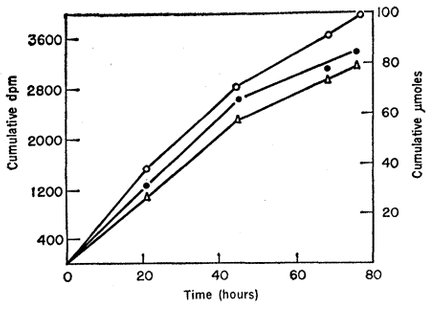

Fig. 1. Evolution of carbon monoxide (μmole, solid circles; dpm, open circles) into reservoir gas and synthesis of phycocyanobilin (μmole, triangles) by *Cyanidium caldarium* cells after administration of [5-C^{14}]-ALA (4.4×10^5 dpm; specific activity, 2.2×10^5 dpm/μmole; total ALA added, 2 μmole) in light. The disintegrations per minute were determined by driving bicarbonate from solution with acid after titration of residual alkali to *p*H 8.5. The liberated $C^{14}O_2$ (derived from $C^{14}O$) was then collected in 5 ml of ethanolamine: methoxyethanol (2 : 1), and counted (*9*) in a liquid scintillation spectrometer. Quench was determined by addition of toluene-C^{14} internal standard.

were derived from a common heme precursor derived from [5-C^{14}]-ALA.

It is known that 8 moles of ALA are necessary for the formation of 1 mole of heme, and that eight labeled carbons from [5-C^{14}])-ALA are incorporated, that is, four in the pyrrole rings and four in the methyne bridge carbons (*1*). If this pattern were followed in the formation of algal heme destined for phycocyanobilin, the specific activity of phycocyanobilin ought to be seven times that of carbon monoxide. In addition, total label incorporated into phycocyanobilin per culture should be seven times that incorporated into carbon monoxide. To explore this relationship further, phycocyanin [$E_{1cm}^{0.1\%} = 7.74$ at 618 nm in 0.1*M* phosphate buffer, *p*H 6.5 (*8*)] was isolated from cells disrupted by high-frequency sound and purified by fractionation with ammonium sulfate and by chromatography on a brushite column (*3*). The specific activity (dpm/μmole) of phycocyanobilin was determined (*8*) (Table 1).

The stoichiometry of carbon monoxide and phycocyanobilin produced per culture were in good agreement. The specific activity (dpm/μmole) of phycocyanobilin was seven times that of carbon monoxide, as was total incorporation of [5-C^{14}]-ALA per culture. In three additional experiments, in which labeled ALA was omitted, *C. caldarium* cells produced 35, 73, and 54 μmole of carbon monoxide, respectively, which corresponds to synthesis of 34, 70, and 53 μmole of phycocyanobilin, respectively.

Thus, carbon monoxide is a normal byproduct of phycocyanobilin synthesis. Evolution of carbon monoxide and synthesis of phycocyanobilin proceed at equimolar rates in *C. caldarium* cells exposed to light. The results, therefore, support the concept that phycocyanobilin arises from precursor heme, and suggest that the mechanism of algal heme catabolism is similar to that described in mammalian systems.

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White Pine Blister Rust: Simply Inherited Resistance in Sugar Pine

Abstract. *Segregation ratios of offspring from disease-free sugar pines suggest that resistance to the white pine blister rust fungus is under major gene control and simply inherited.*

Resistance to disease in cultivated plants is often conditioned by single genes with major effects. Heritable variation in resistance to diseases in forest trees also has been demonstrated, but modes of inheritance in most cases are poorly understood and probably more complex. We report one, if not the first, instance of an effective and simply inherited factor for resistance to a major forest disease in a commercially important species.

Table 1. Blister rust infection on sugar pine progenies from rust-free parent trees.

Parents		No. of seedlings		Healthy (%)
Seed	Pollen	Infected	Healthy	
K36	K17	18	21	54
K16	K17	23	16	41
K60	K17	26	21	45
K60	K10	23	19	45
K36	K73	7	12	63
K70	Wind	2	36	95
Other progenies*		1359	28	2
Controls*		402	0	0

* Combined data from 1962–1964 outplantings, involving 29 hand-pollinated crosses (with K60, K36, and K16 as seed parents in most crosses), 6 wind-pollinated progenies, and controls.

White pine blister rust, caused by *Cronartium ribicola* Fischer, has become chronically epidemic on white pines in north temperate forests the world over, with devastating effects. Ever since this disease was introduced to North America on infected planting stock around 1900, its continued spread and intensification on indigenous white pines has seriously jeopardized the future management and, in some areas, even existence of these economically and ecologically important trees. Attempts to control the spread of the rust by eradication of its alternate hosts (*Ribes* spp.) have been costly and relatively ineffective in areas of high disease hazard. Use of antibiotics to effect therapy of diseased stands has not justified the early enthusiasm it inspired. Presently, production of trees with genetic resistance offers the most feasible and promising approach to curtailing wholesale loss of regeneration over vast areas where climatic conditions favor disease development. In spite of the high susceptibility of all North American species, occasional rust-free individuals do exist in severely infected stands, prompting the hope that some of their apparent resistance might be genetically transmissible. These rust-free phenotypes have provided the initial germ plasm for several breeding programs in eastern (*Pinus strobus* L.) and western (*P. monticola* Dougl.) white pines in the United States and Canada over the past three decades (*1*). Progress in some of these has been encouraging, if slow and difficult. Progeny tests have shown generally low but usable levels of resistance from a relatively small proportion of the parent selections (*1, 2*). Apparently several

different mechanisms of resistance exist, with complex modes of inheritance (*1*).

One of the most valuable native white pine species, sugar pine (*P. lambertiana* Dougl.) is also one of the most susceptible to blister rust. In 1957, the U.S. Forest Service's California Region started a systematic search for disease-free parent trees as potential sources of resistant germ plasm. Trees selected for trial had to be of flowering age and rust free (though occasionally a tree with a few cankers was chosen if its immediate neighbors were heavily infected) in a stand where the disease had been severe for 10 to 15 years and where *Ribes* plants were still present. Since candidates were bred in the wild, the number of different crosses was limited by practical and biological considerations. Pollen from most candidates was tested on the relatively few selections that consistently produced flowers and set seed in adequate amounts. To date, 175 candidates have been selected and propagated in clonal seed orchards, but only 26 of the earlier selections have been adequately evaluated in progeny tests.

After growing 1 year in a nursery, 34 hand-pollinated and 7 wind-pollinated progenies were outplanted from 1962–1964 in a disease garden on a site considered to be hazardous for blister rust infection, in the Klamath National Forest, California. The experimental design was a randomized complete block with five replications of ten seedlings per plot. Bushes of the alternate host (*Ribes sanguineum* Parsh., *R. lobbii* Gray, and *R. roezlii* Regel) were interplanted between every twelfth row of test progeny to increase the inoculum potential. A plot of control seedlings from susceptible, wind-pollinated parent trees was included between every row of *Ribes*. Seedlings were examined for stem and branch cankers each spring. Since the amount of infection observed was not uniform from year to year, evaluation of progeny was not considered final until nearly 100 percent of the control seedlings were cankered.

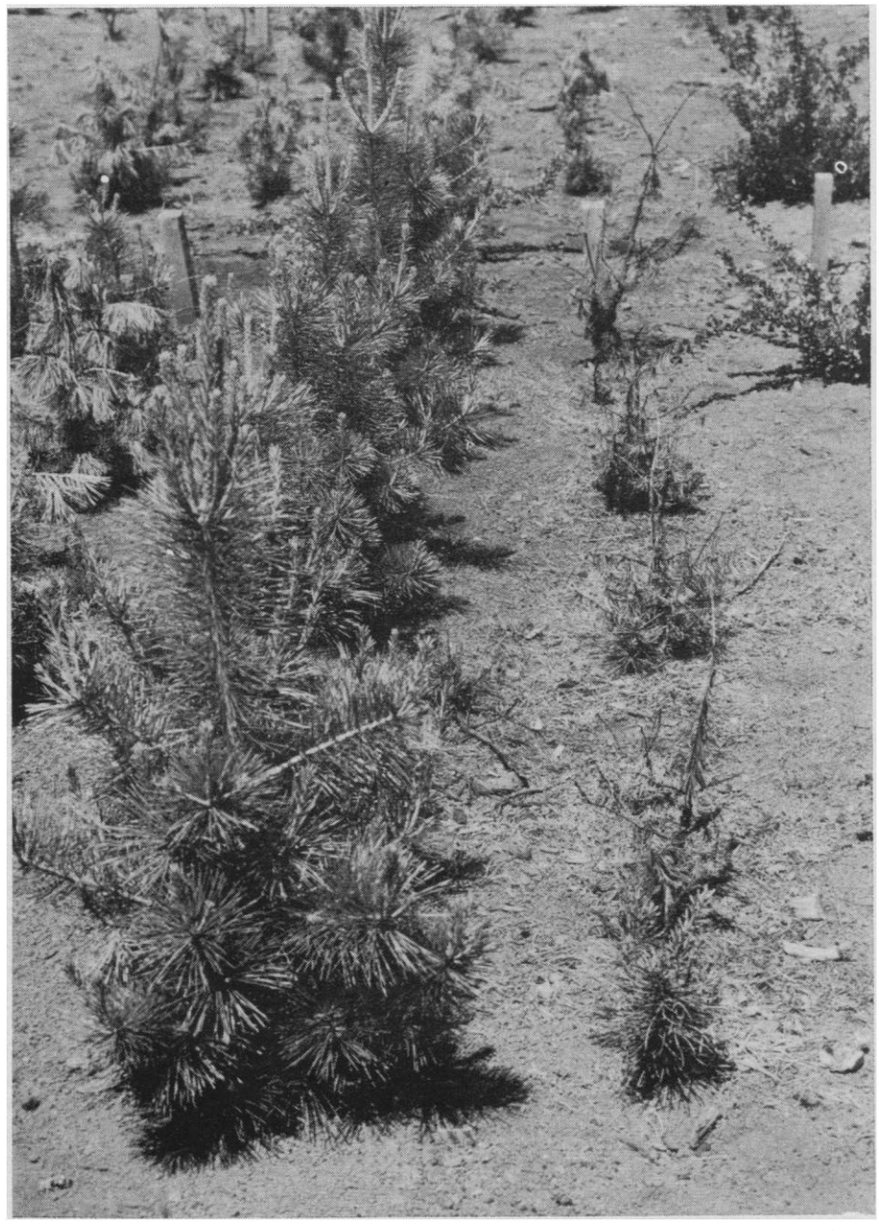

Fig. 1. Progenies of sugar pine completely susceptible (right) and segregating (left) for resistance to white pine blister rust. Dead and dying seedlings of the resistant line have been removed or are concealed. *Ribes* bushes are in background.

Although most of the 26 candidate trees tested were no better than controls, four were outstanding, with 41 to 95 percent of their offspring free from cankers (Table 1). The distinction between resistance and susceptibility was nearly absolute—most susceptible trees were dead and the rest dying from lethal, often multiple, girdling cankers, whereas those with resistance appeared immune, at least to canker development (Fig. 1).

The qualitative nature of resistance and the pattern of infection exhibited by offspring from these four candidates suggest that resistance to blister rust in sugar pine is under strong genetic control and is probably simply inherited. In three different crosses, progeny from one parent (K17) segregated into an approximately 1:1 ratio of resistant to susceptible individuals; those from two others (K10 and K73) showed similar ratios in one cross of each (Table 1). Wind-pollinated progeny from K70 were almost entirely resistant. In a subsequent planting, 4 progenies with K70 as the pollen parent were 95 to 100 percent canker-free, compared to 13 percent in controls. From these data, inheritance of resistance could be explained on the basis of a single dominant gene which is heterozygous in parents K10, K17, and K73, and homozygous in K70; observed ratios were in good fit with those expected, and probabilities for chi-square values were in no case below 25 percent.

Because we tested a relatively small number of crosses and seedlings, our hypothesis is tentative. Since only bark infection was scored, it was not possible

to determine whether the site of resistance is in the needles, which are the primary infection courts, or in the bark. Both tissues are potential sites of resistance in other white pines, and the type of resistance they confer is apparently inherited independently (*1*, *3*). Thus, either one or both mechanisms, if controlled by single genes in sugar pine, could have been responsible for resistance in these progenies and could have produced the ratios observed. In any case, a simple mode of inheritance of resistance seems indicated. This finding has important implications not only for improved rust resistance in sugar pine, but, through hybridization, for other commercial white pine species as well.

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Peroxidase-Mediated Virucidal Systems

Abstract. *Peroxidase (myeloperoxidase or lactoperoxidase), hydrogen peroxide, and a halide such as iodide, bromide, or chloride form a potent virucidal system that is effective against polio and vaccinia virus, particularly at a low pH. The peroxidase–halide–hydrogen peroxide system may contribute to the host defense against certain viral infections.*

The inactivation of viruses by the host organism is achieved through complex mechanisms which are poorly understood (*1*). Interest has centered on the role of antibody and interferon. A peroxidase–halide–hydrogen peroxide system, which has bactericidal and fungicidal activity (*2–4*), is shown here to have potent virucidal properties and thus may contribute to the host defense against certain viral infections.

Myeloperoxidase (MPO) was purified from canine leukocytes (*5*), and lactoperoxidase (LPO) was purified from bovine milk (*6*). Peroxidase activity was determined by the *o*-dianisidine method (*7*). Type I poliovirus (LSc-2ab strain) and vaccinia virus (Wyeth calf lymph strain), grown in tube monolayer cultures of rhesus monkey kidney cells (Flow Laboratories) and stored at −20°C, were diluted in water to an infectivity titer of 10^6 $TCID_{50}$ per milliliter (*8*), dialyzed against water for 1 hour at 4°C, and filtered through a Millipore filter (0.22 μm pore size) just prior to use. A portion of the virus suspension (0.1 ml) was incubated with the components of the reaction mixture (Table 1, Fig. 1) in a water bath and shaken for 1 hour at 37°C in an atmosphere of air. The reaction was stopped by the addition of 0.1 ml of 0.1*M* sodium thiosulfate, tenfold serial dilutions of the reaction mixture were made in phosphate-buffered saline (*p*H 7.0) containing penicillin (1000 unit/ml), streptomycin (1000 μg/ml), and amphotericin B (5 μg/ml), and 0.1 ml portions were added to duplicate monolayer cultures for determination of infectivity.

Infectivity of poliovirus and vaccinia virus was determined by cytopathic effect in tube monolayer cultures of rhesus monkey kidney cells grown in minimum essential medium (MEM) with Hanks salts (Flow Laboratories) supplemented with penicillin, streptomycin (Grand Island), and 10 percent fetal calf serum (Hyland). The cells were maintained in minimum essential medium with Earle salts supplemented with penicillin, streptomycin, and 2 percent calf serum. The monolayers were incubated at 37°C in a roller drum (New Brunswick) until no further cytopathic changes were observed (usually 5 days). The $TCID_{50}$ was calculated by the method of Spearman-Karber (*9*). Infectivity of poliovirus also was determined by plaque assay on monolayers of HeLa cells. Following adsorption for 30 minutes at room temperature, the monolayers were overlaid with MEM, supplemented with 2 percent fetal calf serum, penicillin, streptomycin, and 0.3 percent Agarose (Bausch and Lomb). After 2.5 days the monolayers were fixed with 5 percent formalin, stained with 1 percent gentian violet in 20 percent alcohol, and the plaque-forming units were counted.

Incubation of poliovirus with the complete myeloperoxidase–iodide–hydrogen peroxide system produced at least a reduction of 10,000-fold in infectivity titer as measured either by cytopathic effect or plaque assay (Table 1). Vaccinia virus also was inactivated by this system (Table 1). Comparable results were obtained if acetate was substituted for lactate buffer at *p*H 4.5. Bromide or chloride could be substituted for iodide although a higher concentration of chloride was required. The complete system was virucidal when human MPO was substituted for the canine enzyme (*10*). Lactoperoxidase could substitute for MPO when iodide or bromide was employed as the halide. However, a virucidal effect was not observed when chloride was used in the lactoperoxidase–halide–hydrogen peroxide system (Table 1). The lactoperoxidase–chloride–hydrogen peroxide system was reported to have low bactericidal activity (*3*). Deletion of the peroxidase or H_2O_2 from the reaction mixture or heat treatment of the enzyme (100°C for 30 minutes) completely prevented the antiviral effect when either iodide, bromide, or chloride was the halide employed. A significant, but reduced, antiviral effect was noted in experiments with vaccinia virus (but not with poliovirus) when the halide was absent. The optimum *p*H was distinctly acid (Fig. 1). Although the virucidal activity decreased sharply as the *p*H approached neutrality, a virucidal effect could be demonstrated at *p*H 7.0 with both the myeloperoxidase and lactoperoxidase systems.

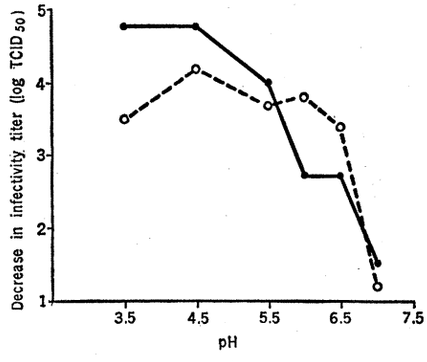

Fig. 1. Effect of *p*H on virucidal activity of peroxidase system. Poliovirus was assayed by cytopathic effect after incubation with iodide, hydrogen peroxide, and myeloperoxidase or lactoperoxidase as described in Table 1. The *p*H was varied as indicated with lactate (*p*H 3.5 to 5.5) or phosphate (*p*H 6.0 to 7.0) buffers.

Iodination of bacteria by the peroxidase–iodide–hydrogen peroxide system