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A NEW SPECIES OF BACTERIA AND THE GALL OF AN APHID

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One of the most mysterious phenomena in biology is the formation of insect-galls on (or by) plants. A common sequence of events is: a "gall-making" insect lays an egg or eggs in some definite part of a plant; that part of the plant then develops into a structure absolutely characteristic of the insect concerned but often decidedly different from anything the plant develops under any other circumstance; this new structure serves as food and protection for the young insect or insects. There are a number of complications which any theory concerning the causative factor or factors of gall formation must meet. Among them are the following.

In some cases the gall forms before the insect egg hatches; in others not until the larva starts to feed on the plant tissue. It has been said that sometimes a gall follows an egg-laying puncture even though an egg is not laid or, if laid, fails to hatch.

Certain plant-lice are associated with gall formation even though they lay no eggs on the plant but reproduce viviparously. In this case the gall has been supposed to be due to the irritation caused by the puncture of the plant tissues by the insects while feeding.

The fact that the galls, in their external appearance and internal structure, are frequently so characteristic of the insects associated with them that the galls give as satisfactory grounds for identification as the morphology of the insects themselves is well illustrated by two common "oak apples," *Amphibolips* (or *Cynips*) *confluentus* and *Amphibolips inanis*. The same part (leaf) of the same or closely related plants (oaks of the black-oak group) is affected and yet in the case of *confluentus* the whole interior of the gall surrounding the larval chamber is filled with a juicy tissue that later becomes like a mass of wool, while in the case of *inanis* the mature gall is, as its name signifies, empty except for the larval chamber held in place by radiating threads of plant tissue. Such structures are never known on oaks except in connection with this group of insects, and these insects are always associated in at least one part of their life cycle with a particular form of gall.

In this connection there is another complication. Many of the Cynipid wasps, to which family *Amphibolips* belongs, have alternating generations. In that case, one generation may be associated with one kind of a gall in one part of its host plant and the other generation with a totally different but equally characteristic kind of a gall in another part of the same host.

If it be true, as seems to be the case, that the plant-louse, *Hamamelistes spinosus*, which is associated with a complicated spiny hollow gall on the witch-hazel, has an alternate generation on another host (birch) where it is not associated with a gall, matters are, if anything, more complicated.

If one generation of a given insect is associated with a gall and the alternate generation is not, it is scarcely surprising that among closely related insects some species are "skilled gall-makers" and others are in no way connected with plant galls. At the same time, gall-insects are confined largely to one family (Cynipidæ) of Hymenoptera, one family (Itonididæ, formerly called Cecidomyiidæ) of Diptera, and one family (Aphididæ) of Homoptera. Also, although a wide variety of plants are affected, willows, oaks, the rose group, and goldenrods furnish the largest number of good examples.

There is every gradation in gall structure from such complicated things as "oak apples" and the spiny gall of the witch-hazel down to simple swellings or slight leaf-foldings. It is, therefore, impossible to say in some cases whether a malformation of the plant should be called a gall or not.

Insects are not the only organisms which, either directly or indirectly, bring about galls. Of the others, the most interesting in this connection are fungi and bacteria. Fungus galls are the more conspicuous but the bacterial root-galls of legumes, for example, are quite as definite and seem to be as truly galls.

The most accepted explanation of gall formation is that some chemical, enzyme or something of the sort, is injected by the female when she lays her egg, or by the plant-louse as it sucks, or is given off by the larva as it feeds, and that this stimulates the plant to gall formation. There is nothing to disprove this and there is even some experimental evidence in its favor. At the same time, the senior author has been wondering for some time if all plant galls, including those supposed to be directly caused by insects, are not fundamentally due to either bacteria or fungi.

It is known that the females of certain wood-boring insects carry with them from one tunnel to another spores of the fungi upon which they feed and the same is true of fungus-growing ants. Likewise, an insect coming from a bacterial gall might carry bacteria with which she unintentionally innoculates the plants upon which she lays her eggs. With this idea in mind, he suggested to the junior author in 1926 a study of the bacteria of galls and of the associated insects. The remainder of this paper is entirely the work of Mr. Brown and, although time has not permitted a wide survey and the case is far from proved as to *spinosus*, his results are certainly interesting and the matter seems worthy of further consideration even if only from the standpoint of bacteriology.

During August and September 1926 five strains of the same bacterium were isolated from the tissues of the *Hamamelistes spinosus* gall on witch-hazel and also from the aphids themselves. Two of these were from inoculations on agar made by Dr. F. E. Lutz at the American Museum's Station for the Study of Insects near Tuxedo, N. Y., two were made by F. M. Brown at the same place, and one by F. M. Brown at Newport, R. I These repeated appearances of the same organism both in the "gall-former" and the gall tissue at different times and in different localities leads us to believe that the organism may be associated with the formation of the gall. Experimental inoculation of witch-hazel leaf-buds by F. E. Lutz and F. M. Brown during the winter, spring, summer and early autumn of 1927 with saline suspensions of the bacteria, broth cultures, and smears of agar growth were in each case negative. The non-success, however, may be due to the loss of virulence of the organism when grown on artificial media, as occurs in many cases. This may or may not be remedied by a passage through the aphid before inoculation in order to restore the strength of the culture.

The *spinosus* organism is a member of the Bacteriaceæ and probably referable to the genus *Erwinia.* A description follows.

Erwinia espinosa, new species. (F. M. Brown)

Soc. Amer. Bact. Index No. 5312–32120–1111.

Rods small and variable in size, 0.2–0.3 by 0.5–0.9 micron. Sluggishly motile except in very young cultures, then quite active. Gram negative.

GELATINE COLONIES.—Punctate, transparent.

GELATINE STAB.—Slow stratiform liquefaction; considerable flocculent growth at bottom of well.

AGAR COLONIES.—Circular, smooth, dirty white; glistening surface.

BROTH.—Turbid, some flocculent sediment; one strain, *R* tended to form a slight fragile pellicle.

LITMUS MILK.—Acid, coagulation, some gas, some reduction in one Strain *Y* after 20 days of growth.

POTATO.—Dry, cream-color to pale yellow, surface bubbled (due to fermentation of dextrose from hydrolysis of starch).

INDOL is formed.

NITRATES are reduced.

SULPHATES are not reduced.

H_2S is formed in varying amounts from none to heavy trace.

CARBOHYDRATES are freely attacked; acid and gas are formed in arabinose, dextrose, lactose, xlyose, levulose, galactose, sucrose; the per cent of gases varies slightly with the strain. No action on rhamnose or pectin. Acid is formed from inulin and with one strain, "*Y*," gas.

STARCH is hydrolyzed.

Aerobic, some facultative ability.

Optimum temperature 25°C–30° C.

Bergey, D. H., *et al.*, 1925, 'Manual of Determinative Bacteriology' was used for all bacterial terminology.