HILGARDIA

*A Journal of Agricultural Science Published by
the California Agricultural Experiment Station*

VOLUME 10

MAY, 1937

NUMBER 14

CONTENTS

THE CONNECTION BETWEEN DEMATOPHORA NECATRIX AND ROSELLINIA NECATRIX

H. N. HANSEN, HAROLD E. THOMAS,
AND H. EARL THOMAS

A BACTERIAL GALL DISEASE OF DOUGLAS FIR, PSEUDOTSUGA TAXIFOLIA

H. N. HANSEN AND RALPH E. SMITH

APPLE MOSAIC

H. EARL THOMAS

UNIVERSITY OF CALIFORNIA • BERKELEY, CALIFORNIA

THE CONNECTION BETWEEN DEMATOPHORA
NECATRIX AND ROSELLINIA NECATRIX

H. N. HANSEN, HAROLD E. THOMAS, AND H. EARL THOMAS

A BACTERIAL GALL DISEASE OF DOUGLAS FIR,
PSEUDOTSUGA TAXIFOLIA

H. N. HANSEN AND RALPH E. SMITH

APPLE MOSAIC

H. EARL THOMAS

A BACTERIAL GALL DISEASE OF DOUGLAS FIR,
PSEUDOTSUGA TAXIFOLIA

H. N. HANSEN AND RALPH E. SMITH

A BACTERIAL GALL DISEASE OF DOUGLAS FIR, *PSEUDOTSUGA TAXIFOLIA*¹

H. N. HANSEN² AND RALPH E. SMITH³

BACTERIAL DISEASES of conifers are exceptionally rare ; as far as we know only two have been reported in the literature to occur naturally on twigs, branches, and upper stems of members of the family *Pinaceae*, and both of these are probably produced by the same organism.

In 1888 Vuillemin,^(a) isolated an organism from galls occurring on twigs of *Pinus halepensis* Mill. and named it *Bacterium pini* Vuill. In 1911 Von Tubeuf^(a) isolated what he considered to be the same organism from galls on twigs and branches of *Pinus cembra* L. Several attempts were made by Vuillemin and by later investigators to produce the disease by inoculating with pure cultures of *B. pini* and by transfer of gall material from diseased to healthy plants, but in no case were positive results obtained.

In 1933 Hansen and Smith^(a) published a brief note recording the finding of bacterial galls on Douglas fir (*Pseudotsuga taxifolia* Britt.) in California. The present paper reports additional studies of this disease, its transmission, and the pathogene involved.

ECONOMIC IMPORTANCE

The disease has been observed commonly in parts of Napa, Lake, Santa Cruz, Amador, and Siskiyou counties in California, in marginal localities for the growth of Douglas fir, which here occurs in mixed stands composed of several species of conifers and three or four species of broad-leaved trees. These marginal localities, though practically worthless for timber production, are much used for recreational areas, sanitariums, summer resorts, and private summer homes. As far as the value of such places may be materially lowered by the presence of dead, dying, and deformed trees, the disease can be considered to be of economic importance. In its present known range, the Douglas-fir gall disease is otherwise of no economic importance, and of only potential interest to the lumbering industries. Should it invade areas where Douglas fir is now the predominating species, it might very readily become an important

¹ Received for publication December 29, 1936.

² Assistant Professor of Plant Pathology and Assistant Plant Pathologist in the Experiment Station.

³ Professor of Plant Pathology and Plant Pathologist in the Experiment Station.

⁴ Superscript numbers in parentheses refer to "Literature Cited" at the end of the paper.

factor in determining the future composition of the forest. If the reproduction of a single member of the stand is attacked, associated species would gain a natural advantage, and they should increase numerically in direct proportion to the havoc wrought by the disease.

DESCRIPTION OF THE DISEASE

The disease is characterized by the occurrence of galls on twigs, branches, and upper stems of the host. New galls are formed only on the younger

Fig. 1.—Douglas-fir trees girdled and partly girdled by bacterial galls. ($\times \frac{1}{10}$.)

trees up to about fifteen years of age, and most frequently on very young trees growing in crowded stands in rather damp situations near streams, ponds, or swamps. The galls most often occur on twigs or small branches but not uncommonly also on the main stems, where they occasionally completely girdle a tree, and thus give rise to an unsightly dead top commonly referred to as “spike top” or, if a secondary leader is formed, it is called a “stag head” (fig. 1). Most of the trees handicapped by having one or more galls on the main stem usually die within a few

years because they are unable to compete successfully with healthy trees. Occasionally an isolated tree with stem galls will live for several years and may even reach small timber size; but since the galls also continue to grow, the trunk is usually so badly deformed that it is useless for anything but firewood (fig. 2). The health of the host does not appear to be

Fig. 2.—Part of Douglas-fir stem, showing old galls. ($\times \frac{1}{10}$.)

seriously threatened by infected twigs since these are gradually shaded out and dropped as the tree grows older.

The galls vary in size from that of a pinhead to several inches in diameter. They are globular in shape, with a rough, spongy, fissured surface which breaks out in a typical more or less cross-shaped pattern (fig. 3, A to D). During the first year, they are much lighter in color than the bark of the host and therefore stand out rather prominently. In older galls, the shape is materially altered and the typical surface markings gradually disappear. These changes in appearance can probably be attributed to insects and saprophytic fungi, which invade practically all galls that are more than one year old.

Fig. 3.—*A*, Natural galls showing typical cross-shaped markings ($\times 1\frac{1}{2}$); *B*, *C*, and *D*, galls produced by experimental inoculation ($\times 1$); *E*, section of natural gall, with arrow indicating point of origin ($\times 1$); *F*, twig of Douglas fir from which bark has been stripped to show wounds caused by *Chermes cooleyi* ($\times 1$).

The gall is composed of hypertrophied tissues, involving both stele and cortex, and is very similar in structure to that of the olive-tree galls produced by the bacterial pathogene *Bacterium savastanoi* E.F.S. There is this important difference, however, that the olive-tree gall can be produced in cortical tissues without involving any of the xylem elements,

Fig. 4.—*A*, Section of a gall showing groups of rapidly dividing cells and also the presence of woody elements among these groups; *B* and *C*, photomicrograph of *Bacterium pseudotsugae* n. sp. in intercellular spaces.

whereas in all the Douglas-fir galls examined, both those occurring naturally and those produced experimentally, the inner core was found to be always woody in nature, and furthermore the point of origin of the galls was always found to be located within the stele (fig. 3, *E*). The internal structure of the galls is characterized by the presence of many more or less spherical groups of rapidly dividing cells, near or in the

centers of which occur comparatively large intercellular spaces which contain the pathogene (fig. 4, *B* and *C*).

PATHOGENICITY OF THE CAUSAL ORGANISM

Douglas firs were inoculated for the first time on May 15, 1932. Bacteria from pure 48-hour-old cultures were smeared on freshly made leaf scars on previous season's growth and inoculated into the cortex in shallow needle stabs and into the wood in deep needle stabs. Observations were made at various intervals during summer and fall, but all the stab wounds, inoculated and controls, appeared to have healed over rapidly and all looked alike. Additional inoculations by the same methods were made on the fifteenth of January, February, March, and April of 1933.

When the January inoculations were examined on April 15, small galls varying from 1 to nearly 5 mm in diameter were found on the inoculated deep-stab wounds. None of the leaf-scar or shallow-cortex wounds showed any signs of gall formation. Upon examination of the trees inoculated in May of the previous year, it was found that all of the deep-stab inoculations had developed small galls and that inoculated leaf scars and shallow stabs had not.

During the summer of 1933, galls developed on all deep-stab inoculations made in January, February, and March but on only one of those made in April. The remainder of these, however, began to show small galls early in April of 1934. This clearly shows that the pathogene can be present in the host for nearly a year before definite symptoms (galls) begin to appear. It is also fairly evident that gall formation and gall growth is limited to the active growth period of the host, which is roughly from late March to July in Berkeley, California, where the experimental work was done.

The causal organism was reisolated from experimentally produced galls and used for inoculations made in August, 1933, and in January, 1934. Of the 10 wounds inoculated in August, only 3 developed galls. This indicates that the organism finds it relatively difficult to establish itself during late summer, perhaps because of the greatly reduced growth rate of the host at that time of the year. The 8 wounds inoculated with the reisolate in January produced galls during the spring of 1934.

TRANSMISSION

Actual contact of the pathogene with xylem elements of the host tissues appears to be essential to gall formation, on evidence of the facts that the point of origin of all galls examined was found to be located in the

stele and that no galls were produced in inoculated leaf scars or in shallow-cortex wounds. These facts would seem to eliminate water, the principal agent of dispersal of the organism causing olive knot, as a carrier, and indicate that insects capable of producing rather deep wounds were responsible for transmission.

Wherever we have found the Douglas-fir gall disease, two insects—the orchard cicada, *Platypedia areolata* Uhler.; and Cooley's chermes, *Chermes cooleyi* Gill.—have been present in spring and early summer. The cicada oviposits in twigs of the Douglas fir and in doing so causes deep wounds through which splinters of wood protrude. The egg cases remain within the wood and can be found there several years after the wound has healed. More than 200 cicada wounds from one to four years old have been examined, however, and in no case was there any evidence of gall formation.

The other possible carrier, *Chermes cooleyi*, is a sucking insect that feeds on the juices of young Douglas-fir twigs. In these it produces deep feeding punctures that penetrate through the bark and cortex into the wood. Figure 3, *F*, shows a twig from which the bark has been stripped to show the typical wounds produced by the insect. In the middle of each of the transverse lines is a small hole penetrating into the wood to a depth of about a millimeter. In the early spring of 1934, all our experimental trees were heavily infested with these insects, and they were observed to feed on some of the experimentally produced galls. In August of the same year, we found three galls which were definitely traced to wounds produced by *Chermes cooleyi*. Von Tubeuf⁽²⁾ suggests that a species of *Chermes* is probably responsible for the spread of *Bacterium pini*.

In the localities where the Douglas-fir gall disease occurs, we find the host associated with the following conifers: *Pinus lambertiana* Dougl. (sugar pine), *P. monticola* Don. (silver pine), *P. ponderosa* Dougl. (ponderosa pine), *Libocedrus decurrens* Torr. (incense cedar), and *Abies concolor* Lindl. and Gord. (white fir). We have never found the disease on any of the above species though branches of some of them were occasionally found intermingled with those of infected Douglas fir. As further evidence that the pathogene is highly specific, it was inoculated into the following plants with negative results: *Pinus halepensis* Mill., *P. lambertiana* Dougl., *P. radiata* Don. (Monterey pine), *Tsuga heterophylla* (Raf.) Sarg. (coast hemlock); and into the following herbaceous plants frequently used to test the pathogenicity of the crown-gall organism, *Pseudomonas tumefaciens* Town.: tomato, begonia, beans, and bryophyllum.

TAXONOMY OF THE CAUSAL ORGANISM

On cultural, morphological, and physiological bases, the causal organism appears to be distinct from previously described plant pathogenes and should therefore be considered a new species. Hence, we suggest the name *Bacterium pseudotsugae*.

TECHNICAL DESCRIPTION OF BACTERIUM
PSEUDOTSUGAE N. SP.⁵

A nonmotile rod with rounded ends, averaging in size $1.9-3.9 \times 0.5-1.5\mu$; frequently occurring in pairs; non-spore-forming; Gram-negative, non-acid-fast; stains readily with analine dyes; facultative aerobe; liquefies gelatin; slight H_2S produced; nitrates reduced; no acid in milk; no ammonia produced; starch not hydrolyzed; no acid and no gas produced in lactose, sucrose, or glycerine; acid but no gas produced in glucose, levulose, galactose, and maltose. *On nutrient agar slant*, growth scanty, flat, glistening, smooth-surfaced; translucent whitish; medium unchanged. *On potato dextrose agar slant*, growth moderate, slightly spreading, with wavy margin, slightly raised, glistening; surface somewhat contoured; whitish, translucent, becoming brownish with age; medium unchanged. *On potato dextrose pepton agar slant*, growth abundant, spreading, with irregular margin, flat, glistening, becoming dull with age; surface contoured; grayish white; medium unchanged. *On potato cylinder*, growth moderate, spreading, viscid, white becoming brown with age; medium turns brown. *In nutrient broth*, growth slight, no surface growth, clouding slight, no sediment. *In potato dextrose peptone broth*, growth abundant; partial ring formed; clouding strong; sediment fairly abundant; flocculent. *In S. A. B. broth*, growth moderate, no surface growth; clouding slight; sediment scanty-viscid. *In Fermi's solution*, growth moderate, no surface growth; clouding slight to moderate; no sediment. *In Conn's solution*, no growth. *In Uchinsky's solution*, no growth.

⁵ We are indebted to Mr. George Zentmyer for testing the physiological reactions of the pathogene.

SUMMARY

A gall disease of the twigs and stems of Douglas fir (*Pseudotsuga taxifolia*), is described and shown to be of bacterial origin. It is suggested that the causal organism is insect-transmitted, the carrier being probably *Chermes cooleyi*. Three species of pine, one of hemlock, and several herbaceous plants were inoculated with negative results. A technical description is given of the causal organism, which is named *Bacterium pseudotsugae* n. sp.

LITERATURE CITED

- ¹ HANSEN, H. N., and RALPH E. SMITH.
1933. A bacterial gall disease of the Douglas fir. *Science* 77:628.
- ² VON TUBEUF, C.
1911. Knospenhexenbesen und Zweig Tuberkulose der Zirbelkiefer. *Naturw. Ztschr. Forst u. Landw.* 9:25-44.
- ³ VUILLEMIN, PAUL.
1888. Sur une bacterioecceidie ou tumeur bacillaire du pin d'Alp. *Compt. Rend. Acad. Sci. [Paris]* 107:874-76, 1184-86.