A GALL SIMILAR TO CROWN GALL, PRODUCED ON GYPSOPHILA BY A NEW BACTERIUM [1]

By NELLIE A. BROWN

Associate pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Galls on the crown and roots of *Gypsophila paniculata* L. were first brought to the attention of pathologists of the Bureau of Plant Industry in the summer of 1932, when a grower submitted specimens of galls that had occurred in an ornamental-plant nursery in the eastern part of the United States. Galls on *Gypsophila* apparently were seen for the first time in 1931 by eastern growers of this important ornamental plant. However, neither the damage to plants nor the financial loss was extensive; consequently the growers did not at that time bring the disease to the attention of Federal workers interested in plant-disease problems.

In 1932, of 12 nurserymen who produced *Gypsophila paniculata* for the wholesale trade, only 2 were familiar with the disease, and one of these minimized his losses. He admitted that he understood the potential danger but stated that his loss had been less than 1 percent. The losses of the nurseryman who submitted the galled plants in 1932 amounted to about 25 percent.

A note regarding the discovery of the disease was published in the autumn of 1932.[2]

THE DISEASE

The galls occur principally on grafted plants in the region of the graft. They are of a soft nodular type, ½ to 3 cm in diameter (fig. 1, *A*), and may extend around the greater part of the stem or root, eventually causing the death of the plant. It is the practice of the eastern *Gypsophila* growers to lift seedling plants in the fall to use later for grafting with the desired variety. Should their field plants be galled in the summer, they are worthless when dug.

Crown gall of ornamental plants, of vegetables, and of fruit trees, which is produced by *Bacterium tumefaciens* Smith and Town., is so well known and wide-spread that no surprise is manifested when a new host plant for the disease is found. Consequently, when the *Gypsophila* gall was received and examined and its outward appearance was observed to be very like crown gall, it was at first thought that *Gypsophila* was a new host for crown gall and that the disease was not new.

The routine work for identification, however, changed this idea. Cross sections examined under the microscope showed water-soaked areas and masses of bacteria streaming from the tissues, characters that are unlike crown gall. No nematodes or fungi were present. The disease was not crown gall, but there was a possibility that it might be related to one of the other bacterial plant tumors, namely,

[1] Received for publication Mar. 19, 1934; issued July 1934.

[2] BROWN, N. A. ANOTHER GALL-FORMING BACTERIUM. Phytopathology 22: 924–925, illus. 1932.

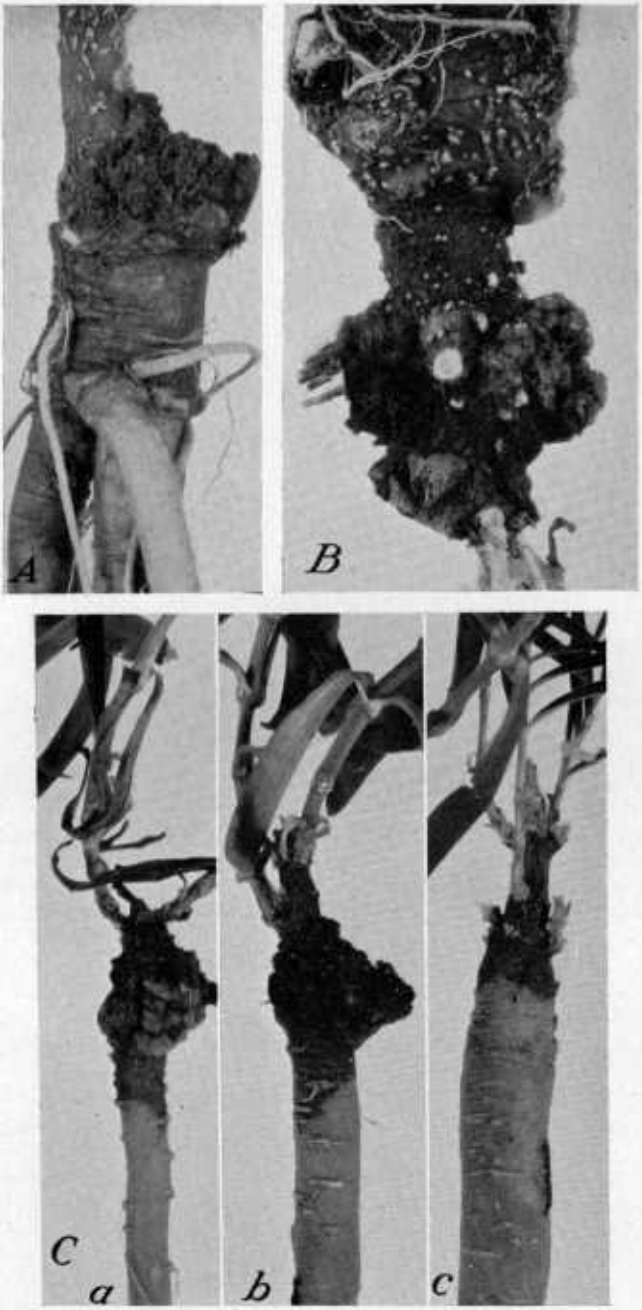

FIGURE 1.—*A*, *Gypsophila paniculata* gall from an Eastern State. *B*, *G. paniculata* gall 5 weeks old, produced by inoculating with an organism isolated from *A*. *C*, Seedling *G. paniculata* plants: *a* and *b*, Inoculated with 2 different colonies reisolated from gall *B*, which produced these galls in less than 1 month; *c*, control plant. Natural size.

the pocket disease of the sugar beet, produced by *Bacterium beticola* (Smith, Brown, and Townsend) Potebnia; the olive knot, produced by *Bact. savastanoi* E. F. Smith; the oleander gall, produced by *Bact. savastanoi* var. *nerii* C. O. Smith; or a canker of ash trees, produced by *Bact. savastanoi* var. *fraxini* Brown.

A further study of the structure of the *Gypsophila* gall, however, showed that it had features unlike these last-named tumors and could not be classed with them. There were no gum pockets in any of the *Gypsophila* galls received for examination. There were a few brown areas in some of them, but whether the discoloration was the beginning of the break-down of the gall tissue due to the invading organism or to a reaction of the gall tissue to the byproducts of this organism is not known. No browning occurred in the galls produced by inoculation. The *Gypsophila* galls are so soft that disintegration occurs very easily.

The galls were studied in cross section (fig. 2) and in the water-soaked areas motile bacteria were seen in the cells. Some of the cells were filled with them, others were partly filled, while many had none. A few of the tumor cells were packed with crystals. The cells near the periphery of the galls contained the greatest number of bacteria. The causal organism of crown gall has not been seen in the natural gall.

The structure of the *Gypsophila* galls was found to be much like that of crown gall. Nests of rapidly developing cells could be distinguished in which parenchyma and sclerenchyma cells were mixed irregularly (figs. 2, *A*; 3, *C*).

ISOLATIONS AND INOCULATIONS

COLONIES

An organism was isolated from several galls, the same type of colony appearing on the plates poured from each gall. The colonies were abundant and apparently consisted of the pathogene responsible for the disease. They appeared in 24 hours, were translucent white in reflected light, circular, 2 to 4 mm in diameter, slightly raised in the center, and finely granular (fig. 2, *B*). Buried colonies were mostly lens-shaped, but some were round. There were no irregular colonies. In 3 days they were a creamy yellow, 4 to 7 mm in diameter, and on thinly sown plates, 8 to 11 mm. Both rough and smooth colonies appeared on the plates isolated from the same gall, but the smooth colonies predominated. Both types of colonies were used for inoculations and produced galls of similar size, although the rough type produced them a little more slowly than did the smooth type.

By means of needle pricks grafted *Gypsophila* plants were inoculated at the crown and also on the stems with several of the isolated colonies. There was a beginning of gall formation at the crown in 7 to 9 days. In 2 weeks light-colored nodular galls 1 to 1½ cm in diameter showed at the crowns. Some of the inoculated plants were slow in showing infection, but in 3 weeks all had definite galls (fig. 1, *B*). When they were 2½ to 3 cm across, quite frequently disintegration began (fig. 4, *A*). Cankers, instead of galls, were produced on the inoculated aerial stems. On the 24 plants inoculated, the infection on crown and stem was 100 percent.

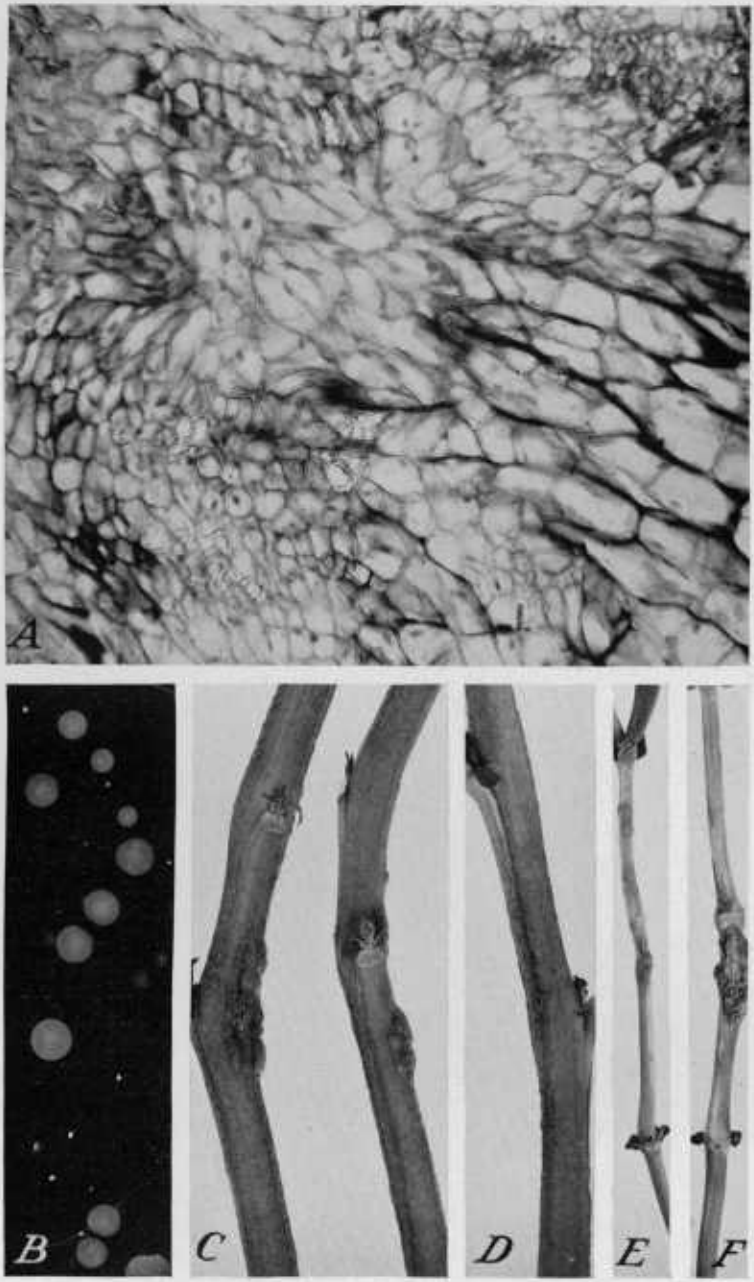

FIGURE 2.—*A*, Cross section of *Gypsophila paniculata* gall showing cell structure. *B*, Agar-plate colonies of the *Gypsophila* gall organism; natural size. *C*, Potato stems showing swellings; stems inoculated 3 weeks with *Gypsophila* gall organism that had previously passed through a potato stem and been reisolated; natural size. *D*, Control punctures on potato stem made at the same time as inoculations in *C*; natural size. *E*, *Saponaria vaccaria* inoculated with *Gypsophila* gall organism 4 days. *F*, *S. vaccaria* inoculated with *Gypsophila* gall organism 11 days. Organism is most active on this host, which is a relative of *Gypsophila*. *E* and *F* slightly reduced in size.

Roots of seedling *Gypsophila* plants inoculated at the crown likewise gave 100 percent infections. The galls formed about as rapidly as on the older grafted plants (fig. 4, *C, a*). Hot moist conditions favored gall development. Platings were made from the galls produced by inoculation, and the organism was recovered. *Gypsophila* plants inoculated with this reisolated strain developed galls of the same type as rapidly as did plants inoculated with the original strain (fig. 1, *C, a, b, c*).

At the time the *Gypsophila* plants were inoculated, stems of tomato (*Lycopersicon esculentum* Mill.) and potato (*Solanum tuberosum* L.), carnations (*Dianthus caryophyllus* L.), garden balsam (*Impatiens balsamina* L.), sugar beets (*Beta vulgaris* L.), Paris daisies (*Chrysanthemum frutescens* L.), nasturtiums (*Tropaeolum* L.), and other plants were inoculated with the same organism. No galls formed on any of them, but well-defined swellings occurred on the potato stems. Isolations were made from the potato swellings, the organism was recovered and outgrowths similar to the natural galls were obtained by inoculating *Gypsophila* plants with this potato isolation (fig. 3, *A*). Potato stems, inoculated with the potato isolation, developed swellings like those caused by the original organism, but neither galls nor cankers (fig. 2, *C, D*). Inoculations made into potato tubers attached to the plant at different stages of growth produced no outgrowths.

The common hosts of *Bacterium tumefaciens*, such as Paris daisy, sugar beet, *Ricinus* L., geranium (*Geranium* L.), and garden balsam, did not prove susceptible to the *Gypsophila* gall organism when they were inoculated with it, nor did *Bact. tumefaciens* produce any trace of outgrowth on roots or stems of *Gypsophila paniculata* (fig. 4, *C, b, c*). The roots and stems of *G. paniculata* were also inoculated with the olive-knot organism (*Bact. savastanoi*) and the ash-canker organism (*Bact. savastanoi* var. *fraxini*) with negative results.

FILTRATES AND PLEOMORPHIC FORMS

The juice of crushed *Gypsophila* galls was passed through Chamberland L 3 filters, and *Gypsophila paniculata* and *Lychnis chalcedonica* L. plants were inoculated with the filtrate. No galls resulted. Filtrates from beef-bouillon cultures were also used for inoculations, with the same result. A portion of the filtrates was held in sterile tubes for several weeks, then cultured repeatedly on hardened agar plates, according to the technic of Hauduroy [3] and of Hadley.[4] With this procedure the filtrates passed through the granular and coccus stages and later reached the normal rod form again, but when the cultures arrived at the rod form the ability to infect the *Gypsophila* plants was lacking. The cultures would not produce galls.

The writer had tried out the method previously with three different strains of *Bacterium tumefaciens*, namely, the hop, daisy, and peach strains. Neither the crushed-gall filtrates nor the beef-bouillon-culture filtrates of the three strains produced galls when inoculated into susceptible plants. Portions of the sterile filtrates were held in tubes for a few weeks to a few months, then cultured for some time on hardened agar plates. From the granules, the coccus form developed, and later from the coccus the normal rods. Inoculations were made into young

[3] HAUDUROY, P. LES ULTRAVIRUS ET LES FORMES FILTRANTES DES MICROBES. 392 pp. Paris. 1929.
[4] HADLEY, P., DELVES, E., and KLIMEK, J. THE FILTRABLE FORMS OF BACTERIA. I. A FILTRABLE STAGE IN THE LIFE HISTORY OF THE SHIGA DYSENTERY BACILLUS. Jour. Infect. Diseases 48: 1–159. 1931.

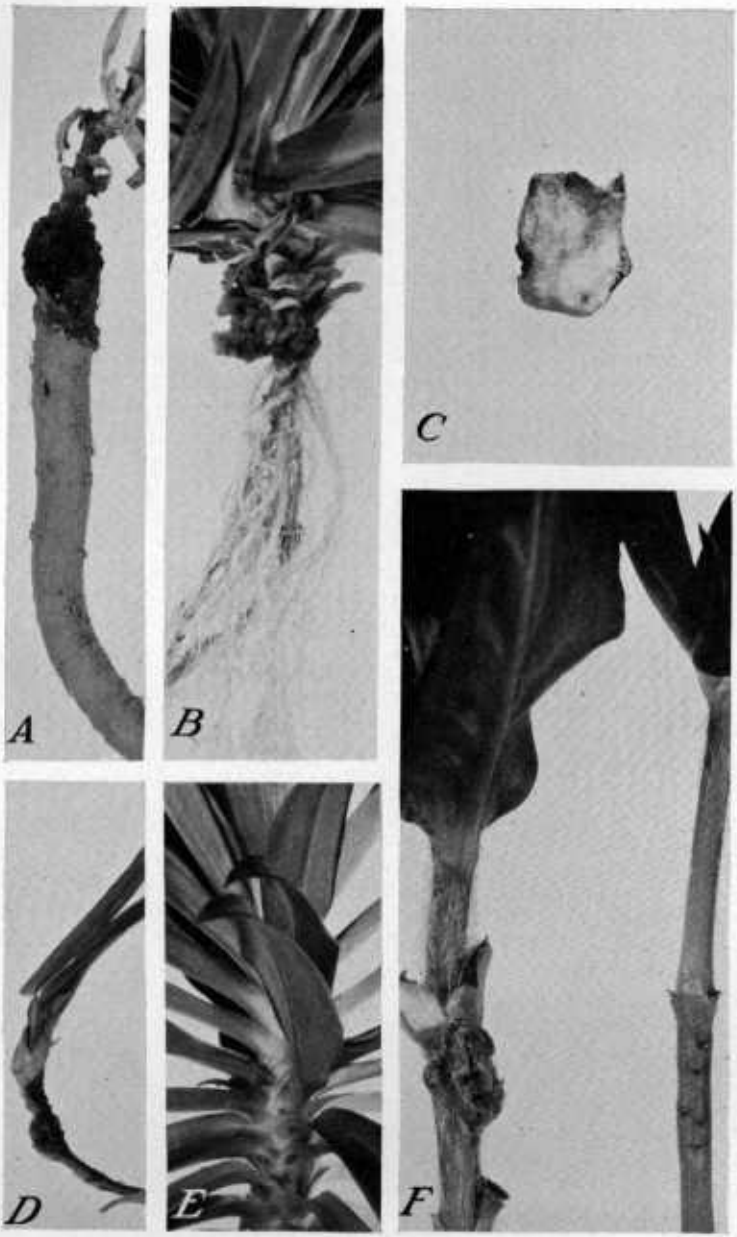

FIGURE 3.—*A*, Gall on seedling *Gypsophila paniculata*, produced by inoculation with a strain of the organism reisolated from a potato stem on which a swelling was produced but not a gall. The potato reisolation, however, produced galls on *Gypsophila* root. Photographed 5 weeks after inoculation. *B*, Gall on *Silene armeria* produced by inoculation with the *Gypsophila* gall organism; time, 2 months. *C*, *Gypsophila* gall 1 month old cut across to show internal structure. *D*, Gall on *Dianthus plumarius* (garden pink) produced by inoculation with the *Gypsophila* gall organism; time, 2 months. *E*, *Dianthus barbatus* (sweet-william) inoculated with *Gypsophila* gall organism 2 months; no infection. *F*, At left gall on *Lychnis chalcedonica* produced in less than 1 month by inoculation with the *Gypsophila* gall organism; at right, control punctures on *L. chalcedonica*. All natural size.

susceptible plants with both coccus and rod forms, but no trace of crown gall resulted.

With the *Gypsophila* gall filtrates the transition stage from coccus to rod came more quickly than with the filtrates of *Bacterium tumefaciens*, and it was hoped pathogenicity accompanied this less tedious manipulation. It did not prove to be the case, however, as no infection followed inoculations into susceptible plants.

THE CAUSAL ORGANISM

CULTURAL CHARACTERS

BEEF-INFUSION AGAR PLATES.—White colonies are visible in 24 hours after pouring plates from macerated gall tissue incubated at 22° to 25° C. In 48 hours they are deep cream to wax yellow and range from 2 to 4 mm in diameter; they are smooth, circular with entire margin, shining, convex, a little thicker in the center. In 4 days the colonies on thinly sown plates are 4 to 11 mm across, and in some there is a margin. At 6 days the color is mustard yellow; later, primuline yellow.[5]

After the organism has been cultured a few weeks in artificial media, plates poured from a 1-day beef-bouillon culture may show more rough than smooth colonies. These are much the same as the colonies that appear from the isolation material. Occasionally there is a rough colony that has a high convoluted surface. The color of week-old cultures is primuline yellow.

BEEF-INFUSION AGAR SLANTS.—There is a thin spreading growth, usually papillate but sometimes smooth, on beef agar slants in 24 hours. Under the hand lens it has a metallic luster on beef agar. The pH is 6.8 at temperatures of 25° to 30° C. At 4 days growth is abundant, butyrous, translucent; at 7 days the metallic luster has disappeared and there are many crystals. The color of the growth is Naples yellow.

BEEF-INFUSION BOUILLON.—Clouding is prompt, being quite definite in 7 hours at 34° C.; at 30° there is good clouding in 24 hours; in 48 hours a yellow pellicle has formed which falls readily.

THAXTER'S POTATO-DEXTROSE AGAR SLANTS.—The growth is spreading but not so rapid as on beef agar; it is rough, butyrous, cream-colored, and continues so when a week old.

POTATO CYLINDERS.—There is a thin cream-yellow growth in 1 day; it is still scanty at 6 days, with the color buff-yellow, and the potato is slightly discolored. After 30 days the color is Naples yellow, but the medium has not darkened further.

COHN'S SOLUTION.—Growth is rapid and heavy in Cohn's solution, and a complete pellicle forms with larger irregular crystals hanging from it. At first the pellicle is white but changes in 7 days to Naples yellow. The medium becomes cream-colored and has a yellow precipitate.

USCHINSKY'S SOLUTION.—Growth is prompt in this medium, and there is a heavy white pellicle in 2 days; in 12 days the pellicle is cream-colored and the medium Naples yellow.

FERMI'S SOLUTION.—Growth occurs readily but is not so heavy as in Uschinsky's solution. There is a white pellicle in 2 days, which changes to mustard yellow in 12 days.

PHYSIOLOGIC CHARACTERS

LIQUEFACTION OF GELATIN.—No liquefaction begins in beef-gelatin stabs until the cultures are a month old. The liquefaction continues slowly and is not completed until after 4 months. The cultures were kept at 14° to 15° C. and three different lots of beef gelatin, having pH 6.5, 7.0, and 7.3, respectively, were used.

Colonies on beef-gelatin plates are cream-colored until 6 days old, when they become mustard yellow. Both smooth and rough colonies occur on all plates, with the rough type more abundant; they are translucent in reflected light, transparent in transmitted light. In 3 weeks there are little hollows around the colonies, and in 4 weeks liquefaction is definitely visible but continues very slowly. In heavily seeded plates the gelatin is entirely liquefied in 4 months; in sparsely seeded plates the liquefaction extends 1 cm beyond the colony. Feathery branched crystals are formed on the plates and in the stab cultures.

[5] The color readings in this paper are based on the colors in the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D.C. 1912.

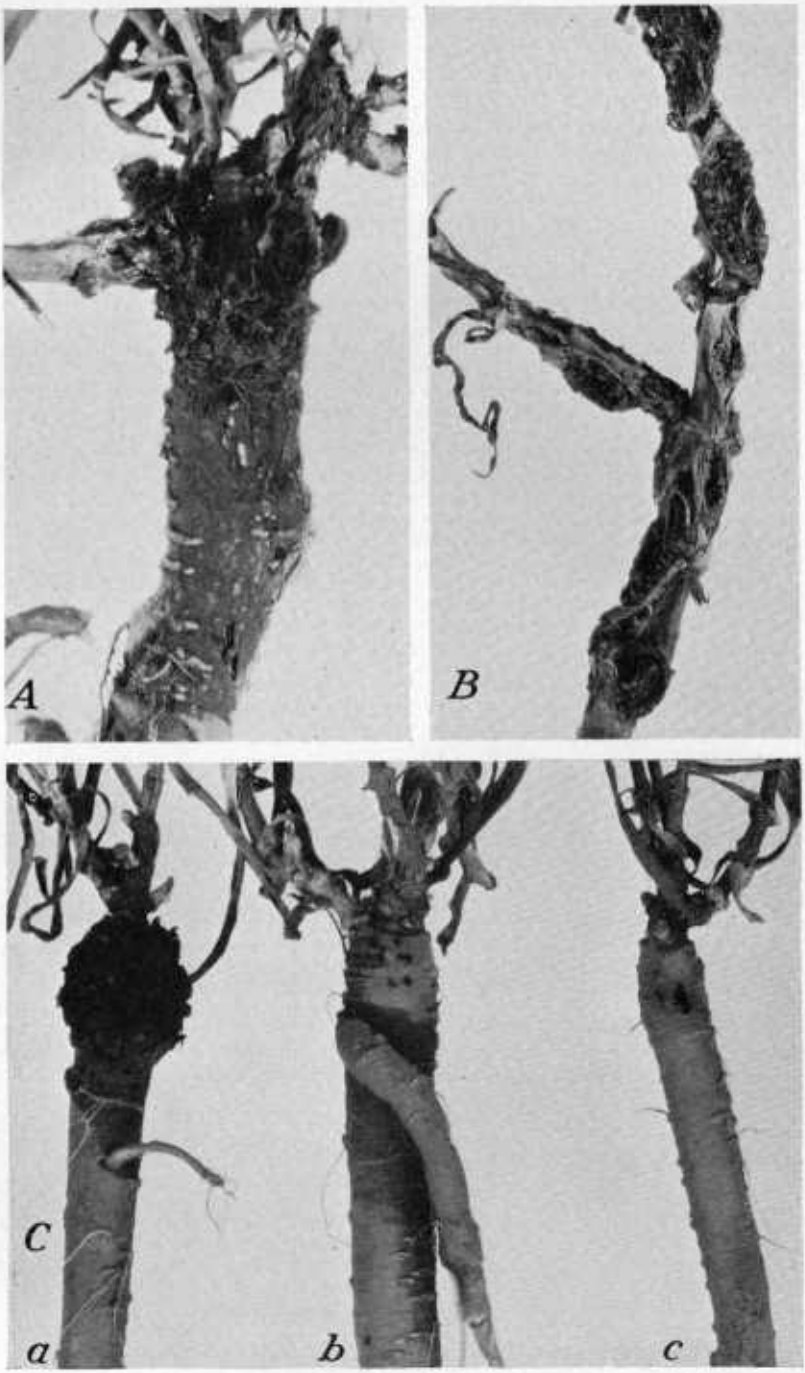

FIGURE 4.—*A*, Gall on *Gypsophila paniculata*, mostly rotted off. Crown was inoculated with *Gypsophila* gall organism July 23, 1932. Photographed August 30, 1932. *B*, Cankers, not galls, produced on *G. paniculata* stems, by inoculation with the *Gypsophila* gall organism; time, 3 months. *C*, Seedling *G. paniculata* roots inoculated with various organisms 2 months: *a*, with *Gypsophila* gall organism; *b*, with *Bacterium tumefaciens* hop strain, no infection; *c*, with *Bact. tumefaciens* daisy strain, no infection. All natural size.

MILK.—Coagulation of milk occurs at 9 days, with very little whey. A light straw-colored pellicle is visible at 6 days. The curd digests slowly, beginning at 17 days and being completed in 90 days, at which time the color of the milk is tawny.

BLOOD SERUM.—A very good growth takes place on blood serum, but there is no liquefaction. The mustard-yellow color at 2 days becomes the deeper primuline yellow at 12 days. The blood serum grays a little at the base. Cultures 2 months old show no trace of liquefaction.

REDUCTION OF LITMUS.—There is a dull pink color throughout litmus-milk cultures in 24 hours, and the original color, pale aniline lilac, is changed to pale lobelia violet; in 48 hours this color is still duller except at the surface of the liquid. In general the color change in comparison with the uninoculated tubes is slight. In 8 days the litmus has faded to lavender-gray and in 10 days it is reduced, the color now being tilleul-buff; there is a yellow pellicle and yellow precipitate of the growing organism. Coagulation usually takes place on the ninth day, sometimes on the eighth day, after litmus milk has been inoculated. Digestion of the curd is slow, not being completed before 3 months. The medium at this time is dull reddish purple, and the organism is still alive.

REDUCTION OF NITRATES.—Nitrates are reduced to nitrites. Tests were made on nitrate-bouillon cultures 3 and 5 days old, with the sulphanilic acid α-naphthylamine test. There was a good red color in all the tubes, indicating the presence of nitrites. Other cultures when 25 and 30 days old were tested with the same result.

INDOL PRODUCTION.—No indol is produced. Tests were made on the organism growing in tryptophane broth at 3, 5, and 30 days, respectively, with the Ehrlich-Böhme method. *Bacillus coli* (Escherich) Migula, which produces indol, grown as a control and tested at the same time, gave a good pink color, a positive test. The *Gypsophila* gall organism showed no pink color.

FERMENTATION OF CARBOHYDRATES.—The organism is not a gas former. It was tested in fermentation tubes in the presence of the following carbon compounds: Dextrose, saccharose, lactose, glycerin, and mannite. A 1-percent solution of each was made in a 1-percent water solution of Difco peptone. Besides heavy growth in the open arm of each tube there was growth in the closed arm of the tube with each compound except glycerin. No gas was produced. Acid was produced in all the solutions but that of lactose. A second test was made with the same result. The pH readings were taken just before inoculation with the *Gypsophila* gall organism, also 20 and 27 days after, as shown in table 1.

The same carbohydrates added to synthetic agar with brom cresol purple as indicator, made according to the formula given in the Manual of Methods,[6] were also tested for fermentation. Growth occurred promptly, as did the acid reaction with saccharose, dextrose, glycerin, and mannite, the yellow color change in the purple medium beginning in 18 hours and being complete in 48 hours. There was growth in the lactose cultures but no color change.

TABLE 1.—*Acid production by the Gypsophila gall organism after 20 and 27 days of growth in 1-percent sugar solutions added to 1-percent Difco peptone*

[Acidity indicated by pH readings]

Number of days of growth	pH of solution containing peptone and indicated carbohydrate					pH of plain peptone water
	Dextrose	Lactose	Saccharose	Mannite	Glycerin	
0	6.5	6.5	6.7	6.7	6.5	6.6
20	4.2	7.0	5.0	5.0	6.4	7.4
27	4.4	6.8	5.0	4.9	6.5	7.3

DIASTATIC ACTION.—There is no reduction of starch. On starch-agar plates streaked with the organism and tested at 5, 9, 12, and 16 days, respectively, there was no clear zone in the medium.

AMMONIA PRODUCTION.—The organism produces ammonia. Tests were made with old and young beef agar, beef bouillon, and peptone-water cultures, using

[6] SOCIETY OF AMERICAN BACTERIOLOGISTS, COMMITTEE ON BACTERIOLOGICAL TECHNIQUE. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA. 130 pp., illus. Geneva, N.Y. 1930.

filter paper saturated in Nessler's solution and suspended in the tubes. When the cultures were heated in a water-bath, browning of the paper began immediately. In cultures 2 weeks old the brown color was much more pronounced than in the 3- and 5-day cultures.

HYDROGEN SULPHIDE PRODUCTION.—The organism may produce a trace of hydrogen sulphide. Agar, beef-bouillon, and potato-cylinder cultures were tested by suspending lead acetate paper in the culture tubes. When the beef-bouillon cultures were 8 days old there was slight darkening at the tip ends of the paper, indicating hydrogen sulphide production; there was slightly more darkening of the paper when cultures were 2 weeks old. There was no darkening of the paper in the other cultures.

The organism was then grown on lead acetate agar. A heavy yellow growth occurred on the red agar, but there was no dark line or any browning indicating the presence of hydrogen sulphide. A second and third test with the lead acetate agar was made which likewise showed no hydrogen sulphide production.

TOLERATION OF SODIUM CHLORIDE.—The organism grows in pH 6.5 beef-infusion bouillon containing 6, 7, 8, or 9 percent of sodium chloride. There is no growth in beef bouillon containing 10 percent of sodium chloride.

OXYGEN RELATIONS.—The organism is a facultative anaerobe. In tests made with shake cultures of agar and gelatin, tiny clumplike colonies grew throughout the medium. When long sterile cover glasses were dropped on hardened agar plates streaked with the organism, growth was more abundant at the edges of the cover glass, but it extended inside the edges, showing the anaerobic tendencies. In agar and gelatin stab cultures it grew at once at the bottom of the tube, and in synthetic-dextrose-indicator-agar shake cultures, the purple color of the medium was changed to red at the bottom of the tube as quickly as at the surface of the medium.

THERMAL RELATIONS.—The organism grows at temperatures ranging from 5° to 40° C. The optimum temperature is about 34°; it does not grow at 0° nor at 42° and only faintly at 5° and 40°.

The thermal death point is between 52° and 53° C., when fresh beef-bouillon cultures, pH 7.0, are exposed in a water bath for 10 minutes.

GROWTH IN BEEF BOUILLON.—The best growth in peptone-beef-infusion bouillon takes place at pH 6.5 to 6.7, although the organism has a wide range, extending from pH 5.1 to 9. There is no growth at pH 4.9 or 9.1. At pH 5.1 there is only a faint growth; at pH 9 there is a fair amount of clouding with pellicle.

EFFECT OF DESICCATION.—The organism is only slightly resistant to drying. Sterile cover glasses smeared with young beef-bouillon cultures and dried at room temperatures (24° to 27° C.) were dead in 5 days.

EFFECT OF FREEZING.—The organism can withstand freezing temperatures for more than 45 days. Immediately after being transferred, beef-agar and beef-bouillon cultures were placed at temperatures of −21.7° to −23.9° C. Some were removed after 7, 9, 12, and 45 days. All showed typical growth within 1 day after the medium melted.

LONGEVITY.—The organism lives for 7 to 8 months in sterile milk and beef bouillon, pH 6.8, at room temperature of 22° to 30° C., whereas it dies on beef agar slants, in Cohn's, Fermi's, and Uschinsky's solutions, after 4 months at the same temperatures. Sterile-milk and beef-bouillon cultures kept at 14° are alive after 11 months.

VIRULENCE.—The organism remains virulent for more than a year. Fifteen months after isolating, transfers descended from the original isolation, including a smooth and rough colony, were inoculated into *Gypsophila paniculata* plants. In 7 days the galls were forming and continued to grow rapidly.

CHROMOGENESIS.—On beef agar the color of this organism is at first a light cream that changes to a Naples yellow in a few days. It is much the same in other solid media. Later the color may be mustard yellow or primuline yellow.

MORPHOLOGY

Grown on beef-infusion agar, the *Gypsophila* gall organism is a short rod with rounded ends growing singly, in pairs, and occasionally in chains of four to several elements; in rare cases there have been more than 25. Grown on beef agar for 1 day and stained with carbol fuchsin, the size is 0.5μ to 1.2μ long by 0.3μ to 0.8μ wide. Grown on the same medium for 2 days and stained with gentian violet, the

size is 0.4μ to 1.03μ long by 0.2μ to 0.62μ wide. The organism is motile on beef agar and in beef bouillon and its motility was demonstrated by staining with Casares-Gil flagella stain. There are several flagella, all bipolar. Capsules are formed, as was shown by staining young beef agar cultures with Ribbert's capsule stain. The tests for endospores showed none.

STAINING RELATIONS

The organism stains well with gentian violet and carbol fuchsin. It is not acid-fast and is Gram-negative. (Hucker's modification of Gram was used.)

TECHNICAL DESCRIPTION

Bacterium gypsophilae, sp. nov.

A motile rod 0.4μ to 1.2μ long and 0.2μ to 0.8μ wide, with several bipolar flagella; capsules present, no spores; Gram-negative, not acid-fast; colonies on beef-infusion agar are circular, either smooth or rough, yellow, butyrous; clouds beef-infusion bouillon heavily in 18 hours; liquefies gelatin slowly, but not blood serum; is facultative anaerobic; coagulates milk; reduces litmus in 9 to 12 days; grows well in Uschinsky's and Fermi's solutions and unusually well in Cohn's solution; reduces nitrates; produces ammonia and a trace of hydrogen sulphide but no indol; no diastatic action; survives cover-glass drying only 4 days; acid without gas produced with saccharose, dextrose, maltose, mannite, but not lactose, and only a slight amount with glycerin; the optimum temperature for growth is over 30° C., the maximum is 40°, the minimum is 5°; thermal death point is between 52° and 53°; optimum reaction for growth is from pH 6.5 to 6.7, limits of growth from pH 5.1 to 9.0; in beef bouillon and in sterile milk lives 8 months at 22° to 28°, over 11 months at 14°; stains readily with carbol fuchsin and gentian violet; pathogenic to *Gypsophila paniculata* and some of its relatives, producing galls on the crown and root, and cankers on the stem.

COMPARISON WITH BACTERIUM BETICOLA

Because of certain points of resemblance between *Bacterium gypsophilae* and *Bact. beticola* and the lesions caused by them, a study of certain cultural, physiologic, and morphologic characters of these organisms was made. A comparison of these characters is shown in table 2.

NATURAL INFECTION AND CONTROL

The *Gypsophila* gall organism is selective in its host plants, as only related plants have been found susceptible to it; however, there are also related plants which are not susceptible. The limitation of this gall-forming ability differs from that of the crown-gall organism, which produces galls on many unrelated plants. The hosts susceptible to the *Gypsophila* gall organism are *Lychnis chalcedonica* (fig. 3, *F*), *Dianthus plumarius* L. (fig. 3, *D*), *Silene armeria* L. (fig. 3, *B*), and *Saponaria vaccaria* L., which is the weed soapwort (fig. 2, *E* and *F*). This last relative seems to be more susceptible to the organism than *Gypsophila paniculata* itself, for infection begins in 3 to 4 days after inoculation and galls are formed very rapidly. It may be that this weed is the natural host of the *Gypsophila* gall organism and it would be advisable not to allow it to grow in the neighborhood of *Gypsophila* plants. Another relative, *Saponaria ocymoides splendens* Hort., is slow to become infected, as the galls did not begin to form on young plants until nearly 3 weeks after inoculation. The relatives, *Cerastium tomentosum* L., *Tunica saxifraga* Scop., *Spergula pilifera* DC., *Dianthus barbatus* L., which is the common sweet-william

(fig. 3, *E*), and the greenhouse carnations are not susceptible. It is an interesting fact that the carnation is not infected by the *Gypsophila* gall organism, for *Bacterium tumefaciens* produces galls thereon quite readily, and occasionally natural *Bact. tumefaciens* galls are found on it. To be quite certain that carnation plants could not be infected by the *Gypsophila* gall organism, inoculations were made at different times of the year and in different growing stages of the plant. Other hosts that did not prove susceptible are sugar beet, tobacco (*Nicotiana tabacum* L.), *Ricinus communis* L., Paris daisy, *Impatiens balsamina*, tomato, geranium, rose (*Rosa* L.), *Bryophyllum pinnatum* Kurz, nasturtium, and two monocotyledons, amaryllis (*Amaryllis* L.) and calla (*Zantedeschia aethiopica* (L.) Spreng.). As stated previously decided swellings but no galls formed on the potato stem.

TABLE 2.—*Comparative cultural, physiologic, and morphologic characters of Bacterium gypsophilae and Bact. beticola*

Character compared	*Bacterium gypsophilae*	*Bacterium beticola*
Colonies in beef-agar plates	Circular; none irregular; butyrous; white first day; deep cream to yellow, 4 to 8 mm in diameter, in 4 days.	Circular; some irregular; viscid; buff-colored first day; yellow, 4 to 6 mm in diameter, in 4 days.
Cohn's solution	Rapid, heavy growth	No growth.
Liquefaction of gelatin stabs	Begins after 30 days; complete after 4 months.	Begins after 7 to 8 days; complete in 20 to 30 days.
Hydrogen sulphide production.	A trace to none	Rapid and good production.
Indol production	None	None.
Reduction of nitrates	Nitrates reduced	Nitrates reduced.
Reduction of litmus milk	Complete in 9 to 10 days; milk coagulated in 8 to 9 days.	Complete in 20 to 30 days; milk coagulated in 10 to 20 days.
Gas production	None	None.
Acid produced with dextrose, saccharose, glycerin, and mannite.	Acidity produced	Acidity produced.
Acid produced with lactose	None	None.
Ammonia production	Ammonia produced	Ammonia produced.
Diastatic action	None	Starch reduced.
Relation to oxygen	Facultative anaerobic	Aerobic.
Relation to acid and alkali	pH range 5.1 to 9.0	pH range 4.8 to 9.1.
Temperature relations	Grows from 5° to 40° C; optimum, about 34°; thermal death point, 52° to 53°.	Grows from 1.5° to 39° C.; optimum, about 29°; thermal death point, 51° to 52°.
Survives cover-glass drying	4 days	7 days.
Color	Ranges from white to yellow	Ranges from buff to yellow.
Size	0.4μ to 1.2μ long; 0.2μ to 0.8μ wide	0.6μ to 2μ long; 0.4μ to 0.8μ wide.
Gram negative or positive	Negative	Variable.
Pathogenicity	Produces galls on *Gypsophila paniculata* but not on sugar beets.	Produces galls on sugar beets but not on *Gypsophila paniculata*.

The *Gypsophila* gall organism produces cankers on stems of *Gypsophila paniculata*, fair-sized cankers forming in less than 1 month after inoculation, large ones in 3 months (fig. 4, *B*). On *Lychnis chalcedonica* stems the infection is of the typical gall type. *Lychnis* crowns inoculated in November in the greenhouse developed galls, which rotted away during the winter. In the spring, when growth started, new galls formed which became larger than the original ones. This occurred with inoculated *G. paniculata* plants also, although the galls were not so soft and did not disintegrate so easily. The organism, like other organisms that produce galls, is a wound parasite, this one getting into the plant from the soil through imperfect grafting or through cultivation wounds. The disease occurs on lands that have been manured and on those that have not.

The rapid development of galls at the crown produces the death of some of the stems, and if the girdling is complete the death of

the entire plant follows. A small gall which has not caused any apparent trouble to the plant may be a decided menace to others later. The method used for propagating *Gypsophila* plants is to graft a desirable variety on seedling roots. If this variety is galled the organism is carried over to the young seedling roots and galls develop. Because of the sensitiveness of the gall organism to weak solutions of mercuric chloride, control measures can be carried on at the time of grafting to reduce the amount of disease occurring in the field. The roots should be dipped in a 1:1,000 mercuric chloride solution for 1½ to 2 minutes to kill the *Gypsophila* gall bacteria that may be on the surface; then with a disinfected knife a well-matched graft should be made and bound with nursery tape.

The sensitiveness of the organism to weak solutions of mercuric chloride was tested out by the poured-plate method. A fresh beef-bouillon transfer was exposed to 1 cc of a 1:1,000 solution of mercuric chloride for various lengths of time and then plates were poured, carrying over a loop of the exposed culture to each agar plate. No *Gypsophila* gall colonies appeared on the plates exposed over 1½ minutes to the mercuric chloride solution. As *Gypsophila paniculata* plants are not very sensitive to a 1:1,000 solution of mercuric chloride they can be treated with it to kill the gall organisms that may be present on the surface. Twenty-five seedling plants 3 to 6 inches tall were soaked in the solution for 1½ minutes, 25 for 3 minutes, 25 for 5 minutes, and 25 for 10 minutes. There was no appreciable injury to the plants from this treatment, even in the 10-minute lot.

In planting out seedlings and grafted plants, in weeding them and in loosening the soil about them during the summer, care should be taken to avoid wounding, for the organism may be present in the soil and may enter the plant through some tiny wound.

SUMMARY

An outbreak of an infectious gall disease occurred in 1932 in an ornamental-plant nursery in the eastern part of the United States. The galls were on the crown and roots of *Gypsophila paniculata.* They were soft and nodular; some were flat and spreading, others globular.

The *Gypsophila* galls when developing do not have the fissures or pockets that occur in developing galls of pocket disease of sugar beets and in those of olive knot. Growth of the galls is favored by hot moist weather, which also favors the disintegration of the old galls, releasing more organisms into the soil. Imperfect grafting and cultivation wounds allow entrance of the pathogene into susceptible tissue. Galls weaken the plants, producing defoliation and death of stems and, where girdling is severe, death of the plants.

An organism, which could be seen readily in sound gall tissue under the microscope, was isolated from the outgrowths and produced galls when inoculated into healthy plants of *Gypsophila paniculata.*

Galls were also produced by inoculation on several species related to *Gypsophila,* but the organism did not produce galls on the sugar beet, which is the host of the pocket disease, nor on such common hosts of the crown-gall organism as the Paris daisy, *Ricinus,* or geranium.

For the pathogene, which is a yellow, polar-flagellate organism apparently unlike any other known gall-producing organism, the name *Bacterium gypsophilae* is proposed. A description of its cultural, physiologic, and morphologic characters is given.

A comparison has been made between the new gall organism and *Bacterium beticola*, the organism causing the pocket disease of sugar beets.

A study has been made of conditions governing the natural occurrence of infection, and methods of controlling the disease are suggested.