Host Specialization of Three Morphological Variants of *Verticicladiella wageneri*

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ABSTRACT

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Inoculations of seedlings and mature trees of ponderosa pine and Douglas-fir with 28 isolates of *Verticicladiella wageneri*, cause of black stain root disease, demonstrated host preferences of three morphological variants of the pathogen. Although each isolate tested was capable of infecting both hosts, isolates of the hard pine variant (isolated primarily from ponderosa, Jeffrey, and lodgepole pines) consistently infected more pine seedlings and older ponderosa pines than did isolates of the Douglas-fir variant (isolated from Douglas-fir). The converse occurred with Douglas-fir. Isolates of the third variant (isolated only from pinyons) were generally intermediate between isolates of the other two variants in pathogenicity on seedlings of both hosts. Few seedlings of sugar pine or western hemlock were infected by any of 10 isolates that were selected from the hard pine and Douglas-fir variants. Two-way analysis of variance showed significant host species × morphological variant interaction in two seedling-inoculation experiments and in one field-inoculation experiment, which demonstrates that these variants preferentially infect and colonize their respective hosts. The variants also differed in survival when colonized blocks were buried in nonsterile soil at 21 and 27 C. The possible role of temperature in the restriction of the hard pine variant to relatively cool sites is discussed.

Black stain root disease on conifers, caused by *Verticicladiella wageneri* Kendr. (teleomorph: *Ceratocystis wageneri* Goheen et Cobb) is widespread throughout western North America on various species of Pinaceae, but the relative prevalence on various host species varies geographically (1,5,10,11,13,14,17,19). In the Southwest, pinyons (*Pinus edulis* Engelm. and *P. monophylla* Torr. et Frem.) are the principal hosts. In other locations, the disease usually occurs on either Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) or on yellow or hard pines. Of the hard pines, *Pinus ponderosa* Laws., *P. contorta* Dougl., and *P. jeffreyi* Grev. and Balf. are most commonly infected. There have been reports of *V. wageneri* on members of the white pine group (*P. monticola* Dougl., *P. strobus* L., and *P. lambertiana* Dougl.) when they were growing near diseased hard pines (12,14,17). Host crossovers, where the pathogen has moved from one host species to another host species, have been recorded (13), but they are rare.

Examination of isolates of *V. wageneri*, as well as the field observations, indicate that the pathogen consists of three host-specialized variants. Wagener and Mielke (19) and Smith (16) recognized cultural differences in isolates from pinyon and hard pines. Recently, we (6,7) described three morphological variants of *V. wageneri* that can be distinguished by mycelial characters. These three morphological variants may be host specific because the pinyon variant has been isolated only from pinyons, the Douglas-fir variant only from Douglas-fir and a single western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and the hard pine variant from pines other than pinyons. Isolates from hemlocks at two locations in Oregon (D. J. Goheen, *unpublished*) are morphologically assignable to the hard pine variant of *V. wageneri*. In spite of morphological evidence for host specialization, Smith (16) found no difference in pathogenicity between an isolate from *P. ponderosa* and an isolate from *P. monophylla* when he inoculated seedlings of their respective hosts. Cobb and Platt (2) found that *V. wageneri* from Douglas-fir and ponderosa pine would each infect seedlings of both hosts, but there appeared to be some host preference.

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In our study, host preferences of the three morphological variants of *V. wageneri* were investigated by inoculating both seedlings and older trees. Because the variants differ in growth rate in axenic culture at warm temperatures (7), we tested the effect of temperature on infection and colonization of seedlings and on survival of the variants in soil.

MATERIALS AND METHODS

Isolates. Twenty-eight isolates of *V. wageneri* (Table 1), representing the three morphological variants, were selected for use in four inoculation experiments. Isolates used for inoculating seedlings in two host-preference experiments (A and B) were chosen to obtain the widest possible range in host of origin and geographic distribution of each variant. Only California isolates were used for inoculations of trees in the field (experiment C) and in temperature studies (experiment D). Hyphal-tipped cultures were obtained from all isolates and inoculum blocks were prepared as described previously (8). Three-centimeter-long segments of ponderosa pine twigs were boiled in malt extract, autoclaved, and incubated with the respective isolates for 8 wk at 18 C.

Seedling inoculations. Two-year-old, bare-root seedlings of ponderosa pine (*P. ponderosa*), sugar pine (*P. lambertiana*), and Douglas-fir, and 3-yr-old western hemlock were lifted from the nursery and stored at 1 C until potting (within 1 mo). Seedlings were potted in January 1982 (experiment A) or February 1981 (experiment B) with a pasteurized mixture of peat and sand (1:1, v/v) in 8-cm-diameter cans and placed on greenhouse benches where they were watered two or three times per week.

Each morphological variant was represented by five isolates. For each isolate, 15 seedlings of each host were inoculated. In experiment A, seedlings were inoculated immediately before potting by securing an inoculum block with masking tape to the taproot 10–13 cm below the groundline. In experiment B, seedlings were inoculated 2 wk after potting by moving the soil away from the taproot, placing the inoculum block against the root ~5–8 cm below groundline and replacing the soil around the inoculum block. Attempts were made to avoid wounding in both inoculation techniques.

Fourteen weeks after inoculation, the seedlings were dissected and examined for the linear extent of brown-black staining, which

is characteristic of black stain root disease (2,4,8,16). A disease severity rating was assigned to each seedling: 1 = healthy, no staining; 2 = no foliar symptoms and less than 15 cm of stain; 3 = no foliar symptoms but greater than 15 cm stain; 4 = extensive stain and chlorotic or dead needles; 5 = dead, with stain. A few inoculated seedlings as well as uninoculated controls with no apparent infection by *V. wageneri* or other pathogen died of transplant shock and were excluded in computing percent infection or mean disease severity rating.

Inoculations in the field. Two sites along the Georgetown Divide (El Dorado County) in the central Sierra Nevada were selected for experiment C. The Gaddis Creek site, on U.C. Blodgett Research Forest, was at 1,300 m elevation in a mixed conifer stand, portions of which had ponderosa pine or Douglas-fir predominating. *Abies concolor* (Gord. et Glendl.) Lindl., *Calocedrus decurrens* (Torr.) Florin. and *Pinus lambertiana* were also present. The second site was above Rock Creek, at 1,000 m elevation, along a relatively dry, exposed ridge where ponderosa pine, Douglas-fir, and sugar pine predominated.

At each site, 18 Douglas-fir and 18 ponderosa pine trees (12–28 cm diameter at 1.4 m height) were inoculated in January 1981. Two major lateral roots on opposite sides of each tree were inoculated, one root with a pine isolate and the other with a Douglas-fir isolate. Each of six isolates (three from Douglas-fir and three from ponderosa pine) was inoculated into six trees of each host species at each site. Roots were exposed and swabbed with 95% ethanol at the point of inoculation, 75 cm from the base of the tree. A 13-mm-diameter hole, ~4-cm deep, was drilled into each root with a bit rinsed in ethanol. Colonized inoculum blocks were inserted into the drilled holes, the holes sealed with molten paraffin, and the roots covered with the original soil and litter.

Inoculated roots of half of the trees were examined 10 mo after inoculation, and those of the remaining trees were examined 6 mo later. Samples of stained wood from the margin of colonization were excised and brought back to the laboratory for isolation of *V. wageneri*. Two samples were excised from a root if the stain extended both distally and proximally from the point of inoculation. No samples were taken from unstained roots. Isolations were made from the excised samples by aseptically placing four chips of stained wood on water agar plates amended with cycloheximide and streptomycin (8). Plates were incubated at 18 C for 14 days.

Temperature effects. In experiment D, seedlings were potted in sieved (5 mm), nonsterile field soil (A12 horizon of a sandy loam, Musick series soil taken from Blodgett Research Forest, El Dorado County, CA) in January 1982. After potting, seedlings were placed in four temperature-controlled rooms within a greenhouse (16) and watered daily. Thermostats in the four rooms were set at 16, 21, 27, and 32 C, but ambient temperatures ranged from 16–20, 20–25, 25–27, and 27–40 C, respectively. Soil temperatures at a depth of ~6 cm were 15–17, 18–22, 22–26, and 25–31 C, respectively. Two weeks after being potted, ten seedlings of each host were inoculated with each isolate at each temperature by placing a colonized inoculum block against the taproot. Twelve weeks after inoculation, seedlings were dissected and examined for black stain. Mortality of both inoculated and control seedlings without black stain was noted in the 27 C and 32 C treatments. These seedlings, apparently killed by transplant shock and higher temperatures, were excluded in compilation of data.

Inoculum blocks in experiment D were recovered from the soil and tested for viability of conidia or hyphae of *V. wageneri*. After rinsing in tapwater, each block was placed in a 5-cm-diameter petri dish to which was added 10 ml of molten, acidified (pH 4.0) water agar amended with 400 μg cycloheximide, 100 μg streptomycin-sulfate, and 70 μg penicillin G per milliliter. Plates were incubated at 18 C for 14 days and examined at ×40 for the presence of conidiophores of *V. wageneri* on the inoculum blocks.

TABLE 1. Hosts and geographic origin of *Verticicladiella wageneri* isolates used in inoculation experiments

Morphological variant and isolate no.	Host of origin	Location	Experiment[a]
Hard pine			
BCL-1	*Pinus contorta*	Monashee, BC	B
CAJ-3	*P. jeffreyi*	Blacks Mtn., CA	A, D
CAP-1	*P. ponderosa*	Gaddis Creek, CA	C, D
CAP-19	*P. ponderosa*	McCloud Flat, CA	B
CAP-36	*P. ponderosa*	Sugar Pine Creek, CA	A, D
CAP-H	*P. ponderosa*	Butcher Corral, CA	C
CAP-I	*P. ponderosa*	Tickey Creek, CA	C
IDP-1	*P. ponderosa*	Boville, ID	B
MOW-1	*P. monticola*	Yaak River, MT	A
ORH-1	*Tsuga heterophylla*	Mt. Hood, OR	A
ORL-1	*P. contorta*	Sisters, OR	B
ORM-S	*T. mertensiana*	Warm Springs, OR	A, B
Douglas-fir			
BCD-1	*Pseudotsuga menziesii*	Cicero, BC	A, B
BCH-1	*T. heterophylla*	Tugwell, BC	A, B
CAD-1	*P. menziesii*	Rock Creek, CA	C
CAD-7	*P. menziesii*	Sugar Pine Creek, CA	A, C, D
CAD-18	*P. menziesii*	Union Valley, CA	B, C
CAD-21	*P. menziesii*	Trinidad, CA	B, D
CAD-32	*P. menziesii*	Greagle, CA	D
MOD-1	*P. menziesii*	Flathead Lake, MT	A
NMD-1	*P. menziesii*	Mescalero, NM	B
ORD-5	*P. menziesii*	Burnt Mtn., OR	A
Pinyon			
CAS-1	*Pinus monophylla*	Onyx Summit, CA	B
CAS-3	*P. monophylla*	Big Bear Lake, CA	B, D
CAS-4	*P. monophylla*	Kennedy Meadows, CA	B, D
CAS-5	*P. monophylla*	Kennedy Meadows, CA	D
COE-1	*P. edulis*	Mesa Verde, CO	B
NME-1	*P. edulis*	Jicarilla, NM	B

[a] Isolates were used in host preference tests on seedlings in the greenhouse (experiments A and B), on mature trees in field inoculations (experiment C), or in studies on temperature effects (experiment D).

TABLE 2. Percentage infection and disease severity rating on seedlings inoculated in the greenhouse with isolates of *Verticicladiella wageneri* from three morphologically distinct variants

Morphological variant[x]	*Pinus ponderosa*		*Pseudotsuga menziesii*		*Tsuga heterophylla*		*Pinus lambertiana*	
	Percent infection	Disease severity rating[y]	Percent infection	Disease severity rating	Percent infection	Disease severity rating	Percent infection	Disease severity rating
Experiment A								
Hard pine	29.3 a[z]	1.6 a	6.8 a	1.1 a	5.3 a	1.1 a	0.0 a	1.0 a
Douglas-fir	26.7 a	1.5 a	39.1 b	1.8 b	8.9 a	1.1 a	7.0 b	1.1 b
Experiment B								
Hard pine	14.7 a	1.5 a	33.3 a	1.7 a				
Pinyon	8.0 a	1.3 a	42.7 a	2.3 a				
Douglas-fir	2.7 b	1.0 b	48.0 a	2.4 a				

[x] Each variant was represented by five isolates, and fifteen seedlings of each host were inoculated with each isolate.
[y] Mean ratings are based on a scale of 1 = healthy to 5 = dead.
[z] Means followed by the same letter are not statistically different ($P = 0.05$) from means of other variant groups on that host in that experiment, according to the test for least significant difference. Arcsine of the square root of the percent infection was used in analysis.

TABLE 3. Analysis of variance mean squares for percentage infection of tree seedlings inoculated with *Verticicladiella wageneri* from two (Experiment A) or three (Experiment B) morphological variants

Source of variation	Degrees of freedom	Mean square
Experiment A		
Host species	3	1,279.5**[b]
Morphological variant	1	1,110.4**
Host species × morphological variant	3	402.2*
Error	32	92.3
Experiment B		
Host species	1	5,117.5**
Morphological variant	2	60.8
Host species × morphological variant	2	451.2**
Error	24	52.1

[a] Fifteen seedlings of each host were inoculated with each of five isolates from each morphological variant (hard pine, pinyon and/or Douglas-fir).
[b] Asterisks (* and **) denote statistical significance, $P = 0.05$, or $P = 0.01$, respectively.

RESULTS

Seedling inoculations. In experiment A, isolates of the Douglas-fir variant infected more Douglas-fir seedlings and had a greater mean disease severity rating on Douglas-fir than did isolates of the hard pine variant (Table 2). The opposite was true on ponderosa pine seedlings, but the differences were not statistically significant. Results with individual isolates were consistent within the morphological variants. Each of the five isolates representing the hard pine variant infected as many or more ponderosa pine seedlings as Douglas-fir seedlings. Likewise, each of the isolates of the Douglas-fir variant infected and caused more severe symptoms on Douglas-fir seedlings than they did on ponderosa pine seedlings.

Few seedlings of western hemlock were infected (Table 2), even by isolates that were obtained from hemlock (Table 1). Likewise, few seedlings of sugar pine were infected, even by the isolate from *P. monticola* (Table 1), a species closely related to sugar pine. Although mortality of some infected ponderosa pines and Douglas-firs occurred 10–14 wk after inoculation, we observed no mortality of hemlock or sugar pine attributable to *V. wageneri*.

Fewer ponderosa pine seedlings were infected in experiment B than in experiment A. However, isolates of the hard pine variant infected significantly more pines than did isolates of the Douglas-fir variant (Table 2). The opposite was true on Douglas-fir seedlings, but the differences were not statistically significant. On both hosts, the pinyon variant was intermediate among the variants in percent infection and disease severity rating (Table 2).

The two-way analysis of variance (18) indicated a significant interaction among morphological variants and host species in experiments A and B (Table 3), ie, the pattern of infection on the hosts differed among the variants. Although there was infection of pine seedlings by Douglas-fir isolates and vice versa, statistically significant host preferences among the morphological variants were evident.

Field inoculations. Stronger host preferences of the Douglas-fir and hard pine variants were found when larger, naturally occurring trees were wound-inoculated than when seedlings were inoculated. However, unexpected infections again occurred, particularly on pines inoculated with the Douglas-fir variant at the Rock Creek site (Table 4). The analysis of variance of percentage infection data showed that host species × morphological variant interaction was the only significant ($P < 0.005$) effect in this experiment.

Because of the substantial tree-to-tree variation in the extent of colonization by the various isolates, comparisons between variants could perhaps best be made by comparing the paired inoculations (with isolates from the two variants) on a tree-by-tree basis. In 25 of the 36 trees inoculated at Blodgett, the compatible isolate (a Douglas-fir isolate on a Douglas-fir or a ponderosa pine isolate on a ponderosa pine) colonized the host more than the putatively incompatible isolate. The opposite occurred on one pine and one Douglas-fir. Nine trees were not infected by either isolate. At Rock Creek, six Douglas-firs were not infected by either isolate. The other 12 Douglas-firs were colonized by the Douglas-fir isolates to a greater extent than by the paired ponderosa pine isolates. Fourteen of the 18 inoculated pines at Rock Creek were infected. The pine isolate colonized the host more than did the Douglas-fir isolate in nine of these cases, but the opposite was true on the other five pines.

Colonization by both morphological variants was generally greater at Rock Creek than at Blodgett (Table 4). At Rock Creek, particularly on ponderosa pine, it appeared that most of the colonization took place during the first 10 mo after inoculation because trees sampled in May 1982 were not colonized more than those sampled the previous November. This may reflect death of hyphae of *V. wageneri* in host tissue prior to the November sampling, possibly during warm summer months.

Isolates of the hard pine variant were reisolated from samples of colonized tissue in lower frequency at Rock Creek than at Blodgett (Table 4). This difference was statistically significant, $P = 0.05$, according to chi-square analysis (18). In contrast, isolates of the Douglas-fir variant were reisolated in roughly the same frequency at the two sites. After reisolation, the morphology of each isolate was identical to that noted before inoculation, regardless of the host from which it was reisolated.

Temperature effects. No seedling maintained at 27 or 32 C became infected. At 16 and 21 C, there were only two statistically significant factors at $P = 0.05$. First, the three variants differed in percent seedlings infected. Secondly, the two hosts differed in susceptibility (percent infection) at the two temperatures, ie, there was a significant host species × temperature interaction. Of the

seedlings that became infected, temperature apparently had little effect on extent of colonization by the variants (Table 5).

Conidia and/or hyphae on inoculum blocks of all nine isolates of *V. wageneri* survived burial for 12 wk in nonsterile soil at 16 C as indicated by subsequent production of conidiophores on inoculum blocks (Table 6). At 21 C, however, isolates of the hard pine variant showed poorer survival than did the isolates of the other variants, and at 27 C, the hard pine variant did not survive. The fungus was not recovered from any block buried at 32 C.

DISCUSSION

The three morphological variants of *V. wageneri* consistently showed host preferences (host species × morphological variant interaction) in our inoculations. Stronger host specificity was expected based upon observations of naturally occurring infection centers and upon examination of 98 isolates of *V. wageneri* obtained from various hosts (6). In no case have we isolated a variant from a naturally infected hard pine, Douglas-fir, or pinyon other than the expected one. Failure to find a similarly strong specificity in our inoculations may be due to a number of factors. These variants may have broader host ranges than our field observations indicate. Infections under field conditions generally would escape detection if the variants are capable of infecting roots of species outside of their respective host ranges without sufficiently colonizing the xylem to induce crown symptoms or death. Feeding activity of insect vectors (3,20) may also play a role in the apparent restriction of the variants to their respective hosts. On the other hand, seedlings may not fully express the capacity for resistance that mature trees have to a variant of *V. wageneri*; the observed host specificity on mature trees may result in mere host preference on seedlings, as has been suggested with other root diseases of forest trees (15,21). Some unexpected infections also occurred when older trees were inoculated but, because inoculated wounds are unnatural, the potential of the morphological variants to infect other hosts under field conditions may have been exaggerated. Regardless, both inoculation studies and empirical evidence show host specialization of the morphological variants.

Because seedlings of pinyon were not available for inoculation and because of concern about introducing the pinyon variant of *V. wageneri* into a new area, we were not able to adequately demonstrate host specialization for this variant. Isolates of the pinyon variant are morphologically distinct, but the evidence for specialization is inconclusive. Interestingly, pinyon isolates tended to infect as many seedlings of Douglas-fir and ponderosa pine as did the isolates originating from those hosts. Smith (16) had similar results with an isolate from singleleaf pinyon.

Under as yet undetermined conditions, hemlocks and white pines can sometimes become infected by *V. wageneri*, especially by the hard pine variant. However, few western hemlock seedlings were infected in our inoculations, even by isolates originating from hemlocks. Sugar pine seedlings were highly resistant to *V. wageneri* in our inoculations, and field observations indicate that sugar pine is only rarely infected. However, the closely related white pines (*P.*

TABLE 4. Percentage infection, extent of colonization, and recovery of *Verticicladiella wageneri* in ponderosa pine and Douglas-fir at two sites in the central Sierra Nevada 10 and 16 mo after inoculation

		Blodgett Forest (1,300 m)				Rock Creek (1,000 m)			
			Extent of colonization (cm)[d]				Extent of colonization (cm)[d]		
Host species[a]	Morphological variant[b]	Percent infection[c]	10 mo	16 mo	Fungus recovery[e]	Percent infection[c]	10 mo	16 mo	Fungus recovery[e]
Pinus ponderosa	Hard pine	77.8	68	119	20/25	55.5	192	170	7/17
	Douglas-fir	16.8	22	125	2/5	44.4	66	54	8/13
Pseudotsuga menziesii	Hard pine	16.7	8	8	2/3	11.1	8	18	1/2
	Douglas-fir	66.7	10	34	11/17	66.7	69	77	12/19

[a] Eighteen trees of each host were wound inoculated at each location. Roots on opposite sides of each tree were inoculated with an isolate from the hard pine variant or an isolate from the Douglas-fir variant.
[b] Each of three isolates from each morphological variant were inoculated into six pines and six Douglas-firs at each location.
[c] Infection was considered successful when at least 5 cm of black stain extended from the inoculation point.
[d] Extent of xylem staining above and below the point of inoculation on infected trees. One-half of the inoculated trees were examined at each sampling time.
[e] Number of samples from which the fungus was successfully isolated divided by the total number of samples tested.

TABLE 5. Influence of temperature on percentage infection and extent of colonization of ponderosa pine and Douglas-fir seedlings inoculated with isolates of *Verticicladiella wageneri* representing three morphological variants

		Temperature[a]				
		16 C		21 C		27 & 32 C[b]
Host species[c]	Morphological variant[d]	Percentage infection	Extent of colonization (cm)[e]	Percentage infection	Extent of colonization (cm)[e]	Percentage infection
Pinus ponderosa						
	Hard pine	33.3	28	16.7	22	0
	Pinyon	46.7	27	13.3	14	0
	Douglas-fir	20.0	12	23.3	11	0
Pseudotsuga menziesii						
	Hard pine	10.0	8	13.3	8	0
	Pinyon	30.0	15	53.3	15	0
	Douglas-fir	36.6	14	43.3	15	0

[a] Soil temperatures ranged from 15–17 C in the 16 C treatment, 18–22 C in the 21 C treatment, 22–26 C in the 27 C treatment, and 25–31 C in the 32 C treatment.
[b] No infection took place in the 27 C or the 32 C treatments.
[c] Ten seedlings of each host were inoculated with each isolate at each temperature.
[d] Three isolates of each morphological variant.
[e] Mean extent of xylem stain above and below the point of inoculation on infected seedlings.

TABLE 6. Influence of temperature on percentage recovery of *Verticicladiella wageneri* from inoculum blocks buried in field soil for 12 wk

Morphological variant[y]	Temperature (C)			
	15–17	18–22	22–26	26–31
Hard pine	100 a[z]	70 a	0 a	0 a
Pinyon	100 a	97 b	45 b	0 a
Douglas-fir	100 a	100 b	27 b	0 a

[y] Three isolates of each variant were tested by burying 20 blocks at each temperature. Successful recovery was determined by conidiophore production on the blocks after 2 wk incubation at 18 C.

[z] Means of percentages within columns followed by the same letter are not statistically different ($P = 0.05$) by the least significant difference test of the arcsine of the square root of the percentages.

strobus and *P. monticola*) can be infected (12,14) and may be more susceptible than sugar pine to *V. wageneri*. The feasibility of using hemlocks (9) and white pines as alternative species in stands affected by black stain warrants further investigation, but at this point they appear to be good choices in most stands.

V. wageneri grows poorly at temperatures above 21 C, and some isolates fail to grow at 25 C (7,11,16). Temperature may play a role in the observed restriction of black stain root disease on hard pines and pinyons to relatively cool, moist sites at high elevations (1,6,13,19). The restriction of the disease on pinyon to the west side of the Rocky Mountains (13) may be due to the generally warmer, drier sites on which pinyon occurs on the east side. During winter months, temperatures of most forest soils should be sufficiently cool for infection and colonization. However, temperatures during summer months may occasionally become unfavorable for survival of hyphae of *V. wageneri*, especially those of the hard pine variant which did not survive as well as the other variants when buried in nonsterile soil maintained at 18–26 C. In field inoculations, isolates of the hard pine variant were reisolated from colonized trees in lower frequency at the 1,000 m elevation (warm) site than at the 1,300 m elevation (cooler) site, but isolates of the Douglas-fir variant were recovered in slightly higher frequency at the warmer site than at the cooler site. Black stain root disease on ponderosa pine is apparently restricted to elevations above 1,300 m in the central Sierra Nevada (1,6), but no such restriction has been seen with the Douglas-fir variant (6) which is less sensitive than the hard pine variant to warm temperatures when grown in axenic culture (6,7) or when buried in soil.

For resource management purposes, the morphological variants should be considered host-specialized. For instance, because the Douglas-fir variant is essentially restricted to Douglas-fir, planting other coniferous species such as pines or hemlocks (9) may be feasible in stands where black stain is a problem on Douglas-fir. Recognition of environmental restrictions on the morphological variants of *V. wageneri* should also be of use in identifying sites where cultural control practices might be appropriate.

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