Physiological Aspects of Parasitism in Mistletoes (Arceuthobium and Phoradendron)

II. The Photosynthetic Capacity of Mistletoe [1, 2]

Richard J. Hull[3] and Oliver A. Leonard
Department of Botany, University of California, Davis

In the preceding paper (7) it was noted that the true mistletoe, *Phoradendron,* derives very little if any carbohydrate from its host, while the dwarf mistletoe, *Arceuthobium,* draws heavily upon the photosynthate of its host. These findings imply that *Phoradendron* is capable of furnishing its own energy requirements but say little regarding the extent of dependence of *Arceuthobium* upon its host for carbohydrates.

The aerial shoots of *Arceuthobium* frequently exhibit an off-green color which Peirce (9) claimed is due to an abundant supply of chlorophyll that is often masked by dark pigments in the epidermal cells. Heinricher (6) described green-colored bodies in the cells of the endophytic system, however, this observation was not confirmed by Peirce (9) or Kuijt (8). A capacity of *Arceuthobium* to provide much of its energy requirements was implied by findings of Weir (16). He conducted a test on 8- to 12-year old lodgepole pines in which 6 infected and 6 uninfected trees were repeatedly defoliated. During the second year the 6 infected trees continued to live while the uninfected trees had died. He suggested that under such conditions the mistletoe may contribute to the nutrition of its host but he added that mutual subsistence on stored materials in the infected region of host stems may explain the results. On the other hand, Buckland and Marples (2) noted that defoliation of western hemlock by the hemlock looper had a much more detrimental effect on dwarf mistletoe infected trees than on healthy trees.

When Rediske and Shea (11) exposed aerial shoots of *Arceuthobium americanum* Nutt. infecting *Pinus murrayana* Grev. and Balf. seedlings to $C^{14}O_2$, they noted a marked fixation of C^{14} in the shoots and movement of label into the host plant. This evidence was used to support their claim that the decline of the host plant normally associated with *Arceuthobium* infection is not a direct result of parasitism by the mistletoe.

Freeland (5) analyzed leaves of *Phoradendron flavescens* (Pursh.) Nutt. for chlorophyll and found that both chlorophyll a and chlorophyll b were present. Apparent photosynthesis was measured and a net rate of CO_2 fixation was determined. The mass of photosynthetic tissue produced by some species of *Phoradendron* has prompted the speculation that a symbiotic relationship might exist between the true mistletoe and its host. Wagener (15) noted that junipers heavily infected with *Phoradendron juniperinum* Engelm. will often have the entire upper portion of their crown replaced by mistletoe foliage. After an extremely cold winter, which killed the mistletoe, severe crown die-back was observed. He concluded that when the functioning mistletoe foliage was killed the very small amount of host foliage remaining was unable to support the crown and it died. *Phoradendron* infections are frequently found to terminate branches of its host (3, 15), that portion of the host branch distal to the site of infection is suppressed and killed with the proximal portion subsequently "sustained" by the mistletoe.

Similar conclusions were drawn from defoliation and girdling experiments involving the European mistletoe *Viscum album* (14). Infections of *Viscum* were observed to prolong the life of denuded branches and even whole trees but such a situation could never be maintained for an extended period of time. The concept of symbiosis was not supported by findings of Seledzhanu and Galan-Fabian (12) who fed $C^{14}O_2$ to foliage of *Viscum* infecting black poplar. They observed fixation of CO_2 by mistletoe leaves during all seasons of the year. Movement of C^{14} into adjacent mistletoe shoots was noted within 2 hours as well as into the endophytic system and to a small extent into the host tissue. The authors concluded that the transfer of organic materials between mistletoe and its host occurs only to a very limited extent.

The purpose of the investigations reported here was to determine to what extent mistletoes can contribute to their own carbon nutrition and possibly to that of their hosts. This has been attempted primarily through $C^{14}O_2$ feeding experiments, in which the photosynthetic products of mistletoe shoots were traced by radioautography and analyzed chemically to determine their similarity to carbon fixation products in other tissues.

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[3] Present address: Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana.

Materials and Methods

The capacity of mistletoes to provide for their own energy requirements was investigated through studies on the photosynthetic activity of mistletoe shoots. *Arceuthobium campylopodum* Engelm., infecting *Abies concolor* (G. and G.) Lindl., and *Arceuthobium americanum* Nutt., parasitizing *Pinus murrayana* Grev. and Balf., were the principal subjects of study. Several species of *Phoradendron* infecting a number of different host plants were used for comparative studies (table II). Translocation patterns between parasite and host were determined by exposing aerial shoots to 50 μc of $C^{14}O_2$ in the light and following the subsequent distribution of photosynthate by procedures of gross radioautography and radioassay similar to those described in Part I (7).

Aerial shoots of *Phoradendron* are reasonably large; thus no problem was encountered in enclosing portions of a plant in a polyethylene bag for $C^{14}O_2$ application. Dwarf mistletoe shoots, on the other hand, are quite small and easily broken, characteristics which render the polyethylene bag impractical as an exposure chamber. In its place, a 1.75 liter polyethylene bottle was adapted for the purpose. Inlet and exit tubes were inserted through the base of the bottle, which was supported by them while the apparatus was in use. The major portion of a moderately sized mistletoe shoot was carefully teased through the neck of the bottle and a gas tight seal around the stem achieved by means of a 1-hole rubber stopper split along one side. The hole and split of the stopper were lined with lanolin paste so that no gas could escape but some movement of the mistletoe shoot was possible. The lanolin sealing material was prepared by mixing a small amount of water with commercial lanolin until a thick creamy paste was obtained. This preparation retained its viscosity through midday temperatures during the 4- to 5-hour exposure period and was completely nontoxic to the plant. The concentration of CO_2 in the bottle after reacting the 0.52 mg of $BaC^{14}O_3$ with lactic acid was about 0.06 % by weight, which does not constitute an excessively rich CO_2 environment.

Labeling experiments were also conducted with detached dwarf mistletoe shoots, the cut ends of which were inserted in small vials of water. Glass bell jars were used for exposure chambers in such tests, which were conducted both in the laboratory and in the field. Both plastic bottles and bell jars were utilized as reaction chambers for studies in which the CO_2 concentration within the chamber containing mistletoe shoots was monitored over a period of time under conditions of light and darkness. Such studies were conducted in the laboratory and in the field using a Beckman L/B Infrared Gas Analyzer, Model 15A.

Mistletoe shoots, exposed to $C^{14}O_2$, were prepared for radioassay and chromatography according to the method described in Part I (7). Separation of the water soluble extract into cationic, anionic and nonionic fractions was accomplished by means of ion exchange resins. Cations from the extract, adjusted to pH 2, were collected on a column of Dowex 50W-X8 (100–200 mesh, hydrogen form) and eluted with 1.5 N NH_4OH. The effluent was adjusted to pH 8.2 and passed through a column of Dowex 1-X8 (100–200 mesh, carbonate form) and the anions subsequently eluted with 1.5 M $(NH_4)_2CO_3$. The 3 fractions were assayed for radioactivity and chromatographed using methods described previously.

The chlorophyll content of mistletoe shoots and foliage of the host plant was determined in 80 % acetone extracts using the procedure outlined by Arnon (1). Absorption spectra of acetone extracts from dwarf mistletoe shoots and host foliage were obtained using a Zeiss PMQII Spectrophotometer.

Results and Discussion

The marked accumulation of host photosynthate in the endophytic system and aerial shoots of *Arceuthobium* has already been described (7). This finding would tend in itself to negate the probability that dwarf mistletoe shoots function significantly as photosynthetic organs and thereby contribute to the energy requirements of the plant. The results of an early experiment, in which the terminal foliage of infected white fir branches was exposed to $C^{14}O_2$ after the mistletoe shoots had been either removed, covered with aluminum foil, or removed and the infected portion of the stem covered, suggest that the aerial shoots may play a part in dwarf mistletoe nutrition (table I). While the data are somewhat erratic, there is a strong indication that when mistletoe shoots are removed, there follows an enhanced accumulation of host photosynthate in the region of the endophytic system and even, to a lesser extent, in the uninfected portions of the stem. This phenomenon was observed during all seasons of the year.

One possible explanation of these findings is that,

Table I. *Distribution of C^{14} in White Fir Branches Infected with Dwarf Mistletoe*

The terminal foliage of the fir branches was exposed to 50 μc of $C^{14}O_2$ for 6 hours. Fourteen days after treatment the plants were harvested. Prior to treatment the mistletoe infections were variously manipulated to enhance transport from the host into the parasite.

Manipulation of mistletoe shoots	cpm/mg dry wt					No. of observations
	TF*	SA	ES	MS	SB	
Shoots untouched	463	36	55	96	5	4
Shoots removed	544	82	143	...	24	5
Infection covered	540	53	94	61	49	3
Shoots removed and infection covered	890	212	355	...	108	4
Uninfected branch	311	14	...	...	3	4

* TF, treated foliage of fir; SA, stem above site of infection; ES, endophytic system and associated host tissues; MS, mistletoe shoots; SB, stem below site of infection.

when the aerial shoots of a dwarf mistletoe plant are removed, the normal route of export for materials collected by the mistletoes endophytic system is cut off, resulting in an accumulation in the endophytic system. A similar concentration in the endophytic system was noted in undisturbed infections during the dormant season, when movement of photosynthate to the aerial shoots occurred more slowly. This concept is further supported by the fact that covering the aerial shoots with aluminum foil to exclude light does not induce the same level of accumulation in the endophytic system as does removing the shoots. Also the level of photosynthate transported to darkened shoots does not appear to be any greater than that to shoots growing in the light. Aerial shoots of dwarf mistletoe covered with aluminum foil for 9 months appeared perfectly normal.

An alternate explanation of these findings might suggest that the removal of mistletoe shoots deprives the endophytic system of part of its energy supply, causing it to draw more heavily upon the photosynthate of its host. This would follow the concept of Rediske and Shea (11), who concluded that *Arceuthobium* contributes significantly to its own nutrition and also to that of its host. Similar suggestions have been offered to explain observations made on *Viscum* (14) and *Phoradendron* (15).

The problem resolves itself to an analysis of the photosynthetic capacity of mistletoe shoots. Aerial shoots of a number of mistletoes, both *Arceuthobium* and *Phoradendron,* were exposed to $C^{14}O_2$ for about 5 hours. After 14 days the translocation pattern of photosynthate was determined by radioautography (fig 1, 2) and radioassay (table II). Fixation of C^{14} was noted in the aerial shoots of both mistletoes. Particularly striking is the difference between the 2 mistletoe genera in the movement of fixed C^{14} from the aerial shoots to the endophytic system. This basipetal transport is quite marked in *Phoradendron* (fig 1) but virtually nonexistent in *Arceuthobium* (fig 2).

Neither genus of mistletoes showed movement of photosynthate from aerial shoots into the host plant. A detailed observation of the region of the endophytic system in *Phoradendron* infections revealed that the C^{14} from the aerial shoots was confined to the mistletoe tissue. Any transfer of carbon between the 2 plants occurs to a very small extent if it occurs at all. The aerial shoots of *Phoradendron flavescens* infecting several deciduous trees (*Juglans* and *Quercus*) were exposed to $C^{14}O_2$ during the dormant season when the host was without leaves. Labeled photosynthate was noted to translocate to the endophytic system but again none could be detected in the host tissue.

The translocation relationship between *Phoradendron* and its host was studied in situations where the mistletoe plant terminated a branch of the host which was devoid of foliage of its own. Such cases are not uncommon in *Phoradendron*-infected trees, usually occurring when the host branch distal to the site of infection dies, leaving the mistletoe to "sustain" the basal portion. Infections of this type often live for many years while the supporting branch thickens and appears to grow normally. When the aerial shoots of such a mistletoe infection were exposed to $C^{14}O_2$, the photosynthate moved into the endophytic system but not into the host (fig 3A and B). Treating the terminal foliage of the host branch, to which the infected branch was attached, with $C^{14}O_2$, revealed movement of labeled photosynthate into the infected branch but not into the endophytic system of the mistletoe (fig 3C).

Thus it appears unlikely that the photosynthetic activity of *Phoradendron* shoots contributes to the nutrition of the host during any season of the year, whether the host is deciduous or evergreen. Host branches, containing only mistletoe foliage, are sustained by photosynthate produced by the host and obtain no benefit from the mistletoe except possibly through the maintenance of proper water relations within the branch and conceivably through the establishment of a suitable hormonal balance. Most of the reports in the literature (14, 15), which appear to

Table II. *Distribution of C^{14} in Mistletoe Infections on Various Host Trees Two Weeks after Exposing the Aerial Shoots of Mistletoe to $C^{14}O_2$*

Mistletoe	Host	cpm/mg dry wt		No. of trials
		ES*	MS	
Arceuthobium				
A. americanum	*Pinus murrayana*	0.3	672	4
A. campylopodum	*Abies concolor*	0.1	99	16
A. campylopodum	*Pinus sabiniana*	0.2	...	6
Phoradendron				
P. bolleanum	*Abies concolor*	26	290	1
P. bolleanum	*Cupressus macnabiana*	24	768	4
P. flavescens	*Juglans hindsii*	5	1708	3
P. flavescens	*Quercus douglasii*	7	1279	5
P. flavescens	*Quercus kelloggii*	20	687	3
P. flavescens	*Quercus wislizenii*	6	576	1
P. juniperinum	*Libocedrus decurrens*	24	547	1

* ES, endophytic system and associated host tissue; MS, mistletoe shoots.

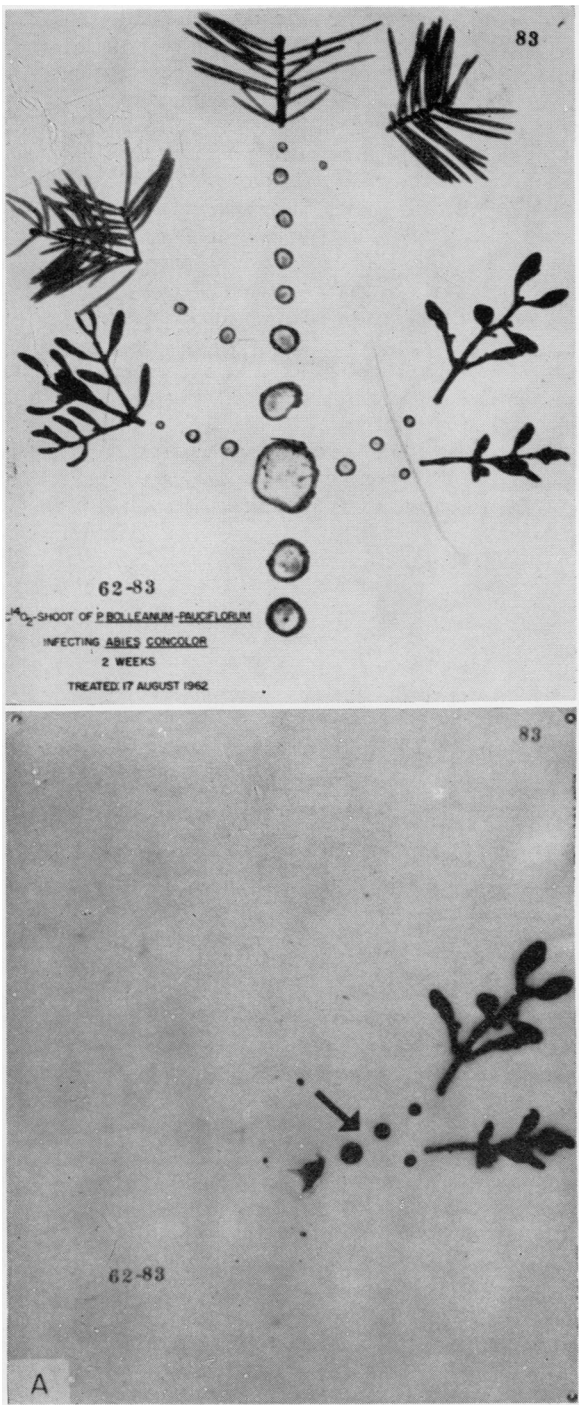

FIG. 1. Mounted plants (*above*) and radioautographs (*below*) of a branch of *Abies concolor* bearing an infection of *Phoradendron bolleanum* var. *pauciflorum*. Portions of the mistletoe shoots (above arrow) were exposed to 50 μc of $C^{14}O_2$ for 5 hours on August 17, 1962 and harvested 14 days later. Note the transport of C^{14} into the endophytic system of the mistletoe but negligible transfer into host tissue.

support a symbiotic relationship between leafy mistletoes and their hosts, can be explained on the basis of concepts described above.

The failure of *Arceuthobium* to export photosynthate from its aerial shoots into its endophytic system, let alone into the host, is in agreement with the fact that large quantities of host photosynthate normally move in the reverse direction. This finding is also in agreement with the fact that differentiated sieve elements have never been observed in the aerial shoots of *Arceuthobium* (4, 8, 13), although the absence of such structures apparently does not restrict movement into aerial shoots. In an effort to induce movement out of dwarf mistletoe shoots, infected white fir branches were defoliated, covered with aluminum foil, girdled near the trunk, or defoliated and girdled prior to exposing the mistletoe shoots to $C^{14}O_2$. These tests were conducted during the summer (July 24) and autumn (October 7). Two weeks after treatment there was no indication of radioactivity moving out of the shoots of any dwarf mistletoe plant tested during either season.

The discrepancy of these findings with those of Rediske and Shea (11) probably can be explained as a result of $C^{14}O_2$ leakage into the atmosphere of the growth chamber used in their study. The uniform labeling pattern obtained by these authors suggests that such a technical error may be involved.

To investigate further the photosynthetic nature of CO_2 fixation by *Arceuthobium*, shoots were exposed to $C^{14}O_2$ under conditions of light and darkness. For these studies, detached shoots were used as well as those retained on the host plant. The results (table III) indicate an 8- to 15-fold increase in carbon fixation in the light over that occurring in darkness. Male and female plants showed no appreciable difference in carbon fixation in either light or darkness. Detaching mistletoe shoots from their endophytic system appeared to reduce the efficiency of both light and dark fixation. For this reason all further studies were conducted on undisturbed infections.

Shoots of *Arceuthobium*, exposed to $C^{14}O_2$ for about 5 hours and sampled at various time intervals thereafter, showed a gradual decline in radioactivity over a 14-day period amounting to about 1/2 to 2/3 of that originally fixed (table IV). During this time the amount of C^{14} occurring in materials insoluble in 80 % ethanol increased, representing about 50 % of the C^{14} remaining in the plant after 14 days. Incubation with clarase for 36 hours failed to release more than 10 % of the insoluble C^{14}. This indicates that starch is not the principal end product for shoot photosynthate. By comparison, C^{14} fixed by *Phoradendron* shoots and converted to an ethanol insoluble material is about 50 % hydrolyzable with clarase.

Thus while both genera of mistletoes photosynthetically fix CO_2 in their aerial shoots, the chemical destination of this carbon appears to be different. Since *Arceuthobium* obtains large quantities of carbon from its host, that fixed by the aerial shoots may enter into a scheme of metabolism unlike that nor-

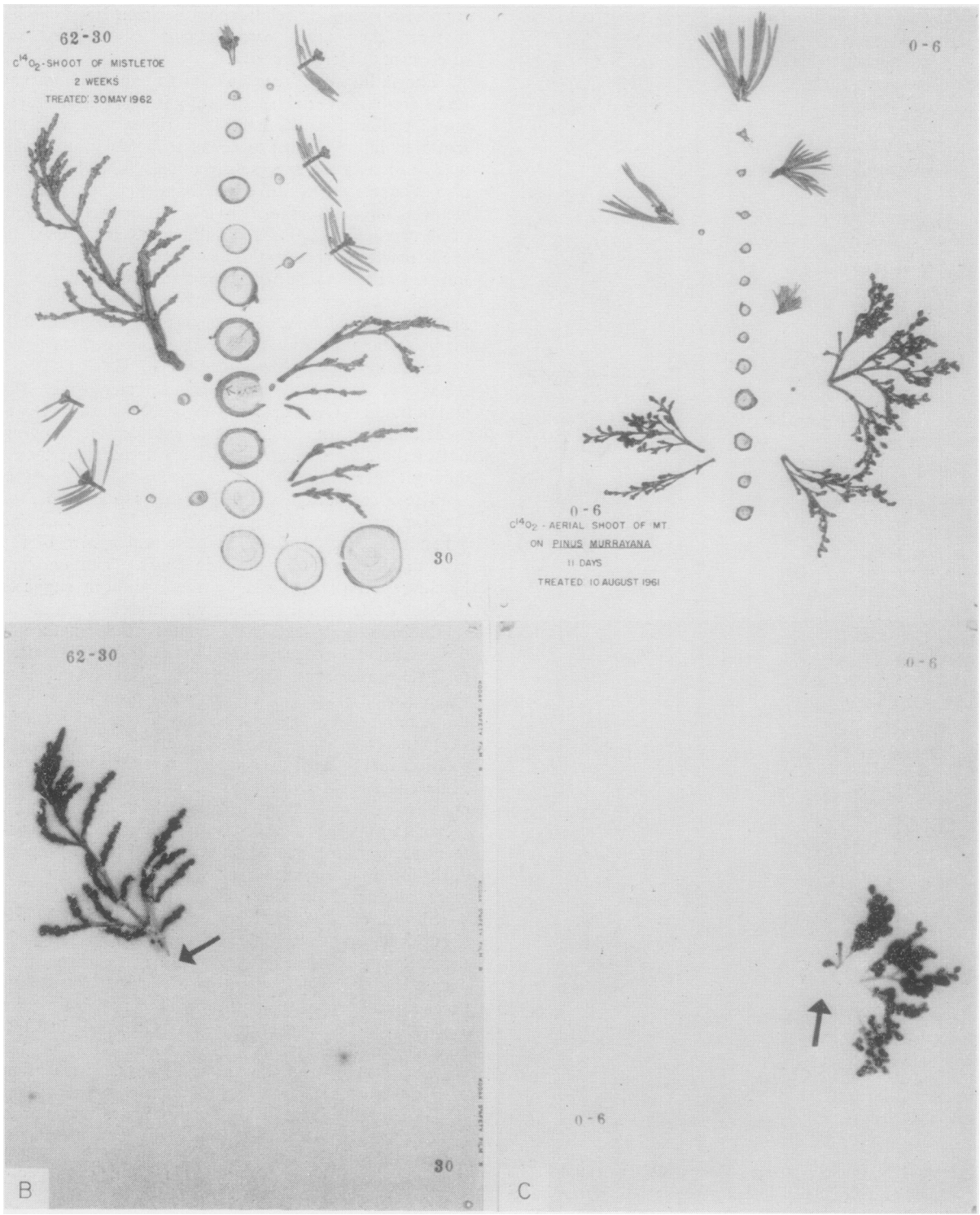

FIG. 2. Mounted plants (*above*) and radioautographs (*below*) of conifer branches bearing dwarf mistletoe infections. Part of the mistletoe shoot (above arrow) was exposed to 50 μc of $C^{14}O_2$ for 5 hours and harvested about 2 weeks later. B) *Arceuthobium campylopodum* infecting *Abies concolor;* treated May 30, 1962. C) *Arceuthobium americanum* infecting *Pinus murrayana;* treated August 10, 1961. In both examples note the lack of basipetal transport of C^{14} from the mistletoe shoots and the absence of radioactivity in the host.

Table III. *$C^{14}O_2$ Fixation by Aerial Shoots of* Arceuthobium campylopodum *Infecting* Abies concolor *under Conditions of Light and Darkness*

Light condition	Sex of plant	cpm/mg dry wt	No. of trials
Shoots detached from host			
Light	Male	125	7
"	Female	127	8
Dark	Male	9	2
"	Female	7	4
Shoots attached to host			
Light	Male and Female	328	6
Dark	Male and Female	42	2

mally associated with photosynthetic carbon fixation.

This speculation was confirmed when the C^{14} occurring in the water soluble extract from $C^{14}O_2$-fed mistletoe shoots was separated into cationic, anionic and nonionic fractions by means of ion exchange resins (table V). A striking difference was noted in the distribution of carbon fed directly to the mistletoe shoots as $C^{14}O_2$ and that obtained through labeled host photosynthate. An unusually high percentage of the carbon photosynthetically fixed by the shoots remains in the anionic fraction, even after 14 days from the time of treatment. This fraction contains mostly organic acids and some phosphate esters. C^{14} obtained from labeled host photosynthate remains primarily in the nonionic sugar fraction. In neither case does any appreciable amount of C^{14} accumulate in the cationic amino acid fraction.

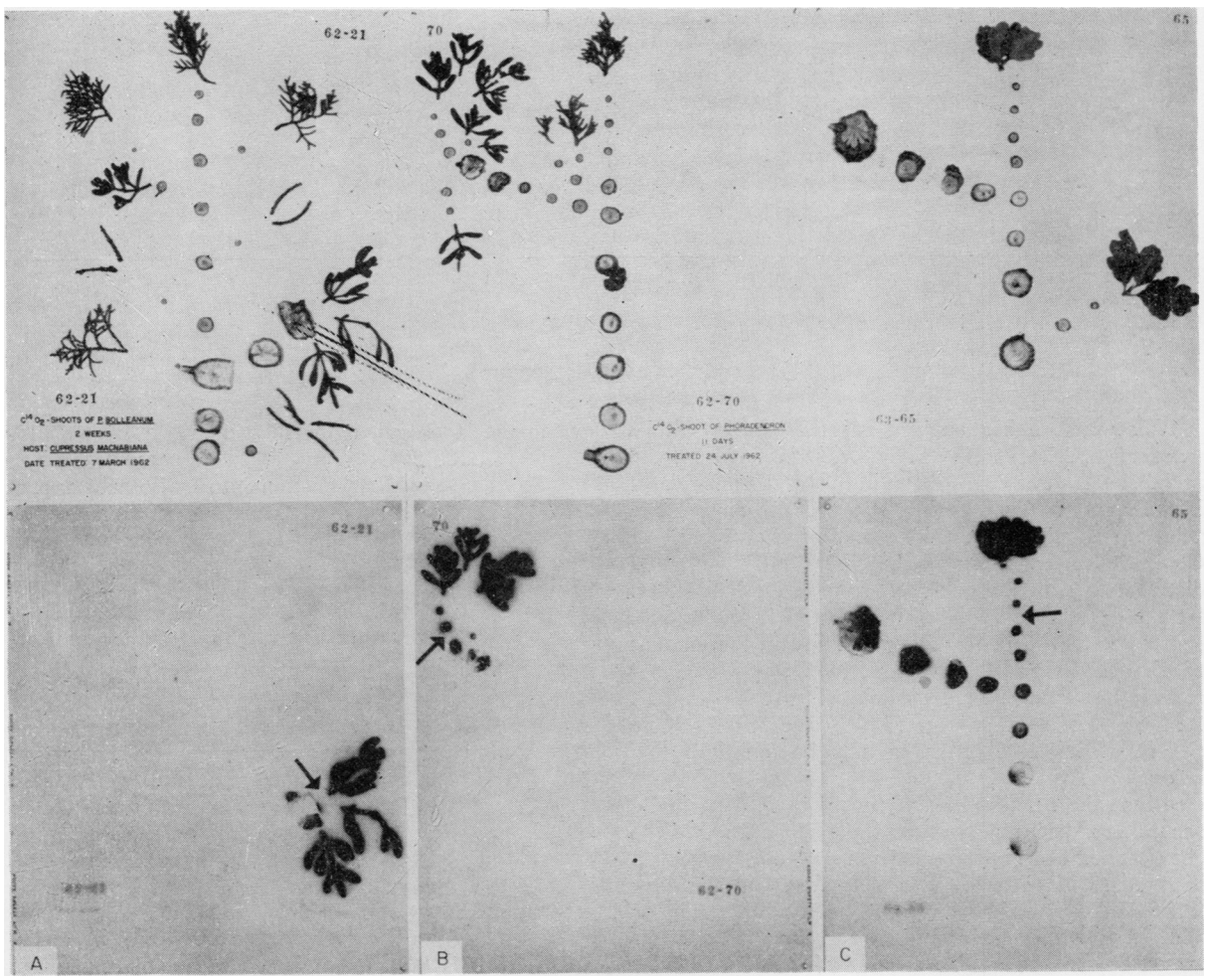

FIG. 3. Mounted plants (*above*) and radioautographs (*below*) of *Phoradendron* infected branches containing the mistletoe on a lateral branch having no host foliage. A) *P. bolleanum* var. *densum* infecting *Cupressus macnabiana;* mistletoe shoots treated with $C^{14}O_2$ (above arrow) on March 7, 1962. Note lack of movement into lateral branch. B) Same as A above; treated July 24, 1962. C) *P. flavescens* var. *villosum* infecting *Quercus douglasii;* oak foliage treated with $C^{14}O_2$ (above arrow) on July 1, 1963; mistletoe shoots removed May 28, 1963. Note movement of label into lateral branch but not into endophytic system of mistletoe.

Table IV. *Fate of C^{14} in Aerial Shoots of Dwarf Mistletoe Exposed to $C^{14}O_2$ for 5 Hours*

Mistletoe	Time after treatment	cpm/mg dry wt	Percent distribution	
			80 % Ethanol	Residue
*A. campylopodum**	0 hr	293	74	26
"	40 hr	363	57	43
"	14 days	99	42	58
*A. americanum***	0 hr	1280	85	15
"	14 days	672	49	51

* *A. campylopodum* infected *Abies concolor.*
** *A. americanum* infected *Pinus murrayana.*

Table V. *Distribution of C^{14} in the Water Soluble Fraction from Dwarf Mistletoe Shoots Exposed to $C^{14}O_2$ or Receiving Labeled Photosynthate from Its Host*

Mistletoe	Time after treatment	Percent of C^{14}			Total* cpm × 10^4
		Cationic	Anionic	Nonionic	
Mistletoe exposed to C^{14}					
*A. americanum***	0 hr	1	36	63	88.6
"	14 days	13	70	16	20.0
*A. campylopodum****	0 hr	6	62	32	12.4
"	40 hr	6	46	48	15.3
"	14 days	10	52	39	3.6
Host foliage exposed to C^{14}					
*A. americanum***	7 days	0.5	7	92	72.9
*A. campylopodum****	14 days	0.3	3	96	5.4

* Counts found only in the water soluble fraction from 1 g dry wt of mistletoe shoots.
** *A. americanum* infecting *Pinus murrayana.*
*** *A. campylopodum* infecting *Abies concolor.*

Chromatographic analysis of the water soluble extract (table VI) shows that carbon fixed by the aerial shoots of *Arceuthobium* in the light accumulates initially in the disaccharide, sucrose, and in organic acids, primarily malic acid. In time the amount of C^{14} in the sugars decreases, becoming more uniformly distributed between sucrose, glucose, and fructose. At the same time label initially found in malic acid decreases, with a concomitant increase in the amount of C^{14} occurring in an unidentified phosphorylated reducing substance here designated as compound-X. At the end of 14 days over 55 % of the water soluble C^{14} remaining in the shoots may be found in this unknown compound.

Since the fixation of CO_2 in malic acid normally occurs as a dark reaction, the light dependence of the phenomenon observed in mistletoe was tested. Shoots of *Arceuthobium* were exposed to $C^{14}O_2$ in the dark for 6 hours and sampled immediately thereafter or allowed to remain undisturbed for an additional 25 hours before being harvested (table VI). Under these conditions no C^{14} was detected in the sugars but as expected over 75 % of the fixed label was present as malic acid. Twenty-five hours later, the percentage of C^{14} in malic acid decreased while that in compound-X increased. This suggests that compound-X is derived from products of the malic acid fixation mechanism, since C^{14} was never detected in this compound when the label was supplied through the host photosynthate.

Since $C^{14}O_2$ incorporation into malic acid apparently occurs in shoots of *Arceuthobium* under either

Table VI. *Percent Distribution of C^{14} in the Water Soluble Fraction of Dwarf Mistletoe Shoots at Various Times after Exposure to $C^{14}O_2$*

Compound	Time after exposure to $C^{14}O_2$				
	0 hr	40 hr	14 days	0 hr	25 hr
*A. campylopodum**	Light			Dark	
Sucrose	39	52	4	0.0	0.0
Glucose	4	10	14	0.0	0.0
Fructose	0.4	3	6	0.0	0.0
Malic acid	34	18	6	80	66
Compound-X	11	15	58	7	34
Others	12	3	13	13	0.0
*A. americanum***					
Sucrose	54		2		
Glucose	7		8		
Fructose	3		3		
Malic acid	31		0.4		
Compound-X	5		70		
Others	1.0		15		

* *A. campylopodum* infecting *Abies concolor.*
** *A. americanum* infecting *Pinus murrayana.*

light or dark conditions, this process differs somewhat from the scheme of Crassulacean acid metabolism as described by Ranson and Thomas (10). Nevertheless, it seems likely that the enzymes operative in Crassulacean acid metabolism may be operative here. Dwarf mistletoe shoots exhibit many characteristics common to xerophytic plants, (thickened cuticle, reduced leaf surface, and a decreased number of stomata), so it would not be surprising for its metabolism to evidence similar adaptations. This aspect of mistletoe physiology is certainly deserving of further study.

Freeland (5) found that leaves of *Phoradendron flavescens* contain both chlorophyll a and chlorophyll b and a net rate of photosynthesis of 0.6 mg of CO_2 per 10 ml of tissue per hour was measured. Similar studies have never been conducted with *Arceuthobium,* or if they have we are not aware of them. Eighty percent acetone extracts, prepared from a number of mistletoes and from the leaves of their hosts, were assayed for chlorophyll by determining their optical density at 663 and 645 mμ. Both fresh- and freeze-dried material was used, but a comparison of the results indicated that the latter was less efficiently extracted by 80 % acetone and yielded more variable results. For this reason only the analyses from fresh material are presented in table VII except for *Phoradendron* and its hosts, for which only data from freeze-dried material are available.

Chlorophyll was found in all mistletoes tested, however the concentration in *Arceuthobium* was only about 1/4 of that found in the leaves of its hosts. The chlorophyll content of *Phoradendron* leaves was equal to or only slightly less than that of the foliage of its hosts. In all cases the ratio of chlorophyll a to chlorophyll b was about the same for mistletoe and host however, this value was subject to considerable variation. Absorption spectra were determined for 80 % acetone extracts of *Arceuthobium* shoots and host foliage. Except for the strong absorption band in the near ultraviolet range, mistletoe extracts qualitatively show the same pigment complex as do similar extracts from pine and fir needles. Based on the pigments present and the fact that sucrose is among the principal products, it appears that photosynthetic CO_2 fixation in *Arceuthobium* possibly occurs via normal mechanisms.

An effort was made to determine the rate of CO_2 fixation by dwarf mistletoe shoots. Detached shoots were placed in a closed chamber, the atmosphere of which was monitored for changes in the CO_2 concentration by means of an infrared gas analyzer. Light was supplied by 2 banks of 20 w Daylight-Mazda lamps, which provided a light intensity of 700 ft-c at the plant surface. This light intensity is comparable to that normally encountered by these plants in the field. When conifer needles were placed in the chamber, a decrease in the CO_2 concentration was recorded in the light followed by an increase when the light was turned off. Mistletoe shoots, showed an increase in CO_2 level in the chamber whether the lights were on or off (fig 4). When the same test was conducted in the field, utilizing shoots attached to their host plant, there continued to be no measurable utilization of CO_2. The rate curve for CO_2 increase did not even show a deflection when the plants were taken from light to darkness (fig 4). It appears that the rate of CO_2 fixation by aerial shoots of *Arceuthobium* is too small to be measured by the equipment used in these studies.

The above findings suggest a method by which the

Table VII. *Chlorophyll Content of the Foliage of Various Conifers and Aerial Shoots of Mistletoes Infecting Them*

Plant species	Total chlorophyll mg/g dry wt	Chlorophyll a / Chlorophyll b
Conifers		
Abies concolor	1.92	2.9
Abies magnifica	1.90	3.1
Cupressus macnabiana	1.24	2.4
Juniperus occidentalis	0.79	2.9
Libocedrus decurrens	1.60	2.1
Pinus monophylla	1.85	3.2
Pinus murrayana	1.70	3.0
Pinus ponderosa	1.90	3.5
Pinus sabiniana	2.20	3.4
Arceuthobium		
A. americanum on *P. murrayana*	0.41	2.1
A. campylopodum on *A. concolor*	0.35	1.9
A. campylopodum on *A. magnifica*	0.30	3.4
A. campylopodum on *P. monophylla*	0.44	3.3
A. campylopodum on *P. ponderosa*	0.24	2.8
A. campylopodum on *P. sabiniana*	0.48	3.4
Phoradendron		
P. bolleanum var. *densum*	0.92	3.3
P. bolleanum var. *pauciflorum*	1.25	2.5
P. juniperinum var. *libocedri*	1.52	2.3
P. juniperinum var. *ligatum*	0.91	2.2

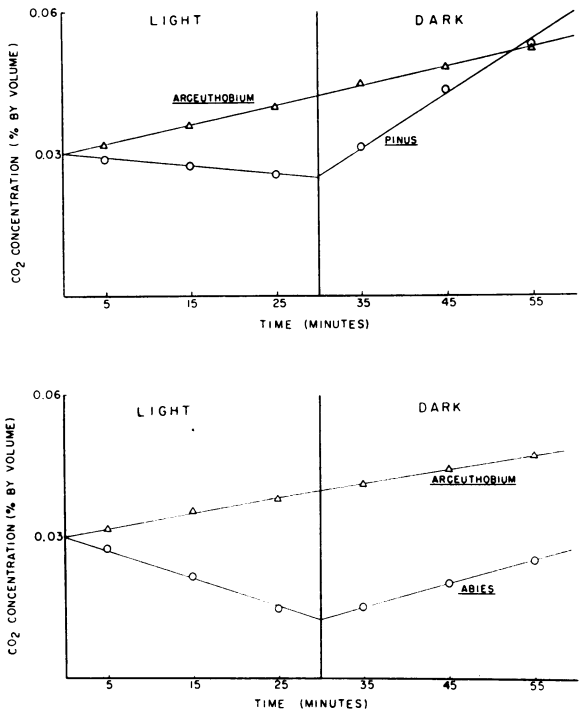

FIG. 4. Variation of the CO_2 concentration within a closed chamber containing shoots of *Arceuthobium* or leaves of its host under conditions of light and darkness. (*Upper*) *Arceuthobium americanum* and *Pinus murrayana*, laboratory test. (*Lower*) *Arceuthobium campylopodum* and *Abies concolor*, field test.

rate of CO_2 fixation may be calculated for *Arceuthobium* shoots. Since there is no net utilization of CO_2 by mistletoe shoots, the concentration of this gas in the reaction chamber will never become limiting. In $C^{14}O_2$-feeding experiments, data are available for the amount of CO_2 introduced into the system, the total amount of radioactivity expressed as dpm, and the volume of the system. After correcting for the reduced atmospheric pressure at the altitudes of the experimental sites, the amount of CO_2 present in the system before introduction of $C^{14}O_2$ can be determined. If it is assumed that the specific activity of CO_2 in the chamber remains constant during the 4- to 8-hour exposure period, a somewhat tenuous assumption, then the rate of CO_2 fixation can be calculated if the total amount of radioactivity fixed and the mass of the tissue are known. Such calculations indicate a rate of CO_2 fixation of 0.024 mg/g dry weight hour for *Arceuthobium americanum* and 0.003 mg/g dry weight hour for *Arceuthobium campylopodum*, infecting lodgepole pine and white fir respectively. The rate of dark fixation for the latter mistletoe is 0.0007 mg/g dry weight hour. Even if these estimates are off by a factor of 10 or more it is apparent that the rate of CO_2 fixation by dwarf mistletoe shoots is indeed low.

Summary

The ability of mistletoe plants to satisfy their own energy requirements was investigated by studying the photosynthetic properties of their aerial shoots. *Arceuthobium campylopodum* Engelm. infecting *Abies concolor* (G. and G.) Lindl. and *Arceuthobium americanum* Nutt. infecting *Pinus murrayana* Grev. and Balf. were the principal experimental subjects. Comparative studies were conducted on several species of *Phoradendron* infecting a number of angiosperm and gymnosperm hosts. Mistletoe shoots were exposed to $C^{14}O_2$ and after a period of time the pattern of carbon translocation was determined by radioautography. The chemical fate of carbon fixed by dwarf mistletoe shoots was determined by standard analytical procedures.

Phoradendron leaves were found to have a chlorophyll content comparable to that of foliage from the host. These leaves photosynthetically fix large quantities of CO_2 with much of the carbon being deposited as starch. Translocation of photosynthate from mistletoe shoots to the endophytic system of the parasite was noted. There was no appreciable transfer of organic materials from the mistletoe to its host even when the infected branch was without leaves.

Aerial shoots of *Arceuthobium* also contain chlorophyll but at concentrations only 1/5 to 1/10 that found in foliage of its host. Carbon fixed by dwarf mistletoe shoots never was observed to translocate into the endophytic system or into the host tissue. Carbon fixed in the shoots of *Arceuthobium* in the light was found to be concentrated in sucrose and malic acid. The rate of CO_2 fixation in aerial shoots of *Arceuthobium* ranged from 0.024 mg of CO_2/g dry weight hour for shoots of *A. americanum* to 0.003 mg of CO_2/g dry weight hour for *A. campylopodum*. Dark fixation of CO_2 occurred in shoots of *A. campylopodum* at about 1/5 the rate of light fixation.

While both mistletoe genera are obligate parasites, it appears that *Arceuthobium* is much more host dependent for its energy requirements than is *Phoradendron*. This greater drain upon the carbohydrate supply of dwarf mistletoe infected trees very likely accounts for the highly destructive nature of *Arceuthobium* parasitism.

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We thank the Pacific Southwest Forest and Range Experiment Station for making available the facilities of the field station at Strawberry without which much of this work would have been impossible.

Literature Cited

1. ARNON, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24: 1–15.
2. BUCKLAND, D. C. AND E. G. MARPLES. 1952. Management of western hemlock infested with dwarf mistletoe. Brit. Columbia Lumberman 36: 50–1, 136, 138, 140.

3. CANNON, W. A. 1901. The anatomy of *Phoradendron villosum* Nutt. Torrey Botan. Club Bull. 28: 374–90.
4. COHEN, L. I. 1954. The anatomy of the endophytic system of the dwarf mistletoe, *Arceuthobium campylopodum*. Am. J. Botany 41: 840–47.
5. FREELAND, R. O. 1943. The American mistletoe with respect to chlorophyll and photosynthesis. Plant Physiol. 18: 299–302.
6. HEINRICHER, E. 1923. Das Absorptionssystem der Wacholdermistel, *Arceuthobium oxycedri* (D.C.) M.B., mit besonderer Berücksichtigung seiner Entwicklung und Leistung. Sitzber. Akad. Wiss. Wien, Abt. 1, 132: 143–94.
7. HULL, R. J. AND O. A. LEONARD. 1964. Physiological aspects of parasitism in mistletoes (*Arceuthobium* and *Phoradendron*). I. The carbohydrate nutrition of mistletoe. Plant Physiol. 39: 996–1007.
8. KUIJT, J. 1960. Morphological aspects of parasitism in the dwarf mistletoes (*Arceuthobium*). Calif. Univ. Publications Botany 30: 337–436.
9. PEIRCE, G. J. 1905. The dissemination and germination of *Arceuthobium occidentale* Eng. Ann. Botany 19: 99–113.
10. RANSON, S. L. AND M. THOMAS. 1960. Crassulacean acid metabolism. Ann. Rev. Plant Physiol. 11: 81–110.
11. REDISKE, J. H. AND K. R. SHEA. 1961. The production and translocation of photosynthate in dwarf mistletoe and lodgepole pine. Am. J. Botany 48: 447–52.
12. SELEDZHANU, N. AND D. GALAN-FABIAN. 1961. Nutrition of common mistletoe. Fiziol. Rast. 8: 547–54. English Transl. in Plant Physiol. (USSR) 1962, A.I.B.S. 8: 436–42.
13. THODAY, D. AND E. T. JOHNSON. 1930. On *Arceuthobium pusillum* Peck. I. The endophytic system. Ann. Botany 44: 393–413.
14. TUBEUF, K. VON. 1923. Monographie der Mistel. R. Oldenbourg, Printer, Munich and Berlin, 832 pp.
15. WAGENER, W. W. 1957. The limitation of 2 leafy mistletoes of the genus *Phoradendron* by low temperatures. Ecology 38: 142–45.
16. WEIR, J. R. 1916. Mistletoe injury to conifers in the Northwest. U.S. Dept. of Agr. Tech. Bull. 360, 39 pp.

The Occurrence of and Effect of Cyanide on Respiratory Drift in the Developing Tung Nut [1]

L. Y. Yatsu [2] and Margaret R. Easterling

United States Field Laboratory for Tung Investigations, Fruit and Nut Crops Research Branch, United States Department of Agriculture, Bogalusa, Louisiana

Respiratory drift is a well-known phenomenon in various horticultural fruits (3, 6). In fruits which show such a drift, a relatively high respiratory rate per unit fresh weight occurs at the time of fruit-set and is followed by a gradual decline in the rate of respiration until the fruits ripen. A sudden rise in respiration coincides approximately with the ripening process, attains a maximum, and then gradually declines until the fruits decay. The peak of respiratory activity, known as the climacteric, is presumed to be the stage in development of fruit marking the beginning of senescence (3).

Blackman and Parija (4) speculated that a phenomenon which they called "a lowering of the organization resistance" occurs at the senescent phase of apples. This early theory has recently received experimental support from the work of Sacher (13), who noted changes in membrane permeability during the climacteric in several fruits.

Sacher theorized that the onset of changes in permeability gradually occurs in more and more cells with an attendant loss in the compartmentalization of cytoplasmic and vacuolar constituents as the climacteric progresses. These changes could cause acceleration of the rate of respiration and other metabolic changes associated with the climacteric. The decline in rate of respiration following the climacteric peak may be attributed to further disorganization of the protoplasm as a result of loss of membrane integrity.

More recently, Bain and Mercer (1) published the results of their work on the Williams variety of pear which tends to support this view. They noted a breakdown of ultrastructure and changes in cytoplasmic membranes which was correlated with electrical studies of the plant cells indicating an increase in permeability of the membranes during the climacteric.

This is a very attractive hypothesis and merits consideration as the explanation of the climacteric in deciduous fruits. However, results obtained in a study of endosperm material from the seed of the tung tree, *Aleurites fordii* Hemsl., indicate that the explanation for the respiratory drift in fruit may not be so simple.

Materials and Methods

From June 15, 1962, about 1 month before oil synthesis had begun, until September 25, samples of

[1] Received April 27, 1964.

[2] Present address: Seed Protein Pioneering Research Laboratory, P. O. Box 19687, New Orleans, Louisiana.