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Survival Factors of Plant Pathogenic Bacteria

M. L. Schuster and D. P. Coyne[1]

INTRODUCTION

The existence of present bacterial species is an important terminal in evolution at this point in time. Bacterial pathogens must survive or the diseases they cause would have disappeared long ago. It is this aspect of plant parasitic bacteria survival that concerns us.

Natural habitats usually do not offer bacteria the same degree of continuity of conditions as is true for agricultural crops (artificial culture). With some crops artificial culture is almost uninterrupted and perpetuation of the pathogen is no problem; other agricultural practices provide some interruption between crops but this interruption may be less important than that which exists in nature. Uniformity of crop germ plasm favors inoculum buildup and perhaps perpetuation of the pathogens.

The growth of most plant pathogens is discontinuous, either because of the seasonal effect upon the pathogen itself or because growth on the host plant is interrupted. An important hurdle for the pathogen is the bridging of any discontinuities in its environment. A successful pathogen must be able to bridge the gaps between successive crops and seasons. A sufficient amount of inoculum must be able to survive the off seasons in order to re-establish the pathogen when favorable conditions again are presented. Facultative saprophytes or facultative parasites are not handicapped as much as the obligate parasites by discontinuous growth. The main effect of growth stoppage of the pathogen is a decrease in the amount of potential inoculum. The term inoculum used in this review refers to bacteria that, when placed in suitable contact with the host, cause disease.

The relative success or failure of a plant pathogen depends in part on the amount of inoculum. In the case of bacterial plant diseases, the bacterial cells themselves are the inoculum. Pathogens, such as bacteria, which have a short disease cycle, can be expected to develop more rapidly toward epidemic proportions because of the rapid inoculum buildup from a small amount of surviving primary inoculum.

The question at this point concerns the minimum amount of inoculum necessary to initiate disease. Large amounts of primary inoculum are of little or no value if successful transmission to a susceptible host plant does not take place. Therefore, it is suggested that the

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source, exit, and transmission of the primary inoculum into new crops should also be considered. This troika is necessary for disease to occur, for survival, and for continuity of the bacterial species. Knowledge of these factors could be important in control by establishment of a "curtain" between host and pathogen. This might be a simpler and cheaper task when in the primary inoculum phase. The population levels are usually at their lowest point during off-seasons and more amenable to control. Phytopathogenic bacteria dissemination is a difficult task to prevent once the disease is established (53).

The longevity of the primary inoculum is an important feature in the success of bacterial pathogens and depends upon its ability to escape or endure adverse environmental conditions. Survival may depend upon external (physical and biological environment) factors as well as the internal makeup and form of the primary inoculum of the pathogen. The combination of these factors affects the minimum concentration of primary inoculum necessary for initiation of a disease upon re-establishment of favorable infection conditions. These are some of the points that need to be considered in survival mechanisms of plant pathogenic bacteria. Since there are about 200 different "species," variations in modes of survival are destined to occur. All of the different species cannot be included here because of limitations imposed by space and time; the actinomycetes are excluded by design (37). Different species were included to illustrate the different types and to establish basic concepts and principles.

The bacterial phytopathogens have been relegated into five genera which are aerobic non-spore-forming rods: *Agrobacterium (A.), Corynebacterium (C.), Erwinia (E.), Pseudomonas (P.),* and *Xanthomonas (X.),* Only Corynebacterium is Gram-positive; most are motile with polar or peritrichous flagella but a few are atrichous.

Some bacterial diseases are locally or generally of great economic importance, for example, vascular wilts of alfalfa (caused by *Corynebacterium insidiosum),* corn *(Erwinia stewartii, Corynebacterium nebraskense),* potato *(Corynebacterium sepedonicum),* beans *(Corynebacterium flaccumfaciens* and vars. *aurantiacum, violaceum),* solanaceous and musaceous plants *(Pseudomonas solanacearum),* fireblight of Rosaceae *(Erwinia amylovora)* and blights of cotton *(Xanthomonas malvacearum),* rice *(X. oryzae),* and beans *(X. phaseoli, Pseudomonas phaseolicola).* There are many other bacterial diseases of lesser importance. Presumably the disease syndromes could differentially affect the survivability of the pathogens, directly or indirectly.

In bacterial disease, primary inoculum sources are the various survival mechanisms adapted by the pathogens during dormancy imposed by the growth periodicity of the host plant or unfavorable periods for infection and development: winter in the temperate zones or the dry periods in the tropics. Mechanisms of survivability could be presented by use of different approaches. Phytopathogenic bacteria

do not form resting spores or structures comparable to other plant pathogens (nematodes or fungi), and remain dormant during the quiescent period in association with different animate or inanimate agencies: (1) seeds; (2) plant residues; (3) perennial plant hosts or parts; (4) insects; (5) epiphytes; (6) soil and other non-host materials. Pathogen longevity will be discussed under natural and artificial environmental conditions.

Pathologists have devoted much time and effort to the growth stage of the life cycle. It is at this period that effects of bacterial growth are most noticeable: leaf spots, blights, wilts, galls and other symptoms that result in plant losses. Knowledge of life cycles and means of survival may provide improved control measures. Presumably the vulnerable period of bacteria is commonly during low population levels. This point is usually between seasons. Primary inoculum survival which is from season to season and secondary inoculum survival refers to survival for days (long-term) and minutes (short-term) (165, 166).

The life cycle of a plant pathogenic bacterium can be divided into three phases: pathogenic, saprophytic and survival phases. Leben (165, 166) has added a fourth, "resident" to include all types of associations of microflora with healthy plants. The pathogenic, saprophytic, and resident phases are considered as growth phases, and are relegated to the secondary inoculum category; the fourth phase could be considered principally as primary inoculum. How is each phase involved in the production of surviving cells?

The research works were selected to provide a cross section of the phenomenon of survival of plant pathogenic bacteria.

PRIMARY INOCULUM SURVIVAL

Association With Seed

"The flowers of all the tomorrows are in the seeds of today" (52). As vehicles to spread new life, seeds can be enemies to the survival of man when they carry unwanted parasites. Seed is considered the ideal agency in which plant pathogens survive periods when the host is lacking. The term seed in this text will also include fruits, such as caryopses and achenes, but exclude vegetative propagules (e. g., seed potatoes).

There are a number of general reference works on seed-borne pathogens. In 1931, Orton (203) indicated that any of the bacterial phytopathogens is likely to be transmitted by seed; he listed 59 such bacterial pathogens of the 128 species then recorded. Kreitlow *et al.* (152) briefly discussed 18 seed-borne bacteria that occur most frequently or have special significance, 6 of which are supposedly controlled by growing seed in the arid West: *X. phaseoli, P. phaseolicola, P.*

pisi, P. syringae, X. campestris, P. lachrymans. In an annotated list of seed-borne pathogens, Noble and Richardson (198) included 95 species and varieties of bacteria. Orton's prediction that all bacterial pathogens are potentially seed-borne appears to be becoming reality as the roster becomes greater (12, 258, 261, 262, 263). Because of the extensive literature on the subject, Baker and Smith (17) and Baker (16) presented the essentials of the field in an expanded outline form, referring only to a few bacterial species.

Apparently, Beach (20) was the first to show accompanying transport of the common blight bacterium on bean seed in 1893. Some workers claim that the first evidence of internal seed transmission of a bacterial plant pathogen was offered in 1892 by Halsted (109) for the common blight bacterium in beans. Interestingly enough, this bacterium was demonstrated to be seed-borne before it was actually identified as *Bacillus (Xanthomonas) phaseoli* by Smith (268) in 1897. Rolfs (235) supplied better proof for internal transmission of *Xanthomonas malvacearum* in cotton seed and association with lint; another early demonstration of internal seed infection by bacteria was furnished by Clayton (48) for *Xanthomonas campestris* on cauliflower; external transport of bacterium was demonstrated by Harding, *et al.* (111). External transmission of a bacterium on seeds was initially shown by Stewart (287) for *Erwinia stewartii* on corn. Smith (270) and Rand and Cash (223) demonstrated internal seed transmission by surface disinfection and isolation of *E. stewartii* from the endosperm of kernels.

Many seeds, especially in temperate zone plants, go through a period of dormancy. The vegetative cells of bacteria are not subject to dormancy, *per se*, therefore it is important in seed transmission that their survival correspond to that of host seed viability. The occurrence of a virulent bacterium on, in, or accompanying a prescribed lot of seed is not an assurance of transmission. Various environmental and seed factors could affect success of seed transmission of pathogenic bacteria under natural conditions. Transport of a bacterial pathogen in seed is important because it is an effective means of transfer in time and space. Besides functioning as a means of survival other implications are involved.

Bacterial survival time on or in seed is an important factor in seed transfer effectiveness. Some die before seeds lose their viability, most survive as long as the seed is viable, others longer.

Seeds are viable for a much longer time than vegetative propagules. For example, Harrington (114) listed bean *(Phaseolus vulgaris)* seed dry storage viability as 22 years and maize *(Zea mays)* kernels for 37 years. Bacteria commonly remain viable longer in association with seeds than separately or in soil. The bacteria for several years can effectively transfer to the seedlings after germination.

Bacterial pathogens that infect *Phaseolus vulgaris* and other legumes serve as excellent examples of bacteria that survive in seeds

beyond the seed viability. *Corynebacterium flaccumfaciens* was found viable from 5 to 24 years (35, 116). Schuster and Sayre (262) isolated virulent *C. flaccumfaciens* var *aurantiacum, X. phaseoli,* and *X. phaseoli* var. *fuscans* from 15-year-old bean seed and *C. flaccumfaciens* var. *violaceum* from 8-year-old seed kept at 10° C. Basu and Wallen (19) found *X. phaseoli* capable of survival in seeds for 3 years at 20–35° C and Zaumeyer and Thomas (323) isolated it from 10-year-old seed. Christow (44) found *X. phaseoli* nonviable in 2-year-old seed and Rapp (225) reported that this pathogen was dead in 2-3-year-old seed and suggested aging of seed as a possible control (36). However, this phenomenon is too variable and unreliable a control method as the discrepancies on longevity of bean bacteria are apparently due to storage, moisture-temperature, bacterial species, strains and length of experiments.

The growth and development of the seed-bean producing industry in the semi-arid West was based on the relative freedom from several bacterial pathogens (38, 175): *P. phaseolicola, X. phaseoli, X. phaseoli* var. *fuscans, C. flaccumfaciens* and *P. syringae.* But the bean industry sustained disastrous economic losses as a result of severe outbreaks of bacterial diseases. The estimate was based on the compilation of direct and indirect losses by W-96 committee in justifying an inter-regional crash research program on bacterial disease of beans. The occurrence of halo blight in commercial fields (207, 257, 309) planted with western-grown seed as well as at Twin Falls, Idaho (105, 207, 309) where seed production is concentrated suggests cause and effect. A pilot 600-acre snap-bean acreage, for example, in McCook, Nebraska was devastated in 1964 by *P. phaseolicola* (257).

Halo blight of beans made a sudden appearance in the 1920's and assumed epidemic proportions almost immediately (36). The transmission of the pathogen in bean seed can be explained in its wide distribution. At the time halo blight appeared in U.S.A., a disease of kudzu vine was reported by Hedges (117) incited by the same bacterium. It is presumed that the organism was imported on kudzu vine and became an important pathogen of bean.

The importance of bacterial diseases and their relation to a healthy bean industry is offered as one of the classic examples of disease control by use of disease-free seed. Recent outbreaks of bacterial diseases of snap and dry edible beans may be attributed, in part, to favorable weather (39, 105) for halo blight and changes in races of the pathogen (105) in Idaho during the 1963–1966 growing seasons.

Three bacterial pathogens in apparently healthy bean plants from certified seed emphasize the problem of control of the bacterial diseases (303). The explanation offered by Grogan and Kimble (104) and Guthrie and Fenwick (105) was that seed contamination might occur during harvesting and processing even though the disease is rare and difficult to detect by field inspection! This may be unrealistic

based on extensive epidemics in the 1960's which imply more than rare amounts of seed infestation! This may appear aphoristic despite the fact that a few infested seeds in 100 pounds of seed may result in considerable losses in "humid" bean production areas; this was illustrated under Wisconsin conditions where a dozen seeds infected with *P. phaseolicola* per acre (0.02%) distributed at random could have promoted a general epidemic (309). Epidemiological work, especially in Canada, has shown that 0.5% seed infection level *(X. phaseoli* var. *fuscans)* can be disastrous. The recent outbreaks of bacterial diseases of snap and dry edible beans may be attributed, in part, to favorable weather and changes in races of the pathogens (105).

Sabet and Ishag (242) considered survival of *X. phaseoli* in *Dolichos* seeds of little practical significance despite the fact that 2% of seed after storage for 549 days was infected, claiming that symptoms occurring on seedlings were atypical. They were atypical for secondary infections but typical for sytemic infections; secondary spread should be possible from systemically infected seedlings, nevertheless. Brown spot caused by *P. syringae* had been isolated from Wisconsin and Idaho grown bean seed; Idaho beans did not exhibit the disease in the field. Wisconsin beans just recently became plagued with this ubiquitous pathogen (123). Sutton and Wallen (295, 296) believe that *X. phaseoli* var. *fuscans* assumed epidemic proportions in Ontario, Canada shortly after introduction of a new bean cv. Sanilac, emphasizing that a new pathogen might be introduced into an environmentally suitable region and become a serious problem. Widespread epidemics can occur when seed for large areas is localized in one area and favorable conditions for disease occur.

Various facets regarding survival in seeds need to be known. Taylor (299) found that a high proportion of bean seeds infected with *P. phaseolicola* failed to produce infected plants and this may account for very low levels of primary infection reported in the field. Taylor devised a quantitative estimation for seed infection, but no answer is available as to factors or level of infection necessary for primary infection. These must be present since 10% of infected plants arose from infected seeds (299). Therefore, of what value is detection of say 3 bacterial cells per seed if it requires 100,000 to initiate seedling infection.

Workers (105, 295, 299, 314) painstakingly devised procedures to detect one infected seed in 10,000 to 40,000 or more seeds, but the basic question regarding number of cells required to initiate seedling infection still remains unanswered. Removal of infected seed based on "discolored" symptoms did not eliminate all infected seed (299); a point not determined by Taylor was whether the "clean" seed would give rise to infected plants. The degree of success of transfer is dependent on cell load on the seed; a heavily infested seed may not germinate or gives rise to a weak seedling that dies before emergence;

P. phaseolicola is more apt to fall in this category than other bean pathogens (Schuster, unpublished). Saettler (243) reported an assay method for internally-borne blight bacteria in bean seed with the aid of a seedling injection test and claims that one infected seed in 500–1000 could be detected. Schuster, Coyne, Hoff (256) found using the watersoaking method (249) that detection of *X. phaseoli* could be illustrated at lower population levels than Saettler's injection method.

Most investigators agree that *X. malvacearum* overwinters principally in cotton seed fuzz which remains on the seed coat after the ginning process. There is, however, considerable disagreement on the occurrence of the organism in seed. Faulwetter (79) at South Carolina showed that internal infection rarely occurs whereas Rolfs (235) at the same experiment station reported up to 24% of seed internally infected from bolls artificially inoculated. On the other hand, Massey (177) reported that internal infection of seed occurs in nature but could not be demonstrated artifically. Rolfs (236) at Oklahoma, Archibald (6), Bain (13), Stoughton (289), Tennyson (301), Brinkerhoff and Hunter (30), Hunter and Brinkerhoff (127), and Hansford (110) found no evidence of the bacterium within the seed. External infections of the cotyledonary margins occur during germination of the cotton seed and this process has been shown to be influenced by environmental conditions. This is comparable to infection of cucumber seed by *P. lachrymans* (317).

Fallen bolls containing seed-cotton furnish a source of overwintering bacteria, and the volunteer seedlings that often emerge from such bolls in the spring may become a source of infection for the planted crop. Brinkerhoff (28) and Schnathorst (247) recovered viable pathogen in the seed after several years even though the pathogen lost viability more rapidly than the seed. A relatively small amount of inoculum is required to initiate an epidemic (30). Conflicting results of survival in seed may be attributed to systemic vs. non-systemic infection. The preponderance of evidence indicates that angular leaf spot is non-systemic; however, Massey (177, 178, 179) believes that it is systemic and that cotyledons are infected while still enclosed in the seed coat. According to Massey (177) the disease organism is never truly vascular but passes through the tissue external to the vascular strands or in the intercellular spaces of the cortical parenchyma. Wickens (315) claims the bacteria is on the seed as a surface contaminant on the micropyle. Acid-delinting as a control measure substantiates the premise that the pathogen is principally borne on lint (248).

Bacterial leaf blight is recognized as one of the most important diseases of rice in the Asian countries. *X. oryzae* overwinters on diseased grains stored in farmhouses as in the case of rice straw. It normally is found in the husk tissues (298, 307) and has not been detected in unhulled rice grains in Japan. It has been located in the glumes and occasionally in the endosperm in severely infected rice in

China (78). One of the most important sources of inoculum in India is infested seed (61, 280). They also reported that seedlings from infected seed were usually diseased. In Indonesia, investigations by Reitsma and Schure (229) showed that rice blight bacterium was not seed-borne.

Two bacterial diseases of corn need consideration from the standpoint of kernel infection and in survival mechanisms. These are Stewart's wilt caused by *E. stewartii* and the more recently discovered Nebraska Leaf Freckles and Wilt induced by *C. nebraskense* (260, 261). Both are seed-borne in nature (83, 130, 260, 261, 269, 270) and both invade the kernels via the vascular system and become surface contaminated from infested debris and bacterial exudate on the inner husks. Both organisms are present in the old vascular tissue of the chalazal region of the endosperm (130, 261) but there is no good evidence of *E. stewartii* in the embryo region; *C. nebraskense* on occasion was found around the embryos but under these circumstances the kernels were severely stunted and did not germinate (260, 261). The vascular elements of the pedicel terminate in the chalazal region; further progression into the kernel is by dissolution of the chalazal areas resulting in lysigenous cavities filled with bacterial ooze (83, 130, 261). Both bacterial species were still viable and pathogenic from one-year-old kernels. Low seed transmission (about 2%) of *E. stewartii* was reported when kernels were planted in sterilized soil (212, 223). Ivanoff (130), on the other hand, found no transmission to seedlings from infected seed in autoclaved and in field soil. Schuster, *et al.* (261) found that from kernels infected with *C. nebraskense* less than 1 percent transmission was realized when planted in autoclaved and untreated field soil. A logical explanation for the low amount of seedling infection from internally infected kernels was that *E. stewartii* (83, 130) and *C. nebraskense* (261) required wounding of host tissues as a prerequisite to infection. The low rate was attributed to the small percentage of infected embryos, which are the biological equivalents of infected seedlings. This can be ascribed to the anatomical structure of the corn kernel: the vascularization terminates before reaching the embryo.

Shelled corn kernels usually have the pedicels attached thus providing a protective cover for their vascular elements and the infected chalazal regions. Because of this characteristic, 10-minute treatments in mercuric chloride, 1:10,000, or 5-minute soak in tetracycline, 100 and 200 ppm, or chlorox, 15%, were ineffective in controlling *C. nebraskense* in the seed based on plate culturing. On the other hand, Rand and Cash (223) and Smith (269) decreased infection by 90 percent after a 10-15 minute soak in mercuric chloride, 1:1000, of *E. stewartii* infected kernels. Reddy, Holbert and Erwin (228) treating sweet-corn seed with chlorophol, 0.25%, or several organic mercurials 0 to 24 hours were not successful in decreasing the amount of in-

fected plants from such treated seed. The percentage of transmission, which was at times over 50% is surprising in light of the reported average transmission of 2%. Since the experiments were field conducted and infection data recorded at the canning stage, insect transmission of *E. stewartii* might have been inadvertently overlooked.

A few pathogens infect the seeds through the vascular elements *(X. campestris, X. incanae); Corynebacterium michiganense*, however, invades the tomato fruit via the vascular elements but seed contamination results during seed extraction process (22). Thus, the tomato canker bacterium accompanies the seed but apparently is independent of it. Rates of transmission to seedlings from infested seed were variable, ranging from 1 to 4% (103). Variable data on rates of transmission of *C. michiganense* to seedlings from infested seed by several workers were reviewed by Strider (293). A few diseased plants in a seed bed or plant bed can result in a high percentage of diseased plants (10, 202). Bryan (33) reported that 1 percent infected seed in the greenhouse resulted in 54% disease in seed bed in the field. Ark (10) suggested that infested seed in a seed bed act primarily to contaminate the soil as they decompose, resulting in root infection of adjacent plants. Schuster and Wagner (264) have found that *C. michiganense* can infect unwounded tomato roots, obtaining 100 percent infected plants.

That seed anatomy might be related to seed infection and transmission has already been demonstrated for two bacterial pathogens of corn. Cyclical infection characteristic of annual legumes is involved in anatomical structure of seed. Testa of the seed contain vascular elements which may be extensive in large seeds. In bean, cotton, cucurbits, and tomato the vascular tissue, the raphe, is a continuation of the funiculus. Seeds with such vascularization have favorable sites for internal transmission of pathogens. The parasites are retained in the raphe and may also invade the embryo, e.g., *P. pisi* (267), *P. phaseolicola, X. phaseoli* and other bean pathogens (217, 321). The bean bacteria may gain entrance through seed openings: hilum, micropyle, and breaks from threshing operations (104, 105, 321). The natural openings through seed coats of tomato into the space between the testa and endosperm cuticle is easily penetrated. Bacteria may enter via the vascular tissues of immature fruits and develop there: *X. incanae* (14). In most annual plants it is possible that bacterial pathogens survive non-pathogenically on or inside the testa of dry seeds comparable to saprophytic bacteria that exist epiphytically on plant parts.

The manner of germination may affect transmission of seedborne bacteria. Seeds with hypogeal germination (Gramineae, pea, sweet pea) could affect infection by *C. fascians* (15), *P, pisi* (267), and *X. translucens* (311), *C. nebraskense* (260, 261) and others. This type of germination could limit transmission of bacteria that only infect aerial parts. Epigeal germination (bean, alfalfa, beet, cabbage, carrot, cot-

ton, garden stock, lettuce, pepper, castor bean and tomato), could understandably favor transmission of seed-borne bacteria that infect the aerial parts exemplified by *X. campestris* in cabbage, *P. phaseolicola* of bean, *X. incanae* of stock, *X. carotae* of carrot, and *C. michiganense* of tomato (10), but may be effective for pathogens infecting subterranean plant organs. These characteristics for five classes of seed-borne pathogens were prepared by Baker (16). The success of seed-borne bacteria is dependent on their location on the seed, anatomical structure and type of seed germination, survivability and the bacterial species itself.

Although about 100 species of bacteria are reported as seed-borne, this section relates only to several. Omitted were many such as *C. sepedonicum*, the contagious ring rot bacterium of potatoes, which can infect tomato seed and thus play an active role in its persistence and spread (156). It is restricted to the vascular spiral and annual vessels of the xylem. Alfalfa seed grown in areas infested with *C. insidiosum* can be an important factor in survival and spread of this important disease (51). However, Cormack and Moffatt (51) made no transmission studies to seedlings but believed the seed-borne organism could be introduced into the soil.

Because of the seriousness of the problem, the American Phytopathological Society appointed a committee on Seed and Plant Materials Certification in 1950 to make recommendations on reducing seed-borne pathogens (112). Leppik (171) made a plea to the First International Congress of Plant Pathology for post-entry surveillance of introduced seed as a safeguard against importation of seed-borne pathogens. Just recently new strains of *X. phaseoli* were recovered from bean seeds from Colombia and Uganda which were much more virulent than Nebraska strains (254, 256), reiterating the concern of Leppik and other plant pathologists. A point worthy of consideration is the inherent disadvantage of localization of pathogen-free seed. Diseases developing in these areas are quickly spread throughout the distribution area. Note the experience of bean seed infections in the United States in the 1960's. Bean varieties with a narrow genetic base are more vulnerable to the pathogen, especially if seed production is confined to one area. This is especially true for snap beans because of the specific demands concerning the type and quality of beans (194, 322). Quixotically, in centralization of seed production the producer may eliminate or minimize important problems for his entire clientele. Reference is made in controlling certain diseases by use of clean seed, to the exclusion of other infection sources. This is being presumptuous since other sources of primary inoculum exist. Some of the seed-borne pathogens do persist in the soil in infested plant residues and thus serve as an infection reservoir for succeeding crops. Nevertheless, other sources for seed-borne pathogens are not required for perpetuation of the diseases.

Association With Plant Residues

This section pertains to survival in dead plant tissues. In the temperate climate, overwintering in plant tissues could be an important consideration whereas in some tropical areas the plant material may not be decomposed between successive crops. Reference will be made principally to survival of pathogens between herbaceous crops. Included will be many pathogens which cannot persist for long in the free state, but may overwinter in the soil in infested plant residues.

Corynebacterium michiganense survives in plant debris in the soil for two to three years according to Bryan (33), Grogan and Kendrick (103), Elenkov (72). Strider (292) found overwintering of the pathogen in air-dried foliages.

Although the primary inoculum source of *X. malvacearum* is infested seed, proof of the presence of the pathogen in old diseased tissue was easily determined. It persists in dry plant tissues for years (179, 235, 236, 237, 289). Trap methods proved successful in isolation when dilution cultures failed due to the predominance of secondary organisms. Supernatant resulting from crushing dry infested tissues in distilled water was poured over water-soaked leaves of susceptible plants. Another successful method was soaking seeds in the suspension for 2 to 3 hours and then germinating them in paper towelling. Estimates of the relative concentration of the bacterium in leaf tissue were determined by employing comparable samples of dry material in 10 ml water blanks. Severity of lesions and duration of the incubation served as barometers of the relative numbers of bacteria present. The angular leaf spot bacterium may frequently overwinter in the field and be the source of seedling infection in spring (313). The bacterium in infested debris buried in soil was infective until debris were throughly decomposed (29). In the arid climate of Sudan, infested debris are a threat to the next cotton crop. The pathogen is viable in infested stems exposed for 4 months to 19.25 cm of precipitation but lost its viability in 72 hours in river water (5). In Tanganyika where rainfall is considerably higher than in the Sudan, *X. malvacearum* barely survived between cotton crops on the soil surface (7); the debris were not infective between 20 to 60 days in debris buried in the field, while on the soil surface infectivity was lost between 52 and 200 days. Work on *X. malvacearum* was reviewed by Brinkerhoff (27).

Despite the fact that disease-free-seed of *P. vulgaris* is commonly employed, certain bacterial problems still exist. For example, common blight occurs where crop rotation is not practiced (193); this is based on circumstantial field evidence as summarized by Zaumeyer and Thomas (323). Hedges (118) was unable to obtain infection by planting bean seed in potted soil with buried lima bean leaves infested with *X. phaseoli* and thus attempts to simulate natural conditions in the greenhouse failed. Direct evidence that *X. phaseoli* survives at least one

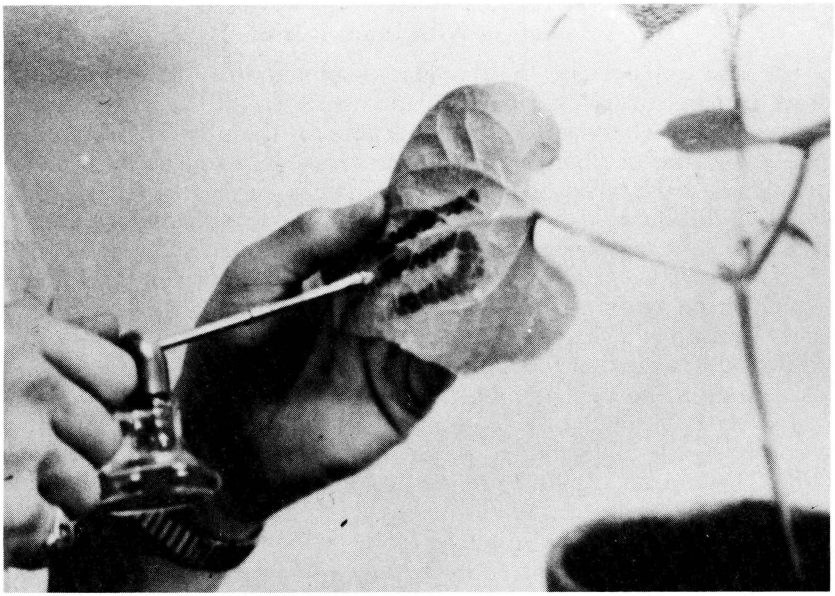

Fig. 1. Watersoaking method used for isolating from supernatant by forcing soakate through underside of bean leaf. Note the "S" where soakate forced into intercellular spaces of leaf by using an atomizer attached to air-line at 15 p. s. i.

winter in infested bean straw was established (259) by using a watersoaking technique (Fig. 1) developed for evaluating reaction of beans to bacterial diseases (249). The procedure entails excising infested bean straw and then soaking in tap water for 12 hours to permit bacteria to exit the tissues. The supernatant is then forced through the stomata of the abaxial side of the leaves. Positive symptoms appear in two weeks indicating that *X. phaseoli* remains virulent in bean straw from fall harvest to spring planting. A realistic field demonstration confirmed these findings by placement of infested straw on new ground just before land preparation and planting (250).

P. phaseolicola can overwinter in stems, pods, and leaves on the soil surface (105, 195). New virulent strains of *P. phaseolicola* survive through the winter in central Nebraska fields (257). In extensive studies, Schuster and coworkers (251, 252, 253, 255) demonstrated the importance of infested bean straw on the over-wintering of several bean bacterial pathogens *(P. phaseolicola* Race 1, Race 2, and Nebr. 16, *X. phaseoli, X. phaseoli* var. *fuscans; C. flaccumfaciens, C. flaccumfaciens* var. *aurantiacum* and *P. syringae)* (Fig. 2). Placement of infested bean straw had an important bearing on viability and/or pathogenicity of the eight bean pathogens (Table 1). In general, infested straw on soil surface favored survival compared to debris buried 8 inches in the soil. Infested straw kept below the soil surface

Fig. 2. Survival studies of bacteria in infested plant residues were conducted in sunken clay tile in Lincoln and Scottsbluff.

eliminated most of the bacterial species. Recovery during the second season, 22 months later, was not successful. In a later experiment, using 3 times more infected straw, *C. flaccumfaciens* and *C. flaccumfaciens* var. *aurantiacum* were recovered after 22 months in the field. *P. phaseolicola* Race 2 and Nebraska 16 were recovered in larger numbers than Race 1—which difference may be significant in a epidemiological sense.

Table 1. Effect of placement of infested bean straw on survival of bacterial pathogens 10 months in the field at two locations, Scottsbluff and Lincoln, Nebraska, 1968, as indicated by disease index percentage.

Bacterium	Top of soil		Buried 8"	
	SB[a]	LNK[b]	SB	LNK
Pseudomonas phaseolicola, race 1	33[c]	16	0	0
Pseudomonas phaseolicola, race 2	67	33	0	0
Pseudomonas phaseolicola, Nebr. 16	67	33	0	0
Xanthomonas phaseoli	100	83	0	0
X. phaseoli var. *fuscans*	50	50	16	0
Corynebacterium flaccumfaciens	33	67	0	0
C. flaccumfaciens var. *aurantiacum*	67	100	0	0
Pseudomonas syringae	67	75	0	0

[a]SB = Panhandle Station.

[b]LNK = Nebraska Agricultural Experiment Station, Lincoln.

[c]Disease index percent based on number and severity of infection of Red Kidney plants inoculated with water soakate from the respective items.

Greenhouse tests confirmed field experiments in that infested bean debris kept in dry condition favor survival (242, 251, 253, 255). Air-dry soil favored survival and retention of pathogenicity of the several bean bacterial pathogens as compared to the moist series (Table 2). Race 1 of *P. phaseolicola* did not survive as well as the Nebraska 16 strain under either the moist or air-dry series. Brinkerhoff and Fink (29) found that in air-dried and moist soils the difference between these two moisture levels was slight after 8 days; but *X. malvacearum* lost its viability sooner in nonsterilized soil than autoclaved soil. Under furrow irrigation in the semi-arid West, infestation with dried bean dust may be an important type of seed contamination and can occur even though halo blight is rare or difficult to detect by field inspection (104, 105). The pathogen is viable for 13 months in bean dust (104).

From overwintered soybean stubble collected in the spring of 1969 from 18 randomly selected fields in eastern Nebraska, virulent *P. glycinea* and *Xanthomonas phaseoli* var. *sojense* were recovered from 13 and 5 locations, respectively. The next season a survey indicated bacterial blight more common than pustule in eastern Nebraska although both were relatively unimportant. *Pseudomonas tabaci*, which is considered a requisite for pustule development, was not recovered from soybean stubble nor observed in the field. In field experiments, *P. glycinea* was found to field carryover in soybean straw (57, 99, 148). Graham (99) also determined that *X. phaseoli* var. *sojense* and *P. tabaci* survived the winter in infested soybean leaves on the soil surface and could initiate infection the following spring. Apparently the two main soybean bacterial pathogens can overwinter in the field in the northern and southern parts of the United States; different recovery methods were employed by the workers. Kennedy (146) found survival of *P. glycinea* was greater when in frozen infected soybean leaves than suspended in any of several substances, indicating a possible difference between the bacteria in leaves and artificial culture.

The perpetuation of *X. oryzae*, the causal agent of rice blight, from

Table 2. Effect of air-dry (0.95% W.H.C.) vs. moist (30% W.H.C.) soil on survival of bean bacterial pathogens for a six-week period at temperatures of 80–120° F. Ground infested bean straw was mixed in two soils.

Bacterium	Air-dry	Moist
P. phaseolicola, Race 1	16[a]	0
P. phaseolicola, Nebr. 16	67	33
X. phaseoli	33	16
X. phaseoli var. *fuscans*	33	16
C. flaccumfaciens	67	8
C. flaccumfaciens var. *aurantiacum*	100	50
P. *syringae*	67	33

[a]Disease index percent based on number and severity of infection of Red Kidney plants inoculated with water soakate from the respective items.

crop to crop and season to season and the sources of primary inoculum have been of main concern. In Japan, rice stubble and straw from infested fields were reported as sources of inoculum (298). According to Mizukami and Wakimoto's review (187), *X. oryzae* can easily survive until spring in dried rice straw kept in farmhouses, but the bacteria survive only one or two months when the straw is plowed in the soil. The bacterium, in piled straw protected from rain, survives until the next spring. In the warm areas of Japan, bacteria can survive in rice stubble left in the field whereas in northern Japan where the stubble withers in the winter the pathogen cannot overwinter. This is contrary to expectations. In the Philippines, *X. oryzae* was not recovered from rice stubble 18 days after the "kresek" stage when maintained in pots after removal of all but the basal 3 cm (201). The warm, humid climate of the tropics and the current trend for continuous rice cropping throughout the year favor the perpetuation of *X. oryzae* as most of the existing varieties are susceptible.

Survival studies with bacterial spot *(X. vesicatoria)* revealed the overwintering of this parasite in infested debris in tomato fields of central Nebraska (2), in Indiana (154), etc. (213). This pathogen was introduced in tomato transplants grown in the South. This disease might have prevented the establishment of a new industry in central Nebraska because of its occurrence in several pilot tomato acreages.

A comparison of two primarily vascular parasites of *Zea mays* is pertinent. *E. stewartii* was not recoverable from soil exposed to natural infection from a diseased crop (215, 223, 227). Ivanoff (130) in Wisconsin, however, isolated the bacterium from overwintered corn stubble by means of his selective medium technique developed to isolate this bacterium from the soil. In Michigan, *E. stewartii* could not be isolated after repeated plantings from the soil (83) but could be isolated on Ivanoff's selective medium from diseased corn stalks for a limited length of time but not over winter. Differences in survival may vary in different parts of the country but are differences in Michigan and Wisconsin winters sufficient to explain the results? A concerted effort to clarify this point should be made on survival of *E. stewartii* in corn residues in the northern and central states. Using different isolation techniques this statement is justified on the basis of recent work on a similar vascular disease of corn, Leaf Freckles and Wilt (260, 261). Pepper (212) and Robert (233) summarized information on Stewart's wilt of corn.

Leaf Freckles and Wilt caused by *C. nebraskense*, is similar in many respects to Stewart's wilt in that both invade primarily the vascular elements as well as the parenchyma. Both produce profuse amounts of bacterial slime in the stalks, leaves, etc. *C. nebraskense* was found to overwinter in the field in naturally infected corn residues for each of five consecutive years, 1970–1974. In controlled experiments during these years, virulent *C. nebraskense* was recovered from different plant

parts, particulary when maintained on the soil surface as well as from certain plant parts incorporated in the soil. In two previous tests, in 1970 and 1971, recovery of the pathogen was realized from infested leaves maintained on the soil surface but not at an 8 inch depth. Recovery was possible from infested stems kept at 4 and 8 inch depths. By using the water soakate and the corn plant as the selective medium, viability as well as pathogenicity were determined. Perhaps this method might be useful in clarification of *E. stewartii* survival in corn debris on or in the soil (130). Negative results with pure cultures of *C. nebraskense* are similar to those obtained with *E. stewartii* (83, 215, 223, 227).

Survival *per se* of an organism is not the complete answer. Mere survival without exit and transmission does not provide for disease induction or perpetuation of the pathogen. Proof is necessary that under natural conditions infection can result from infested residues, etc. Transmission to a susceptible host is essential to implicate the survival source. For example, corn residues infested with *C. nebraskense* serve as a source of primary inoculum under field conditions. Farming practices, conducive for disease development, were simulated in a non-infested area to demonstrate that infection can and does occur from infested plant residues. In fact, *C. nebraskense* was recovered the second season in 3 of 15 combinations, viz, stalks, cobs, and leaves maintained on the soil surface resulting in survival index of 32, 27, and 15 percent, respectively. Thus the pathogen can survive outdoors for two seasons.

Association With Perennial Host

Survival of pathogenic bacteria in perennial plants requires different conditions than in annuals upon recurrence of favorable infection periods. In the perennial host, bacteria remain in the living but perhaps dormant tissues during the off-season in temperate climates. In the tropics or where intervals between some crops are short the dry or dormant period may be of short duration or absent. Certain pathogens, such as *X. juglandis* (273), *P. mors-prunorum* (54), *X. citri* (211), *E. amylovora* (183, 214, 304), and *X. pruni* (56) overwinter principally in holdover cankers or blighted twigs, which may produce a bacterial exudate furnishing initial spring inoculum. It is significant that varieties which are most subject to fire blight blossom infection, have, as a rule, the most overwintering source of primary inoculum (183).

Although small percentages of *E. amylovora*-induced cankers were found to contain viable bacteria, it is generally agreed that a small number of holdover cankers until blossoming time is sufficient to initiate a spring epidemic of blight under proper conditions. Percentages of blight cankers harboring viable *E. amylovora* ranged from 2.0

(240) to over 50 in California. In Arkansas, Rosen (240) was unable to confirm survival in cankers and blighted twigs.

Viable *E. amylovora* in a blight canker is limited in the region immediately surrounding the periphery of the discolored margin (134). Rosen's (240) report that cankers with well-defined, suberized, and cracked margins are fully capable of maintaining the parasite over winter is a contradiction on his part.

Apparently certain aspects in epidemiology of fire blight differ between Arkansas and the more northern apple-growing regions of the U.S.A. (239, 241, 304, 305). Marked differences in climatic conditions exist between these two regions and the possible influence of the different climatic factors on the disparate induction of bacterial exudate. Survival in holdover cankers and blighted branches does not adequately explain serious occurrences of fire blight in orchards where the disease has not been noted for one to several years (141). Virulent *E. amylovora* may exist as a natural resident in healthy stems, shoots and buds of apple and pear (141) and move into newly developed shoots with or without producing blight symptoms. A serious case of fire blight of resistant pears in Northern Arkansas associated with a severe hailstorm, strongly suggested that the causal organism was internal (140, 142, 304). An explanation offered for occurrence of epidemics in orchards where the disease has not been observed is the wind transmission of aerial bacterial strands (131, 141) which might clarify the misunderstanding of *E. amylovora* survival. In a comparable situation, catkin buds of walnut provide a perennial source of infection, and when mature shed contaminated pollen; this served as an explanation for the occurrence of *X. juglandis* in epidemic form, with but a few cankers in evidence (9).

Two alfalfa pathogens, *Xanthomonas alfalfae* and *Corynebacterium insidiosum* overwinter in the host (45). Wounds are necessary for infection by the wilt bacterium *C. insidiosum* but not the other pathogen. Winter frost action and mowing provide adequate injury for entry of the wilt pathogen (135, 209). In determining longevity of wilt-infected plants, using the inner invaded vessels as guideposts, it was found that based on annual ring growth some infections had occurred 17 years before; usually infected plants succumb after the second year but apparently inoculum source in the spring can arise for many years from persistent infected alfalfa plants. This demonstrates how a non-epidemic disease could assume an epidemic character as a result of survival mechanism.

Bacterial ring rot bacterium *(Corynebacterium sepedonicum)* of potato *(Solanum tuberosum)* is perpetuated from season to season, chiefly in tubers from wilted plants (23, 71). In storage, tubers whose eyes are in contact with adjacent tubers may become diseased (282). Bruised tubers in storage favor the amount of infection by contact (23). Apparently healthy tubers from a ring rot field may carry the

pathogen and produce as high as 30% infected plants (23, 282), that may or may not wilt, and yield a crop with a high percentage of infection (23).

In many herbaceous perennials bacteria invade the dormant organs of the diseased plant, which is in this manner permanently and cyclically infected. Infected corms can provide inoculum for soft rot of calla lily *E. carotovora* (190) and leaf and corm disease of gladiola *(X. marginata)* (180). Infected rhizomes can function as survival agents for iris rhizome rot *(E. carotovora)* as can infected cuttings of *X. begoniae* (40), *X. pelargonii* (32) and bulbs for yellow rot of hyacinths caused by *X. hyacinthii* (269). *Agrobacterium tumefaciens* is disseminated into new areas on nursery stock and exit into the soil from surfaces of galls.

Epiphytes as Inoculum Sources

Although considered as poor saprophytes in natural soils, phytopathogenic bacteria manifest a diversity of facultative combinations with higher plants. Evidence is accumulating that different plant organs and different plant species sustain a characteristic epiphytic bacterial flora (164, 167, 216). These epiphytes have been found on roots (rhizoplane), buds (gemmiplane) (167) and leaves (phylloplane). Epiphytic organisms were reported initially by Burri in 1903 and Duggeli (67) and may also assume this role and survive on seed surfaces (164). In 1953, Khudzakov (151), reported the migration of epiphytes from seed to fully formed plants in the field. Epiphytic organisms may also assume this role and survive on seed surfaces. Recent investigations have stressed the occurrence of phylloplane bacteria.

Epiphytic bacteria may be a source of primary inoculum. A most interesting report was that by Hagedorn, *et al.* (108), who recovered *P. syringae* throughout the year from leaf surfaces of healthy *Vicia villosa* and associated natural outbreaks of bean brown spot with the epiphytes on nonsusceptible hairy vetch. At the same Wisconsin station, *P. syringae* was isolated from bean debris overwintering until April but not May (123).

In Nebraska, Schuster (253, 255) experienced no difficulty in *P. syringae* recovery in May and June from overwintered bean debris. The discrepancy might be in the sensitivity of recovery methods, strains of the pathogen or environmental differences. That the pathogen would have to resort to resistant nonhost plant species for its survival was unexpected. Haas (106) found under artificial inoculations *X. phaseoli* var. *fuscans* survived on the phylloplane of primary leaves of *Phaseolus vulgaris* cv Sanilac but disappeared quickly from the unifoliate leaves. Mew and Kennedy (182) made an interesting observation: different varieties of *Glycine max* differentially supported strains of *P. glycinea* epiphytically; on susceptible leaves the bacterium increased 1000-fold within two weeks but remained unchanged or

declined on leaf surface of resistant cultivars. Race specificity of *P. glycinea* correlates with the resident phase of the pathogen on the phylloplane of soybeans.

Leben and co-workers (164, 166, 167) showed that three pathogens have a resident phase of host plants, namely, *X. vesicatoria* on tomato, *P. glycinea* on soybean, and *P. syringae* on beans. The phylloplane growth of *P. mors-prunorum* is suggested as a primary factor in providing inoculum for infection of branches of stone fruit trees in the autumn (54, 55). This and another important pathogen of stone fruits, *X. pruni*, have a foliar stage that alternates with an active winter stage in the branches or stems. Although the role leaf surface infections play in the annual cycle of the disease is still obscure, fewer cells of *P. mors-prunorum* reside on leaves of resistant cherry varieties than on susceptible ones; this was substantiated using two species of bacteria on cherries and pears with survival on natural host (54). *X. citri* survived overwinter as leaf epiphytes on 17 non-susceptible weeds in citrus groves (97).

Bud epiphytes may serve as primary inoculum source; this would be especially important in perennials although they can be found in annuals (164). Goodman (91) had recovered overwintering *Erwinia amylovora* and *E. herbicola* from healthy apple buds; *E. herbicola* was inhibitory for *E. amylovora* (92).

While research of the aerosphere flora is limited, that of the rhizosphere and vicinity of roots is more extensive. Epiphytic bacteria migrate from seed to fully formed plants in the field. Certain phytopathogenic bacteria possess the singular capability of surviving in the rhizoplane or root surfaces of non-host plants. *P. tabaci* and *P. angulata* colonize root surfaces of wheat, clover, vetch and certain weeds and thus might be their overwintering sites; these organisms may persist in the soil indefinitely, apparently in association with roots. *X. vesicatoria* was found to overwinter on wheat roots, but not *P. phaseolicola* and *X. phaseoli* var. *sojense* (62).

Stanek and Lasik (281) discovered *X. phaseoli* var. *fuscans* colonizing bean roots but it disappeared after two weeks; root exudates retarded the pathogen and seed exudates stimulated its growth. Apparently an alteration in plant metabolism occurs during the transition from the cotyledonary stage to the phytosynthetic assimilation stage. Diachun and Valleau (62) initiated recovery studies from wheat roots one month after seeding and found that in aseptic tests with plants less than a week old the bean and soybean bacteria survived during the early development of the wheat. *X. malvacearum* recovered from roots of 14 different weed species in blighted cotton fields were not important in overwinter survival of the pathogen since samples collected in the winter gave negative results (276). Perhaps *X. citri* found the year around on rhizomes and roots of *Zoysia japonica*, though in low numbers, could be important in survival although the

strains on citrus and *Z. japonica* differ physiologically (97). Outdoors *X. citri* survived on the surface of *Calystegia japonica* rhizomes for six months and five months in the soil. Proof is still lacking that the rhizoplane bacteria on this common weed can provide primary inoculum for citrus infections. The question was broached as to whether the rhizoplane bacteria represent a partial and possibly transitory extension of the phylloplane phase. This might depend on the interactions of the pathogenic bacterium, host, and other microorganisms involved (89, 106, 281). Gibbins (89) believes there is a paucity of data on relationships between pathogenic and nonpathogenic bacterial epiphytes on leaves and that it is necessary to investigate mixed populations of microorganisms in the different aspects of their biology.

Association With Insects

Insects are *socius criminis* with certain bacterial pathogens in inciting plant diseases. A few bacteria have adopted a specialized means of survival and persist in a unique manner. These pathogens transmitted primarily by insects are capable of survival within the bodies of insects that transmit them. Specialized relationships have been summarized (42, 161) between the bacterial pathogens and the transmitting insects.

Since seed and soil transmission have proved inadequate to explain the prevalence and dissemination of Stewart's wilt the study of insect vectors has been a particularly fruitful field of research. The geographical distribution of this disease coincides with the prevalence of the 12-spotted cucumber beetle, *Diabrotica undecimpunctata howardi*, the corn flea beetle, *Chaetocnema pulicaria*, and the toothed flea beetle, *C. denticulata.* The dissemination of the wilt organism by means of the mouth parts of the adults of the 12-spotted cucumber beetle was not considered important as a factor in the spread of the disease (223). However, the insect harbored the organism in its alimentary tract for considerable periods of time but was not an efficient vector. Field observations and direct tests in the Maryland region demonstrated that most of the late spring and summer infection occurred by direct transfer of bacteria by corn flea beetles.

Overwintering of *E. stewartii* was based on isolations from 40 insect species (74, 215). Successful recovery of the pathogen was only from the intestinal tracts of overwintered adults of the corn flea beetle, *C. pulicaria.* Seventy-five out of every 100 of these insects from different hosts and localities contained the wilt bacterium. Robert (233) found that 10-20% of the beetles emerging from hibernation carry *E. stewartii* and up to 75% of beetles feeding on corn in midsummer may be carriers. Insects *(Phyllophaga* spp., *Diabrotica longicornis, Hylema platura)* will allow invasion by *E. stewartii* of seedlings from infected seed due to

root injury (47, 130). Since over 350 species of insects feed on corn, of which 160 cause noticeable damage (155), it is possible that insects other than those mentioned could act as transmission and survival agents.

There is an apparent relationship between winter temperatures and prevalence of the disease (107, 284, 285). Low temperatures reduce the number of pathogen-harboring insects which overwinter successfully. Haenseler (107) studied temperature records from 1910 to 1936 in New Jersey and arrived at conclusions similar to those of Stevens but found evidence of a lag period after a mild winter indicating time necessary to build disease to epidemic proportions. Disease forecasting is based on the sum of the mean temperatures for December, January and February. In mild winters (mean temperatures above 37 to 38° C) large numbers of beetles survive. Cold winters (sum of mean temperatures below 32° C) reduce the populations and limit the disease development. Pepper (212) summarized research on Stewart's wilt of corn.

C. nebraskense, causal agent of Leaf Freckles and Wilt of corn, is very similar in gross symptomology to Stewart's disease. Attempts to associate corn flea beetle *(C. pulicaria)* with overwintering of the disease proved negative (261). The similarity between the two diseases should be a challenge to further elucidate the epidemiology of the diseases. In fact LFW could become a problem in other corn growing areas if a relationship similar to Stewart's disease was established with certain insects such as the corn flea beetle.

The cucurbit wilt bacterium, *E. tracheiphila,* is completely dependent on cucumber beetles for its survival between seasons. Smith (271) concluded that striped cucumber beetle *(Acalymma vittatum)* was possibly the only disseminator of the disease organism. By careful research primary infection was shown not to be associated with soil or seed. Insects serve not only in transmission but the hibernating adult striped cucumber beetle, *A. vittatum* and the 12-spotted cucumber beetle, *D. undecimpunctata howardi,* also may harbor the pathogen overwinter in their intestinal tracts (64, 220, 221). Primary infection in the spring always originates from the feeding punctures of such overwintered beetles. The bacterium was recovered from a relatively small percentage of the overwintered beetles tested but only a small number is required to establish centers of infection for secondary spread. The dependence of the pathogen on the insects is therefore complete because without the two species of cucumber beetles there would not be any disease. Feces from infective beetles may contain virulent bacteria and serve as primary inoculum if dropped into fresh wounds (221). The overwintered adults of *A. vittatum* feed on plants of the Rosaceae and wild cucumber in the spring before migration to cucurbit seedlings (98). Thus, from these other hosts first infected, fresh inoculum source is present in their intestinal

tracts; therefore, in actuality infection of cucurbits may be from secondary spread and/or primary inoculum source. Any weather conditions that affect the abundance of cucumber beetles must also influence the prevalence of bacterial wilt of cucurbits.

The potato blackleg organism, *E. atroseptica*, can live in all stages of the seed-corn maggot, *H. platura*, and might persist by means of this insect, even though it has alternative methods of persisting in tubers and in the soil (157, 158, 159, 160). The insect intensifies the disease incidence not only because it transports the pathogen but places it into ideal infection courts in the plants. Since *E. atroseptica* is a facultative anaerobe, and cork formation is not favored in wet soils, infection is increased by wet, poorly drained soils. Leach (157, 159, 160) has demonstrated that the bacterium is present in the intestinal tracts of both adult flies and larvae. The pathogen can survive pupation which means that the adult on emergence can contaminate eggs as they are laid. *E. atroseptica* survives in the cast-out linings of the fore and hind intestines and in the lumen of the mid-intestine of the pupa. Survival in the fore and hind intestines must be entirely saprophytic. Leach (161) believes that soft rot of crucifers associated with cabbage maggot *(H. brassicae)* is in the same category as potato blackleg.

Survival of bacterial pathogens in the alimentary canals of insects is not an assurance of transmission. Beetles in the subfamily Chrysomilidae commonly regurgitate their food and have been shown to transmit plant pathogenic viruses (58, 82, 272). It is logical that in representatives *(A. vittatum; D. undecimpunctata howardi)* of this subfamily the mechanism of transmission could be by regurgitation. Other related beetles *(C. pulicaria, C. denticulata)* in the subfamily Galerucinae also regurgitate and presumably transmit internally borne bacteria during their trophic responses. Feces, regurgitated fluids, and crushed bodies were found to carry the bacteria.

It is doubtful that symbiosis is involved for the insect-pathogen relationship of bacterial wilt of cucurbits and Stewart's wilt of corn. The vectors of both are biting insects and the bacteria in plant tissues on which they feed would enter and pass through the intestinal tract. This does not connote any symbiotic relationship. In the *E. carotovora* seedcorn maggot association, the survival of bacteria in the pupa does suggest symbiosis. The association cannot be considered a parasitic type because the tissues on which the bacteria develop are cast-off tissues. Therefore, while there is an apparent symbiotic relationship, *E. atroseptica* has a favored position.

The relationship between *Daucus oleae,* olive fly, and *P. savastanoi*, cause of olive-knot disease, is in a category separate from those cases just presented. One is the entry of the pathogen into the egg through the micropyle and the other is the diverticulum of the esophagus in the pupa. The diverticulum is a reservoir for the pathogen from which the alimentary tract of the fly becomes contaminated. The

specialized organs do indicate a more highly developed association than previously reported. The olive fly is not essential to survival and transmission of *P. savastanoi* (42).

What may account for some of the variation in field incidence and severity of some of these diseases? A case in point is that strains of *E. stewartii*, for example, of different virulence (181, 222) could be a fruitful field for investigation to ascertain the effect of virulence on survival in the intestinal tract and passage through wild host plants. A similar situation may occur in cucurbit wilt (224). Indiscriminate feeders, such as the cucumber beetles, would be an interesting area for study with respect to above aspects. Other areas for study include the bacterial dosage, pathogen retention and mode of inoculation on efficiency of transmission. Does the pathogen multiply in the vector and is it symbiotic with or pathogenic for its survival host? Aposymbiosis is a method to determine symbiosis in bacterial hibernating insects by use of antibiotics. For example, antibiotics eliminated pseudomonads in maggots, especially *P. savastanoi*, the microsymbiote of *D. oleae*. Streptomycin applied to adult flies prevented larval development in olives. Thus it is possible to treat plants simultaneously against hibernating pathogens and symbiote-dependent insects (153). What is the relationship of spiroplasmas (59) and plant pathogenic Rickettsia-like bacteria and insects, in the epidemiology of disease such as Pierce's disease of grapes, etc. (81, 125)? Could the leafhopper be a winter carrier of the Rickettsia-like bacterium (90)? Auger, *et al.* (11, 12) and Mircetich, *et al.* (184) found an interrelation between Gram-positive bacteria causing Pierce's disease of grapevines and almond leaf scorch and the leaf hopper, *Draeculacephala minerva.* Mycoplasma-like organisms which resemble bacteria survive in insects.

Association With Non-host Materials

Many plant parasitic bacteria may reside in non-host materials, such as soil or plant parts. After parasitizing the plant, bacteria may by various means gain entry into the soil. Whether the bacteria survive in host parts or are free-living in the soil is of some concern in order to gain a better comprehension of the survival mechanisms. Thus, it behooves us to differentiate between survival in infested plant tissues or residues with survival in the soil. A case in point is the assumption that *X. phaseoli* (20, 250) survived in the "soil," with the exact inoculum source not ascertained.

Buddenhagen (34) categorized the role of the soil in the ecology of phytopathogenic bacteria into three groups. In one group the soil phase is a rapidly decreasing one, commonly not contributing to the perpetuation of the pathogens. Perhaps most phytopathogenic bacteria qualify in this category although data are incomplete to place

every pathogen: *E. stewartii* (130), *E. amylovora* (8), *E. tracheiphila* (220), *C. nebraskense* (261), *X. citri* (85, 169), *X. vesicatoria* (213), *X. vasculorum* (200), and *E. rubrifaciens* (244, 245). Several other xanthomonads were found to persist overwinter in natural soil, but probably in debris of diseased plants: *X. campestris, X. malvacearum, X. juglandis, X. vesicatoria* (2), *X. oryzae* (187), *X. translucens* (311), and *X. phaseoli* and *X. phaseoli* var. *fuscans* (250, 251).

Citrus canker has been one of the worst diseases ever introduced into Florida. *X. citri* underwent a rapid and continuous decline in numbers in different non-sterile soil types tested and vanished in about two weeks (85, 169, 210). Similar results were obtained with infested leaf debris.

Although Peltier and Frederich (210) and Goto (95) believe that *X. citri* gains entrance into the outer bark tissue through the lenticels and remains dormant through the winter months, the latter worker contends that the pathogen overwinters in the soil. Peltier and Frederich (210) attempted direct soil isolations on germinating citrus seeds in infested soil. Goto (96) claims that seedlings in infested soil with high *X. citri* numbers would not become infected but could easily be detected at concentrations of 10^2 cells/ml with an elaborate leaf infiltration technique. To what extent the results of the workers from U.S.A. and Japan reflected the sensitivity of the isolation method should be examined. Both Lee (169) and Fulton (85) used a very sensitive method involving inoculation of punctured leaves with soil infusion, not unlike the multineedle inoculator used by Goto. Both Lee and Fulton employed extensive and varied conditions for spread of the bacterium from soil to plants. Recovery of *X. citri* from soil under severely infected grapefruit trees or after removal of infected trees was negative (85, 169). Yet Goto (96) has no direct evidence that *X. citri* at low concentrations in soil or non-host plants serves as a source of primary inoculum in the orchards under natural conditions. The work by Lee (169), Fulton (85), and Peltier and Frederich (210) is substantiated by eradication of citrus canker in citrus areas in United States by systematic destruction of diseased grove and nursery trees and by use of strict sanitation. Inspite of the claims by Goto and co-workers *X. citri* may not possess adequate survivability to maintain sufficient populations in the soil phase for long duration, or infection is barely established from this inoculum source. Because of the contagious nature of this epidemic citrus canker it seems highly improbable that survival in soil and non-host plants could serve as primary inoculum source, providing favorable conditions for transmission and infection exist.

Corynebacteria do not survive very long in the free state but overwinter in the soil. Insufficient survival in natural soil is common for *C. sepedonicum* (23, 277), *C. insidiosum* (196), *C. nebraskense* (261), and *C. flaccumfaciens* (36). Strider (293) in his review contends that *C.*

michiganense, cause of tomato bacterial canker, can persist in soil for a long period of time; this is well documented based on recommendations throughout the world. The pathogen kept in tubes of air-dried soil persisted out of doors for five years. Circumstantial evidence of survival overwinter for several years in soil in the field is commonly reported, but there is a dearth of experimental evidence. *C. fascians* is common in the soil (132), but there is some uncertainty regarding its biology in the soil (34) as well as its taxonomic position (170).

Certain pseudomonads are incapable of persisting in the free state in natural soils for extended periods, e.g., *P. syringae* (172, 255), *P.pisi* (267), *P. phaseolicola* (172, 255, 319), *P. solanacearum* race 2, *P. tabaci* (4, 100), *P. glycinea* (99), *P. lachrymans* (41), *P. mors-prunorum*, *P. savastanoi* (161), *X. pruni*, and others. These pathogens have been liberated from the soil phase requirement as a result of associations with other survival agencies.

Another category is characterized by organisms whose numbers gradually decline when returned to the soil; their long term occurrence in the soil is host-dependent with their populations in the soil increasing or gradually decreasing according to cropping practices. Pathogens with an extended soil phase include *A. tumefaciens*, *P. solanacearum* race 1, and *E. carotovora*. This group, according to Buddenhagen (34), might be considered as "resident visitors" and the first group as "transient visitors." The pathogens, *P. solanacearum* and species of Agrobacterium, can be considered as causal agents of true soil-borne diseases, yet both are wound parasites. Host wounding, due to various agencies, is perhaps a common phenomenon.

With the exception of a few bacterial species, their survival in the soil in a free state is uncertain. One of the most successful pathogens capable of surviving in soil is the common and important *P. solanacearum* race 1. Kelman (143) has made a comprehensive review of this pathogen on 33 plant families with Solanaceae containing the largest number of susceptible species. This brown rot organism survives four to six years under bare fallow (274, 316) or survived up to 10 years in soils cropped to non-susceptible plants (238). This disease may occur in the first planting of a susceptible crop on virgin land; this has been attributed to the presence of susceptible weed hosts in the natural flora (274). A gradual decline in some soils under cultivation has been reported due to the pathogen's low tolerance to desiccation, increase in microbial antagonism, exposure to sunlight and absence of weed hosts.

However, the bacterium persists in soil for varying lengths of time. These reports have been based on the occurrence of brown rot in plants replanted in the field; the variable conclusions may be due to the divergent differences of soil factors and bacterial strains (34). The pathogen enters the plant through wounds in the roots and invades the xylem inducing wilt and possible death of the plant (144); races 2

and 3 are transmitted independently of the soil but the principal source of inoculum is the soil. Cogent data of soil factors influencing *P. solanacearum* survival are needed. Loss of viability in infested plant residues is associated with rate of desiccation of the tissues. Comparable results were obtained with persistence in potato tubers and exposure to cold storage temperature. This is not in agreement with Smith's (274) report that the bacterium can survive *in vitro* exposure to −77° C, so factors other than low temperatures in the above conditions must be involved.

Because of the dearth of good experimental evidence the complete ecology of *P. solanacearum* still remains unclear (56). It has a wider host range than any of the other bacterial plant pathogens: 200 plant species (143). Because of its host range, its occurrence as a native pathogen in indigenous plants, and its ability for adaptation are somewhat more consistent with a pathogen typified as a root inhabitant (87, 274). The distribution of *P. solanacearum* is based more on the crop disease it incites than as a species. It may be widely distributed as a harmless rhizoplane organism.

A. tumefaciens, the crown gall bacterium, which is practically universal in its distribution, is a wound parasite and is able to live in the soil long enough to infect crops the following year. The bacteria under favorable conditions are continually passed off into the soil from infected hosts. There is experimental evidence that *A. tumefaciens* can be re-isolated from artificially infested non-sterile soil without plant cover up to 669 days (206). The crown gall bacterium gradually declines in numbers in natural soils and is favored by lower temperatures, alkaline and moist conditions (63). Capability of two strains of *A. tumefaciens* to survive in field soil is important. The keto-negative strain did not survive as well in artificially infested field soil as the keto-positive strain and rapidly declined to a level at or below that of the background non-infested control soil (137). Recent workers (139, 150) question the validity of separation of three species of Agrobacterium and feel that if the genus is to be retained that it consist of one species, *A. radiobacter*, with pathogenicity indicated by a varietal epithet. Taxonomy and nomenclature of other plant pathogenic bacteria are currently re-evaluated and the re-evaluation is directed towards identification of avirulent as well as virulent strains (37, 68, 170, 302). Riker and Hildebrandt (231) conclude that the economic importance of true crown gall, except in isolated cases, is commonly very slight and that its chief value lies in its academic interest. Field observations of *A. tumefaciens* suggest long survival in the soil, but, as with *P. solanacearum* this interpretation is confounded by the extensive host range, survival in plant residues and by the presence of pathogenic strains (320) capable of independent distribution in plant material. There is, however, some evidence of prolonged soil persistence preferably in warm and moist climates. The *Erwinia* soft-rotters

may be included in the category with *P. solanacearum* and *A. tumefaciens*. Research might, however, show them to be root epiphytes.

The third ecological group of phytopathogens is typified by bacteria whose populations are for the most part produced in the soil, including root epiphytes, and whose relation to plant disease is ephemeral and irregular. These include the green fluorescent *Pseudomonas* soft rotters of plant organs in or near the soil (65, 66). This group would also include rhizoplane bacteria and the true soil saprophytes (185.) Some of the *Erwinia (Pectobacterium)* soft rotters also might be included here (308). Vorokevich (306) believed that *Erwinia* soft rotters were not capable of persisting for long in the soil, but survive in diseased plant residues until they are decomposed. Evidence on soil relations of soft rotting *Erwinia* species is conflicting (34, 149).

A common explanation for poor survivability of plant pathogenic bacteria in soil is that they are inhibited by antagonistic microflora. Brian (26) reported antibiotic production in this respect. Patrick (208) demonstrated that many soil microorganisms (actinomycetes, bacteria, fungi) are antagonistic for phytopathogenic bacteria in culture. He found a good negative correlation between the number antagonistic toward a pathogen and its ability to survive in the soil. A notable exception, *A. tumefaciens*, was explained in the stimulation zone by antibiotics in the inhibition zone in culture. Of 1200 organisms, 120 produced large inhibition zones using 28 species of plant pathogenic bacteria. It is difficult to transpose the test results from laboratory to the soil environment with its mixed microflora and unlimited chances for interactions. Addition of organic matter to soil increases other organisms and decreases *A. tumefaciens* due to antagonism (63). In mixed cultures, antagonistic bacteria decreased infection by several pathogens (37, 133, 300).

A limited study has shown that bacteriophages are common in soil association with diseased plants (53, 288). They have been held responsible for reducing bacterial pathogens in the soil. This appears highly unlikely and improbable (56). Phages are not usually important in bacterial ecology because of the improbability of finding conditions necessary for phage action to occur in the soil. The requisites for control are high bacteriophage concentrations and a very low concentration of sensitive bacterial cells. Anderson (3) reported that under favorable *in vitro* situations the lowest initial concentration of a virulent bacteriophage required to eliminate a single cell of *Salmonella typhi* was in the vicinity of 10^7 particles/ml. He concluded that in nature changes in bacterial numbers due to wholesale elimination of sensitive cells were improbable. Crosse (56) found that from soils enriched with concentrated suspensions of plant pathogenic bacteria, *P. syringae* and *P. mors-prunorum* have rarely exceeded 10^2 phage particles/ml of soil suspension. Since this yield is equivalent to the lysis

of only two or three cells, it is clear that the initial concentration of bacteriophage is very low and the chances of absorption onto sensitive cells occurring, therefore, very remote. Sutton and Wallen (295) failed to detect *X. phaseoli* in soil from infested fields and to isolate phages of this pathogen without phage enrichment; this suggested low chance of contact between phage and susceptible *X. phaseoli.* Conditions for bacteriophage to significantly effect occurrence of *X. phaseoli* strains are improbable (295). The practical application of bacteriocins would probably fall in the same category. The possible use of obligately parasitic bdellvibrios (283, 286, 288) or the predacious protozoans and free-livng nematodes might be studied with respect to their bacterial survival.

Certain bacteria can survive on non-host materials other than soil. A classic example is *C. sepedonicum* which persists on planting equipment (69, 218), harvesting and grading machinery (230), sacks, and storage bins (218) and is very resistant to heat and desiccation. Planting and harvesting equipment can be survival sites for other organisms: *X. malvacearum* (247); and *P. phaseolicola* (104). The non-host agencies should not be taken too lightly, especially with contagious organisms, such as the potato ring rot, bean halo blight, and cotton blight bacteria.

SECONDARY INOCULUM SURVIVAL

Survival of metabolically active bacteria for short duration is of less concern to plant pathologists than longer survival of primary inoculum. Both are affected by chemical, physical and microbiological factors.

Inanimate Factors

Animate and inanimate factors also affect survival of secondary inoculum. Free water is required for bacterial reproduction and locomotion. Metabolically active bacteria vary in their susceptibility to desiccation depending on species and drying conditions.

Actively growing asporogenous bacteria existing independently succumb quickly when exposed to desiccation. Jones (136) and Smith (269) found that survival of metabolically active bacteria on glass slides depended on conditions of drying. Jones found that potato soft rot bacteria died within minutes after water suspensions were dried. This was confirmed for *Pseudomonas glycinea* (166).

Relative humidity during drying affects survival. Survival of *Erwinia amylovora* was favored by 40-90% R.H. in air-borne particles (278). A number of workers have found that low relative humidities

limit reproduction of many kinds of macro- and microflora on plant surface, including phytopathogenic bacteria. Leben (164) reported that high humidity favors epiphytic growth in general.

Plant pathogenic bacteria exposed on the aerial plant parts probably are quickly decimated by the ultraviolet rays of the sunlight. Ultraviolet irradiation kills bacteria *(Xanthomonas phaseoli* var. *sojense)* on the leaf surface but not in the intercellular leaf spaces (18). The effect of UV rays is influenced by relative humidity (232). Jones (136) found soft rot bacteria sensitive to sunlight and suggested control by exposure of potato tubers to sunlight. There are numerous references on the UV effects and relative humidity on survival of bacteria but a dearth of research on phytopathogenic types exists. Other inanimate factors, temperature and chemicals at the survival site, influence survival as well as other competitive microorganisms. The interactions among inanimate factors are complex with a lack of experiments on pathogen survival.

Animate Factors

After infection and during disease development phytopathogenic bacteria are subject to the effects of other microorganisms. The pathogen perhaps is essentially free from influences of other microorganisms during the initial invasion. As the disease develops nonpathogenic microorganisms initiate decay of the lesions and the pathogens therein; this decay is more rapid when infection is in proximity to the soil. Many kinds of microflora effect, or many kinds of microfauna, ingest, bacterial pathogens. The survival time would depend on the rate of decay of the plant tissues. Interactions between bacterial species in the lesions can influence the population buildup and consequently survival (24, 25, 126) or prior inoculation with avirulent mutants may protect against virulent pathogens (92).

Research reviewed indicates that the survivability of active independent cells of pathogenic bacteria is apt to be of short duration. According to Leben (165) life expectancy in an aerial environment would be expected to be short due to UV irradiation and desiccation; bacteria washed from an active lesion on foliage probably would die quickly due to desiccation or ultraviolet irradiation or would be exposed to other competitive organisms. Relationships among epiphytic microorganisms have been discussed at a symposium (88, 216).

Survivability of metabolically active independent cells in the soil environment is likely to be short. The soil source could be from an active lesion on leaf or root. Gray and Williams (102) in their review suggested that microorganisms in the soil are inactive, by and large, because of the dearth of energy sources and unfavorable conditions. Brown (31) found that bacteria in the soil are in a state of reduced metabolism (bacteriostasis) most of the time.

DISCUSSION

Obviously, without means of distribution and the capability to exist through the winter in the temperate zone or the dry periods in the tropics, a plant pathogenic bacterium would not long survive. The common pathogens are universal, perhaps because they have succeeded in both dissemination and maintenance.

The mechanisms pathogens adopt in overwintering are few and appear uncomplicated. The survival through unfavorable conditions can be in association with animate (seeds, vegetative propagules, perennials, plant residues, insects) or inanimate (soil or non-host materials) agencies. Any one of the survival sites is not mutually exclusive for individual species. In survival in the host plants some pathogens have become so dependent that they become quite vulnerable when this association fails.

Although plant pathogenic bacteria are non-spore formers, many are usually tolerant to desiccation and survive for relatively long periods of time under dry conditions. Are these bacteria protected by some agency or material? Survival of primary inoculum in nature is in association with living or dead plant tissues and permits survival during occasional or recurrent stress (1). In some survival agencies bacterial exudate, ooze or slime has been considered as offering a protective effect. This substance is commonly found in infected seed, cankers, or in living or dead plant parts. *C. flaccumfaciens,* for example, was shown to survive about five years in "dried bacterial ooze" (115). In fact the time of survival and viability of this and other bean bacterial pathogens exceeds that of the bean seed.

Workers have studied the production of bacterial exudate, slime, ooze, or polysaccharides in culture with that in infested host plants and found them comparable: *X. campestris* (297), *E. amylovora* (120), *X. phaseoli* (80, 162, 318), *E. stewartii* (93), *E. carotovora* (80), *A. tumefaciens* (80, 121, 122), *C. michiganense* (219), *C. insidiosum* (279), *C. sepedonicum* (279), *E. rubrifaciens* (244), and *P. solanacearum* (128). Hedrick (119) reported that 16 species of pathogens in five genera, *Bacterium, Corynebacterium, Erwinia, Pseudomonas,* and *Xanthomonas,* produced exudate on media containing sucrose or glucose in basal casein hydrolysate.

Although exudates are produced by many phytopathogenic bacteria, ooze produced by *E. amylovora* has been given much attention with respect to survival. It was believed that *E. amylovora* was very susceptible to desiccation although the fire blight bacterium was capable of surviving for a long time in dried exudate (214, 312). Rosen (241) found that the pathogen survived for more than 300 days at different temperatures and relative humidities. Hildebrand (120) recovered virulent cultures of *E. amylovora* from dried exudate after 15 and 25 months, while the organism survived only 13 days in moist

exudate. This fact brings up an interesting point. In the spring when the exudate becomes moist the opportunity for infection is short indeed. It survived only 13 days in nutrient media in which capsules or slimy layers were absent around the bacterial cells (120). *E. amylovora* remains viable up to 12 months in aerial strands, another form of ooze production. Both aerial strands and ooze are composed of about four-fifths matrix and one-fifth bacterial cells. The aerial strands, because of their ease of spread by wind, could explain severe outbreaks of fire-blight not accountable to survival in holdover cankers and blighted branches.

Hedrick (119), Leach *et al.* (162), Feder and Ark (80), Corey and Starr (50), and others believe that the bacterial exudates behave as a hydrophilic colloid. Its high water-holding capacity may aid in bacterial survival during unfavorable conditions and seasons. This has been reported for *E. amylovora.* Leach, *et al.* (162) found that appreciable numbers of *X. phaseoli* cells survived in exudate for as long as 1325 days under a variety of conditions.

A common denominator favoring survival in plant residues was maintenance under dry conditions. Diseased debris associated with seed likely would be maintained dry and therefore not exposed to decomposition. Under these conditions bacterial cells may survive as long as seed remains viable. Storage of seed under different conditions of humidity may well affect rate of debris decomposition and consequently length of survivability. Along this line Kennedy, Laurence and Venette (147) found that at higher moisture levels death of naturally infected seed was reduced to 50 percent before pathogenic bacteria could no longer be detected. Residues on or near the soil surface favored survival over incorporation in the soil. By inference, the pathogens are protected in dry undecayed residues from antagonistic microflora (208) and voracious bacterial feeders among the microfauna which include the protozoa (291) and the free-living nematodes (204). Moisture is necessary for movement of these microfauna. Estimates for protozoan and nematode consumption amounted to 9×10^{14} bacterial cells/M^2 and about one ton of bacteria hectare/year, respectively. These microfauna, which multiply rapidly and are attracted to bacteria in greater numbers in decaying plant tissues, might be, therefore, involved in bacterial survival. The problem is to keep bacteria in an active state. Usually moisture may be the factor that disappears and bacteria assume a "dormant" state. Thus, control may be realized by maintaining the survival site in a moist condition which is possible under irrigation systems. *P. solanacearum* and *A. tumefaciens* both survive better under moist conditions and for this reason are "true" soil pathogens.

In addition to living plants providing special sites, the debris of dead plants may provide loci more favorable than the soil matrix. Lucas (174) showed that even within dead materials different sites

may exist, and that the morphology of an individual piece of straw may influence the species colonizing it. After some decomposition of colonized materials in soil some species may disappear while others, both fungi and bacteria (294) continue to locate a specially favorable site for survival in the resulting humus. It has been suggested that this may be due in part to protection afforded by organic matter and Record and Taylor (226) indicate that this medium may help protect bacterial cells against adverse conditions. The rate of decomposition of infested plant parts may have a bearing on longevity. For example, rapid drying of bacterial slime seems to be important since *X. malvacearum* tends to disappear from decaying tissue (29). Virulent *C. nebraskense* was recovered with greater facility and for a longer period of time from corn plant parts less amenable to decay. Persistence of organic constituents will vary in the tropics and temperate regions (46). In tropical soils organic matter is decomposed very rapidly whereas in the temperate zones it reaches a fairly stable equilibrium level; repeated addition of organic manures does not cause any permanent increase. Obviously, perpetuation of bacterial pathogens in dead residues will be differentially reduced in the two climatic zones in period of time.

Presumably some bacterial phytopathogens may have been soil inhabitants. During the process of change, due to any one of several variations resulting from mutations, etc., the free-living types may have assumed a parasitic habit and in due time became dependent on the plant for their nutritional requirements. Under natural conditions these bacteria are essentially obligate parasites since they presumably lost their saprophytic capability. It might be argued that survival and nutritional requirements are not the same. However, effect of plant host nutrition could be an area for investigation.

The root pathogens, when compared with antibiotic-producing soil saprophytes, are more susceptible to antibiotics, and thus possess a low competitive saprophytic ability. Why have the root parasites not developed an adaptive tolerance as a result of selective pressures (150)? It may be aphoristic that by escaping into plant tissues for the greater period of the year in the case of pathogens of annuals, and for longer periods for parasites of perennials, the period of exposure to the selective pressure of the antibiotic producing saprophytes is reduced. Tolerance to antibiotics would seem to help survival of a root parasite and certainly should be a helpful adaptation and likely to favor natural selection. An interesting study could involve effect of bacterial interactions on survival in synergistic and antagonistic relationships.

Survival of the fittest, to parody Darwin, was a real test for nonpathogenic survival in the soil. Burkholder (36) offered the rather erudite statement, "Bacterial plant pathogens are not soil-inhabiting organisms and are apparently unable to stand the competition in

nature." Many pathogens have a soil phase even if only one of a rapidly declining population. Soil matrix may be protective in function during survival because of its colloidal properties. The fact bacterial pathogens are asporogenous should not preclude their survivability. For Chen and Alexander (43) and Robinson *et al.* (234) found certain asporogenous soil bacteria not protected by colloids survived in extremely dry conditions for long periods; they found that a higher percentage of drought-tolerant than drought-sensitive bacteria were able to grow under dry conditions. They found that the bacteria remained viable if collected in the stationary phase and became nonviable if harvested in the exponential phase. The explanation proposed that the effect of age on the osmotic fragility and viability of the organisms may result from differences in their internal osmotic pressure. Understanding the physiological basis for their durability might shed some light on the reason most plant pathogens are poor soil inhabitants.

A criticism offered concerns the efficiency techniques to detect low population levels. A case in point is the brief that *X. citri* survives in the soil (96) despite all the earlier work to the contrary; however, the responsibility of proof that the low soil populations serve as primary inoculum rests with the critic. It is unrealistic to assume that with the exception of a few pathogens soil populations are very high. The success of *P. solanacearum* and *A. tumefaciens* is perhaps due to their wide host range and versatile nature; the latter survives in greater numbers in cool, moist soils (63), whereas many others in residues are favored by dry conditions. The basic question is why many bacterial pathogens are poor survivors in the soil.

Many refined techniques have been developed to detect surviving bacteria but the authors speculate about their relations to actual natural conditions. Jenkins, *et al.* (133) found serology more sensitive in detecting *P. solanacearum* in soil than direct isolation methods but the question unanswered is the relationship of the methods and natural infection. The partial vacuum method (94) or use of additives are very artificial. Watersoaking method or its modifications tend to duplicate nature in detecting viable as well as pathogenic cells (49, 62, 249). To ascertain survival, injury to leaves as exemplified by the leaf clip spray or root injury are required to duplicate nature for certain diseases (130, 173, 261). To approach natural conditions realistic field experiments need be performed simulating commercial planting in every way possible. To determine survival, infested residues, nonhost materials, vegetative propagules and seed, lend themselves to natural field tests. In greenhouse tests it is almost impossible to simulate population levels and environmental conditions that occur under field conditions. A question has been raised as to the validity of artificial isolation methods to detect very low levels of bacterial populations (70, 77, 130, 137, 138).

In general, what relations do epiphytes actually have on perpetuation of pathogens? A few cases of gemmiplane and phylloplane are so involved. Some pathogenic bacteria have the unusual capacity to persist on the root surfaces or in the leaves of nonhost crops and weeds. Perhaps the few pathogens that are "true soil-borne" pathogens have the proclivity of survival as rhizoplane rather than saprophytically alone. Many beliefs are based on artifacts and there is a dearth of basic studies regarding soil microbiology.

The exact nature for poor soil survival of many pathogens could be antagonistic microorganisms or they perhaps lost their saprophytic ability during change from the saprophytic to parasitic habit. There may be varying degrees of saprophytism depending on bacterial species. Possibly pathogenic bacteria can multiply in or on dead tissues from host or nonhost plants in natural habitats. The degree of success might depend on the level of nonsterility of the dead tissue. Since pathogenic bacteria readily reproduce on artificial media it seems likely that the main problem on survival is more of a case of antibiosis or poor competitive ability. Some pathogens readily survive in dry host tissue but disappear when decomposition occurs —indicating that saprophytism is not necessarily essential for long-term survival. For transmission of overwintered bacteria to occur with the onset of the growing season a happy medium must be attained whereby reactivation of pathogenic bacteria by moistening of dead tissue does not cause death of all the bacteria due to decaying organisms or stresses. Further critical study on these points may be applied to our advantage.

A criticism levelled at plant pathologists is that they cannot recognize a pathogen unless a disease is in evidence. However, Clark's (46) explanation may be more to the point, "microbial ecology has sometimes appeared to be the art of talking about what nobody really knows about in a language that everyone pretends to understand."

Critical investigations are needed concerning pathogenic variability in bacterial survival. We commonly assume that soil bacteria usually change in the direction of parasitism. Virulent isolates change to avirulence in culture. Why not in nature? But plant pathologists cannot readily recognize avirulent isolates! Differential selection pressures are in operation in nature affecting persistence of bacterial strains and species (56, 76, 77). It is possible that the greater vulnerability to antibiotics of root pathogens cf to soil saprophytes is in part responsible for their low competitive "saprophytic" ability (26). Differential persistence has been noted for races of *X. malvacearum* as a result of interaction of different host varieties (28). Buddenhagen (34) suggests that races of pathogens best adapted to off-season saprophytic survival are not necessarily the most virulent. In other words the more aggressive strains would have no advantage in the off-season survival; however, these strains during their pathogenic phase

might affect "saprophytic" survival because of their large population buildup. We have found that the more virulent strains of *P. phaseolicola* and *C. flaccumfaciens* are better adapted for survival, and that two equally virulent strains of *C. nebraskense* differed in survivability overwinter. Many factors must be involved as implied by different workers and the success of strains or species are not necessarily associated with degrees of virulence. Interactions with other biological and environmental factors complicate the problem.

Current phytopathogenic bacteria must have evolved successful survival mechanisms, otherwise they would be extinct. Bacteria have developed for many generations in environments as competitive as those experienced by any other form of life; rapid reproduction must be an important factor in their survival. It appears that through evolution bacteria have gained capabilities to grow rapidly and to adjust their enzyme activities to achieve this end. Man attempted to eliminate primary inoculum sources and had a modicum of success. The best example is the employment of eradication to control citrus canker between 1914–1927; recently this method appeared successful in California for *X. malvacearum* (248). Other general controls of primary inoculum consist of exclusion (disease-free seed, potato tubers and cuttings), cultural practices and resistant cultivars; specific examples will not be cited. Control of bacterial diseases has never been as satisfactory as for fungus diseases.

A question with regard to survival concerns the origin of plant diseases. In this regard, Hollis (124) and Tesic (302) gave considerable thought and admirably discussed the origin of different disease types based on the probability that affinities exist between types of associations. Buddenhagen (34) suggested ways in which competitive saprophytic ability of bacterial pathogens may have been altered with respect to relevance in perpetuation of the life cycle. How plant parasitic bacteria are derived is equally provocative and speculative. Presumably in some instances a pathogen may be the product of long evolution. In others, such as halo blight of beans, a root parasite of kudzu vine found beans to its liking and reached epidemic proportions in a few years (36, 117); the kudzu vine relation to bean halo blight is hypothetical and the initial origin is still undetermined.

Crosse (56) points to a localized origin of a bacterial pathogen at some distant point of time. A case very close to "home" in point of time and space is the new bacterial pathogen *C. nebraskense* Schuster, Hoff, Mandel, Lazar, 1972. The disease, Leaf Freckles and Wilt of corn, was first noted in two locations in south central Nebraska in 1969. Although the complete epidemiology of the new corn disease in Nebraska is not known, it appears to have a localized origin. The primary source of inoculum of Leaf Freckles and Wilt is corn stubble maintained on or near the soil surface; seed transmission is negligible. The cultural practices, minimum tillage and corn monoculture, had

been widely practiced in south central Nebraska for a decade before the sudden and widespread appearance of the new disease. Is it possible that the corn roots had been in a "bacterial soup" long enough for buildup of the inoculum to occur and/or opportunity of adaptation of soil bacteria or of another vascular parasite in the direction of parasitism for corn. It is possible that *Corynebacterium insidiosum*, which was a common disease of alfalfa in the area where *C. nebraskense* was first found, could have mutated or had been transformed by action of pesticides or other agents (Fulkerson, personal communication); *C. insidiosum* has been shown genetically variable by Fulkerson (84) and is very closely related to *C. nebraskense* (261). It is highly improbable that the new bacterium appeared *de novo* in less than a decade and then assumed serious proportions. Because of the narrow corn germplasm base, the pathogen, of course, was again favored (Compton, personal communication). Perhaps the organism was present as an epiphyte and/or as a parasite of green foxtail *(Setaria viridis L.)* or shattercane *(Sorghum bicolor* (L.) Moench), common weeds. Because of the novelty of the disease which is not confounded by many years of change to modified farming practices, our reconstruction of its evolution may be essentially correct.

The predominance of non-pathogenic over pathogenic associations of bacteria-higher plant relations is well established; bacteria are not endowed with a means of projecting themselves from the soil, so that bacteria resident in the soil would be those resulting from root invasion. With the countless numbers of rhizoplane bacteria, there should be numerous diseases from this source. Roots of plants during their evolution have been submerged in "bacterial soup" so long that current survivors are tolerant to most of the soil-borne bacteria, assuming that some are pathogenic. The disease via the soil may be considered the lowest in the evolutionary scale because the plant is stationary. Later the pathogen becomes adapted to aerial parts via the root or epiphytic route. Ascending the evolutionary scale order would be pathogens that survive in residues in the soil, seeds, vegetative propagules, perennial plants and perhaps those surviving in the insect.

Some phases of survival need elaboration. Certain investigations have been performed with regard to individual species, but cogent data are needed on a wider spectrum of species. The effects of host and non-susceptible crops on survival would be desirable, the latter with regard to superficial contamination on different plant parts as well as maintenance inside plant tissues. For example, *X. phaseoli, C. flaccumfaciens* var. *aurantiacum*, overwintered inside weeds (86, 253). We need to determine the relative degrees of competitive saprophytic abilities of pathogens and the substrates utilized by them. Do different bacterial strains possess the ability to colonize different plant parts? What are the active and inactive stages of survival in soil, etc?

We need to determine the precise environmental conditions in which activity and survival occur. The soil is heterogeneous, therefore, different niches must be present. What effect does cultivation have on survival in contrast to non-cultivation. Debris may differ from mineral soil matrix as a site for survival. The initial soil heterogeneity affects survival values with regard to duration and tolerance to adverse conditions and sensitivity to toxic materials. Factors inimical to survival might differentially affect the bacteria in their active and inactive phases; the period when active and inactive phases begin and end are perhaps not definitive or the same for all pathogens. Nutrient deficiencies and true competition with other organisms could operate only during the active phases; the inactive stage which has no demands on nutrients would not be deprived. Antibiosis could operate during the active stage and possibly to some extent during the dormant stage. Senescence of inactive stages may be expected to occur in soil, etc., as in culture and thus may account for progressive decline in populations. Survival in the free state in soil could be misinterpreted for bacteria may occur not as single cells but as microcolonies in a mucilaginous matrix. Long-time survival requires cells in aggregates or associated with living plant tissues in protected locations. The bacteria may assume L-forms or resting cells induced by antibiosis or unfavorable conditions (191, 205).

Bacterial cells in a state of low metabolism are more likely to survive than are actively metabolizing cells. The term, hypobiosis, refers to cells in a state of reduced metabolism (166, 172). Hypobiotic or dormant cells may survive for long periods without addition of nutrients and under physical and chemical stresses that would induce death of actively metabolizing cells. Cells have arrived at the hypobiotic state as a result of aging of diseased plant parts. Such cells would survive in dried plant parts of annuals and would represent a small proportion of the cells that were alive within the infected parts. Dormant cells differ from actively metabolizing ones in sensitivity to desiccation in the exponential growth phase than when seven days old (266) and also differed in morphology (199). Cells in protected locations of "healthy" living plant parts may be in a dormant state. The nature of hypobiotic cells requires more detailed study.

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