

Effect of Infection by *Verticicladiella wagnerii* on the Physiology of *Pinus ponderosa*

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ABSTRACT

The effects of *Verticicladiella wagnerii* infection on host tree physiology were studied on ponderosa pine seedlings growing under field conditions. The study was carried out at an elevation of 1,300 m in the Sierra Nevada Mountains of California from July to October 1969. Data analyzed were obtained at approximately 2-week intervals during the noon period from 1100-1300 hr. Despite soil temperature reaching 24 C during the summer, approximately 80% of all seedlings inoculated became infected. One month after inoculation, dramatic decreases in net photosynthesis and transpiration occurred in diseased seedlings together with marked increases

in foliar water stress and closure of stomates despite the absence of visual symptoms. No real differences in dark respiration were observed. These effects continued until the end of the study when most of the inoculated seedlings had died. These findings elucidate some of the fungus-host interactions of the root stain disease, which is commonly observed in parts of the mixed conifer forests in California. The importance of the fungus is discussed, particularly from the standpoint of its predisposing trees to bark beetle attack. *Phytopathology* 61:920-925.

Additional key words: respiration ratios.

Verticicladiella wagnerii Kendrick, cause of the root stain disease (18), has recently been discovered in several new locations in California, particularly on ponderosa pine (*Pinus ponderosa* Laws.). The fungus usually infects one or more roots of a tree, presumably via contacts with infected roots of adjacent trees, colonizes the outer rings of the xylem (17), and grows through tracheids up the roots into the base of the trunk. *Verticicladiella wagnerii* attacks trees of all sizes, and clearly presents a threat to ponderosa pine, particularly where it occurs in young, relatively even-aged, pure stands. In addition to direct damage, this fungus commonly predisposes trees to attack by bark beetles. Infected ponderosa pines more than 20 cm in diameter at breast height (ca. 1.5 m aboveground) are almost invariably attacked by *Dendroctonus brevicomis* LeC. and/or *D. ponderosae* LeC. before death due to root disease. Smaller infected trees are not usually attacked because of beetle preference for larger hosts; however, these smaller trees and seedlings are usually killed by the fungus alone.

The occurrence and spread of *V. wagnerii* is clearly of considerable importance to forest management in California. The pathogen obviously lowers host resistance (or possibly increases host attractiveness) to bark beetles, but the mechanism by which resistance is lowered is not known. The beetles rarely infest a tree before the fungus invades the root collar or main stem. However, at this stage diseased trees often show no apparent foliar symptoms or change in growth rate. Studies on ponderosa pines larger than 20 cm in diameter have indicated that diseased trees are under greater moisture stress than healthy trees, and that oleoresin yields and pressures are reduced. The size of infected trees and their remote location make detailed studies on the effect of disease on host tree physiology difficult. Therefore, the initial study reported here was made on seedlings planted in a forest environment.

The objectives of the study were to determine the effects of disease on rates of net photosynthesis, dark respiration, transpiration, foliar water stress, and stomatal aperture of the host seedlings growing in a natural environment. Although the effects of disease on seedlings are not necessarily the same as those on older trees, the results of this study can aid in understanding the physiology of disease caused by *V. wagnerii*, and may lead to elucidation of the processes involved in predisposition of trees to bark beetle attack.

MATERIALS AND METHODS.—The study was located at the University of California Blodgett Forest Research Station, situated at an elevation of 1,300 m in the Sierra Nevada Mountains, a location where the natural occurrence of *V. wagnerii* has recently been confirmed. One-year-old ponderosa pine seedlings grown from locally collected seed were planted in spring 1969 in a forest clearing and under 50% shade cover provided by lumite fabric. On 17 July 1969, 80 seedlings in a block of 130 were inoculated with a culture of *V. wagnerii* isolated from ponderosa pine. Inoculum consisted of colonized ponderosa pine twigs 2 cm in length and 0.6-1.0 cm in diameter. The twigs, placed in 125-ml Erlenmeyer flasks with 10 ml 2% potato-dextrose broth and sterilized by autoclaving, were inoculated with 0.5 ml of a spore and mycelial suspension. They were incubated at 18 C for 1 month prior to inoculation of seedlings. A colonized twig section was placed against the main root of each seedling about 8 cm below the soil surface. Sterilized twig sections were placed against the roots of the remaining 50 seedlings which served as controls. All seedlings were mulched with pine needles and were watered frequently to reduce soil temperature and maintain soil moisture.

On seven occasions between 20 July and 13 October 1969, all the current season's foliage of five healthy controls and five diseased seedlings was concurrently monitored for 2 consecutive days to evaluate the ef-

fects of disease on the physiological functioning of the seedlings. Net photosynthesis, dark respiration, and transpiration were continuously monitored during each 2-day sampling period with two infrared gas analyzers, using procedures similar to those of Neuwirth (16) and Keller (11, 12). A 12-line gas selector switch permitted sequential sampling of 10 seedlings; two lines were used for continuous monitoring of ambient CO₂ concentration. Switching intervals of 30 sec provided consecutive recordings of gas exchange every 6 min. A flow rate of 2.5 liters/min was used, and CO₂ concentrations within the cuvettes were always within 10% of ambient, the acceptable limit described by Larcher (14).

Sampling cuvettes were constructed from tubular Plexiglas, and were fitted with overhead water filters to minimize buildup of artificially high cuvette air temperature. Small fans were installed within each cuvette to reduce boundary layer effects. Ambient air was introduced into all cuvettes from a common intake at 3.5 m aboveground to avoid the wide variation in ambient CO₂ concentration which occurs between adjacent sampling points near the surface of the ground.

Ambient air temperature and air and leaf temperature within each cuvette were measured with shielded thermocouples and monitored 25 times/min on a 24-line, strip-chart recorder. Solar radiation was monitored by an Eppley pyrliometer. Soil temperature at a depth of 8 cm was monitored by four tempscribe units.

On 1 day in each sampling period, plant water stress and stomatal aperture were measured hourly during the photosynthetic period, using fascicle samples. Stress was evaluated by the Scholander pressure bomb technique (19), and stomatal aperture was measured by the infiltration pressure method of Fry & Walker (8).

All comparisons of gas exchange at each sampling date were based on 40 measurements for each seedling within the period from 1100-1300 hr on 2 consecutive days. During this period, environmental conditions were most stable and rates of gas exchange fluctuated least. After each sampling run, each seedling was harvested and the oven-dry wt of all current-year foliage was determined.

All seedlings in each sample were examined to determine the extent of colonization by *V. wagnerii*. Isolations were made from discolored xylem to confirm identity of the pathogen. A rating system of 0-4 was used to describe the extent of colonization based on vertical and transverse extension of vascular discoloration, root and rootlet mortality, extent of resinosis, and foliar chlorosis.

Significance of differences between mean results was tested using the "Student" t-test.

RESULTS.—Soil temperature in July-August ranged daily between a min of 18 C at night and a max of 24 C during the day, with a 10-hr period above 20 C. By October, min night soil temperature was 16 C and daily max was 23 C, with 6 hr above 20 C. Despite these high soil temperatures, about 80% of the inoculated seedlings became infected with *V. wagnerii*. Variable rates of development of the pathogen among

TABLE 1. Average disease rating of *Pinus ponderosa* infected with *Verticicladiella wagnerii* seedlings and average needle infiltration pressure and photosynthesis:transpiration ratios of healthy and diseased plants at each measurement date

Date	Avg disease rating of infected seedlings	Avg needle infiltration pressure (atm)		Photosynthesis:transpiration ratio (mg CO ₂ /g H ₂ O. 10 ⁻³)	
		Healthy	Diseased	Healthy	Diseased
20-21 July		0.3	0.3	4.6	4.2
6-7 Aug.		0.3	0.3	4.4	4.4
12-13 Aug.	0.8	0.3	1-2	4.4	4.2
30-31 Aug.	2.0	0.3	2-3	5.1	1.8
10-11 Sept.	2.7	0.4	2-3	4.0	0.6
26-27 Sept.	3.3	0.3	2-3	4.2	—5.7
12-13 Oct.	3.3	0.4	2-3	3.9	—11.8

different seedlings resulted in a wide range of treatment effects in each sample. On the first two sample dates, 20-21 July and 6-7 August, each sample of five inoculated seedlings included one plant which was subsequently found to be uninfected. These seedlings were eliminated from the analyses, and the results presented for the first two dates are based on samples of four seedlings each. All subsequent results are from five-seedling samples.

The average disease rating of the infected seedlings monitored during the study is shown in Table 1. Although a more quantitative appraisal system would have been preferable, the rating system used provides an indication of rate of disease development. One month after inoculation, seedlings were rated slightly-to-moderately colonized, and external visual symptoms of infection were completely absent. In four out of five seedlings, the pathogen had spread around 60-90% of the stem circumference, and vertical extension was 2.5-5 cm. After 55 days (10 September), transverse spread of infection in all sample seedlings was 80-100% of the circumference, and vertical extension was typically 10 cm. The extent of colonization increased during the study period, the effects being mainly evident by rootlet mortality. Foliar chlorosis was slow to develop, and was apparent only by direct comparison with healthy plants. Toward the end of September, considerable chlorosis was evident; by the end of November, all remaining diseased seedlings had died.

Net photosynthesis.—Rates of net photosynthesis of the healthy seedlings growing in a relatively uniform field environment were remarkably stable for most of the photosynthetic day (Fig. 1-a). Rates exhibited by diseased seedlings were not as uniform despite the stable environment (Fig. 1-a, b). Average net photosynthesis of healthy control seedlings throughout the summer was characteristically about 2.2 mg CO₂/g dry wt foliage per hr (Fig. 2). Substantial reduction in average rate of net photosynthesis by seedlings infected with *V. wagnerii* was first detected on 12-13 August, 1 month after inoculation (Fig. 2); however, this reduction was not significant at the 95% level. Subsequently, net photosynthesis of diseased seedlings

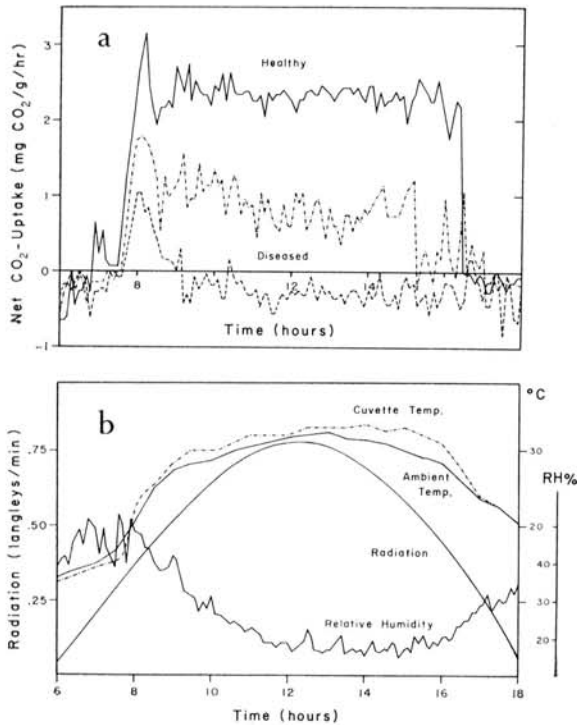


Fig. 1. a) Net photosynthesis of three seedlings of *Pinus ponderosa* monitored on 30 August showing influence of increasing degree of infection by *Verticicladiella wagnerii* on net CO₂ uptake. b) Environmental conditions monitored on 30 August.

declined rapidly. Two months after inoculation, rates of respiration exceeded rates of photosynthesis, and some seedlings were beginning to show chlorosis. By 12-13 October, 3 months after inoculation, net CO₂ uptake of diseased trees was negative throughout the entire day, and was equivalent to net respiration averaging 1.5 mg CO₂/g per hr during the sampling period.

Dark respiration.—The rates of respiration of diseased and healthy seedlings were compared, using data collected between 2300-0100 hr. The average rates of dark respiration were similar throughout the summer, but the healthy control plants exhibited a much wider range of values (Fig. 3). In every case, the most actively photosynthesizing seedlings during the day also exhibited the highest average rates of respiration at night. Differences between mean values of healthy and diseased seedlings at each sampling date were not significant.

Transpiration.—Rates of transpiration of healthy control seedlings averaged ca. 0.45 g H₂O/g dry wt foliage per hr, with a tendency towards increasing rates as the summer progressed (Fig. 4). Approximately 1 month after inoculation (12-13 August), marked decreases in rates of transpiration occurred in the diseased seedlings. At that date, differences between mean rates of transpiration of healthy and diseased seedlings were significant at the 95% level. Two and one-half months after treatment (28 September), transpiration

of diseased seedlings reached a min of 0.12 g H₂O/g per hr.

Healthy seedlings maintained photosynthesis:transpiration ratios of about 4-5 throughout the summer (Table 1). Inoculated plants had similar ratios at the beginning of the study. However, as colonization increased in the diseased seedlings, the ratios declined until, at the end of the study period, values of almost —12 were obtained, indicating drastic decline in vigor.

Foliar water stress.—Before sunrise and at night, foliar water stress of healthy seedlings was characteristically between 6.0-6.5 atm. Maximum stress in those healthy plants throughout the midday periods in summer was quite uniform, and averaged 12-14 atm for each five-seedling sample (Fig. 5). The inoculated seedlings showed a marked increase in water stress 1.5 months after treatment (31 August); the difference between means at this date was significant at the 95% level. After a period of 2 months, average midday foliar stress of diseased plants was ca. 30-36 atm.

Stomatal aperture.—Midday infiltration pressures of healthy seedlings were consistently between 0.3 and 0.4 atm throughout the summer (Table 1). However, infiltration pressures of *V. wagnerii*-infected seedlings increased 1 month after inoculation, and by 31 August ranged between 2.5-3.5 atm. According to Fry & Walker (8), an infiltration pressure of 3 atm in Douglas fir (*Pseudotsuga menziesii*) indicates stomatal closure. It is most probable, therefore, that in this study the stomates of the healthy plants remained open, whereas those of the infected seedlings were closed during the midday sampling period.

DISCUSSION.—Studies on the effects of infection on host tree physiology can be carried out most simply in the laboratory. However, results of such studies are often difficult to extrapolate to field conditions. The present study is an initial analysis of the *V. wagnerii*-*ponderosa* pine interaction using seedlings growing in their natural environment. Due to limitations of gas exchange instrumentation, the results presented are based on the concurrent performance of five diseased and five healthy seedlings at each sampling date. This sample size is not sufficient to accurately estimate experimental error; however, the ranges of values obtained and the differences between means indicate the magnitude of the interactions involved.

One difficulty in comparing physiological behavior over a complete growing season is that current-year needles continue elongating until midsummer, and the net photosynthetic efficiency is continually changing during this development period (4). To minimize this problem, the study was initiated in mid-July. Some experimental error probably arises from the development of an artificial cuvette environment. The measurement of air temperature within and outside cuvettes showed that during the midday period, the sample foliage was exposed to temperatures up to 3°C greater than ambient. Rates of photosynthesis and transpiration determined by gas exchange procedures are influenced by any resultant buildup of humidity levels within cuvettes (13). This artifact was minimized by

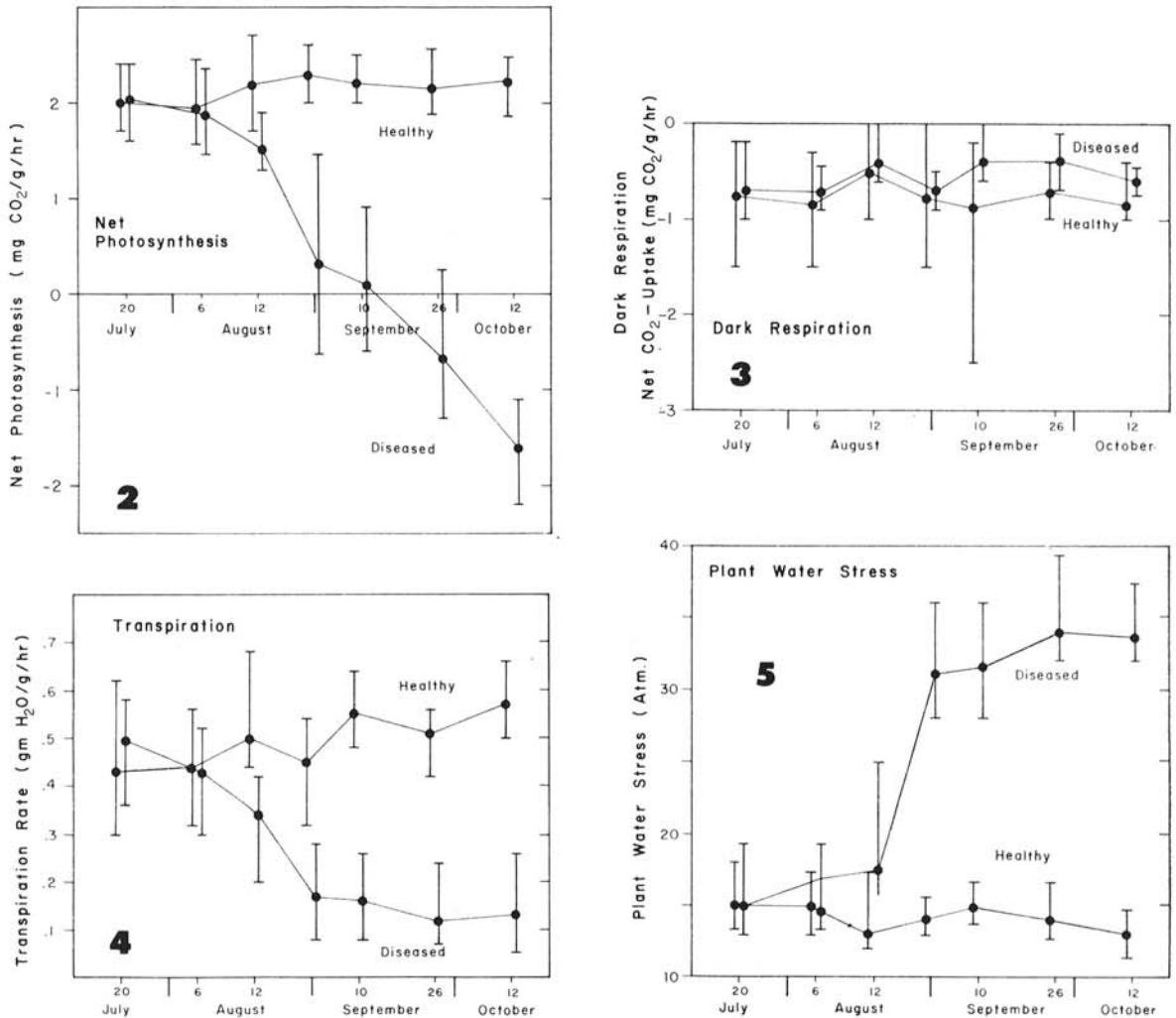


Fig. 2-5. Effect of infection by *Verticilladiella wagenerii* on physiology of *Pinus ponderosa* seedlings. 2-4) Bars = range of values obtained from 40 measures from each of five seedlings. 5) Bars = the range of individual measures from each of the five seedlings.

using an optimal flow rate, but the effect cannot be eliminated unless cuvettes are available in which internal environment can be controlled.

Despite max soil temperature reaching 24 C almost every day, ca. 80% of the inoculated seedlings became infected by the fungus. This high proportion of successful infection was not expected, as studies by Smith (17) in the laboratory showed that infection of ponderosa pine seedlings at temperatures of 16 and 21 C was 90 and 30%, respectively, while at 27 and 32 C, no seedlings became infected. Similarly, little colonization of the sapwood was expected within 6 weeks after inoculation, but in this experiment, substantial colonization occurred within 25 days. This unexpectedly high rate of colonization may be partly explained by the fact that actual temperature within seedling stems at ground level (as measured by inserted thermocouples) was 3-7 C lower than soil temperature.

A period of ca. 1 month elapsed after inoculation

before colonization was sufficient to markedly affect the physiological functioning of the host seedlings. At that stage, no external symptoms of infection were evident. Subsequently, drastic decreases in net photosynthesis occurred, and within 2 months the seedlings were utilizing considerably more food materials in respiration than were being manufactured by photosynthesis.

Previous studies have shown that rates of net photosynthesis are affected when plants are in advanced stages of infection (1, 20). However, in this study, net photosynthesis in diseased seedlings was reduced substantially at least 1 month before the initial appearance of foliage symptoms. There was no evidence that the pathogen invaded the foliage of any seedling during this study, and colonization was still confined to the roots and underground stem on 31 August. The most plausible explanation for this early reduction seems to be associated with the demonstrated development of

foliar water stress, probably due to reduced water uptake. This conclusion supports the earlier work of Beckman et al. (2). Mean rates of net photosynthesis of healthy plants during the 2-hr sampling period at noon were remarkably stable and uniform. The wider ranges of values obtained for infected trees, compared with those for the controls, are indicative of the different degrees of infection among the seedlings.

Increases in amount of vascular colonization by *V. wagnerii* with time were accompanied by decreases in host transpiration as well as in net photosynthesis. While infection by pathogens such as mildews and rusts increases transpiration (9), at least for a time, root diseases and vascular wilts may cause a reduction in transpiration due to a disruption of the water economy of the host. Beckman et al. (2) reported that the rates of both photosynthesis and transpiration fluctuated in banana plants infected by *Pseudomonas solanacearum* in the same way as in noninfected plants under moisture stress. They concluded that such an effect was related to the loss of turgor by guard cells with resultant stomatal closure. Dimond & Waggoner (7) reported much the same effect upon *Fusarium*-infected tomato plants. These diseased plants transpired less than one-half as much as healthy plants, but the difference between diseased and drought-hardened plants was much less; the stomates of the latter plants remained closed during the day, whereas those of the healthy plants were open. Smith (17) reported vascular disruption in *V. wagnerii*-infected seedlings. He showed that tyloses occasionally developed from ray parenchyma and protruded into the lumina of adjacent tracheids. This apparently causes an effect similar to that associated with vascular wilts in other plants.

The possible trend of slightly increasing transpiration of healthy seedlings as the summer progressed was probably associated with gradual decreases in radiation. In July, mean radiation load at noon was 1.63 calories/cm² per min, whereas by October this value had dropped to 1.35 calories/cm² per min. Reduced radiation load was shown to reduce foliar water stress, which in turn probably resulted in increased stomatal opening and transpiration in healthy plants.

The relative magnitude of foliar water stress in plants is a good indication of their reaction to a given environment. Our measures indicated that the seedlings were not being exposed to adverse conditions. Under the 50% shade cover, healthy seedlings developed a stress of 12-14 atm during the noon sampling period, whereas the diseased trees typically developed stresses of 30-36 atm during September-October. Healthy seedlings growing in the sun develop stresses of ca. 15-20 atm, and Cleary (5) showed that if ponderosa pine seedlings are subjected to drought conditions, stresses reach 40 atm or greater which, if sustained, typically lead to death of the plant. Our results show that ca. 1 month after inoculation, decreases in net photosynthesis and transpiration were accompanied by a marked increase in foliar water stress. This relationship, and the ease with which water stress measures can be obtained,

make this technique the best single procedure for determining relative health and vigor of seedlings.

Numerous studies (9, 20) have shown that an increase in respiratory rate is a common response to infection, especially by obligate parasites. Less is known concerning effects of facultative parasites (3). On the basis of previous reports, an increase in respiration by *V. wagnerii*-infected seedlings was expected. However, measurements of dark respiration during the 2-hr periods at midnight consistently showed no significant differences in mean rates of respiration between healthy and diseased seedlings. Additionally, an examination of all gas exchange data from throughout the night yielded no evidence of differences. It was observed, however, that healthy plants which had the highest rates of CO₂ uptake during the day also had the highest rates of CO₂ evolution at night.

The reason for the lack of change in respiration rate of diseased seedlings is not entirely clear. In our study of intact seedlings, the foliage was not infected by the fungus, and low stress and cool night temperatures were apparently conducive to normal rates of respiration.

Photosynthesis:transpiration ratios are currently used as ecological indices of the success with which a plant is exploiting its environment (12). The healthy plants in this study maintained a ratio of ca. 4, a value similar to those obtained by Keller (12). A slight diminishing in the ratio toward the end of the summer was due to slight increases in transpiration as radiation load diminished. On the other hand, the ratio for diseased trees rapidly decreased as colonization by the fungus increased. Negative values were obtained 2 months after inoculation because net CO₂ uptake became negative during the midday period. The ratio, therefore, clearly shows the abnormal physiological functioning of diseased plants.

Numerous observations have indicated that trees under moisture stress or other conditions which result in a subnormal physiological state are prone to bark beetle and other insect infestations (6). Madden (15) showed that at the time when a wood wasp, *Sirex noctilio*, is attracted to distressed trees, the diffusion of CO₂ from the stem increases and the phloem shows a greater degree of enzymatic oxidation. Graham (10) reported an increased attractiveness of cut logs to ambrosia beetles which appears to be associated with metabolic changes in the host tissue. Thus, it is highly probable that certain metabolic changes induced by fungus colonization of host tissue is also associated with increased susceptibility and/or attractiveness of ponderosa pines to the bark beetles, *Dendroctonus brevicornis* and *D. ponderosae*.

Our study showed that infection of seedlings by *V. wagnerii* can induce severe reductions in photosynthesis and transpiration, which occur concurrently with a drastic increase in water stress and stomatal closure. Other detrimental changes must also occur, although rate of dark respiration appeared to be unaffected. Based on the success of this initial study, more inten-

sive studies will be made on both seedlings and mature trees growing in the natural environment.

LITERATURE CITED

1. ALLEN, P. J. 1954. Physiological aspects of fungus diseases of plants. *Annu. Rev. Plant Physiol.* 5:225-248.
2. BECKMAN, C. H., W. A. BRUN, & I. W. BUDDENHAGEN. 1962. Water relations in banana plants infected with *Pseudomonas solanacearum*. *Phytopathology* 52:1144-1148.
3. BOLLARD, E. G., & R. E. F. MATTHEWS. 1966. The physiology of parasitic disease, p. 417-550. *In* F. C. Steward [ed.]. *Plant physiology, a treatise*. Academic Press, N.Y.
4. CLARK, J. 1961. Photosynthesis and respiration in white spruce and balsam fir. N.Y. State Univ. Coll. Forest. Syracuse Tech. Publ. 85. 72 p.
5. CLEARY, B. D. 1968. Water stress measurements and their application to forest regeneration. *Western Reforestation Proc. San Francisco, Calif.* 3 p.
6. COBB, F. W., JR., D. L. WOOD, R. W. STARK, & J. R. PARMETER, JR. 1968. Photochemical oxidant injury and bark beetle (Coleoptera:Scolytidae) infestation of ponderosa pine. *Hilgardia* 39:141-152.
7. DIMOND, A. E., & P. E. WAGGONER. 1953. The water economy of *Fusarium*-wilted tomato plants. *Phytopathology* 43:619-623.
8. FRY, K. E., & R. B. WALKER. 1967. A pressure-infiltration method for estimating stomatal opening in conifers. *Ecology* 48:155-157.
9. GOODMAN, R. N., Z. KIRÁLY, & M. ZAITLIN. 1967. The bio-chemistry and physiology of infectious plant disease. Van Nostrand Co., Princeton, N.J. 354 p.
10. GRAHAM, K. 1968. Anaerobic induction of primary chemical attractancy for ambrosia beetles. *Can. J. Zool.* 46:905-908.
11. KELLER, T. 1966. Über den Einfluss von transpirationshemmenden Chemikalien auf Transpiration, CO₂-Aufnahme und Wurzelwachstum von Jungfichten. *Forstwissenschaftliches Centralblatt* 85:65-79.
12. KELLER, T. 1970. Wuchsleistung, Gaswechsel, Überlebensprozente und Schneeschimmelpilzbefall gedüngter Ballenpflanzen an der oberen Waldgrenze. *Mitteilungen Schweiz. Anstalt für das forstliche Versuchswesen* 46:5-31.
13. LANGE, O. L., W. KOCH, & E. D. SCHULZE. 1969. CO₂-gas exchange and water relationships of plants in the Negev Desert at the end of the dry period. *Berichte Deut. Bot. Ges.* 82:39-61.
14. LARCHER, W. 1969. Physiological approaches to the measurement of photosynthesis in relation to dry matter production by trees. *Photosynthetica* 3:150-166.
15. MADDEN, J. L. 1968. Physiological aspects of host tree favourability for the wood wasp, *Sirex noctilio* F. *Ecol. Soc. Australia Proc.* 3:147-149.
16. NEUWIRTH, G. 1966. Assimilation, Atmung und Transpiration unterschiedlich gedüngter junger Koniferen während des Frühjahrsaustriebes in Abhängigkeit von Klimafaktoren. *Archiv für Forstwesen* 15:629-639.
17. SMITH, R. S., JR. 1967. *Verticilladiella* root disease of pines. *Phytopathology* 57:935-938.
18. WAGENER, W. W., & J. L. MIELKE. 1961. A staining-fungus root disease of ponderosa, Jeffrey, and pinyon pines. *Plant Dis. Repr.* 45:831-835.
19. WARING, R. H., & B. D. CLEARY. 1967. Plant moisture stress: Evaluation by pressure bomb. *Science* 155:1248-1254.
20. WOOD, R. K. S. 1967. *Physiological plant pathology*. Blackwell Sci. Publ. Ltd., Oxford. 570 p.