RELATION OF WATER-SOAKED TISSUES TO INFECTION BY BACTERIUM ANGULATUM AND BACT. TABACUM AND OTHER ORGANISMS

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INTRODUCTION

The epidemiology of certain leaf spot diseases of tobacco and other plants is still the subject of much speculation, even though distinct advances in the knowledge of the subject have been made in recent years. Blackfire or angular leaf spot of tobacco, caused by Bacterium angulatum Fromme and Murray, is, for example, a widespread and often a serious disease of this crop. Nevertheless, artificial inoculation with this pathogen under ordinarily favorable conditions of environment for infection with many parasites either fails to produce any symptoms, or, at most, produces only small incipient lesions not comparable to the severe necrosis which often develops under field conditions. This situation remained obscure until recently, when it was shown by Clayton (3) that the highly necrotic or "epidemic" form of wildfire (Bact. tabacum Wolf and Foster) and blackfire is dependent upon the tissues becoming water-soaked by rain followed by the infection of these areas by the pathogen. The water-soaking explanation of infection, as contrasted with the older conception of leaf wounding by rain as a predisposing factor, marked a distinct step in the understanding of certain diseases of tobacco, particularly the blackfire disease. The experimental method in the present investigation differs from that used by Clayton in that water soaking was induced by internal rather than external water pressure.

The demonstration of the relation of water-soaked tissues to infection by organisms and development of disease now presents new problems on the nature of parasitism and of predisposition and susceptibility of plants to disease. In a practical sense, it also raises questions as to the reliance that can be placed on certain types of control measures such as sanitation and eradication. It will be shown in this paper, for example, that water soaking permits infection of normally immune plant species with various bacterial pathogens, and to some degree with bacteria which are not normally pathogenic. The determination of the host range of an organism, and even the definition of parasitism, may become difficult under such circumstances.

METHODS AND MATERIALS

The chief modification of method used in the present studies beyond those commonly employed or previously described consisted essentially of water soaking the plant tissues by means of the application of a high water pressure to the root system or the cut stem end of plants. The principle involved was perhaps first used by De Bary, and has been...
frequently employed since in physiological investigations. The apparatus as used in this laboratory was briefly described in 1924 in connection with plant virus studies (6). This equipment consisted essentially of a brass container connection to the pipe line of an ordinary water supply under pressure, together with the necessary valves and drain. This container is fitted with a neck to hold a no. 4 rubber stopper, which is securely held in place by means of a packing box similar to that used around valve stems. Rubber stoppers with holes of different sizes were made to hold plant stems of different sizes. These stoppers may be split down one side, so as to slip readily around the stem of the plants. A split brass washer on the top of the stopper permits the packing-box nut to be screwed down sufficiently by hand without displacement of the stopper. The contact around the plant stem may be quickly and almost perfectly made in this manner and will stand 100 pounds of water pressure without leaks (fig. 1).

The soil was washed off the roots before the root system was placed under pressure. If desired, the root system need not be used, the cut end of the stems only being placed under pressure. Water soaking in some plants starts very quickly, and may cover 50 to 100 percent of the leaf area in 15 minutes. With other species or with individual plants 30 to 60 minutes of exposure may be required to secure a limited amount of water soaking. After the plants were water-soaked to the desired degree, they were atomized with a water suspension of the organism to be tested, then removed from the apparatus and the roots or stem ends placed in a flask of water. The plants were then placed in a chamber with a moisture-saturated atmosphere, where the water-soaked conditions could often be maintained for several days if desired. Evident symptoms usually developed after 18 to 48 hours in the moist chamber, but in cases of uncertain infection or none the plants were left in the moist chamber for as long as 4 days.

Tomatoes (Lycopersicium esculentum Mill.) were frequently used as the test plant for parasitism because of the ease with which they were handled and water-soaked, but tobacco was used regularly, particularly in connection with the symptom studies on the blackfire and wildfire organisms. Aside from species of Solanaceae, the other plants used were usually chosen at random from species available at the time in the greenhouse or garden.

The organisms used in the experiments were secured from authoritative sources, largely from workers actively engaged in studies of the organism in the Departments of Plant Pathology or Agricultural Bacteriology at the University of Wisconsin.\(^2\)

**EXPERIMENTAL RESULTS**

The infection experiments have been conducted for the most part without any special consideration to tobacco (Nicotiana tabacum L.) as the typical host to Bacterium angulatum. It may be recalled that Bact. tabacum, the causal organism of wildfire, was reported as possessing a wide host range (8) but that according to Clayton (2) only species of Nicotiana should be regarded as true hosts. Since the close relationship of Bact. tabacum and Bact. angulatum has come to be accepted

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\(^2\) Thanks are due to several associates for furnishing these cultures, and especially to A. J. Braun, who maintained suitable cultures of Bacterium angulatum and Bact. tabacum for these studies.
FIGURE 1.—The equipment used for water soaking plant tissues by means of a high water pressure on the root system or cut stems of plants. The packing-box nut holding the split rubber stopper is shown at the base of the stem.
and the relation of water soaking to infection has been established, the host range of one or both of these organisms will no doubt need reconsideration. If we should limit the true hosts to those only on which infection may be secured artificially by ordinary spraying inoculation, together with those which are found to be infected under natural conditions, we should even then possibly need to consider the true hosts of Bact. angulatum and Bact. tabacum as extending beyond the genus Nicotiana. Manifestly, the influence of both the internal and external water relations of the host may be considered in host-range determinations.

INFECTION WITH BACTERIUM ANGULATUM WITHOUT WATER SOAKING

Some of the conditions aside from external environment which favor infection of tobacco with Bacterium angulatum have been frequently suggested, among these being the nutritional balance and height of topping of the crop (4), but these factors according to Clayton are not clearly separable from the modified susceptibility of the plants to water soaking (8).

The writer's experiments, first carried on with Nicotiana tabacum (var. Wisconsin Havana Seed) under greenhouse conditions and without water-soaked tissues, were often made unreliable because of the failure to secure infection in sufficient degree to form a satisfactory basis for interpretation of results. N. tabacum is relatively very resistant to Bacterium angulatum as compared with N. glutinosa L. (fig. 2). For example, a group of 46 inoculations to tobacco under a wide range of temperature (10° to 40° C.) and relative humidity (50 to 100 percent) conditions yielded only 6 plants definitely infected, whereas out of 54 N. glutinosa plants under the same conditions, 51 were definitely infected. It is significant that distinct infections on N. glutinosa were secured at both the temperature and humidity extremes, and that the atmospheric environment following spray inoculation on the whole appeared to play but a minor role in the amount of infection which developed. Pretreatment of N. tabacum for 12 hours or more in a humid atmosphere before inoculation was somewhat more reliable and effective in favoring infection than was the treatment after inoculation.

Repeated spray inoculations in the greenhouse to both Nicotiana tabacum and N. glutinosa on 27 soils (including soil from all tobacco-growing sections of the United States) differing widely in physical structure and chemical fertility yielded no results which could be definitely correlated with soil type, soil fertility, or rate and vigor of growth of the host. In 10 trials performed at different times, however, it was fairly evident that certain 2 or 3 individual soils regularly yielded plants considerably more susceptible to infection than did other soils, the reason for which could not be determined. Still other modifications, such as inoculation by wiping Bacterium angulatum over the leaf surface with cheesecloth so as to break the trichomes, failed to yield good infection. The result of all experiments of this type in the absence of water soaking was the failure to secure at will the large angular or blotchy necrotic symptoms characteristic of heavy field infection on ordinary tobacco. However, good infection of the small necrotic or incipient type may be fairly easily secured on nearly all species of Nicotiana (N. repanda is most resistant or immune) and on species of several other genera with Bact. angulatum in the absence of
water soaking. The following species in particular often yielded fair infection: Tomato (*Lycopersicum esculentum* Mill.), potato (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.) (fig. 3), jimsonweed (*Datura stramonium* L.), apple-of-Peru (*Nicandra physaloides* (L.), Pers.), cucumber (*Cucumis sativus* L.), and pokeweed (*Phytolacca decandra* L.). Other solanaceous species such as black nightshade (*S. nigrum* L.), eggplant (*S. melongena* L.), and physalis (*Physalis pubescens* L.) failed to yield even minute amounts of infection under the above experimental conditions. It is of some interest to note that the sterile hybrid of *N. tabacum* (resistant) × *N. glutinosa* (susceptible) is distinctly intermediate in reaction to *Bact. angulatum*.

**INFECTION WITH BACTERIUM TABACUM WITHOUT WATER SOAKING**

The accumulated experience and observation over a period of years indicates that *Bacterium tabacum* is a more virulent parasite than is *Bact. angulatum*, though admittedly comparative morphological, physiological, and serological studies of the two organisms indicate their close relationship (1). The toxin-producing ability of *Bact. tabacum* is, of course, a major difference, and Clayton (3) has come to the conclusion from his water-soaking experiments that this is the chief difference between the two organisms as far as infection and pathogenicity are concerned.

Observations in the seedbed and in the field have led the writer to conclude that water soaking is by no means as essential for infection and severe expression of disease with *Bacterium tabacum* as with *Bact. angulatum*. Inoculation of tobacco in the greenhouse by mild spray-
ing has also regularly shown greater virulence in *Bact. tabacum* as measured by the number of infections which develop and the size of the necrotic area. In seeking further experimental proof of this difference, tobacco leaves were inoculated by gently wiping bacterial suspensions from cultures over the leaf surface with cheesecloth, thus breaking the leaf hairs and perhaps occasionally the cuticle. When inoculations are made in this manner *Bact. tabacum* yields heavy infection both as regards number of infections and subsequent necrosis, whereas *Bact. angulatum* yields little or none. Since it might be argued that the wildfire toxin from the culture, and not the bacteria, is responsible for the resultant symptoms, the bacteria were centrifuged out of the suspension in three changes of water, thereby removing the toxin. Wiping inoculation with the bacteria alone, evidently free from perceptible amounts of toxin, yielded almost equally numerous though slower infections (fig. 4, A). On the other hand, when the bacteria were removed by heat (the toxin according to Clayton (2) being thermostable) and the same method of inoculation employed, there were no symptoms of disease.

These results seem to indicate that, given a favorable external environment of reasonable duration, especially as regards moisture, *Bacterium tabacum* is quite able to enter the cells through wounds, or to enter the stomata and produce lesions of considerable size; whereas *Bact. angulatum* may fail completely to infect under like conditions. Furthermore, this greater virulence does not seem necessarily to be connected with the toxin-producing property of *Bact. tabacum*. It is by no means contended, however, that water soaking does not greatly facilitate infection and the rate of progress of *Bact. tabacum* in the tissues, resulting in extensive necrotic areas, though it seems clear from later experiments that the toxin itself is not of any particular advantage in causing such necroses of the tissues.

**WATER SOAKING WITH ARTIFICIAL INTERNAL WATER PRESSURE**

The physiology of water soaking of the intercellular spaces of plant tissues by water pressure is not sufficiently understood as yet to warrant extensive discussion. Some species water-soak much more readily than others, e. g., tomatoes much more readily than tobacco. Great differences in individual plants of one variety grown under like conditions may exist, and this difference seems to bear comparatively little relation to the vigor of the plant. A very stunted yellow and red slow-growing tomato, for example, may water-soak quite as easily as a larger, vigorous, rapidly growing plant.

Tomatoes are the most convenient and most susceptible plants with which the writer has worked in infection trials on internally water-soaked tissues. For that reason they have invariably been used as control plants when other species were inoculated to verify both the pathogenicity of the culture used and the favorableness of the subsequent environment under which the plants were placed. Control tomato plants not water-soaked but inoculated were also invariably used in each separate trial.

The greatest interest in connection with *Bacterium angulatum* naturally centers around ordinary tobacco as the host. Comparatively young plants with 8 to 10 leaves but with somewhat elongated internodes (which may be induced by crowding of plants on the bench)
FIGURE 3.—Lesions of *Bacterium angulatum* on pepper secured by ordinary inoculation methods. Many other slightly susceptible hosts outside the Nicotianas have been demonstrated by the same method.

FIGURE 4.—Inoculation of tobacco by the leaf-wiping method, using *Bacterium tabacum* centrifuged free from toxin (A) and *Bact. angulatum* (B). The infective power of the former in the absence of water soaking is illustrated by the chlorotic areas resulting from numerous young infections. Spots on B are due to mechanical injury from wiping.
are most convenient for the equipment used. Twenty to forty
minutes of water pressure was often required to water-soak 50 percent
or more of the leaf area. The upper leaves usually water-soak more
quickly than the older basal leaves, and the intercellular spaces nearest
the midrib commonly fill first. However, the soaking is by no means
uniform in the tobacco leaves, and the result is usually a wide variety
of angles and patterns of varying sizes scattered over the leaf surface,
which when allowed to proceed to certain degrees often bears a marked
resemblance to the shape and distribution of angular leaf spot disease
as it often occurs under field conditions (fig. 5).

If such water-soaked plants are taken out of the apparatus without
inoculation and the roots or cut end of the stems placed in water at
atmospheric pressure, transpiration at the lower humidities will
rapidly remove the excess water in the leaves, but in a saturated
atmosphere the water-soaked condition may remain up to 48 hours or
more. The uninoculated recovered plants, even though kept under
water pressure for 12 hours, show no sign of any physical internal or
external injury to the tissues. The freedom from injury may be
convincingly demonstrated by spraying water-soaked leaves of an F₁
hybrid (Nicotiana tabacum × N. glutinosa) with the virus of ordinary
tobacco mosaic. If lesions of microscopic size are present, this host
will develop marked necrotic lesions of virus infection. This absence
of injury to the leaf surface by the internal water-pressure method has
some advantages over the externally applied sprays from the point
of view of illustrating the basic facts of infection.

The plants were sprayed once or twice with a DeVilbiss atomizer
while under pressure, then set into milk bottles or Erlenmeyer flasks
in water sufficient to cover the root system or stem end and placed in
a saturated humidity chamber at about 25° C. Exposure in this
chamber for as short a duration as 3 hours was sufficient to yield
subsequent infection with some organisms on the tomato, but ordi-
narily the plants were left in the chamber until they showed signs of
infection, which incubation usually required from 24 to 72 hours.

INFECTION WITH BACTERIUM ANGULATUM IN WATER-SOAKED TISSUES

When tobacco leaves water-soaked by internal pressure are inocu-
lated with Bacterium angulatum over the entire surface, the necrotic
lesions resulting often correspond closely to the water-soaked areas,
and are usually of the irregular and angular type, resembling natural
field infection as it often occurs (fig. 6). The blackfire lesions on
leaves that are water-soaked by spraying the leaf surface (externally
applied water pressure) more rarely show the sharply angular lesions,
indicating the delimiting effect of the leaf veins on the lesion. No
significance can as yet, however, be attached to this observation.
Infections secured by the internal water-pressure method demonstrate
in a convincing manner that the organisms enter through the stomata,
in the absence of such cuticular wounding as may result from external
sprays or storms. Clayton (3) suggests that the bacteria are shot
directly into the stomata by the force of sprays or rain, and this is
borne out in part by the fact that the careful dropping of water sus-
pensions of the organism on the water-soaked area of the leaf is not as
likely to yield infection as is light spraying.
Figure 5.—A tobacco leaf from a plant water-soaked by the water-pressure method. The angular and speckled character of the lighter colored water-soaked areas is shown.
FIGURE 6.—A leaf of tobacco sprayed with *Bacterium angulatum* following water soaking by the water pressure method. The necrotic spots correspond roughly to the water-soaked areas and resemble the "epidemic" type of blackfire.
Bacterium angulatum, finding the water-filled stomatal chambers and intercellular spaces suitable for growth, multiplies rapidly and soon causes the cells to collapse, thus forming large necrotic areas (fig. 7). This action of the bacteria results in the epidemic or field type of symptom as contrasted with the small or incipient type of lesion secured from inoculations without prior water soaking. The incipient lesions may, however, also arise from the pathogen’s gaining a foothold in a water-soaked area of very small size. However, as Clayton (3) points out, for such lesions to develop, the water soaking
must be of sufficient duration to permit the organism to gain a foothold. The writer has found considerable variation in the time required for infection to occur in different host species as well as in individual plants of the same variety.

The greatly increased susceptibility of tobacco to *Bacterium angulatum* by the water-soaking method led the writer to try this organism on other hosts similarly treated. The results were most striking and unusual in many respects. The tomato plant, for example, proved much more susceptible than tobacco to the blackfire organism, and the leaves often collapsed completely in 24 to 48 hours, along with parts of the leaf petioles and younger portions of the stem (figs. 8, 9). Other solanaceous plants tried, such as potato, eggplant, and datura, were not so markedly susceptible. On the other hand, when still other plants selected at random were used, it became clear that infection with *Bact. angulatum* by this method was not limited to genera or families but spread into a wide variety of unrelated families (figs. 10, 11).

The following species in particular were strikingly infected, some almost as severely as the tomato: Rose (*Rosa* sp.), poinsettia (*Euphorbia pulcherrima* Willd.), locust (*Robinia pseudo-acacia* L.), golden flax (*Linum flavum*), hollyhock (*Lonicera morrowi* Gray), apple (*Malus sylvestris* Mill.), garden pea (*Pisum sativum* L.), marigold (*Tagetes patula* L.), geranium (*Geranium sp.*), hemp (*Cannabis sativa* L.), bean (*Phaseolus vulgaris* L.), ragweed (*Ambrosia retroflexus* L.), English ivy (*Hedera sp.*), alfalfa (*Medicago sativa* L.), clover (*Trifolium pratense* L.). Species on which infection was not secured with *Bacterium angulatum* included barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), cabbage (*Brassica oleracea* L.), stock (*Matthiola incana* R. Br.), buckwheat (*Fagopyrum esculentum* Moench), snapdragon (*Antirrhinum majus* L.), cactus (*Zygocactus truncatus*), lemon (*Citrus sp.*). Over one-half of the species selected at random from the garden and greenhouse were readily infected with *Bact. angulatum* when inoculations were made to water-soaked tissue. Inoculations

![Figure 8](image-url)
FIGURE 9.—Typical lesions of *Bacterium angulatum* on older water-soaked tomato leaf, which has not wholly collapsed.
A great variety of plants are equally susceptible after water soaking.
on leaves of these species when not water-soaked yielded no symptoms in this series of trials.

INFECTION WITH BACTERIUM TABACUM IN WATER-SOAKED TISSUES

As is well known, the artificial inoculation of tobacco with *Bacterium tabacum* on tissues that are not water-soaked yields results which are distinct from those secured with *Bact. angulatum*. Under practically all expressions of the two diseases resulting from natural field infection, it is also possible to recognize wildfire because at least some halos are invariably present, and the angular and frequently black lesions of blackfire are characteristic. However, symptoms on tobacco from the two organisms on tissues artificially water-soaked by the internal pressure method are much less distinct and it becomes more difficult and sometimes impossible to separate them on the basis of symptoms.

When tomatoes water-soaked by the internal-pressure method are inoculated with *Bacterium tabacum*, the symptoms are identical with those secured with *Bact. angulatum*. The leaf tissue collapses, turns black, and no typical halos are produced, although should the leaf tissue fail to collapse, some general yellowing of the water-soaked area may be visible. Presumably the cells are killed before the toxin has sufficient time to form and act on the surrounding tissue, and the host or conditions are not sufficiently favorable to support the continued development of the organism and its toxin at the margins of the infected area. The advantage, if any, that the toxin furnishes to the parasitism of *Bact. tabacum* appears to be entirely absent in water-soaked tissues. The only difference that the writer has noted between the two organisms on tomato is the greater virulence of *Bact. tabacum* as indicated by the shorter duration of the water soaking required for the necrotic action to take place. Attenuated (1) strains of wildfire showing no toxin production are quite as virulent as are the normal strains.

Inoculations with *Bacterium tabacum* were made on water-soaked leaves of the same species used in the *Bact. angulatum* infection trials described above. Results almost identical in symptom expression with those found in the *Bact. angulatum* tests were secured in all instances, suggesting again the great similarity and close relationship of these two organisms.

INFECTION WITH OTHER ORGANISMS IN WATER-SOAKED TISSUES

The results with the blackfire and wildfire bacteria on water-soaked tissues can hardly be subject to sound interpretation without giving some consideration to the behavior of other plant parasites, or even saprophytes, under similar circumstances. The field of investigation at once becomes too extensive to be adequately surveyed in a preliminary paper, but a limited number of trials has shown that *Bacterium angulatum* and *Bact. tabacum* are not entirely unique in the above-described respects.

The results with some of the other organisms used have not been so uniformly consistent as those with *Bacterium tabacum* and *Bact. angulatum*, perhaps for the reason that the tomato is not a favorable host plant. Some probability of contamination of inoculated test plants by other organisms, where the same incubation chamber is used simultaneously for different organisms, has also been encoun-
FiouEE 12.—A water-soaked tomato leaf inoculated with *Bacterium phaseoli*. The symptoms closely resemble those secured with *Bact. angulatum* and *Bact. tabucum*, but the progress of necrosis is much slower.
tered. Water-soaked uninoculated controls under these conditions have, however, invariably been free from symptoms. Fifteen other bacteria and two fungi have been tested, mostly in a limited way, especially on water-soaked tomatoes. Eight out of nine trials with *Bact. phaseoli* Smith on tomatoes gave definite and distinctive symptoms. The symptoms were somewhat less severe and slower in developing, but were much like those secured with *Bact. angulatum* or *Bact. tabacum* (fig. 12). Infection was also secured with *Bact. phaseoli* on potato, hemp, alfalfa, and marigold, but not on tobacco. *Bacillus carotovorus* Jones collapsed water-soaked tomatoes rapidly with symptoms readily distinguishable from those previously noted. *Bact. tumefaciens* Smith and Townsend, *Bact. punctulans* Bryan, and *Aplanobacter insidiosum* McC. yielded only small areas of blackened

![Figure 13](image-url) *Macrosporium solani* on water-soaked tomato leaf (*A*) and on leaf without water soaking (*B*).

lesions of some uncertain character; *Bact. stewarti* Smith failed to give any signs of infection on tomato in three trials.

Infection experiments with fungus parasites on water-soaked tissues have been very limited thus far. Infection with *Macrosporium solani* Ell. and Mart. and *Septoria lycopersici* Speg. on tomato was favored by water soaking before inoculation (fig. 13). The latter fungus also evidently attacked potato readily under these conditions. Less conclusive but more surprising were the results sometimes secured with common bacterial organisms not belonging in the category of plant parasites. *Bacillus coli* (*Escherich*) Migula, *Pseudomonas fluorescens* (Flügge) Migula, *B. radiobacter* Berij. and Van Deld., *B. ruber balticus* L. and N., *B. aerogenes* Kruse, and *Proteus vulgaris* Hauser produced mild necrotic areas on water-soaked tomatoes.

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* B. coli has previously been reported as attacking plant tissue. See references in Elliott, Charlotte. Manual of Bacterial plant parasites. Williams and Wilkins Co., 1930.
Other organisms, including *B. prodigiosus* (Ehren.) Flügge, *B. subtilis* (Ehren.) Cohn, and *Staphylococcus aureus* Rosenbach, failed to produce any symptoms on tomato under similar conditions. In the above group of saprophytes particularly the writer is not prepared to state that true infection was definitely secured, and it should be noted here that in no single case in these preliminary trials have Koch's postulates been carried to completion.

**DISCUSSION OF RESULTS**

It is apparent from the results presented that the infection secured with *Bacterium angulatum* on water-soaked tissues of normally immune species cannot be regarded as a form of saprophytism. The species evidently must be susceptible to attack by certain organisms, since many other species and organisms fail to respond in a similar manner to water soaking.
Since large numbers of organisms have been uniformly applied over the water-soaked areas, we know as yet comparatively little about the rate and distance of the development of the organism through the tissues. It is most likely that the progress from any one point of infection is comparatively localized, and it is obvious that the progress of the organism is limited by the area and the duration of the water soaking. The evidence taken altogether therefore goes to show that Bacterium angulatum is a comparatively weak parasite, and, as pointed out earlier by the writer (5), the significance of water soaking to infection is likely to be greatest with the less virulent parasites or the relatively more resistant hosts. It is perhaps of little consequence where the boundaries of the parasitism of organisms or the lines of immunity of the hosts are theoretically drawn. This subject resolves itself eventually into a matter of definition. It is significant, however, that these preliminary experiments suggest that a wide variety of normally immune host species may under some circumstances be temporary hosts in nature to Bact. angulatum, Bact. tabacum, and other organisms, thereby harboring them in such a manner that even the most thorough measures of sanitation and eradication of other known sources of infection may not suffice. Although there is reason to believe that the bacteria in question may persist in such host tissue in a dormant condition for a considerable time (7), the proof of this relation remains to be established. It is also possible that many of our so-called nonparasitic leaf spots in nature may be caused by organisms capable of infecting only water-soaked tissue. Isolates from such diseased tissue would naturally fail to give infection upon reinoculation by ordinary methods, and thus lead to erroneous conclusions, as suggested by Clayton (3) for the so-called nonparasitic blackfire described by Valleau (10).

It is believed that the experimental method of internal water soaking, as described, along with the external method of water soaking as used by Clayton (3), may have a wide application in furthering the present understanding of infection and progress of disease in plants. It is not unlikely that in nature a combination of both external and internal water pressure plays a role in predisposition to disease. It follows that more careful observations of water soaking as it occurs in nature, both as a result of beating rain (3) and root pressure (5), should be more generally made in relation to the epidemiology of plant diseases.

**SUMMARY**

The intercellular spaces of tobacco and other plant species were water-soaked by applying water pressure to the root system or cut stems, after which they were inoculated with Bacterium angulatum and other organisms. Tobacco is normally very resistant to infection with Bacterium angulatum, but when tissues are water soaked, either by external or internal application of water pressure, and this condition is of sufficient duration, the tissues become very susceptible to the organism. No other set of environmental conditions for infection brought about the severe or "epidemic" type of this disease.

It is shown that this situation is not peculiar to tobacco or to Bacterium angulatum and Bact. tabacum. A wide variety of plant
species becomes equally susceptible to attack by these same organisms when the tissues are water-soaked. Excellent necrosis was secured on such plants as tomato, alfalfa, bean, pea, hemp, rose, apple, locust, flax, marigold, and poinsettia. These plants are normally immune to infection with these organisms. Other plant species tried were immune in the water-soaked condition.

Other plant parasites, such as Bacterium phaseoli, not normally capable of affecting tomato, for example, are capable of causing necrosis when inoculated into water-soaked tissues of this plant. A small amount of necrotic action was also secured on water-soaked tomatoes sprayed with such saprophytic species as Bacillus coi.

So far as can be determined, water soaking by the internal water-pressure method does not wound or injure the tissues, showing rather conclusively that the bacteria enter through the stomata, and that cuticular or epidermal wounding caused by rainstorms is not a fundamentally necessary condition for heavy field infection with Bacterium angulatum and Bact. tabacum as was previously supposed.

Modifications in the present conception of parasitism and immunity as regards definition of the terms are suggested by the results secured with water-soaked tissues. It is also likely that the results may have some practical bearing upon our present understanding of the sources of overwintering of certain plant parasites, and hence may modify the present theories of applying sanitary and eradication measures of disease control.

LITERATURE CITED


