A comparative study was made of Pinus ponderosa Laws, and Arceuthobium campylopodum Engelm. f. campylopodum to determine (1) whether or not the parasite or parasitized tissues accumulate abnormal concentrations of minerals, (2) whether or not there was evidence of a blockage of mineral translocation in the phloem because of the parasite, and (3) whether or not changes occurred in host foliage which could be interpreted as mineral deficiency.

Also, comparative analyses using chromatographic methods were made of simple carbohydrates and free amino acids. Quantitative analyses of carbohydrates also were carried out. Manometric methods were employed to evaluate the ability of host and parasite to utilize exogenously supplied carbohydrates and amino acids. Substances identified by chromatography in the host and parasite
served as substrates in the respiration studies to determine their usability.

The results showed the parasite and swollen bark ramified by the parasite to have higher contents of nitrogen, phosphorus, and potassium than bark adjacent to the swellings and sometimes more than the host foliage. Magnesium content was greatest in the parasite. No difference occurred between fusiform swellings and adjacent bark. Calcium was the exception in that it did not accumulate in the parasite. No evidence was found that mineral translocation was blocked in the phloem except for calcium. Calcium was more abundant below the infection than above. No evidence was found which would substantiate the theory that the parasite caused mineral deficiencies in the host's foliage.

Neither was the movement of sugars, as indicated by quantitative analysis, interrupted by the mistletoe or the swollen bark from which it grew.

The host contained raffinose which was not found in the parasite, and the parasite contained an unknown substance, possibly a uronic acid, a methylpentose or a deoxy sugar, which was not found in the host.

Dwarf mistletoe and its host contained virtually the same amino acids, the chief exception being the presence of cysteic acid and the
absence of glycine in the parasite.

Dwarf mistletoe was found to use a variety of sugars in its metabolism, including those identified from the host. Respiration of pine tissues was not stimulated by the addition of carbohydrate. Tissues of both host and parasite responded to the addition of amino acid and amides.
PHYSIOLOGICAL RELATIONSHIPS BETWEEN DWARF MISTLETOE AND PONDEROSA PINE

by

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TABLE OF CONTENTS

Introduction 1
Review of Literature 4
Materials and Methods 10
   Field Procedures 11
   Laboratory Procedures, Analytic 14
   Laboratory Procedures, Manometric 18
Results of Analyses 21
   Mineral, Sugar and Amino Acids 21
      Phosphorus 21
      Potassium 21
      Magnesium 24
      Total Nitrogen 24
      Calcium 27
      Qualitative Sugar Determinations 27
      Quantitative Sugar Determinations 29
      Identification and Distribution of Free Amino Acids 31
   Utilization of Exogenously Supplied Carbohydrates 38
      Dwarf Mistletoe Tissues 38
      Pine Tissues 38
   Utilization of Amino Acids 41
      Dwarf Mistletoe Tissues 41
      Pine Tissues 43
Discussion 45
   Mineral Analyses 45
      Utilization of Exogenously Supplied Substrates 51
Bibliography 60
LIST OF FIGURES

Figure

1  Branch infected with a female dwarf mistletoe plant. Arrows indicate the position from which tissues were taken for analyses. Symbols are described on pages 12 and 14.

2  Chromatogram of amino compounds of bark from healthy branches of ponderosa pine.

3  Chromatogram of amino compounds of bark from "basal" samples of infected ponderosa pine branches.

4  Chromatogram of amino compounds of fusiform swellings on infected branches of ponderosa pine.

5  Chromatogram of amino compounds from "distal" samples of infected branches of ponderosa pine.

6  Chromatograms of amino compounds of dwarf mistletoe tissues from infected branches of ponderosa pine.

7  Stimulation of the respiration of dwarf mistletoe tissues by the addition of carbohydrates.

8  Stimulation of the respiration of dwarf mistletoe tissues by the addition of amino acid and amides.

9  Stimulation of the respiration of pine tissues by the addition of amino acids and amides.
LIST OF TABLES

Table

1  Distribution of phosphorus in dwarf mistletoe and pine.  

2  Distribution of potassium in dwarf mistletoe and pine.  

3  Distribution of magnesium in dwarf mistletoe and pine.  

4  Distribution of total nitrogen in dwarf mistletoe and pine.  

5  Distribution of calcium in dwarf mistletoe and pine.  

6  Distribution of reducing, total, and invert sugar in dwarf mistletoe and pine.  

7  Differential occurrence of amino compounds in dwarf mistletoe and pine.  

8  Ability of dwarf mistletoe tissues to utilize exogenously supplied substrates expressed as percent of utilization of glucose.
Nutrition of the mistletoes has long been a little investigated subject for debate among biologists. It is believed in a very general way that the degree of dependence on the host is inversely proportionate to size and differentiation of the parasite.

The true mistletoes, Phoradendron, Loranthus, and Viscum, for example, primarily parasitize deciduous hosts, and themselves bear fleshy evergreen leaves. In some, the leaves are greatly reduced; the photosynthetic function being assumed by highly branched systems of green stems. The dwarf mistletoes, Arceuthobium, are vegetatively even more reduced. Shoots are sparsely branched and but several centimeters long. The leaves are reduced to scales. The several species are restricted to coniferous hosts.

Cannon (7) felt that dwarf mistletoes were total parasites depending on their hosts for water, minerals, and organic constituents. Heil (17) believed the dwarf mistletoes to be dependent only for water and minerals since chlorophyll was found in the aerial shoots of the parasite. Gill (13) felt it inconceivable that these parasites could manufacture all the food they required. Some students thought the relationships to be symbiotic. Weir (36), for example,
showed that infected branches remained alive longer than noninfected branches when both were defoliated.

Contrarily, growth studies showed that dwarf mistletoes cause large reductions in terminal growth and radial increment when present in at least two-thirds of the crown (16, 23).

Recognition of the seriousness of these pests has focused attention on the dearth of knowledge concerning their physiology. Early physiological studies were limited to isolation and identification of one or several compounds or to comparisons of osmotic pressures of cell sap of host and parasite in the true mistletoes, but not in the dwarf mistletoes.

Recent and continuing studies using radioisotopes are shedding new light on host-parasite relationships of both dwarf and true mistletoes and will be discussed later in some detail. Nevertheless, our knowledge of the physiology of these important pathogens is inadequate to account for their interesting occurrence and development or to provide a basis for disease control. It is a simple matter with the dwarf mistletoes, and the true mistletoes as well, to obtain large quantities of parasite material entirely free of interfering host tissues. Thus it should be possible to characterize discrete segments of the physiology of each entity.

With a knowledge of similar or contrasting features of host
and parasite metabolism it may be possible to devise effective con-
trols based on function.

This study was undertaken to increase our knowledge of the
physiological relationship between the dwarf mistletoe, *Arceuthobi-
um campylopodum* Engelm. f. *campylopodum* and ponderosa pine,
*Pinus ponderosa* Laws., by quantitatively comparing minerals and
carbohydrate constituents of host and parasite, and qualitatively
comparing carbohydrates and free amino acids in the two. And
finally, to determine the extent to which the substances identified
can be utilized interchangeably by the host and parasite as respira-
tory substrates.
REVIEW OF LITERATURE

The accumulation of a variety of substances in both host and parasite as a consequence of infection by obligate parasites is an established phenomenon. The fact that the true mistletoes have higher contents of some minerals than their host was known prior to 1900. Comparative analyses of Viscum and its host were published in 1923 (26) as were analyses of the parasites dodder and broomrape and their hosts. The first evidence that minerals accumulated at infections of obligately parasitic fungi was the classic work of Gottlieb and Garner (15) showing the localization of radioactive phosphorus $^{32}$ at the site of rust infection on wheat plants. They found that although there was no consistent difference in the total amount of $P^{32}$ taken up from solution by rust infected and healthy wheat plants, the portion of leaves bearing lesions had a higher $P^{32}$ content than the uninoculated basal portions. They were unable to determine whether the $P^{32}$ was localized in the host tissue or the fungus mycelium.

Gill and Hawksworth (14) compiled the results of several workers to show a comparison of the ash content of Viscum album and its hosts. The shoots of the parasite contained more phosphorus, sulfur, potassium, and nitrogen than did their hosts. The opposite was true for calcium which was lower in the parasite than in the
hosts. These distributions of ash were interpreted by Gill and Hawksworth as showing a selective uptake by the parasite.

Very few data are available describing mineral distribution in the dwarf mistletoes and their hosts. Hull and Leonard (19) reported the uptake of $P^{32}$ and Sulfur $^{35}$ by A. campylopodum f. campylo-podum on Pinus sabiniana Dougl. Previously (24) they reported accumulation of a number of herbicides in tissues parasitized by mistletoe.

Rediske and Shea (29) in establishing the fact that photosynthesis takes place in the dwarf mistletoe, A. americanum Nutt., showed that radioactive photosynthate from the host was accumulated by the parasite and concluded that the disease syndrome associated with dwarf mistletoe infection was an indirect result of blockage of translocation of photosynthate to stem and roots. They showed that the chief constituent of translocated photosynthate was sucrose and that the labelled carbon accumulating in the parasite was in the sucrose molecule. They also stated that the only substance labelled by allowing the parasite to fix $^{14}C_{2}O_{2}$ was sucrose.

Hull and Leonard (20), working with A. campylopodum f. abietinum Gill, on Abies concolor Lind. and Gord., were unable to find any consistent blockage of translocation but showed that the carbon $^{14}$ isotope accumulated in the parasitized tissue and the
aerial shoots of the parasite when the isotope was introduced into the host foliage.

The fact that true mistletoes accumulate high concentrations of minerals, and the observation that infection by dwarf mistletoes causes swelling and brooming in the host led Hawksworth (16) to speculate that the effects of dwarf mistletoe on its host are brought about by the accumulation of large quantities of water, minerals and other nutrients in the parasite and parasitized bark at the expense of the host. His proposal was not accompanied by data.

Rediske and Shea (29) did not believe the loss of water and minerals to be great enough to account for all the adverse effects of dwarf mistletoes on their hosts.

Hardly any organic constituents, either synthesized by the parasite or accumulated from the host, have been identified from the dwarf mistletoes. Rediske and Shea (29) reported sucrose to be the main sugar. They state that other sugars were present which did not incorporate isotopic carbon$^{14}$. These sugars were apparently identified but were not listed in the publication. Hull and Leonard (19) reported that Greenham identified free and bound amino acids in a dwarf mistletoe and its host but failed to name either the host or parasite, or the identity of the amino acids.

It appears from the literature that we still don't know the
answer to the basic question: "What is the host supplying to the
dwarf mistletoe"?

The findings of Hull and Leonard (19), who compared the para-
sitism of leafy and dwarf mistletoes, indicate a dependence of the
dwarf mistletoes on their hosts for water, minerals and carbohy-
drates, whereas, leafy mistletoes are dependent only for water and
minerals. They could find no translocation of photosynthetic pro-
ducts between the host and parasite with several species of *Phora-
dendron* on their respective hosts.

Seledzhanu and Galan-Fabian (31), working with a *Viscum*
species on black poplar, reported translocation of photosynthetic
products between host and parasite.

Several papers and reviews have appeared in the recent litera-
ture which should alter our thinking about the source of nitrogen
available to the dwarf mistletoes. Bollard (6) reviewed the litera-
ture concerning the transport of substances through the xylem of
trees and emphasized that nitrogen is transported in organic form,
usually as amino acids and amides. Barnes (3) examined the sap of
seven conifers and a large number of hardwoods and found that in
the conifers, as well as in some hardwoods, the primary nitrogen-
ous substance was glutamine. Previously it was thought that the
transportable nitrogen was in the form of nitrate. With this new
information, one must recognize the distinct possibility of the parasite utilizing host amino acids and amides from the xylem as a nitrogen source.

With the evidence (29) that sucrose is the only substance that was radioactive after long periods of $^{14}O_2$ fixation, it would be of interest to know if this substance is the main respirable substrate for the parasite. James (22, p. 98-99) provided ample evidence that the main respirable substrate of healthy vegetative plant tissues is a carbohydrate of one kind or another. In support of this view he pointed to "the universal presence of carbohydrates in plant tissues, their disappearance during active respiration, and the frequent occurrence of unit R.Q.s." He felt also that positive results of the feeding of sugars to respiring tissues was good evidence for this contention. However, he pointed out that negative results have little value and that positive results must be interpreted with care.

Palladine (28) reported a series of sugar feeding experiments using etiolated broad bean leaves. He found that fructose was more readily utilized than was sucrose. Maltose and raffinose were utilized less readily than sucrose. Numerous experiments of this type were carried out by Spoehr and McGee (33).

Beever (4) has used radioactive carbohydrates to determine their utilization in potato tissue slices. He was able to demonstrate
the utilization of several hexoses, pentoses, and sedoheptulose. This was evidence for the presence of the pentose cycle in these storage tissues.

The literature abounds with references to nitrogen metabolism of plants but is primarily concerned with the utilization of \( \text{NO}_3^- \), \( \text{NO}_2^- \), and \( \text{NH}_3 \). No studies have been made on the nitrogen requirements of obligate parasites due to the difficulty of interpreting such data. Although Staples and Ledbetter (33) showed by microautoradiographic procedures that tritiated glycine is incorporated into the mycelium of the bean rust fungus, to the author's knowledge, no organic nitrogenous compounds such as amino acids have been tested on the dwarf mistletoes to determine their usability as nitrogen sources or respirable substrates.
MATERIALS AND METHODS

Typical dwarf mistletoe plants from the selected source area were characterized by aerial shoots ranging in color from the more common yellow-green to dull orange and purple. Male plants of this dioecious species are rather consistently green or yellow. The stems of plants of both sexes have conspicuous nodes. The internodes elongate throughout the season forming stems up to 15 cm long. Female plants tend to be loosely branched and more open, whereas the males are regularly compact. Berry-like fruits mature in the fall of the year following pollination the previous fall.

The absorbing system of *Arceuthobium* occurs as peg-shaped "sinkers" embedded in the xylem rays, and as a much branched system of tissues in the host cortex extending from the point of original infection parallel to the surface of the branch. New sinkers arise inward and shoots arise outward from the tissues of the parasite ramifying the bark. A fusiform swelling results from tissues of host and parasite which are intimately associated. The swollen host-parasite complex will be referred to in this paper as the fusiform swelling. It must be recognized that the living tissues of host and parasite in this complex are physically inseparable.
Field Procedures

Experimental material consisted of dwarf mistletoe-infected branches from ponderosa pines, five to twenty feet tall, which are part of a new stand on cut-over land near Sisters, Oregon. Samples were from trees having no more than one-half of their branches infected. Branches bearing dwarf mistletoe plants or fusiform swellings were collected at random except that no branch was taken if a second dwarf mistletoe plant occurred between the base of the candidate branch and the roots of the tree. Duplicate experimental material was collected near Camp Sherman, Oregon.

Field collections were made on June 14, July 24-25, and September 5-6, of 1962. At the time of the June sampling pine buds had burst but new needles had not elongated sufficiently to be sampled. At this sampling, dwarf mistletoe shoots had started to elongate but were not fully extended. In July, the internodes of the pine appeared to have elongated fully and the needles appeared almost mature. The dwarf mistletoe stems had elongated and small fruits were well developed on the female plants. Needles were fully mature at the September sampling and fruits of the parasite were approaching maturity. A number of male plants had reached anthesis.

In preparing the June collection, various portions of the bark
as outlined below and illustrated in Figure 1 were stripped from branches in the field, placed in polyethylene bags, and packed in ice for transport to the laboratory. In the collections of July and September, the branches were cut into segments and placed in plastic bags, packed in ice, and taken to the laboratory where the bark was removed. Samples of needles were handled in a similar manner.

Bark was removed from portions of the stem as follows:

(1) Basal samples, B, (Fig. 1) included the bark proximal to the swelling from a distance of 1-2 cm below the apparent swelling to a distance not exceeding 15 centimeters.

(2) Fusiform swelling samples, F, included all bark lying between approximately two centimeters of the ends of the fusiform swellings.

(3) Dwarf mistletoe samples, M, included aerial portions of both male and female plants of the parasite.

(4) Distal samples, D, included bark between points 1-2 cm and 15 cm outward from the fusiform swelling.

(5) Needle samples, N, in June, from healthy and diseased branches, included only needles of the previous season; July and September samples from healthy and diseased branches included needles of both the previous and current seasons.
Figure 1. Branch infected with a female dwarf mistletoe plant. Arrows indicate the position from which tissues were taken for analyses. Symbols are described on pages 12 and 14.
(6) Samples of bark from healthy trees, H, were removed from 15 cm lengths of branches of noninfected trees.

The June collection consisted of one large composite sample of each of the portions of the branches described above. In July and September, three replicate samples were taken from the selected areas, each replicate being divided into the portions described above.

**Laboratory Procedures, Analytic**

To prepare materials for chromatographic and sugar analysis, 25 gm samples of tissue (fresh weight) were diced, placed in 135 ml of 95% ethanol, brought to a boil, and allowed to cool. The samples were then blended in the alcohol with an Omnimixer\(^1\) for two minutes and filtered through Whatman No. 5 filter paper on a Buchner funnel. The residue was washed with 80% ethanol until a volume of 250 ml of alcohol was obtained. The residue was discarded and the alcohol extracts were stored at 0°C.

Plant material remaining after removal of the 25 gm samples described above was placed in open petri dishes and dried in an oven at 105°C for at least 24 hours. The dried material was ground in a Wiley mill, passed through a 20 mesh screen, and stored in

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\(^1\) VanSorvall, Inc., Norwalk, Conn.
50 cc French square bottles.

For mineral analyses 100 mg samples of the dried material were placed in Coors evaporating dishes and pre-ashed with 5% alcoholic sulfuric acid as described by Ulrich, et al. (35, p. 28). The samples were ashed in a muffle furnace for 3½-5 hours at 575°C. The ash was removed from the furnace, cooled, and dissolved in 0.1 N nitric acid. These solutions were passed through fluted Whatman No. 12 paper and diluted to 100 ml with distilled water in volumetric flasks. Aliquots of these solutions were used for potassium and phosphorus determinations. A separate sample was ashed for the determination of calcium and magnesium. In this case, the ash was dissolved in 0.1 N HCl and made to a final volume of 100 ml with 0.1 N HCl.

In general, the inorganic components were determined by flame photometric or colorimetric methods. Potassium, calcium, and magnesium were determined on a Beckman D, U. flame photometer according to the methods of Chapman and Parker (8, p. 201-210). Phosphorus was determined by the method of Fiske and Subbarrow (10). Total nitrogen was determined by the microkjeldahl procedure (1, p. 805-806).

Carbohydrate was quantitatively determined as follows. Ten ml of an alcohol extract was pipeted into an evaporating dish and
placed on a water bath at 60°C. The alcohol was evaporated slowly and the level of fluid in the dish was held nearly constant by the addition of distilled water. When the alcohol odor could no longer be detected, the dish was removed, the contents filtered under suction, and diluted to 25 ml and 5 ml of this solution was used for the determination of the reducing sugar present. Somogyi's reagent was used for reducing sugar determinations (2, p. 89). A separate aliquot of the last dilution was treated with 1% invertase, allowed to stand for 3½ hours at room temperature and reducing sugars again determined. The difference between the first and second determination multiplied by 0.95, which compensates for the amount of water taken up by the hydrolysis of sucrose, gives the amount, in milligrams, of invert sugar.

Paper chromatography of amino acids and sugars was as follows. Twenty-five ml aliquots of the alcohol extracts were reduced under vacuum at 45°C until the alcohol odor could no longer be detected. The remaining extract was placed on a Dowex 50W-x4 ion exchange column and washed with at least three wet bed volumes of distilled water. This procedure removed the amino acids and other cations. The effluent from the column was reduced almost to dryness at 45°C under vacuum and dissolved in 10 ml 80% ethanol. This solution was used for identification of sugars by paper
chromatography. Thirty to fifty microliters of each extract were spotted on 18x22 inch Whatman No. 1 chromatographic paper and developed one way in N-butanol-acetic acid-water (4:1:5 v/v). Spots were detected using a phloroglucinol spray and an analine-diphenylamine dip method described by Block (5, p. 183, 184).

The amino acids remaining on the column were removed by passing at least three wet bed volumes of 3 N NH₄OH through the column. The eluant was then reduced almost to dryness at 65°C under vacuum and dissolved in 10 ml of 80% ethanol. This solution was used for two dimensional paper chromatography. One hundred microliters were spotted on Whatman No. 1 paper, 18x22 inches.

After spotting, the chromatograms were placed in the chromatographic chamber. The methods of development were those of Li (25) with the exception of the following minor modifications. The chamber was saturated with NH₄OH by placing a pyrex baking dish containing 100 ml of 3% NH₄OH solution in the bottom of the chamber. Papers were allowed to equilibrate for 12 hours. A phenol-water solvent (920:360 w/v) was introduced and allowed to flow down the paper for 24 hours. The papers were removed and allowed to dry at 65°C for at least five hours. The papers were developed in the second dimension by a N-butanol-acetic acid-water (top layer), (250:60:250 v/v) solvent and allowed to flow for 30-36 hours. Spots
were detected by spraying the air-dried paper with 0.3% ninhydrin in 95% ethanol and heating at 75°C for 15 minutes. Amino acids were identified by use of a chromatograph of known amino acids, differential sprays and co-chromatography.

Laboratory Procedures, Manometric

Branches bearing dwarf mistletoe infections were collected in the Sisters, Oregon area. The branches were cut and the severed ends were immersed in water. Branches were transported to the laboratory, covered with large polyethylene bags, and stored in the cooler at 40°F. Aerial portions of the parasite were removed periodically for experimentation.

Parasite tissue was placed in a 400 ml beaker, and the beaker was covered with cheese cloth. Tap water was jetted into the beaker for 2-4 hours to agitate and wash the tissues. After washing, the tissue was blended in an Omnimixer at 80-100 on a Powerstat \(^1\) for about one minute. This treatment provided uniform pieces of material. The blended material was then poured into a large beaker, and the cell fragments and other light debris were decanted 10-15 times in tap water. The tissue segments were then transferred to an Erlenmeyer flask fitted with an aeration tube connected to a faucet aspirator. The tissues were aeriated in one-fifth Hoagland solution

\(^1\) The Superior Electric Company, Bristol, Conn.
(Solution 1)(18, p. 31) for not less than 12 hours with occasional changes of Hoagland solution.

After aeriation, the dilute Hoagland solution was decanted, and the tissues were washed in water and resuspended in dilute Hoagland's solution. They were then poured into folded filter papers and allowed to drain.

After draining, small amounts (ca 0.3 gm fresh weight) of tissue were transferred by means of forceps to Warburg reaction vessels. The vessels contained 2 ml of .05 M phosphate buffer, ph 6.0. After tissue transfer, 0.2 ml of 20% KOH was added to the center well and 0.5 ml of the substrate to be tested was added to the side arm of the flask. The flasks were then fitted on the manometers, placed in the water bath at 30°C and allowed to equilibrate for twenty minutes at 120 oscillations per minute. The manometers were then closed and readings were taken at 10 minute intervals. Endogenous respiration was recorded for 30 minutes at which time the substrates in the side arms were tipped in.

Sugar substrates were made up to give final concentrations of 0.06 M. Amino acids were prepared to give final concentrations of 0.01 M. The sugars tested included sucrose, glucose, fructose, galactose, raffinose, ribose, xylose, mannose, and rhamnose. Glucuronic acid and succinic acid were also tested.
Pine tissues from several sources were compared to determine whether or not variation occurred in the qualitative respiration. By and large, pine tissues from diverse sources were consistent in their reaction to added substrates.

Pine materials were brought into the laboratory for sectioning. Because of ease of handling only tissues of the current season's growth were employed. Needles were carefully removed and the stem tissue was cut into slices 0.5 mm thick with a razor blade. The slices were aerated vigorously in one-fifth Hoagland solution for at least 12 hours with frequent changes of solution. After overnight aeriation, the pine slices were washed several times in distilled water and resuspended in one-fifth Hoagland solution. They were then handled in the manner described for mistletoe tissue.

At the end of an experiment, tissues were removed from each vessel, dried in the oven at 100°C for 24 hours and weighed; thus giving a dry weight value for each flask. This allowed a comparison of the flasks on a common basis.
RESULTS OF ANALYSES

Mineral, Sugar and Amino Acids

Phosphorus. The results of phosphorus analyses are shown in Table 1. Aerial shoots of the dwarf mistletoe contained phosphorus in concentrations over three times those of host bark and twice those of host foliage. The highest concentration occurred in the aerial portions of the parasite. Parasitized bark of the fusiform swelling contained significantly more than the adjacent bark samples. Highest phosphorus concentrations in the aerial shoots occurred in June and appeared to decline through the summer. Phosphorus levels in the bark adjacent to the swelling also declined through the summer. There were no differences in phosphorus content of bark from noninfected branches as compared to bark adjacent to infections. Similarly, there were no differences in phosphorus when needles from infected and noninfected branches were compared.

Potassium. The results of potassium analyses shown in Table 2 reveal that the aerial shoots of the parasite accumulated concentrations more than three times those of the pine foliage and approximately six times those of bark samples adjacent to the swelling. Infected bark from the swelling contained higher levels of potassium than did the noninfected bark from infected stems. There
Table 1. Distribution of phosphorus in dwarf mistletoe and pine.

<table>
<thead>
<tr>
<th>Tissue Source</th>
<th>Identification Symbol</th>
<th>Sampling Period (Percent of Dry Matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>June</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>B ^1</td>
<td>0.08^2</td>
</tr>
<tr>
<td>Fusiform swelling</td>
<td>F</td>
<td>0.15</td>
</tr>
<tr>
<td>Distal</td>
<td>D</td>
<td>0.08</td>
</tr>
<tr>
<td>Healthy</td>
<td>H</td>
<td>0.06</td>
</tr>
<tr>
<td>Parasite</td>
<td>M</td>
<td>0.24</td>
</tr>
<tr>
<td>One Year Old Needles from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseased branches</td>
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</tr>
<tr>
<td>Healthy branches</td>
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<td>0.12</td>
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<tr>
<td>New Needles from:</td>
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<td></td>
</tr>
<tr>
<td>Diseased branches</td>
<td>N</td>
<td>0.14</td>
</tr>
<tr>
<td>Healthy branches</td>
<td>N</td>
<td>0.15</td>
</tr>
</tbody>
</table>

^1 Symbols are explained in detail on pages 12 and 14.

^2 Values are the results of one determination from a composite sample.

^3 Values represent the averages of three replicates.
Table 2. Distribution of potassium in dwarf mistletoe and pine.

<table>
<thead>
<tr>
<th>Tissue Source</th>
<th>Identification Symbol</th>
<th>Sampling Period (Percent of Dry Matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>June</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>B</td>
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</tr>
<tr>
<td>Fusiform swelling</td>
<td>F</td>
<td>0.41</td>
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<tr>
<td>Distal</td>
<td>D</td>
<td>0.26</td>
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<tr>
<td>Healthy</td>
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<tr>
<td>Parasite</td>
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<td>One Year Old Needles from:</td>
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<td>Diseased branches</td>
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<td>Healthy branches</td>
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<td>New Needles from:</td>
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<td>Diseased branches</td>
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<tr>
<td>Healthy branches</td>
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</table>

1 Symbols are explained in detail on pages 12 and 14.
2 Values are the results of one determination from a composite sample.
3 Values represent averages of three replicates.
were no significant differences in potassium content of noninfected bark from infected stems and bark from noninfected stems. Neither were there differences in the amount of potassium between foliage samples from infected stems and the healthy controls.

**Magnesium.** The amount of magnesium in the aerial portions of the parasite was greater than bark of the fusiform swelling or bark adjacent to the swelling (Table 3). No differences in the quantity of magnesium occurred between bark samples adjacent to the fusiform swelling. There were no differences between basal, distal and healthy samples. There were no differences in foliage samples from infected and noninfected branches.

**Total Nitrogen.** A summary of the nitrogen analyses is shown in Table 4. Nitrogenous substances accumulated in the parasite proper and in the infected bark. The quantity occurring in a unit of parasite was comparable to the total nitrogen of a comparable unit of host foliage. Swollen regions of the branch showed values intermediate between the aerial portions of the parasite and bark bordering the swellings. Nitrogen values are nearly constant throughout the season.
Table 3. Distribution of magnesium in dwarf mistletoe and pine.

<table>
<thead>
<tr>
<th>Tissue Source</th>
<th>Identification Symbol</th>
<th>Sampling Period (Percent of Dry Weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>June</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>B</td>
<td>0.09</td>
</tr>
<tr>
<td>Fusiform swelling</td>
<td>F</td>
<td>0.09</td>
</tr>
<tr>
<td>Distal</td>
<td>D</td>
<td>0.07</td>
</tr>
<tr>
<td>Healthy</td>
<td>H</td>
<td>0.07</td>
</tr>
<tr>
<td>Parasite</td>
<td>M</td>
<td>0.15</td>
</tr>
<tr>
<td>One Year Old Needles from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseased branches</td>
<td>N</td>
<td>0.07</td>
</tr>
<tr>
<td>Healthy branches</td>
<td>N</td>
<td>0.08</td>
</tr>
<tr>
<td>New Needles from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseased branches</td>
<td>N</td>
<td>0.06</td>
</tr>
<tr>
<td>Healthy branches</td>
<td>N</td>
<td>0.06</td>
</tr>
</tbody>
</table>

1 Symbols are explained in detail on pages 12 and 14.
2 Values are the results of one determination from a composite sample.
3 Values represent averages of three replicates.
Table 4. Distribution of total nitrogen in dwarf mistletoe and pine.

<table>
<thead>
<tr>
<th>Tissue Source</th>
<th>Identification Symbol</th>
<th>Sampling Period (Percent of Dry Matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>June</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>B^1</td>
<td>0.37^2</td>
</tr>
<tr>
<td>Fusiform swelling</td>
<td>F</td>
<td>0.84</td>
</tr>
<tr>
<td>Distal</td>
<td>D</td>
<td>0.37</td>
</tr>
<tr>
<td>Healthy</td>
<td>H</td>
<td>0.37</td>
</tr>
<tr>
<td>Parasite</td>
<td>M</td>
<td>1.02</td>
</tr>
<tr>
<td>One Year Old Needles from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseased branches</td>
<td>N</td>
<td>1.02</td>
</tr>
<tr>
<td>Healthy branches</td>
<td>N</td>
<td>1.02</td>
</tr>
<tr>
<td>New Needles from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseased branches</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Healthy branches</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

1 Symbols are explained in detail on pages 12 and 14.
2 Values are the result of one determination from a composite sample.
3 Values represent averages of three replicates.
Calcium. The results of calcium analyses are presented in Table 5. Calcium, differing from phosphorus and potassium, was not accumulated by the aerial shoots of the parasite or by the fusiform swelling.

Samples collected in June were not amenable to statistical analysis. Calcium contents of samples collected in July and September were analyzed by an analysis of variance. The material collected in July showed significantly more calcium in samples from below the swelling than in samples from above. The differences were highly significant in the September material. This difference in calcium was the only instance in the mineral analyses where the content of an element was significantly higher on one side of the swelling than the other.

Qualitative Sugar Determinations. Chromatograms of the alcohol extracts of pine bark were characterized by the presence of five spots revealed by the analine-diphhylamine dip. Spots were identified as sucrose, glucose, fructose and raffinose, all of which have been reported previously as constituents of various pine species. A fifth spot was tentatively identified as dehydroascorbic acid on the basis of its $R_f$ value.

In extracts of parasite tissue, four spots were detected, three of which were identified as sucrose, glucose, and fructose. The
Table 5. Distribution of calcium in dwarf mistletoe and pine.

<table>
<thead>
<tr>
<th>Tissue Source</th>
<th>Identification Symbol</th>
<th>Sampling Period (Percent of Dry Matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>June</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>B</td>
<td>0.18</td>
</tr>
<tr>
<td>Fusiform swelling</td>
<td>F</td>
<td>0.10</td>
</tr>
<tr>
<td>Distal</td>
<td>D</td>
<td>0.11</td>
</tr>
<tr>
<td>Healthy</td>
<td>H</td>
<td>0.14</td>
</tr>
<tr>
<td>Parasite</td>
<td>M</td>
<td>0.12</td>
</tr>
<tr>
<td>One Year Old Needles from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseased branches</td>
<td>N</td>
<td>0.02</td>
</tr>
<tr>
<td>Healthy branches</td>
<td>N</td>
<td>0.05</td>
</tr>
<tr>
<td>New Needles from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseased branches</td>
<td>N</td>
<td>0.05</td>
</tr>
<tr>
<td>Healthy branches</td>
<td>N</td>
<td>0.06</td>
</tr>
</tbody>
</table>

1 Symbols are explained in detail on pages 12 and 14.
2 Values are the results of one determination from a composite sample.
3 Values represent the averages of three replicates.
fourth spot had an $R_f$ value greater than fructose but less than the host substance tentatively identified as dehydroascorbic acid. Differential sprays characterized the unknown from the parasite as either a deoxy sugar, a uronic acid, or a methylpentose.

A spot occurring in the same position as sucrose and tentatively identified as galactose was detected by the use of the phloroglucinol spray which is specific for pentoses with only fructose and galactose interfering (5, p. 183). If this finding can be confirmed, it would indicate that at least five sugars are present in the extracts of parasite tissue.

The tentative identification of galactose is based on two points. They are: (1) its $R_f$ value in the N-butanol-acetic acid-water solvent system, and (2) its reaction with the phloroglucinol reagent.

Quantitative Sugar Determinations. No consistent accumulation of reducing sugars was found in the parasitized tissues or aerial shoots of the parasite (Table 6). Differences were not apparent between samples taken from positions adjacent to the infection. The amounts of reducing substances rose toward the end of the season.

Values for total sugar appear to be similar to those for reducing sugar, and they reflect the rise in total activity toward the end of the season. This rise is not seen in the invert sugar determination. Invert sugar determinations for the June and September
Table 6. Distribution of reducing, total, and invert sugar in dwarf mistletoe and pine.

<table>
<thead>
<tr>
<th>Tissue Source</th>
<th>Identification Symbol</th>
<th>Sampling Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>June</td>
</tr>
<tr>
<td>REDUCING SUGAR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>B¹</td>
<td>21.70²</td>
</tr>
<tr>
<td>Fusiform swelling</td>
<td>F</td>
<td>18.20</td>
</tr>
<tr>
<td>Distal</td>
<td>D</td>
<td>19.37</td>
</tr>
<tr>
<td>Healthy</td>
<td>H</td>
<td>26.40</td>
</tr>
<tr>
<td>Parasite</td>
<td>M</td>
<td>18.20</td>
</tr>
<tr>
<td>TOTAL SUGAR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>B</td>
<td>26.38</td>
</tr>
<tr>
<td>Fusiform swelling</td>
<td>F</td>
<td>31.05</td>
</tr>
<tr>
<td>Distal</td>
<td>D</td>
<td>26.38</td>
</tr>
<tr>
<td>Healthy</td>
<td>H</td>
<td>36.89</td>
</tr>
<tr>
<td>Parasite</td>
<td>M</td>
<td>22.88</td>
</tr>
<tr>
<td>INVERT SUGAR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>B</td>
<td>4.44</td>
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<tr>
<td>Fusiform swelling</td>
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<td>12.20</td>
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<tr>
<td>Distal</td>
<td>D</td>
<td>6.65</td>
</tr>
<tr>
<td>Healthy</td>
<td>H</td>
<td>9.96</td>
</tr>
<tr>
<td>Parasite</td>
<td>M</td>
<td>4.44</td>
</tr>
</tbody>
</table>

¹ Symbols are explained on pages 12 and 14.
² Values represent the results of one determination from a composite sample.
³ Values represent the average of three replicates.
collections show a trend toward higher concentrations in tissues of the fusiform swelling.

Identification and Distribution of Free Amino Acids. Development of the chromatograms revealed differences in occurrence of amino acids between various portions of the host and between the host and parasite. The free amino acids found are listed in Table 7. A spot in the cysteine position was present in chromatograms of "healthy" and "basal" bark (Fig. 8, 11) but was not found in the extracts from the parasite and the fusiform swellings (Fig. 9, 12). Cysteic acid was found in the parasite and the fusiform swelling but not in the "basal", "distal", or "healthy" bark.

Glycine was present in the healthy bark and in the bark extracts adjacent to the fusiform swelling, but not from the parasite or fusiform swelling.

The amino acids identified in host and parasite were: aspartic acid, glutamic acid, serine, asparagine, glycine, threonine, alanine, glutamine, proline, γ-amino butyric acid, valine-methionine, and phenylalanine-leucine(s). Those tentatively identified were lysine-arginine, cysteic acid, cysteine, pipecolic acid, and hydroxyproline.
Table 7. Differential occurrence of amino compounds in dwarf mistletoe and pine.

<table>
<thead>
<tr>
<th>Amino Compound</th>
<th>Identification number on chromatogram</th>
<th>Source of Extract</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Basal</td>
<td>Fusiform</td>
<td>Parasite</td>
<td>Distal</td>
<td>Healthy</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Serine</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycine</td>
<td>4</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Asparagine</td>
<td>5</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Threonine</td>
<td>6</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alanine</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glutamine</td>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lysine-Arginine(^1,2)</td>
<td>9</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proline</td>
<td>10</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Valine-Methionine(^2)</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenylalanine-Leucine(s)(^2)</td>
<td>12</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Cysteic Acid(^1)</td>
<td>13</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(\gamma)-Aminobutyric Acid</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pirocolic Acid(^1)</td>
<td>15</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>16</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Cysteine(^1)</td>
<td>17</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^1\) Identified by position on the chromatogram.

\(^2\) Identified from one position. Spot on chromatogram could be the result of the presence of one or both compounds.

\(^3\) Refers to infected and noninfected branches since no differences occurred between the two.
Figure 2. Chromatograms of amino compounds of bark from non-infected branches of ponderosa pine. See Table 7 for identification of numbers.
Figure 3. Chromatogram of amino compounds from "basal" samples of infected ponderosa pine branches. See Table 7 for identification of numbers.
Figure 4. Chromatogram of amino compounds of "fusiform swellings" from infected branches of ponderosa pine. See Table 7 for identification of numbers.
Figure 5. Chromatogram of amino compounds from "distal" samples of infected branches of ponderosa pine. See Table 7 for identification of numbers.
Figure 6. Chromatogram of amino compounds from dwarf mistletoe tissues from infected branch of ponderosa pine. See Table 7 for identification of numbers.
Utilization of Exogenously Supplied Carbohydrates

**Dwarf Mistletoe Tissues.** A variety of simple carbohydrates were tested for their ability to stimulate respiration of the tissues of the aerial portions of the parasite above the endogenous level. Sugars identified in the parasite by paper chromatography were tested, as well as a number of sugars not found in host or parasite. The most commonly tested substrates are listed in Table 8, and the utilization of these compounds is compared to the utilization of glucose.

In the majority of trials, fructose was more readily utilized than other carbohydrates, although in some trials little difference occurred between glucose, fructose or galactose. The descending order of utilization appeared to be fructose, galactose, glucose, mannose, xylose, raffinose, ribose, glucuronic acid, and sucrose.

Sucrose was poorly utilized in all trials. The position of raffinose varied, but was usually very close to sucrose. Xylose was utilized more readily than ribose in almost all trials, and the values of the pentoses were intermediate between the hexoses and sucrose.

**Pine Tissues.** The endogenous rate of respiration in slices of one year old pine stem was very high compared with the rate in dwarf mistletoe tissues. The same sugars studied in carbohydrate
Figure 7. Stimulation of the respiration of dwarf mistletoe tissue on the addition of carbohydrates.
Table 8. Ability of dwarf mistletoe tissues to utilize exogenously supplied substrates expressed as percent of utilization of glucose.

<table>
<thead>
<tr>
<th>Compound Tested</th>
<th>Percent of Utilization as Compared to Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARBOHYDRATE</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>100</td>
</tr>
<tr>
<td>Fructose</td>
<td>121</td>
</tr>
<tr>
<td>Galactose</td>
<td>111</td>
</tr>
<tr>
<td>Raffinose</td>
<td>92</td>
</tr>
<tr>
<td>Ribose</td>
<td>91</td>
</tr>
<tr>
<td>Xylose</td>
<td>94</td>
</tr>
<tr>
<td>Sucrose</td>
<td>85</td>
</tr>
<tr>
<td>Mannose</td>
<td>98</td>
</tr>
<tr>
<td>Glucuronic Acid</td>
<td>87</td>
</tr>
<tr>
<td>Succinic Acid</td>
<td>91</td>
</tr>
<tr>
<td>AMINO ACIDS</td>
<td></td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>112</td>
</tr>
<tr>
<td>Asparagine</td>
<td>126</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>186</td>
</tr>
<tr>
<td>Glutamine</td>
<td>200</td>
</tr>
<tr>
<td>Glycine</td>
<td>117</td>
</tr>
<tr>
<td>Proline</td>
<td>126</td>
</tr>
<tr>
<td>Alanine</td>
<td>118</td>
</tr>
<tr>
<td>Arginine</td>
<td>118</td>
</tr>
<tr>
<td>Leucine</td>
<td>87</td>
</tr>
<tr>
<td>Valine</td>
<td>104</td>
</tr>
</tbody>
</table>

1 Compounds found by analysis to be present in host tissue.
2 Compounds found by analysis to be present in the parasite.
utilization by mistletoe were tested with pine tissues to determine the respiration response to addition of the substrates. Trials with material collected in the early summer indicated some utilization of the supplied compounds but the response was low and variable. Trials with material collected in late August and September showed no positive response to the added carbohydrates. A few materials appeared to inhibit respiration of the pine slices.

**Utilization of Amino Acids**

**Dwarf Mistletoe Tissues.** A number of amino acids and amides were tested for their ability to stimulate respiration of dwarf mistletoe tissues. They included the free acids identified by paper chromatography in host or parasite. Those tested were aspartic acid, asparagine, γ-aminobutyric acid, glutamic acid, glutamine, glycine, alanine, arginine, leucine, valine, isoleucine and phenylalanine.

Typical tissue responses to the administration of amino acids are shown in Figure 8.

Glutamine consistently stimulated the greatest response in the parasite tissue while asparagine was utilized at a higher rate than aspartic acid. The positions of glutamic acid and asparagine were occasionally reversed from that shown in Figure 8. Amino acids
Figure 8. Stimulation of respiration of dwarf mistletoe tissues by the addition of amino acids and amides.
other than those shown in the figure were capable of stimulating the respiration of the tissues. These are listed in Table 8 along with their utilization expressed as percent of glucose utilization. Table 8 was prepared from representative data collected from a number of trials. Variations in the rates of respiration were common and occurred from plant to plant although the order of utilization was reasonably constant.

Pine Tissues. Pine tissues were characterized by a rapid uptake of oxygen when glutamic acid was introduced into the buffer solution bathing the tissue slices. Glutamic acid was consistently utilized preferentially over other substrates tested. The results of a representative trial are presented in Figure 9. Substrates listed in the order of decreasing stimulation were: glutamic acid, glutamine, glycine, proline, alanine, leucine, aspartic acid, and asparagine. Phenylalanine appeared to be slightly inhibitory.
Figure 9. Stimulation of the respiration of pine tissues by the addition of amino acids and amides.
DISCUSSION

Among the significant findings of this investigation are: (1) conformity of results with data obtained from investigations of host-parasite relations in highly diverse associations: bacteria-higher plants, fungi-higher plants, higher plants-higher plants; (2) differentiation of certain metabolic patterns of host and parasite; (3) disclosure of the ability of the parasite to utilize as respiratory substrate, a number of constituents found in the host as well as in the parasite; and (4) differences in the preference exhibited by a given tissue to metabolize certain exogenously supplied substrates.

Mineral Analyses

_Arceuthobium campylopodum_ was found capable of intercepting and accumulating minerals from the translocation stream of ponderosa pine. These results agree with those of a number of workers who have shown accumulation of minerals at infection sites. Gottlieb and Garner (15) found phosphorus to accumulate in the vicinity of rust infections on wheat plants. Shaw and Samborski (32) found accumulation of organic as well as inorganic compounds, many of which did not occur naturally in the host plants. Hussin (21) showed accumulation of minerals in galls on tomato induced by _Agrobacterium tumefaciens_ (E. F. Sm. and Towns.) Conn.
Work with the rusts and mildews (32) and with tomato crown gall (21) supports the view that the accumulation occurs in the host tissues. However, the work of Staples and Ledbetter (34) with rusted bean leaves showed accumulation of tritiated glycine to result from incorporation into the fungal hyphae forming the uredia.

The mineral contents of the fusiform swellings at dwarf mistletoe infections, which comprise tissues of both host and parasite, were found to have values intermediate between those of the parasite tissue and the adjacent host tissues. The values occurring are regarded to result from high concentrations in that portion of the swelling made up of tissues of the parasite; not from accumulation in the host.

The pattern of selective absorption of minerals in the dwarf mistletoes appears to be the same as reported for other parasitic spermatophytes, for example, *Viscum* on *Pyrus*, *Orobanche* on tobacco, and *Cuscuta* on *Sambucus*. In the latter two, Nicoloff (27) found high contents of phosphorus and potassium and low contents of calcium in the parasites as compared to their hosts. Nitrogen levels of these parasites were equivalent to their hosts. Nitrogen levels in *Viscum* (26) were considerably higher than in adjacent host bark.

It has been shown with *Striga asiatica* Kuntze (30) infecting
corn that radioactive phosphorus is rapidly translocated from the
host into the parasite as was sulfur$^{35}$. Calcium$^{45}$, however, was
retained largely by the host with little translocation into the tissues
of the parasite.

Gäumann (12) felt that the removal of large quantities of
nutrients by the mistletoe results in serious damage to the host, a
conclusion in agreement with that of Hawksworth (16). Although the
data from this study show large amounts of nutrients to be tied up
in the parasite and parasitized tissues of the host, the true magni-
tude of the amounts of nutrients being retained by these tissues
cannot be realized from the presentation of results based on a per
unit dry weight basis since it gives no idea of the total amounts of
substances involved. Also, the data give no indications of mineral
deficiencies being caused by the presence of the parasite which one
might expect if large quantities of minerals are being intercepted
before reaching the terminal region of the branches.

The work of Hussin (21) showed that in severely galled tomatoes,
no mineral deficiencies are observed in tissues from the galls as a
result of the redistribution of minerals. His plants bearing galls
generally were smaller but mineral content of the foliage was some-
times higher than that in the gall free plants.

No blockage of translocation or localized accumulation of the
minerals assayed for occurs in the phloem of the infected pines examined except for calcium which accumulates below the fusiform swelling. However, analyses of needles distal to mistletoe infections gave no indication of calcium deficiencies when compared with the foliage from noninfected stems. The foliage from the sampled branches bearing dwarf mistletoe appeared normal in all respects. Tissues of the parasite and fusiform swelling were lower in calcium than was the host; a situation observed by Hussin with galls on tomato and by early workers who analyzed *Viscum*, *Orobanche*, and *Cuscuta* (26, 27).

Physically, the dwarf mistletoe plants occupy a very favorable position with respect to interception of materials translocated in the host. Sinkers are strategically located to intercept substances transported through the xylem, whereas, the portion of the parasite located in the bark of the host occupies a position favorable for the interception of phloem translocates.

As seen in Tables 1, 2, and 4, large quantities of potassium, phosphorus, and nitrogen accumulated in the parasite and fusiform swelling. Hull and Leonard (2) demonstrated accumulation of sulfur in *Arceuthobium campylopodum* on Pinus sabiniana. Sulfur probably is also accumulated by *A. campylopodum* on ponderosa pine if similarities in selective absorption of other elements may serve as an indicator.
With the dwarf mistletoe in direct competition with its host for nutrients, it seems possible that growth of the host could be supressed by lack of specific nutrients. These effects would be greatest where nutrients are inadequately supplied by the soil or where the dwarf mistletoe infestation is excessively heavy.

The results of analyses showing accumulation of carbohydrates in the fusiform swelling, but not in the aerial portion of the parasite, are at variance with the finding of Rediske and Shea who worked with _A. americanum_ on small lodgepole pines. They found large amounts of their administered carbon$^{14}$ accumulated as sucrose in the parasite, and they also indicated accumulation of the isotopic carbon$^{14}$ distal to the infection. In my findings, there is no evidence of accumulation of sugar distal to the site of the infection. However, sugars did accumulate in the fusiform swellings of the branches.

Although analyses for total nitrogen show comparable amounts of this element in host foliage and aerial portions of the parasite, three observations lend indirect evidence that the quantities of amino compounds in the free form are greater in the dwarf mistletoe than in the host: (1) passage of extracts of the host and parasite through a Dowex 50 (H$^+$ form) column gives visual evidence that greater quantities of amino acids are retained on the column from extracts
of parasite and fusiform swelling than from extracts of either host bark or foliage; (2) the amount of the extract required for detection on a paper chromatogram is smaller with extracts from the parasite than with extracts from the host; (3) visual estimations of spots derived from equal size aliquots show that spots from extracts of parasite and fusiform swelling are larger and more intense than those from extracts of the host.

Inspection of the qualitative distribution of free amino acids in the host bark and parasite can lead to interesting considerations. Glutamic acid, aspartic acid, serine, alanine, glutamine, valine-methionine and γ-aminobutyric acid were found in all portions of host and parasite. Since phenylalanine-leucine(s) were found in all portions except the bark extracts distal to the mistletoe, it is felt that the inability to detect these substances on all chromatograms resulted from insufficient amounts of the extract being spotted, or from excessive discoloration of the paper in the phenylalanine-leucine(s) area caused by breakdown products of the phenol solvent. The spot tentatively identified as hydroxyproline was not detected in the distal extracts. This is felt to result from an insufficient amount in the extract and the insensitivity of the ninhydrin to this acid.

Spots tentatively identified as cysteine are of interest since
they are found only in the extracts of bark from healthy branches and bark proximal to the mistletoe, whereas, cysteic acid, a possible conversion product of cysteine, is found in extracts of the fusi-form swelling and the parasite but not in extracts of bark from healthy branches or bark proximal and distal to the mistletoe. This suggests that the translocation of cysteine, a component of healthy bark, is being intercepted by the parasite and converted to cysteic acid. Neither cysteine nor cysteic acid is found in the pine foliage. The occurrence of lysine-arginine in the host but not in the parasite could suggest an incorporation or utilization by the parasite. This interpretation seems plausible, especially in view of the stimulation of dwarf mistletoe tissue respiration by the addition of arginine.

The occurrence of glycine in bark adjacent to the swelling but not in the swelling or the parasite suggests a rapid incorporation or transformation by the parasite of glycine which results in an undetectably small glycine pool in the parasite.

**Utilization of Exogenously Supplied Substrates**

Dwarf mistletoe is capable of utilizing a number of metabolites found in the free form in host and parasite. Shaw and Samborski (32) indicated that rusts and mildews could stimulate their host to
accumulate a variety of organic substances even though some did not occur naturally in the host or parasite. Coupled with this accumulation was an increase in the rate of respiration and a shift in the pathway through which glucose was resired. Rogers and Nelson (30) have shown a strong accumulation of carbohydrate by Striga asiatica when $\text{C}^{14}\text{O}_2$ was administered to parasitized corn, but they did not attempt to show the utilization of this carbohydrate.

In my studies, respiration of dwarf mistletoe was stimulated by the addition of a variety of substances. Whether or not the substances were used directly or functioned indirectly by stimulating the oxidation of endogenous reserves was not determined.

It is assumed that the added carbohydrates are utilized directly, in the manner shown possible by Shaw (32), Beevers and Gibbs (4), and others employing radioisotopes.

Use of the supplied substrates by tissues of dwarf mistletoe was not in the expected order of preference. Rediski and Shea (29) showed that in A. americanum, sucrose was the only sugar labelled after prolonged exposure of the dwarf mistletoe to light and $\text{C}^{14}\text{O}_2$. They also showed sucrose to be the main sugar translocated from the host into the parasite. In the experiments reported here, preferential utilization of sucrose by A. campylopodum did not occur.

Sucrose was poorly utilized when compared to the
monosaccharides such as glucose, fructose, and galactose. Although cell permeability probably plays a part in the low response of the tissues to added sucrose, it by no means explains the entire situation. If a straight line relationship exists between molecular weight and utilization, the pentoses would have been the most rapidly utilized of the substrates tested. This was not the case. Also, raffinose, a trisaccharide, often stimulated a better response than did sucrose, a disaccharide.

The evidence suggests that dwarf mistletoe tissues contain a system for the non-specific activation of hexoses. The data give no indication as to the pathways by which these substances are being metabolized.

The utilization of pentoses by the tissue of the parasite suggests operation of a pentose utilizing pathway. The presence of the pentose phosphate pathway is well established in many plants and, although no evidence is presented here, it is reasonable to consider that the pentoses, ribose and xylose, are being utilized through this route.

The occurrence of galactose in the tissues of the parasite as shown by the analyses, plus the rapid utilization of galactose as shown by respirometry studies, makes it difficult to accept the findings of Rediske and Shea, concerning the incorporation of carbon
wholly into sucrose, as a generality that can be applied to all host-parasite combinations.

The occurrence of carbohydrates in the host differing from those in the parasite is of interest from two view points. Daly, et al. (9), showed that rust infection on safflower stems and beans caused the appearance of several carbohydrate constituents not normally present in the tissues of healthy hosts. Although he could not show that the different compounds arose from fungal metabolism, the presence of these compounds in spores of the fungus lends credance to his view that they are of fungal origin. This situation then would be analogous to the data presented here where differences occur in the carbohydrate constituents present in the host and parasite.

The occurrence of compounds accumulating in fungus cultures has been used by Foster (11) as a basis for an interesting and provocative article. Foster contends that pathways for the oxidation of carbohydrates in higher plants can usually be established only by difficult techniques and are found often by discrepancies in the data obtained from complex experiments. On the other hand, he considers the fungi to give excellent indications of new metabolic pathways by accumulation of intermediate metabolites in the fungus and culture medium. The parasite in my studies presents behavior similar to Foster's experience with the fungi. The occurrence of
an unidentified carbohydrate in the parasite and not in the host indicates the possibility of a different pathway of carbohydrate metabolism. This type of finding is of interest in considering possible control through the administering of specific antimetabolites which could interrupt parasite metabolism without interfering with host metabolism.

Nitrogen is an essential element for all plants and is used in a great many cellular building blocks. The results of the nitrogen analyses show that the parasite is accumulating large quantities of nitrogen which must occur at the expense of the host. There is no known literature which indicates the form of nitrogen utilized by the dwarf mistletoes. From the work of Bollard (6) and Barnes (3) it seems that almost all nitrogen found in the aerial portions of trees, and that translocated through the xylem, is organic in form, primarily amides and amino acids. This evidence strongly suggests that the nitrogen sources of the parasite are amides and amino acids of the host. The anatomical adaptations of the parasite would lend themselves to an efficient interception of these nitrogenous substances not only in the xylem but the phloem as well. These compounds utilized by the parasite would be withheld from the host.

Table 7 lists the free amino acids and amides found in host and parasite, showing that the components of both are very similar
qualitatively. This similarity supports the view that the parasite is intercepting host metabolites directly, although by no means would this be conclusive. Further information could be gained from protein hydrolysate analyses which are beyond the scope of this study.

Since it has been shown in previously mentioned work that obligate parasites tend to accumulate or cause their hosts to accumulate a wide variety of substances, one cannot, on the basis of chromatographic analysis, determine whether the occurrence of similar amides and amino acids in host and parasite is manifestation of accumulation of these materials or of utilization of the substances present by the parasite in its metabolism. While the addition of amides and amino acids to tissues does not exactly duplicate conditions in the living plant with the parasite in situ, the stimulation of respiratory response does show that the tissues of dwarf mistletoe can respond under the conditions of the experiment.

Respiration of mistletoe above the endogenous level is stimulated by the addition of a number of amino compounds. Fructose, the most utilizable of the sugars, was employed as a standard for comparison. Respiration exceeding the endogenous level was interpreted as either direct or indirect utilization by the tissues.

It is of interest that nitrogen rich compounds such as glutamine, asparagine, and arginine (arginine is not shown in Figure 8,
but its position fell between fructose-alanine and glutamate) stimulated the greatest response from the dwarf mistletoe tissues. Glutamine was preferentially utilized by dwarf mistletoe as opposed to glutamic acid which was used preferentially by the pine tissues. Arginine, which was utilized rapidly by the parasite tissues, caused only small stimulation in the pine tissues.

From the evidence presented, it appears that the amino acids accumulated by the parasite are not relegated to inert pools but, under experimental conditions, are utilized either directly or indirectly in the metabolism of the parasite.

Pine tissues have a very high rate of indogenous respiration even after samples are aerated overnight in dilute Hoagland solution. Although tissues tested in June showed erratic response to the addition of carbohydrates to the medium in which they were bathed, erratic behavior was not noted in tissues examined in August. Samples of pine tissues taken from mid-summer to early fall failed to respond to exogenously supplied carbohydrates. James (22, p. 98-99) has pointed out that negative results can not be interpreted to support or reject the utilization or nonutilization of a supplied substrate since it would be impossible to decide if the lack of response was due to impermeability of the cell membranes or to lack of metabolic systems to accommodate an added constituent.
However, the lack of response to exogenously supplied carbohydrates in the pine tissues examined in the late summer and fall is of interest from the standpoint of proper timing of chemical treatments in the event that a specific antimetabolite effective against the dwarf mistletoe should be found.

Contrary to their lack of response to carbohydrates, pine tissues utilized exogenously supplied amino acids. Glutamic acid stimulated rapid oxygen uptake as did glutamine. Proline, a derivative of glutamic acid, was utilized at one half the utilization rate of glutamic acid which was utilized more actively than a number of other amino compounds. Phenylalanine, which possibly was present but not positively identified on the paper chromatograms, appeared to be slightly inhibitory.

Spoehr and McGee (33) found that administering amino acids to detached leaves of sunflowers greatly increased the rate of respiration. They felt that this rise was due to an increase in carbohydrate utilization since the analyses showed a decline in leaf carbohydrate. The results of my experiments on amino acid utilization suggest similar situations in pine and dwarf mistletoe. The supplied amino acids could serve as a nitrogen source, thus stimulating the breakdown of carbohydrates to supply carbon skeletons for amino acids.
Although differences appear between the host and parasite in the order in which they utilize amino compounds, my data are not sufficient to determine whether or not these different substances are metabolized through different pathways.
BIBLIOGRAPHY


