91%)
(3)	161,000	14,700 (11%)	124,000 (89%)

a % figures based upon total recovered activity from both fractions, some ^{14}C activity loss in (2) and (3) due to emulsion layer between chloroform/H_2O.

TABLE III

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

Exp.	Initial Activity in 10 mls dpm	Activity Retained by Cation Col. in dpm	(%)	Activity Retained by Anion Col. in dpm	(%)	Activity of Neutral Eff. dpm	(%)
(1)[a]	68,000	8,400	(12%)	13,500	(20%)	46,100	(68%)
(2)[a]	47,000	7,600	(16%)	9,700	(21%)	29,700	(63%)
(3)[b]	132,000	34,000	(26%)	30,000	(25%)	68,000	(51%)

a Cation Resin: Amberlite IRC-50

b Cation Resin: Dowex-50 W

310 µl CO_2 $g^{-1}h^{-1}$. With the lights on, CO_2 is still produced, but at a reduced rate of 210 µl CO_2 $g^{-1}h^{-1}$. Table IV shows a series of measurements performed on six different collections of A. tsugense shoots. Most of the experiments have repeat measurements on the same tissue. The mean production of CO_2 from dark respiration is 301 µl CO_2 $g^{-1}h^{-1}$. With the lights on, a reduced rate of 213 µl CO_2 $g^{-1}h^{-1}$ is obtained. The difference of 88 µl CO_2 $g^{-1}h^{-1}$ represents CO_2 fixation due to apparent photosynthesis of the tissue. It is necessary to qualify this figure as apparent photosynthesis, since CO_2 production in the light from photorespiration could be occurring (Tolbert 1971, Beevers 1971). The ratio of CO_2 fixed to CO_2 produced from respiration appears to be about 25-30%, and presumably the balance of the carbon requirements of A. tsugense is derived from the host. By comparison, Tsuga heterophylla twigs measured under the same conditions showed a dark respiration rate of 240 µl CO_2 $g^{-1}h^{-1}$ produced, and -910 µl CO_2 $g^{-1}h^{-1}$ fixed in the light. As is the case with most autotrophic green plants, hemlock can fix many times the CO_2 produced by respiration.

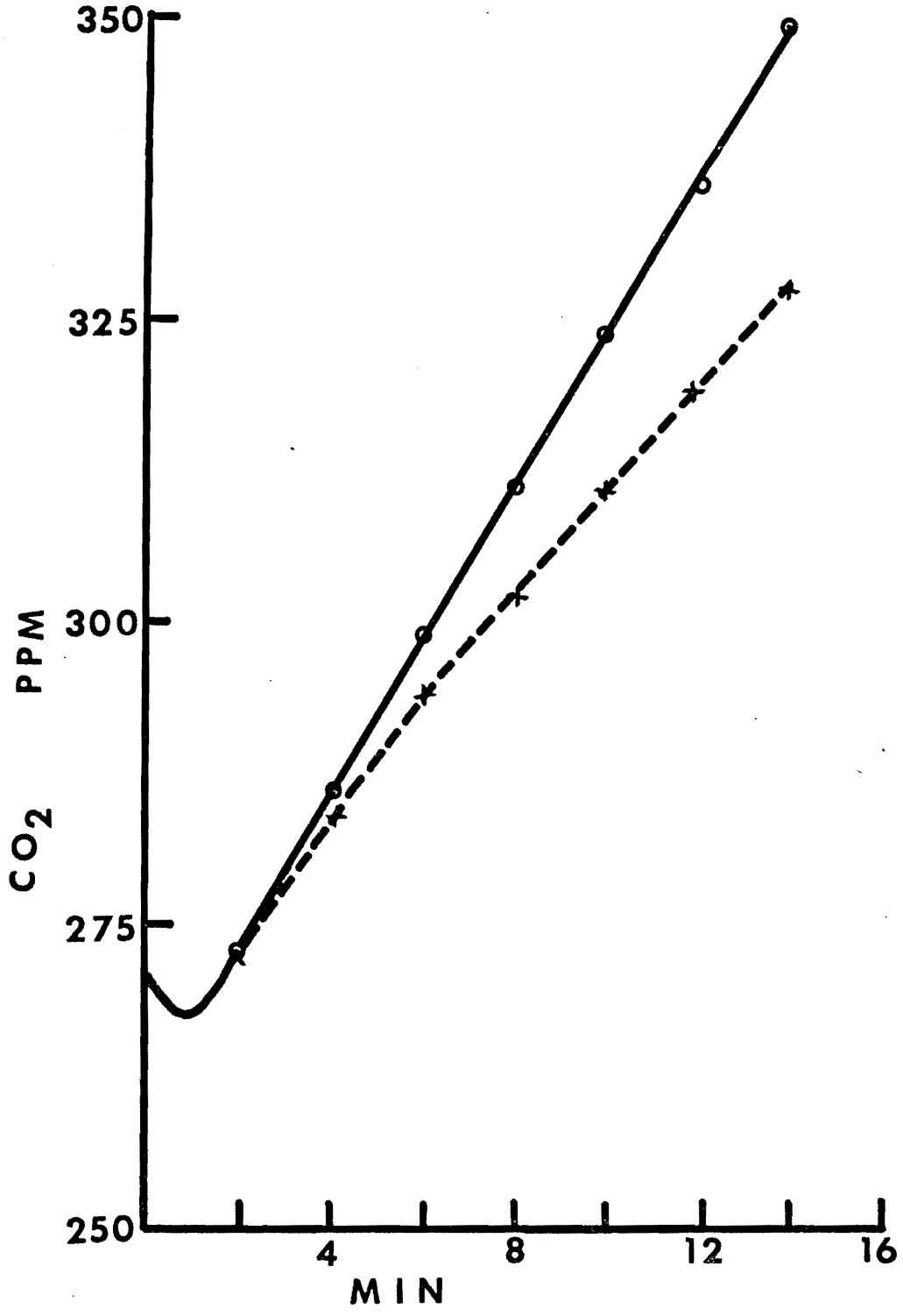

Figure 3. Rate of CO_2 evolution by Arceuthobium tsugense as determined by infrared gas anlysis on 8 May 1973.
——— = Dark -------- = Light

TABLE IV

PHOTOSYNTHESIS AND RESPIRATION OF A. TSUGENSE AERIAL SHOOTS [a]

Exp.	CO_2 Evolution in Dark	CO_2 Evolution in Light	App. P.S.[b]	App. P.S./Resp.
(1)	300	230 250	60	20%
(2)	320 290			
(3)	250 260	180 210 140	78	30%
(4)	320 350	220	115	34%
(5)	310 300	210 210 250	82	27%
(6)	310 310	210 240	85	27%
	$\bar{y}$=301 s=28	$\bar{y}$=213 s=32	$\bar{y}$=88 s=32	$\bar{y}$=28%±8

a Results from IRGA experiments, Spring 1973. All values except left and right-hand columns are µl CO_2 $g^{-1}h^{-1}$.

b App. P.S. = Apparent Photosynthesis.

DISCUSSION

McDowell (1964) floated tissue segments of A. campylopodum Engelm. forma campylopodum in dilute Hoagland's solution and measured O_2 consumption by Warburg manometric methods. His endogenous rate of respiration (110 µl O_2 $g^{-1}h^{-1}$) is only one-half the value I obtained, but the highest respiration rates obtained by the addition of amino acids (210 µl O_2 $g^{-1}h^{-1}$) compare favorably with those of the present investigation. Warburg manometric data of 245 µl O_2 $g^{-1}h^{-1}$ (Spring, 25°) and IRGA results of 301 µl CO_2 $g^{-1}h^{-1}$ (Spring 21-23°) are in close agreement, but are brought even closer if one uses an R.Q. of 1.1 in calculations. Since more CO_2 is produced than O_2 consumed, the figure of 301 µl CO_2 would be about 10% lower when expressed as µl O_2.

The role of lipid metabolism in A. tsugense is uncertain at this time. The presence of lipid droplets in the cytoplasm of parenchyma cells (Tainter 1971, and personal observations of A. tsugense), coupled with the incorporation of about 10% of the ^{14}C-photosynthate into the lipid soluble fraction suggests that lipids may play an important role in cell metabolism. Respiratory quotient values of plants are known to fluctuate widely depending upon season and temperature (Beevers 1961), and it is necessary to measure R.Q. values of Arceuthobium with respect to those variables before definite conclusions can be drawn. An R.Q. value greater than one could indicate lipid synthesis at the expense of carbohydrate. A seasonal analysis of lipid content and variations in lipid constituents would be most helpful in resolving this problem.

Huli and Leonard (1964a,b) tested several ideas concerning the

effect of Arceuthobium spp. upon their hosts. No evidence was found to support the existence of a "physiological girdle" which would disrupt normal phloem transport of the host. Their evidence did suggest that the endophytic system of dwarf mistletoe could intercept labeled host photosynthate and transport it into the aerial shoots. The present study permits us to calculate the magnitude of carbon withdrawal from the host. A dark respiration rate of 300 µl CO_2 $g^{-1}h^{-1}$ is equivalent to 12.3 µmoles CO_2 or 0.5-0.6 mg CO_2 $g^{-1}h^{-1}$ fresh weight. Photosynthetic CO_2 fixation could contribute 25-30% of this amount, and the balance must be obtained from the host. Although this may not be an unusual rate of carbon utilization, the biomass of parasite tissue in a heavily infected tree can be substantial. A. tsugense forms non-systemic or localized infections with much of the parasite tissue found in the aerial shoots (individual shoots may exceed three grams). It would be most instructive to compare rates of host translocation to rates of carbon utilization by a large infection of 30-50 grams, remembering that an individual tree may have many such infections. The present investigation cannot determine whether the localized removal of reduced carbon compounds by the parasite is a significant factor contributing to the detrimental influence of dwarf mistletoe on the host, but it provides a starting point for further studies which could resolve the question. IRGA determinations of carbon utilization by intact aerial shoots of different species of dwarf mistletoe would be especially useful if correlated with similar measurements of host metabolism under field conditions. It seems quite probable that different species of Arceuthobium vary in their dependence upon the host.

REFERENCES CITED

Anonymous 1966. Liquid scintillation counting. Nuclear-Chicago Pub. 711580

Beevers, H. 1961. Repiratory metabolism in plants. Row, Peterson and Co. Evanston, Ill.

_____ 1971. Photorespiration: assessment, p. 541-543. In M. D. Hatch, C. B. Osmund, and R. O. Slayter (ed.) Photosynthesis and photorespiration. Wiley-Interscience, New York.

Freeland, R. O. 1943. The American mistletoe with respect to chlorophyll and photosynthesis. Plant Physiol. 18: 299-302.

Greenham, C. G., and O. A. Leonard 1965. The amino acids of some mistletoes and their hosts. Amer. J. Bot. 52: 41-47.

Hawksworth, F. G., and D. Wiens 1970. Biology and taxonomy of the dwarf mistletoes. Ann. Rev. Phytopath. 8: 187-208.

Hull, R. J., and O. A. Leonard 1964. Physiological aspects of parasitism in mistletoes (Arceuthobium and Phoradendron).

_____ and _____ 1964a. The carbohydrate nutrition of mistletoe. Plant Physiol. 39: 996-1007.

_____ and _____ 1964b. II. The photosynthetic capacity of mistletoe. Plant Physiol. 39: 1008-1017.

Krueger, K. W., and R. H. Ruth 1969. Comparative photosynthesis of red alder, Douglas fir, sitka spruce, and western hemlock seedlings. Can. J. Bot. 47: 519-547.

McDowell, L. L. 1964. Physiological relationships between dwarf mistletoe and ponderosa pine. Ph.D. Thesis, Oregon State Univ., Corvallis.

Rediske, J. H., and K. R. Shea 1961. The production and translocation of photosynthate in dwarf mistletoe and lodgepole pine. Amer. J. Bot. 48: 447-452.

Smith, I. 1960. Chromatographic and electrophoretic techniques. Vol. I Chromatography. Interscience Pub., New York.

Tainter, F. H. 1971. The ultrastructure of Arceuthobium pusillum. Can. J. Bot. 49: 1615-1622.

Tolbert, N. E. 1971. Leaf peroxisomes and photorespiration, p. 458-471. In M. D. Hatch, C. B. Osmund, and R. O. Slayter (ed.) Photosynthesis and photorespiration. Wiley-Interscience, New York.

Umbreit, W. W., R. H. Burris, and J. F. Stauffer 1959. Manometric techniques. Burgess Pub. Minneapolis.