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AXILLARY GALL OF QUEENSLAND MAPLE.

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SUMMARY.

The occurrence, morphology and previous observations concerning axillary gall of Flindersia brayleyana are discussed, and investigations described which showed that the disease is caused by a bacterium closely allied to Xanthomonas tumefaciens.

INTRODUCTION.

Queensland maple (*Flindersia brayleyana* F.v.M.) is a characteristic tree of the northern Queensland rain-forests and is of considerable importance as a producer of cabinet and general-purpose timbers. Plantations of this species have been established on cutover areas of the Atherton Tableland by the Queensland Forest Service and for many years large numbers of plants were normally raised each year in the northern Queensland forest nurseries for planting-out purposes.

In 1927, two seedling plants in the Gadgarra nursery on the Atherton Tableland were noted with gall-like growths in the axils of the leaves. This condition subsequently developed in all buds and the plants became stunted and eventually died. In 1928, the damage amounted to 3 per cent. of all seedlings; in 1930, 25 per cent. of the 55,000 plants raised were attacked and actual losses due to axillary gall amounted to 13 per cent. In the next year losses amounted to almost 50 per cent. of the stock produced, and high percentages of affected plants have appeared in subsequent seasons.

Some investigations were carried out at this time, when it was thought from the high population of jassids and thrips present that the trouble was

probably due to insect damage. However, no conclusive results were obtained. Isolations of organisms from gall tissue were made and a number of non-specific fungi and bacteria were obtained.

Work carried out concurrently in the area by J. Harold Smith (Entomologist) demonstrated that seed sown in insect-proof enclosures produced seedlings with just as high a percentage of affected plants as plants in unenclosed

Plate 1.
Plantation of *Flindersia brayleyana* affected by axillary gall.

seed-beds and thus showed that the effect was not due to the jassids and thrips which were suspected of causing the trouble. Insecticidal dusts and sprays were also used about this time but without effect on gall incidence.

In 1937 the author found gall damage in plantation trees and in naturally occurring trees of all ages in the virgin rain-forest. The damage caused to the plantation trees resulted in death of leading shoots and consequent development of numerous secondary leaders, causing a bunched and stag-headed appearance and reduced growth (Plate 1). The new buds also showed evidence

of stimulation and subsequent gall development. No galled plants had been put out in the plantations from the nursery. Seedling plants exhibiting gall symptoms were obtained for further investigation in Brisbane.

APPEARANCE OF DISEASED PLANTS.

The development of the condition has been traced. In a Queensland maple seedling there are two elongate-cordate cotyledons oppositely placed; the true leaves are alternate, with the laminae 2–3 inches long and a petiole

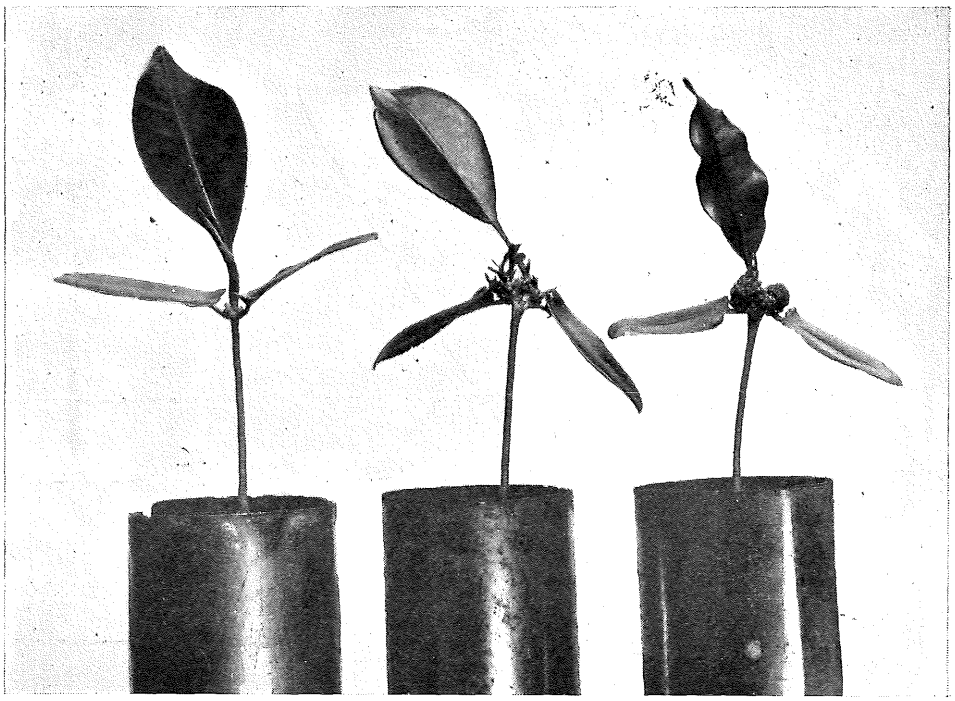

Plate 2.

Tubed seedlings of *Flindersia brayleyana* affected with varying stages of axillary gall.

½-inch long; the nodes are ¼–½-inch apart. In a normal healthy seedling the growth is confined to the terminal shoot. The first symptom of the trouble is the appearance of a small bud in the axil of a cotyledon.

In the case of an affected plant the axillary buds swell and the axil of the leaf is filled with swollen nodular tissue. Later a number of abortive shoots appear on the surface of the tumour; one or two of these may be almost normal in appearance so that instead of one growing point there may be several in each leaf axil. The growth of the terminal growing point is retarded and the plant becomes dwarfed and crooked. In severe cases of gall the stem

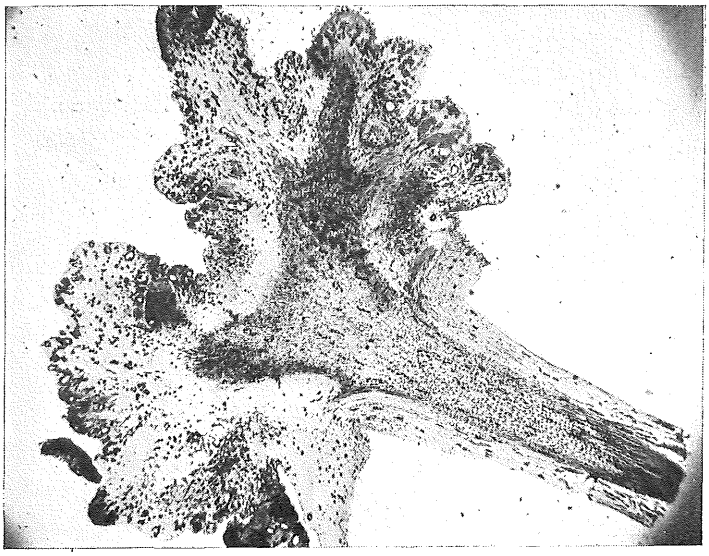

Plate 3.

T.S. through two oppositely placed axillary galls of *Flindersia brayleyana.* × 10.

Plate 4.

Aborted growing point of *Flindersia brayleyana* seedling affected by axillary gall. × 10.

is devoid of leaves and carries a gall up to 1 inch in diameter. After this stage is reached the plant soon dies, and in many cases death occurs before such severe malformation takes place.

It is common for galls to appear in more than one leaf axil; their usual size is $\frac{1}{4}$–$\frac{1}{2}$-inch in diameter. Typical naturally-affected maple seedlings exhibiting gall development are shown in Plate 2.

MORPHOLOGY OF THE GALLS.

The gall consists of an irregular proliferation of the axillary bud. In section it is seen to consist largely of parenchymatous tissue intersected by a number of vascular bundles each of which contains a few lightly lignified elements. A few layers of cork cells line the periphery. The vascular bundles terminate at growing points which are situated at the base of deep hairy depressions in the gall surface. Lateral division of the tumour cells apparently exceeds longitudinal division. A transverse section through two oppositely placed axillary galls is shown in Plate 3. Detailed structure of an affected growing point is shown in Plate 4.

Preliminary microscopic examination failed to reveal any pathogen. Necrotic areas were visible in some cases around what were presumably punctures caused by insects.

ISOLATIONS FROM GALL TISSUE.

Attempts to obtain cultures of organisms from the tissues resulted in the production of numerous colonies of fungi and bacteria. All organisms occurring with any degree of consistency were inoculated into healthy plants, but with negative results. The wide variety of organisms obtained in cultures was presumed to be connected with the difficulty in sterilizing the surfaces of the gall due to its irregular shape and deep depressions and the presence of insect punctures.

FURTHER INVESTIGATIONS.

Because of the negative results obtained from isolations and the similarity of the growth to the soft type of crown gall caused by *Xanthomonas tumefaciens*, more searching microscopic examination of the tumours was made.

Galls were impregnated with silver (Bielschowski's silver nitrate method) and sectioned. The sections were counterstained with eosin. Microscopic examination revealed rods, Y forms and branched forms (Smith, 1912). The bodies found usually occurred inside the tumour cells at the juncture of normal and tumour tissue and appeared to be distinct from the mitochondria. The cells containing the bodies occurred in small groups. Microscopically the appearance suggested a similar new growth to that evidenced by crown gall.

Further isolations were then made. In these cases, however, the galls were first sterilized externally with 1:1,000 mercuric chloride solution, washed in sterile water, and cut with a sterile scalpel. Small pieces of tissue were removed from near the proximal areas of the abnormal growth, macerated in sterile water, and dextrose agar plates poured with the maceration.

In a number of cases whitish bacterial colonies were obtained as well as various other bacteria and fungi. The white colonies grew vigorously on potato dextrose agar plates and slopes.

Microscopically the organisms forming the white colonies were shown to be Gram-negative bacteria, motile, with one polar flagellum, and measuring 0·6 to 0·85 $\times$ 2·5 to 3μ.

At the same time and in the same way isolations were made from crown gall of the peach. The organisms obtained were similar to those isolated from maple gall.

Cultures of both organisms were used as inoculum for nasturtium (*Tropaeolum*), Bryophyllum and laboratory-grown maple seedlings. All seedlings were grown from sterilized seed under glasshouse conditions.

The inoculations were carried out by smearing buds with a pure culture of the organism being used and pricking the plants through the smear. In other cases the organisms were introduced as suspensions by means of a hypodermic syringe.

Five days after inoculation with the *Xanthomonas tumefaciens* the nasturtium plants showed signs of tumour growth, by developing swelling at the points of inoculation.

Twelve days after inoculation gall formation was evident on two of the maple plants inoculated with the crown-gall organism.

On the same day one nasturtium plant inoculated with the maple gall organism showed signs of gall formation and three days later nine of the 25 seedlings inoculated with this organism exhibited the initial symptoms of maple gall at the site of inoculation.

Check inoculations using only sterile water caused no gall appearance except in one or two cases where a swelling due to wound callus appeared but did not develop any further.

The artificially induced galls on all the maple seedlings, whether of crown gall or maple origin, developed as typical maple galls and sectioning of the tissues showed a similar picture to that appearing in normally occurring maple gall.

In the case of both organisms the galls on nasturtium grew into similar soft gall structures.

Isolations from the artificially induced galls produced by inoculating maple seedlings and nasturtium seedlings with the maple gall bacterium were successful in demonstrating the presence of the organism used in the inoculum.

The maple gall organism was also used to inoculate axillary buds of aseptically grown mandarin seedlings in the glasshouse, and axillary galls were produced on two of the 12 seedlings used (Plate 5).

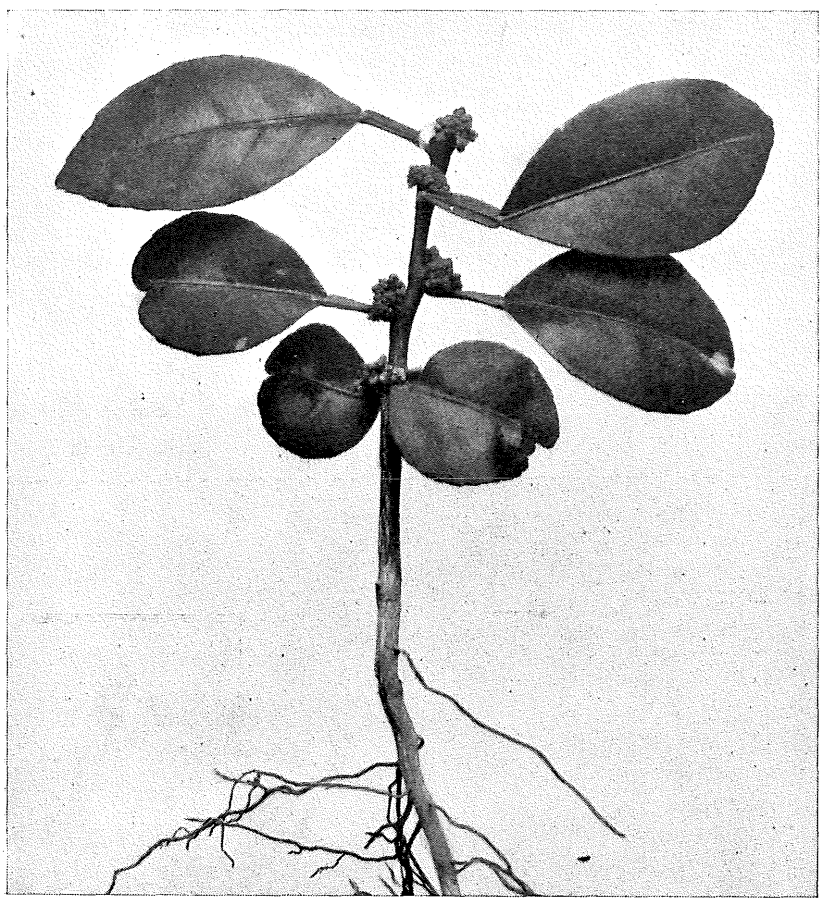

Plate 5.
Mandarin seedling artificially infected with axillary gall organism.

METHOD OF INFECTION.

No definite information concerning the method of natural infection which occurs has been obtained but there are indications that the trouble is soil-borne. As mentioned above, insect-proofing of seed-beds has not prevented the occurrence of the disease and its close affinity with the genuine crown-gall organism points to the soil as its source.

When the trouble was prevalent at Gadgarra, no occurrences were observed at the Wongabel nursery, a few miles distant. Gadgarra is a natural maple locality; the Wongabel area is not. It was observed on another occasion that where soil imported from a maple-bearing area was used as a seed cover a very high incidence of gall formation occurred in the treated beds as compared with other beds.

Experiments involving soil sterilization were laid down at the Gadgarra nursery in two separate seasons, but in each case the trials were vitiated by the occurrence of flooding due to the advent of the rainy season which occurs about seed germination time. No further observations have been carried out pending the resumption of production of maple plantation stock.

CONCLUSION.

It is considered that the investigations here described have successfully demonstrated that axillary gall of Queensland maple is due to infection by a species of *Xanthomonas* closely allied to *X. tumefaciens*.

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