IDENTIFICATION, CHARACTERIZATION, AND SOIL MITE VECTOR RELATIONSHIPS OF *Pseudomonas marginata* (MCCULLOCH) STAPP

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

IDENTIFICATION, CHARACTERIZATION, AND SOIL MITE VECTOR RELATIONSHIPS OF Pseudomonas marginata (MCCULLOCH) STAPP

By

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Chairman: Dr. R. E. Stall
Major Department: Plant Pathology

Florida isolates of Pseudomonas marginata (McCulloch) Stapp, the causal organism of bacterial neck-rot and scab of gladiolus (Gladiolus hortulansus Baily) were collected and subjected to pathogenicity, physiological, nutritional, and bacteriophage-typing tests. Comparisons were made with authentic type culture isolates and data in existing literature to evaluate their usefulness of the tests for identification and characterization of naturally occurring pathogenic isolates of P. marginata.

A method was developed by which large numbers of P. marginata isolates could be rapidly evaluated for pathogenicity. Environmental factors such as presence of free-water and high temperature were found necessary for significant disease development.

Isolates of P. marginata were found to be positive for tobacco hypersensitivity when tested at high inoculum levels (10^9 cells/ml)

and at optimal bacterial growth temperatures of ca. 35 C. The non-fluorescent character of this bacterium's pigmentation was confirmed. The majority of pathogenic isolates formed a white precipitate when cultured on nutrient agar. This characteristic proved useful as a marker in mite/bacteria retention studies.

A bacteriophage was isolated from a neck-rot diseased gladiolus and found to be specific for isolates of P. marginata collected in this study. Bacteriophage typing proved useful in screening for pathogenic isolates of P. marginata. The bacteriophage was observed by electron microscopy and partially characterized.

Three species of soil-inhabiting, astigmatid mites common to diseased gladioli in Florida were studied as potential vectors of P. marginata. Anoetus feroniarum DuFour, Rhizoglyphus robini Claparede, and Tyrophagus putresentiae Schrank were observed under laboratory conditions for their feeding preference and population dynamics on selected fungal and bacterial substrates isolated from diseased gladioli. Anoetus feroniarum exhibited a preference for bacterial substrates. The hypopus stage was observed to be the most readily attracted stage of this mite's life cycle. Rhizoglyphus robini exhibited a primary affinity for forma specialis of Fusarium oxysporum, with a secondary affinity for Stromatinia gladioli. Rapid population increases were noted when A. feroniarum fed on P. marginalis and P. marginata, and when R. robini fed on Fusarium. Tyrophagus putresentiae was observed in low populations on both fungal and bacterial substrates confirming the polyphagous feeding habit of this mite in that it feeds on other things.

Anoetus feroniarum and R. robini were investigated for their ability to acquire and disseminate P. marginata. The hypopus was

considered a possible source of dispersion of P. marginata under natural conditions. Both species of mites were forcibly exposed to pure cultures of P. marginata and tested for passage of this bacterium internally and externally on their body. Neither species gave evidence of retaining viable cells longer than 5 days. The hypopus was eliminated as a source of P. marginata inocula. Dissemination by A. feroniarum of P. marginata from diseased gladioli was negative. This evidence tends to eliminate A. feroniarum as an active vector of P. marginata. It is suggested that this mite species occupies an ecological niche in which it subsists on bacterial flors. Rhizoglyphus robini, while not attracted to, nor feeding on P. marginata, was positive for a low level dissemination of P. marginata from neck-rot diseased gladioli. This species possesses mouthparts more adapted for possible infliction of wounds in soft tissues of young, newly developing cormels. Information gained in this study suggests further study of R. robini, as well as other prevalent soil-inhabiting insects and nematodes as possible short-term vectors of P. marginata in the rhizosphere of newly developing gladiolus cormels.

INTRODUCTION

A leaf and corm disease of gladiolus (Gladiolus hortulansus Bailey) was reported by McCulloch (52) in 1921. The causal organism was described and named Bacterium marginatum (Pseudomonas marginata (McCulloch) Stapp). McCulloch termed the leaf symptom of the disease, neck-rot, and the corm symptom, scab. Since then, the disease has been recognized in all areas where gladiolus is grown (3, 17, 18, 40, 56).

Neck-rot, although infrequent, is probably the most damaging phase of the disease. Upon infection under environmental conditions of free-water and warm temperatures, the parenchymatous tissue of the basal or neck region of newly emerging gladiolus leaves is subject to rapid bacterial colonization and subsequent tissue destruction. The entire above-ground portion of diseased plants may be destroyed (53).

Even though more prevalent, the scab symptom of the disease is much less damaging than that of neck-rot. Scab includes small sunken lesions on the gladiolus corm or cormel, a modified stem. While destruction of the corm is unusual, the scab lesions make the corms unsightly for consumer acceptance (87) and can be a source of inoculum for the disease (53).

Best control of the disease has been obtained by combining the practices of sanitation, timing of plantings, and careful irrigation (25,57). With a hot-water treatment (5,69) and the use

of chemical compounds as corm dip treatments (59,64,65,87), significant reductions of most gladiolus diseases were noted. This included neck-rot and scab which decreased to levels which were considered to be not economically significant (87). Hot-water treatment, and/or chemical treatment of gladiolus corms was thought to affect the survival of the organism since McCulloch (53) demonstrated that this organism was capable of surviving on stored corms for up to 9 months after harvest.

However, sporadic outbreaks of scab and neck-rot still occur, especially in areas where extended periods of rainfall result in water-logged fields. This is particularly true in the gladiolus production areas of south Florida, where in 1965-66 a severe outbreak of bacterial neck-rot was noted (83). Thus, even with use of procedures for reduction of bacterial inoculum in gladiolus planting stock, the incidence and severity of neck-rot and scab can increase to serious proportions when moisture and temperature relations are favorable.

Pseudomonas marginata has been demonstrated to over-season in soil (66). Workers (26,87) have considered the importance of soil-inhabiting insects and mites as possible factors in the etiology of the gladiolus disease. Bulb mites (various species of the genus Rhizoglyphus Fumouze and Robin) have been frequently reported in association with diseased bulbous plants (33,36,73,84). Recently, bulb mites were implicated as possible vectors of the causal bacterium (28,29). Data were presented which indicated that application of certain pesticides to gladiolus planting beds reduced the incidence of bacterial scab (27,29). The impact of this information

was such that recommendations for control of neck-rot and scab of gladiolus now include the application of soil pesticides before planting corms (27,59,60).

A vector relationship for Rhizoglyphus mites and P. marginata was suggested from research in Florida (22), but observations were only of an associative nature. An objective of this study was to investigate whether soil-inhabiting mites from areas of gladiolus production in Florida were capable of vectoring the causal organism of neck-rot and scab. Also, since conflicting reports occur in literature on the characteristics of the causal bacterium, a comprehensive study pertaining to the proper characterization of the causal organism of bacterial neck-rot and scab of gladiolus in Florida was needed. Over 40 bacterial isolates, including authentic type-culture isolates as well as isolates from diseased gladioli from Florida were collected and compared as to their pathological and physiological characteristics. Once criteria for proper identification of pathogenic isolates were determined, a series of experiments were designed to evaluate the ability of three species of soil-inhabiting mites as vectors of the bacterium, P. marginata.

LITERATURE REVIEW

Causal Organism

The causal organism of bacterial neck-rot and scab of gladiolus was identified and named Bacterium marginatum by McCulloch in 1921 (52). Bergey renamed it Phytomonas marginata (McCulloch) Bergey in 1923 and Stapp gave it the present binomial Pseudomonas marginata (McCulloch) Stapp in 1928 (20). McCulloch (53) and Stapp (78) have described this bacterium as being a short rod varying in sizes of 0.8-1.8 u by 0.5-0.6 u, aerobic, gram negative, not acid fast, and produced no spores but did form capsules. Bacteria were motile by 1-4 polar flagella which were 3 to 8 μ long. This bacterium liquefied gelatin, did not reduce nitrates, and produced slight amounts of idol, hydrogen sulfide, and ammonia. The optimum temperature for growth was reported to be 30-32 C with a maximum and minimum of 40 and 8-9 C, respectively. The thermal death point was about 53 C.

McCulloch (53) mentioned a "trace of green in some cultures after 8 weeks" in Uschinsky's solution, and a "pale, yellowish-green" after 3-4 weeks in Fermi's solution. However, in both Bergey's Manual of Determinative Bacteriology (7th ed.) (12) and the Manual of Bacterial Plant Pathogens (20) a green, fluorescent pigment produced in culture was listed as a characteristic of this bacterium. These two references have probably contributed to the confusion of whether P. marginata is a fluorescent or non-fluorescent pseudomonad. Stanier et al. (77) in

1966 received a fluorescent bacterium (ATCC-17556) labeled as P. marginata. Misaghi and Grogan (62) in 1969 published results of two bacterial isolates labeled as P. marginata and included them as fluorescent, phytopathogenic pseudomonads. They recorded the following test results for the LOPAT determinative scheme for fluorescent plant-pathogenic pseudomonads of Lelliott et al. (51): Levan-variable; Oxidase-positive; Potato Rot-negative; Arginine dihydrolase-positive; and Tobacco hypersensitivity-negative. Nitrate reduction was also recorded as negative for the two isolates tested. However pathogenicity of the two isolates was not tested. In 1970, Sands et al. (71) reported that an isolate, NCPPB-316, labeled as P. marginata, was incorrectly labeled because of nonpathogenicity and nutritional spectrum. This isolate was arginine dihydrolase-positive, oxidase-positive, hypersensitive-negative, and produced a green-fluorescent pigment. In 1970, Ballard et al. (8) reported on the taxonomy of four aerobic pseudomonads: P. cepacia (Burkholder) Starr and Burkholder, P. marginata, P. allicola, and P. caryophylli (Burkholder) Starr and Burkholder. Among other findings, they concluded that P. marginata was a non-fluorescent pseudomonad, and agreed that strain ATCC-17556 labeled as P. marginata and studied by Jessen (42) and Stanier et al. (77) was misnamed, and was really P. fluorescens biotype A. In this study, Ballard et al. (8) compared nine strains of P. marginata and eight strains of P. allicola and declared on the basis of phenotypic characterizations that the two nomenspecies were indistinguishable. They characterized these two species as being gram-negative rods and motile by polar multitrichous flagella. None of the strains produced fluorescent pigment, while all accumulated poly-B-hydroxybutyric acid (PHB) as

an intracellular reserve material. They were oxidase-positive, although some strains gave a very weak reaction. They grew at 41 C, but not at 4 C. They produced slime in mineral media containing 2 or 4% sucrose, gave a positive egg-yolk reaction and hydrolyzed Tween 80 and gelatin. None hydrolyzed starch, or PHB. None of the strains produced gas anaerobically in a complex medium containing nitrate. None synthesized the enzymes of the arginine dihydrolase system constitutively. The cleavage of protocatechuate by toluene treated cells grown with benzoate was of the ortho type. The nutritional spectra of the strains of these nomenspecies were essentially identical. They were versatile, with all strains using at least 78 of 136 substrates tested. Thus, while P. allicola was first isolated from onion bulbs in 1942 (14), Ballard et al. declared these two species synonymous, and on grounds of priority suggested P. marginata (McCulloch) Stapp as the correct specific designation.

Sands et al. (71) have developed an interim determinative scheme for the plant pathogenic pseudomonads. Four groups were distinguished, with P. marginata (allicola) being placed in group III. This group is primarily characterized as being non-fluorescent, accumulates PHB and utilizes D-arabinose, D-fucose, cellobiose, and L-threonine. A non-fluorescent pigment is present, arginine dihydrolase not present, oxidase-positive or weakly so, and utilizes mesaconate and nicotinate.

The Diseases Bacterial Neck-Rot and Scab of Gladiolus

Since the original work of McCulloch, neck-rot and scab of gladiolus have been recognized wherever gladioli are grown (3,17,18, 40,56). Her publication in 1924 (53) remains the classic description.

Bacterial Neck-Rot

Of the two phases of the disease, neck-rot can be the most damaging. Upon entry through natural openings or wounds, P. marginata is capable of colonizing and destroying parenchyma tissue in the basal or neck region of the gladiolus. Lesions may appear on any part of the leaf, but are usually confined to the fleshy, basal neck region. Tiny specks which are bright reddish-brown, and slightly elevated are the first visible symptoms of the disease. Upon enlarging and coalescing these spots produce necrotic areas in which all of the parenchyma tissue may be destroyed. The fibrovascular bundles are not primarily affected and stand out prominently in the sunken areas. Disease development is usually on the lower parts of leaves, from ground level up to a height of 6 to 8 in. Plants may often have only slight exterior symptoms of disease, but a surprising amount of rot when leaves are pulled apart. While the vascular systems of the leaves seem not to be directly affected, eventually the vessels become blocked with a brownish gum-like substance and the above parts of the leaves die. In case of severe disease, the whole plant falls over, bending at, or near, the ground level. In this stage of the disease the basal parts are dull brown, fibrous, and soft-rotted, or dry-rotted, depending on the amount of moisture present.

Bacterial Scab

The scab symptom of the disease occurs on the modified stem, the corm (Fig. 1). While corms from gladioli affected with neck-rot are not always diseased, they often show definite lesions on both husks and body of the corm. In the early stages the husk lesion is pale yellow or brown, circular, oval, or sometimes an

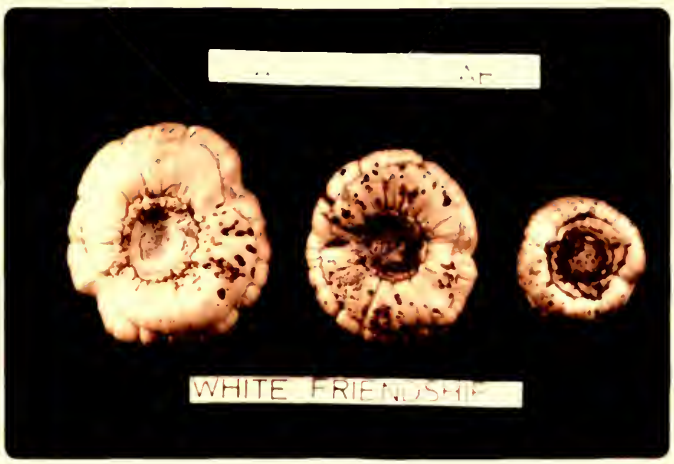
elongated streak. The lesion becomes almost black with the husk often splitting longitudinally and disintegrating at its base. The ragged margins have the texture and color of burned tissues. In many cases, there is a direct relation between husk and corm lesions with a corm lesion occurring just below the husk infection.

Corm lesions begin as pale yellow, water-soaked circular spots. The epidermis shows no visible injury and all tissues are firm. The color darkens, varying on corms of different hues, from light yellow-brown, to dark brown, to almost black. Eventually, the corm lesion becomes a shallow depressed pit surrounded by a definite and somewhat elevated margin. These depressions are usually only 2 to 6 mm in diameter, but when numerous they often coalesce and form large, irregular sunken areas. These pits do not extend deeply into the flesh of the corm but are rather easily removed, leaving a clean, saucer-shaped cavity lined with healthy cells. The formation of periderm or cork cells beneath the lesion are thought to wall off any further lesion development. While this disease does not usually result in the destruction of the corm, the bacterium is capable of overseasoning in the scab lesions, thus providing a source of inoculum for infection of newly emerging shoots.

Incidence of Neck-Rot and Scab in Florida

While not considered economically serious during the months of greatest gladiolus culture in Florida, neck-rot and scab of gladiolus has been epidemic (57), even though the recorded incidence of neck-rot and scab is sporadic. According to records kept by the Division of Plant Industry and Consumer Services of the Florida Department of Agriculture, approximately 70 cases of the gladiolus

Fig. 1. Gladiolus corms of 'White Friendship' exhibiting bacterial scab caused by Pseudomonas marginata.


WHITE FRIENDSHIP

disease incited by P. marginata have been reported from 1956 to October, 1970. Wehlburg (83) stated that neck-rot has been regularly reported from north Florida, but was not a problem in south Florida until 1966. During the 1965-66 season several fields in south Florida suffered over 50% loss. This severe loss was correlated with frequent rainfall and unusually high temperatures during the fall growing season.

Control of Bacterial Neck-Rot and Scab

The most common bactericidal compounds used on plants contain copper, to which the gladiolus is extremely sensitive. Preventative sprays, then are not completely effective in controlling foliar bacterial diseases of gladioli (60). Control of bacterial neck-rot is based on cultural techniques. In Florida, recommendations (60) include using spray pressures under 200 lb/sq. inch, avoidance of overhead irrigation, and delaying of flower spike cutting and cultivation when plants are wet. Morsodren (2.2% methylmercurydicyandiamide) a mercury containing bactericide, has been the only compound available for use as a foliar spray treatment of gladiolus plants after flower cutting and topping procedures.

Control measures for scab of gladiolus have consisted largely of pre-planting corm treatments with various mercury compounds. McCulloch (53) believed that gladiolus corms disinfected and planted in clean soil would yield a scab-free crop. She recommended immersion of corms for 2 h in mercuric chloride (1-1000) or Formalin (1-80). Miles (61) in 1933 reported that Calogreen, a form of mercuric chloride was the most efficient disinfecting agent for the scab organism. Mercury containing compounds such as Emmi,

Calomel, and New Improved Ceresan have been tested, recommended, and used as pre-plant corm dip treatments. However, these compounds can no longer be used (58).

The development of a hot-water treatment (5,69) provided a method for reducing disease organisms and insects carried in or on gladiolus cormels. However, this method was only efficient when fully dormant, preconditioned cormels were submerged in water at 135 F for 30 min. Larger corms could not be effectively treated. Bald (5) stated that virus diseases and P. marginata were not eliminated by heat treatment, and Jefferson et al. (41) noted that plants from hot-water treated cormels were more susceptible to scab. They reasoned that either the 135 F did not eliminate the scab organism, or that the cormel micro-flora was so reduced that natural antagonists of the scab organism were not present in numbers sufficient to retard or control P. marginata. In certain cases, combinations of chemical treatments plus hot-water treatments were tried. Magie (60) recommended a 3 h soak in streptomycin (500 ppm) before the hot-water treatment.

In 1954 Young (87), in Oregon, reported increased control of scab by treating corms with fungicide-insecticide combinations. The addition of 20% aldrin was thought to control wireworms which injured gladiolus corms and provided infection courts for the scab organism. This was the first work which indicated that the scab organism might be spread by soil insects. Shortly thereafter, Forsberg (26), in Illinois, reported scab control was associated with control of white grub (Phyllophaga spp.). This was accomplished by placement of aldrin under corms in the planting furrows.

Subsequently in a series of experiments Forsberg (27,28,29) reported that use of various pesticides was correlated with reduced scab incidence. This indicated that P. marginata was being transmitted by soil insects of unknown identity.

In 1959, Forsberg (28) reported the presence of bulb mites, Rhizoglyphus echinopus, on scabby corms, but none were found on clean corms. In addition, P. marginata was said to have developed on PDA plates inoculated with bulb mites. Severe scab developed on plants grown in soil infested with the cultured mites. Only a few minor lesions developed on plants in soil to which a broth culture of P. marginata was added. The bulb mites were all dead 5 days after 20% aldrin granules were scattered over the agar surface.

In 1965, Forsberg (29) reported on experiments which indicated that R. echinopus eliminated the fungal organism Stromatinia gladioli in soil, but spread the bacterial scab organism P. marginata. These conclusions were based on the following: (1) the development of only a few minor scab lesions when soil was infested with P. marginata alone, (2) Stromatinia rot developed on corms in steamed soil, artificially infested with cultures of S. gladioli, (3) gladioli in steamed soil artificially infested with P. marginata, S. gladioli, and bulb mites, resulted in only scab symptom development on new corms, and (4) gladiolus growers in Illinois who use aldrin or heptachlor insecticides experienced a reduced incidence of scab, but an increase in amount of Stromatinia rot on new corms. Since then, Forsberg and Appleby (30) have continued evaluating newly developed pesticides for their efficacy in controlling scab. Their results indicated that scab was effectively controlled when Dasanit, Disyston,

Dyfonate, and Thimet were used. However, the pesticide Temik, even when applied at double rate, was ineffective in reducing scab incidence.

Relationship of Soil-Inhabiting Mites and Bulb Crops

The relationship of soil-inhabiting mites and various bulb crops has been questioned for many years. McCulloch (53) in her original description of bacterial scab, stated that "the part played by insect transmission of the disease also needs to be considered." In a few cases she observed mites in connection with lesions of husks and corms, and stated that "mites had made perforations in dark husk spots and small burrows in the corm spots." She concluded that "if mites are able to penetrate normal gladiolus tissues, they probably aid in distributing the infection."

Hodson (36) in 1928 found Rhizoglyphus echinopus associated with bulb crops. McDaniel (55) in 1931 reported that R. hyacinthi (Banks) preferred solid, healthy, gladiolus corms, and avoided wet, decayed corms. This mite fed on and "inoculated" healthy corms with bacteria inducing rot. Gambrell (32) in 1934 reported R. hyacinthi in association with diseased gladioli. In 1937 Garman (33) published a bulletin on the study of the bulb mite R. hyacinthi. In 1951 Andison (3) in British Columbia reported R. echinopus and R. solani causing economic damage to bulbs of narcissus and lily. In 1952 and 1956 Bald and Jefferson (6,7) in California related injury to gladiolus with the root mite R. rhizophagus, while Jefferson et al. (41) in 1956 reported R. solani as a primary pest of gladiolus. Following the previously discussed report of Forsberg (29) in 1965, Wiese and Meyer (84) in 1967 reported R. echinopus in amaryllis and

narcissus bulbs in South Africa. Schread (73) in 1969 discussed the bulb mite R. echinopus, and described mites laying eggs on the surface of bulbs, in injured and decaying tissue, and between bulb scales. He stated that "mites chew their way into bulbs and roots, destroying tissue as they advance."

Mite Observations in Florida

Engelhard (21) and Engelhard and Overman (23,24) evaluated chemical treatments for control of mites, nematodes, and fungi in gladiolus corms and on corm debris in the sandy soils of south Florida. In 1969 Engelhard (22) reported that three mite species (Histiostoma sp., Tyrophagus palmarium, and a mite of the R. solani group) attacked bulb crops grown in Florida. He suggested that Rhizoglyphus mites might be important vectors of the scab organism. In 1971 Poe (68) reported three species of mites (Lasioseius subterraneous, Rhizoglyphus robini, and Anoetus feroniarum) as part of the microfauna of diseased gladiolus corms. The Rhizoglyphus mites were found feeding on decaying tissues on corms and in neck areas. Anoetus appeared in association with moist lesions on corms, but rarely on necrotic areas of the neck. Lasioseius mites were observed preying upon other mites and considered to be a predator species.

Thus, species of Rhizoglyphus, Tyrophagus, and Anoetus mites have been implicated, or suggested as possible vectors of the scab organism. However, these observations have been only of associative nature. While both Rhizoglyphus and Tyrophagus species have been cited as crop pests or contaminants of stored food products (28, 38, 75), Woodring (85) considers Rhizoglyphus mites as saprophytic scavengers feeding on rotting tissue, bacteria, or fungi.

Taxonomically Rhizoglyphus, Anoetus, and Tyrophagus mites all belong to the class Arachnida, subclass Acari, order Acariforms, and suborder Astigmata. The Astigmata are divided into two superfamilies, and are a fairly homogenous assemblage of slow-moving, weakly sclerotized mites which range in size from 200 to 1500 μ long. They have evolved to a non-predaceous existence and may be considered a highly advanced group. Respiration is considered integumental throughout the suborder, although traches-like ducts have been observed in some species. These mites are cosmopolitan and have achieved success as fungivores, saprophages, predators, graminivores, and animal parasites (49).

The superfamily Anoetoidea contains a single family the Anoetidae or slime mites. This is the family in which Anoetus feroniarum DuFour is taxonomically positioned. Anoetids are distinguished world-wide and are characteristically found living in highly organic, wet substrates. While certain species live completely submerged in water, the majority of these mites live within a thin water film and are thought to feed on Microorganisms present in their semi-aquatic habitats (39). Decaying vegetable or insect cultures have been sources of rapidly increasing anoetid populations.

The superfamily Acaroidea contains 12 families which include both free-living and phoretic species. The family Acaridae contains a large assemblage of saprophagous, graminivorous, fungivorous, and phytophagous species which may be found from extremely wet to fairly dry habitats, generally feeding on organic debris of plants or animals. Both Rhizoglyphus and Tyrophagus species previously mentioned belong in this family (49).

Mite Life Cycle Characteristics

The life cycle of the free-living mites of the Supercohort Acaridia "usually consists of the egg and larval stage, followed by at least two nymphal stages (protonymph and tritonymph) before reaching the adult stage. However, the life cycle often includes the formation of a third nymphal stage, the deutonymph or hypopus. Structurally, this form of the mite is heavily chitinized, dorso-ventrally flattened, and bears one or two convex dorsal shields. The mouth parts are reduced and non-functional. The hypopus is a non-feeding migratory form resistant to, and apparently formed as a result of environmental stress (49,85). They are transported, or will migrate, to areas of higher nutritional levels and lower environmental stresses before completion of life cycle (67,82). However, R. echinopus is reported to form the hypopal stage regularly, independent of environmental conditions (82).

Schread (73) observed eggs of R. echinopus hatching in 7 days, and described both immature and adult mites as being white with a faint, yellowish tinge. The legs and mouthparts were brown, with a pinkish hue. Weise and Meyer (84), in South Africa, determined life cycles of 17-27 days at temperatures of 60-75 F, 9-13 days at 70-80 F, and torpor at the low temperature of 55-60 F and high temperature of 95 F. Garman (33) reported life cycles from 9 days at 80 F, to 27 days at 60 F. Female mites were recorded as living for 1-2 months, and laying as many as 100 eggs each.

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF BACTERIA ISOLATED FROM NECK-ROT AND SCABBY GLADIOLUS CORMS IN FLORIDA

Pathogenic isolates of the neck-rot and scab organism were needed for this research. Leaf, husk, and corm tissue were collected from diseased gladioli and a series of bacterial isolates were obtained. These isolates were compared with authentic cultures of P. marginata in regards to pathogenicity, physiological, and phage typing tests. These tests were conducted to (1) substantiate previously cited results pertaining to isolates of P. marginata; (2) to determine any characteristics that might be suitable for use in identifying pathogenic isolates of P. marginata; (3) to utilize significant characteristics as possible markers in mite-vector studies; and (4) to determine species uniformity or variability that might affect taxonomic schemes important for identification.

Materials and Methods

Source of Isolates

From a series of bacterial isolations from diseased gladioli, 33 isolates were selected as test isolates. For comparative purposes authentic isolates of P. marginata were obtained from the International Collection of Phytopathogenic Bacteria (ICPB), Davis, California; American Type Culture Collection (ATCC), Rockville, Maryland; and the Florida Type Culture Collection (FTCC) of Bureau of Plant Pathology, Division of Plant Industry and Consumer Services,

Florida Department of Agriculture, Gainesville, Florida. Other isolates of various bacterial species also used in comparative tests were obtained from stock cultures maintained by Dr. R. E. Stall, in the Plant Pathology Department, University of Florida, Gainesville, Florida (Appendix 1).

Maintenance of Isolates

All isolates were maintained in sterile distilled water in screw-capped tubes at 10 C. The isolates were examined at 6-month intervals for viability and purity by streaking onto plates of nutrient agar (NA).

Determination of Bacterial Concentrations

Cultures of test isolates Br-1, F-1, and S-D were shaken for 18-24 h in nutrient broth (NB) and centrifuged at 2000 g for 5 min. The pellets were resuspended and diluted with sterile, 0.42% buffered saline (Oxoid Buffered Saline A) to give bacterial suspensions with light transmittance values of ca. 25, 40, 50, and 75% as determined with a Bausch and Lomb Spectronic 20 colorimeter set at a wavelength of 600 nm.

The number of viable bacterial cells in these suspensions was determined by a dilution plate technique. Bacterial suspensions were diluted by adding 0.05 ml to 0.45 ml of 0.42% buffered saline (BS) and diluted in 10-fold series up to 8-10 times. Appropriate dilutions were poured over dry NA plates and incubated for 48h at 30 C. Three replications were poured for each dilution. Colonies were counted and the numbers converted to cells/ml values. Average values were calculated from the three replicates.

Determination of Optimum Growth Temperature

Twenty-five ml of NB were dispensed into 125 ml Erhlenmeyer

flasks which had been adapted with side-arm glass tubes for replacement into a Bausch and Lomb Spectronic 20 colorimeter. Before inoculation, the NB flasks were allowed to equilibrate in incubators set at specific temperatures to be tested. Flasks of NB were inoculated with 0.5 ml of 10^8 cells/ml of bacterial isolates, Br-1 or F-1, and placed on shakers in incubators held at three temperatures.

Growth of bacteria was determined at regular intervals by turbidometric methods. Each flask was tilted so that the side arm was filled with the bacterial suspension before being placed into the colorimeter for turbidometric determination. This was done by measuring light absorbance at 600 nm wave-length being passed through the bacterial suspension.

Pathogenicity Tests

Gladiolus pathogenicity tests

Inoculum for pathogenicity tests was prepared by culturing each isolate in BN for 24 h and then centrifuging at 2000 g for 5 min. The resulting pellet was resuspended in BS and diluted to give a reading of 50% light transmittance at 600 nm with a Bausch and Lomb Spectronic 20 colorimeter. This gave a final concentration of approximately 1 X 10^8 viable cells/ml. From this suspension other inocula were made by concentration or dilution.

Different inoculation methods were tried. One method consisted of infiltrating suspensions of bacteria into intercellular areas of the gladiolus leaf at the neck region by hypodermic injection using a syringe fitted with a 27-gauge needle. The inoculated area was sometimes outlined with a felt-tip pen. Another method consisted of dusting the leaves with 600 mesh carborundum and then rubbing with a cotton swab saturated with inoculum of 10^8 cells/ml.

Gladiolus plants growing in a greenhouse bench were used in the first tests. Later, pot-grown plants were used so they could be placed in a moist chamber.

Gladiolus cultivars used in pathogenicity tests included Beverly Ann, Spic and Span, Traveler, and White Friendship. Before planting, all gladiolus corms were soaked for 15-30 min. in a 50% wettable powder suspension of benomyl fungicide (75% methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate) at a rate of 2 gr/0.8 liter water.

Relative humidity in the moist chamber was kept at 100% and free water was deposited on the leaves by means of a mist cycle of 3 min. in every 10 min. Time in moist chamber was varied with experiment. After removal the plants were maintained on a greenhouse bench at ambient moisture and temperature ranging from 23-27 C at night to 28-32 C in the day.

The inoculated plants were examined daily for up to 10 days for neck-rot disease symptoms (Fig. 2). Several parameters were used to describe disease development: (1) days for inoculated tissue to first darken, (2) days to first sign of disease beyond infiltrated area and (3) days required for collapse of inoculated leaf. Each plant was dissected by peeling off each successive leaf until the infloresence stalk was exposed and observed in conjunction with the newly developing corm. Observations as to location and extent of disease symptoms were made. A rating system was devised in which the number of days 1-7, were assigned values of 6 to 0. Leaves in which symptoms advanced beyond the outlined infiltrated zone by one day were rated 6; and those that showed no symptom advance

Fig. 2. Gladiolus plants of 'White Friendship' exhibiting symptoms of neck-rot caused by hypodermic infiltration of 10^7 cells/ml of Pseudomonas marginata F-1. Note the water-soaking and browning of leaves resulting in the eventual collapse of plant at the neck region.

Fig. 3. Tobacco leaf showing hypersensitive reaction (HR) induced by inoculation of four isolates of Pseudomonas marginata. Inocula was 10^8 cells/ml and incubation temperature was 30 C. Note the incomplete HR induced by isolates 1 and 2.

Fig. 4. Tobacco leaf showing hypersensitive reaction (HR) induced by inoculation of four isolates of Pseudomonas marginata. Inocula was 10^8 cells/ml and incubation temperature was 37 C. Note the incomplete HR induced by all four isolates.

at the end of the 7th day were rated 0. Thus, each leaf was rated on a scale of from 6-0, and the average of three leaves per replication was calculated. These data were subjected to an Analysis of Variance and treatment significance was noted at the 5% level. Treatment means were tested by the Duncan's Multiple Range Test and significance at the 5% level noted.

Onion pathogenicity tests

Some of the bacterial isolates included in this study were tested for pathogenicity to onion, Allium cepa L. cv. Burpee 6906-2 (white bunching onion sets).

The method followed was that used by Kawamoto and Lorbeer (43). Approximately 0.5 ml of inoculum of 10^8 cells/ml of each bacterial isolate was injected into the lacunar cavity of young onion leaves. Inoculated plants were placed on the greenhouse bench and observed daily for disease symptoms on inoculated leaves.

Tobacco Hypersensitivity Tests

In all experiments, unless otherwise noted, bacterial cultures were shaker-grown for 18-24 h and then centrifuged at 2000 g for 5 min. Inoculum was standardized as described for pathogenicity tests.

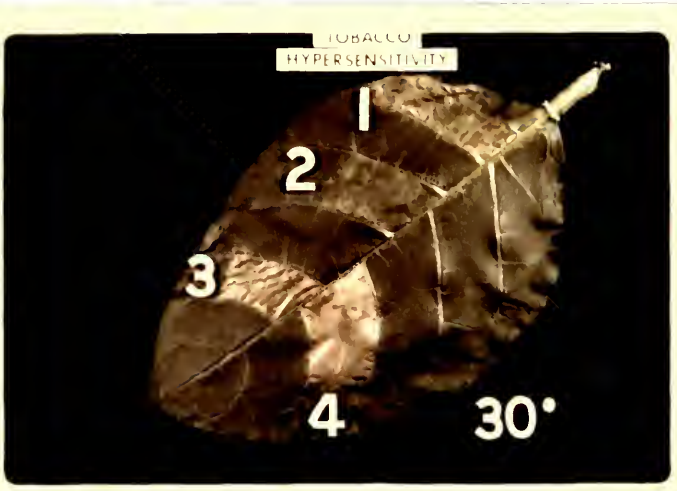
A hypodermic syringe fitted with a 27-gauge needle was used to infiltrate intercostal areas of fully expanded leaves of tobacco, Nicotiana tabacum L. 'F_2C_1', or 'Samsun'. In initial experiments the inoculated tobacco plants were maintained in a greenhouse at ambient temperatures. In subsequent experiments the test plants were placed in Sherer Model CEL 4-4 growth chambers at specific constant temperatures. In the growth chambers, a photoperiod of 12 h illumination

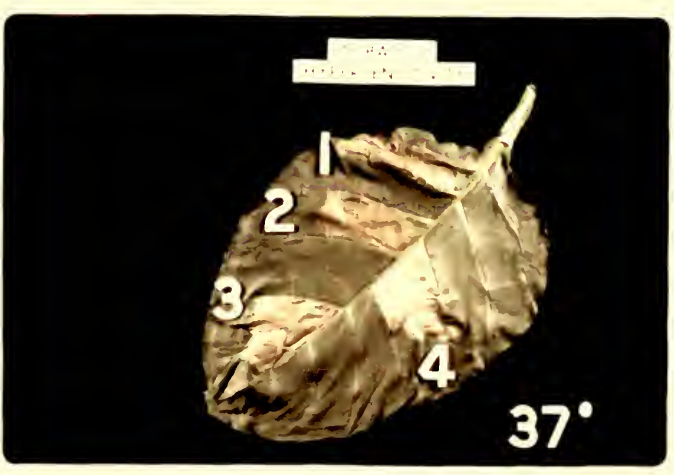
with 8-20 watt cool-white fluorescent lamps was used. Plants were watered twice daily to avoid any moisture stress.

Inoculated areas were examined periodically up to 72 h after inoculation. Various degrees of necrosis occurred. Incomplete hypersensitivity (±) was used to describe the reaction if all of the inoculated area did not collapse and become necrotic. If all of the inoculated area collapsed and became necrotic, complete hypersensitivity (+) was used. If only a chlorosis appeared, this was considered a negative hypersensitive reaction (-) (Fig. 3 and 4).

To help assess the degree of necrosis in inoculated leaves, electrolyte loss from inoculated leaf tissue was determined. Ten discs of infiltrated leaf tissue were removed with a cork borer 15 mm diameter and placed in 20 ml of sterile distilled water. The discs were held under the surface of the water by means of a plastic screen. Immediately, the electrical conductivity of each sample was measured with a Yellow Springs Instruments Model 31 conductivity bridge. Each sample was then subjected to a vacuum of 22 inches Hg for 2 min. and then mechanically agitated for 1 h at room temperature. At this time conductivity was again measured and the value recorded was the difference between the first and second measurements.

Bacterial populations in the leaf tissue were determined by removing five discs per leaf with a cork borer 4 mm diameter, crushing the leaf discs in tubes of 1 ml of BS, and running a 1:10 dilution series as described previously. Bacteria in the dilution series were poured over dry NA plates and the colonies counted after 48 h of incubation at 30 C. Since 62.5 mm^2 of leaf area was added to 1 ml of BS, data were recorded as cell numbers per ml.


TOBACCO
HYPERSENSITIVITY
1
2
3
4
30°


1
2
3
4
37°

Bacteria Size and Flagellar Arrangement

Twenty-four h NB cultures of isolates Br-1, F-1, and S-D were kept stationary for 4 h. Samples were carefully removed with a Pasteur pipette, and a drop of each culture was spotted on individual 200 mesh formvar and carbon-coated copper grids. After blotting each sample was negative stained for 30 sec. with one drop of 2% phosphotungstic acid (PTA). After blotting and drying at room temperature, each sample grid was examined using a Phillips 200 transmission electron microscope. The position and number of flagella were observed and counted. Sizes of bacteria were approximated using the magnification table supplied by Phillips Service. Measurements of ± 5% accuracy were obtained and recorded.

Physiological Tests

Hugh-Liefson test

The medium used was that of Hugh-Liefson (37). Tubes of media, covered with 1 cm of sterile mineral oil, were stab-inoculated with loops of bacterial suspensions from 24-48 h NA cultures. Erwinia carotovora (Jones) Holland 70-1 was used as a positive check. Inoculated tubes were incubated at 30 C and observed for color changes 48 h after inoculation. A color change from blue-green to yellow was considered a positive test for fermentative utilization of sucrose.

Production of fluorescent pigment

Plates of medium B (KMB) of King et al. (46) were used to detect production of a water-soluble, yellow-green fluorescent pigment. Bacterial isolates on NA master plates were transferred to KMB plates by replica plating (50). Plates were incubated for 24-48 h at 30 C and examined under long-wave ultra-violet illumination. Questionable isolates were later streaked onto individual KMB plates and re-examined.

Production of white precipitate in nutrient agar media

Upon observing that several isolates produced a white precipitate (WP) when growing, all bacterial isolates were tested for this character. Cultures of each isolate in NB for 24 h were streaked onto separate NA plates and incubated at 30 C. Plates were examined 24-96 h after inoculation for WP formation in the NA around the bacterial growth.

A second method consisted of transferring, by replica plating (50), small numbers (3-5) of single, discrete bacterial colonies to NA plates. After incubation for up to 96 h, these plates were examined for WP formation around each bacterial colony (Fig. 5).

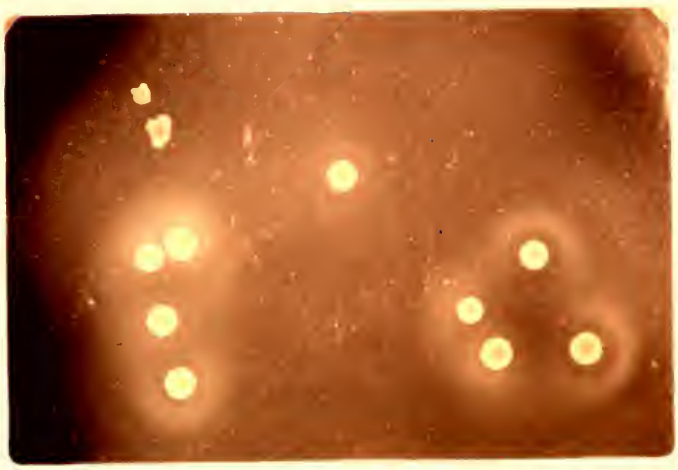
Levan production

Plates of NA containing 5% sucrose (51) were inoculated with bacterial isolates from NA master plates by replica plating. P. lachrymans (Smith and Bryan) Carsner 69-1 was included as a positive check. Questionable isolates were later streaked onto individual levan plates and re-examined. Large, white, dome-shaped, mucoid colonies were interpreted as positive production of levan.

Oxidase reaction

The method of Kovacs (48) was used to determine the presence of oxidase. Determinations were made by smearing bacterial growth from 24-48 h NA cultures with a loop onto Whatman's No. 2 filter paper soaked with tetramethyl-p-phenylenediamine reagent. Cultures showing deep purple within 10 sec were recorded as positive. Those showing color within 60 sec. were recorded as weak, and those showing no color as negative.

Fig. 5. Yellow-cream colonies of Pseudomonas marginata F-1 cultured on nutrient agar. Note the presence of white precipitate in medium surrounding bacterial colonies.



Potato activity and soft rot tests

Several methods were tested in an effort to accurately ascertain soft rot activity of the isolates. In all tests E. carotovora 70-1 was used as a positive check.

In the first method transverse slices, approximately 5-6 mm thick, were cut from fresh, washed, surface sterilized, unpeeled potato tubers with a sterile knife. A potato slice was placed in a sterile petri dish on filter paper moistened with sterile, distilled water. Inoculations were made by smearing copious amounts of bacteria from 24-48 h NA or KMB cultures onto the surface of the potato slice. After incubation at 30 C, the slices were visually examined for signs of tissue maceration.

In the second method a series of four wells, 3 mm in diameter, were made in each potato slice, as prepared above, with a metal microtiter transfer loop. This produced four cavities per potato slice which were inoculated with 0.05 ml of 24 h NB cultures of various bacterial isolates. Incubation was at 30 C and the slices were examined at 24 h intervals.

In the third method potato discs, 16 mm in diameter, were cut from washed, surface sterilized, unpeeled potato slices. Eight to 10 discs were placed per sterile petri dish containing filter paper moistened with sterile, distilled water. Each disc was inoculated with a loopful of bacteria from 24 h NA cultures. Incubation was at 30 C, and observations made at 24 h intervals.

Pectolytic activity

Sodium polypectate gels of three pH levels, ca. 5.0, 7.0, and 8.4, were prepared by the method of Hildebrand (34). One loopful (0.05 ml) of 24 h NB shake-cultures of each bacterial isolate was

spotted, four per plate, on the gel surfaces. After incubation for 24 h at 30 C, the gel plates were examined for pit depressions. Pit sizes in mm were recorded by measuring the pit radius beyond the bacterial colony margin.

Arginine dihydrolase

Thornley's arginine medium 2A (80), minus agar, was used to test for the constitutive presence of the enzyme, arginine dihydrolase. In order to detect the formation of alkali from arginine anaerobically, the broth medium was covered with mineral oil before it was inoculated with bacteria from a 24-48 h NA culture. A color change from orange to red was recorded as a positive test.

Nitrate reduction

Tubes containing 5 ml of NB plus 1% KNO_3 were inoculated with bacteria and shaken for 36 h. Tests for the presence of nitrites were performed using modified Griess-Ilosvay's reagents (76). One ml of 0.6% (w/v) solution of dimethyl-d-naphthylamine and 1 ml of 0.8% (w/v) solution of sulfanilic acid, both in 5 N acetic acid were added to each tube. The development of distinct pink, or red, within 30 min. was recorded as a positive test. Negative tubes were further tested by the addition of powdered zinc. If, indeed, the nitrate was not reduced by the bacteria, the addition of zinc allowed for reduction of nitrate to nitrite.

Growth in dL-B-hydroxybutyrate as sole carbon and energy source

Bacterial isolates were cultured in the chemically defined medium utilized by Stanier et al. (77). This consisted of a standard mineral base, with $(NH_4)_2SO_4$ at a low concentration of 0.2 g/liter, and furnished with dL-B-hydroxybutyrate as sole carbon and energy source.

A duplicate series of isolates were cultured in NB as a check against isolates which would not grow in dL-B-hydroxybutyrate.

Accumulation of poly-B-hydroxybutyrate (PHB)

Utilizing the methods of Burdon (13), bacterial smears on alcohol cleaned slides were air-dryed, heat fixed, and stained with an alcoholic solution of Sudan Black for 5-10 min. The excess dye was removed by rinsing in xylene. After drying the smears were counterstained with 0.05% safranine for not more than 10 sec. at which time the stain was washed with distilled water. Each isolate was examined microscopically at 100X magnification under oil immersion for the intracellular presence of dark purple-blue stained bodies indicative of poly-B-hydroxybutyrate (PHB) accumulation (31). A bacterial isolate, labeled P. alboprecipitans Rosen FTCC-1869, was used as a positive check, and P. lachrymans 69-1 was used as a negative check.

Phenol cleavage test

The determination of ortho, or meta cleavage of a phenol (16) by bacterial test isolates was accomplished by the method described by Stanier et al. (77). Test isolates were individually streaked onto plates containing 0.2% p-hydroxybenzoate as a growth substrate. The 48 h growth of each isolate was removed from the PHB medium, suspended in 0.02 M tris buffer at pH 8.0, toluenized, and 2 ml samples mixed with 20 μ moles of catechol. Positive checks, P. cichorii (Swingle) Stapp 72-8 and P. lachrymans 69-1 were also tested in this manner. Toluene and distilled water were included as negative checks.

Since none of the samples changed to a bright yellow, indicating meta cleavage, each tube was then incubated for 1 h on a

shaker at room temperature, and then observed after 12 h for development of "an unusual deep purple" Rothera reaction. The development of this color is considered strong presumptive evidence of ortho cleavage (45).

Nutritional tests

Selected substrates from the work of Ballard et al. (8) in differentiation of P. cepacia Burkholder, P. marginata, P. allicola Starr and Burkholder, and P. caryophylli Starr and Burkholder were tested for utilization by isolates of P. marginata. The standard mineral base (SMB) of Misaghi and Grogan (62) was used. The non-nitrogenous organic compounds D-(-)-tartrate, glycollate, levulinate, and mesaconate were tested as single sources of carbon and energy. Each of these compounds was tested in SMB containing 0.1% (w/v) ammonium sulfate. Nicotinate and trigonelline, compounds containing both carbon and nitrogen, were added singly to the SMB to test for their utilization as sources as sources of both carbon and nitrogen. Control plates, lacking a carbon or nitrogen source, were prepared from SMB. The YDCB medium of Misaghi and Grogan (62) was used as a complete medium. All organic substrates were used at concentration of 0.1% (w/v). All test media contained 1.5% (w/v) Ion agar No. 2 (Colab), and was autoclaved and adjusted to pH 7.2 before plates were poured.

In the tests for utilization of compounds, bacteria were transferred to plates by the replica-plating technique of Lederberg and Lederberg (50). NA master plates, hand patched with not more than 12 bacterial isolates per plate, were incubated for 48 h. Each master plate was used for inoculation of not more than nine test media,

including an initial check plate of SMB, and a terminal YDCB plate as a check on whether patches had been transferred properly. Plates were incubated at 30 C and scored visually for growth after 24-48 h. Growth on the compounds were tested at least three times with each isolate. Sands (71) observed that carry-over of nutrients on the replica-plating cloth sometimes allowed a small amount of growth. Therefore, all plates were compared to the initial mineral base plate. Only growth exceeding that on the mineral base plate was considered positive. Some isolates were rated for growth 7-21 days after inoculation.

Antibiotic sensitivity

Lawns of P. marginata isolates Br-1 and F-1048B were prepared by the double-layer plating method (2). After solidification of the top layer, antibiotic impregnated Multi-disk (Colab) sensitivity discs were placed on each of the lawn surfaces. Plates were incubated for 24 h at 30 C, and then examined for clear zones indicating inhibition of bacterial growth. The zones were measured in cm at their widest diameter.

Bacteriophage Studies

Isolation of bacteriophage

Phage enrichment flasks were prepared by the usual method (4). One-hundred g of soil was obtained from around roots of a diseased gladiolus plant in a field plot at the Agricultural Research and Education Center, Bradenton, Florida. This was the same plot from which the pathogenic isolate, Br-1, was isolated from a diseased flower stalk of a gladiolus plant. Additional enrichment flasks were prepared with 100 ml of raw sewage substituted for the soil.

One-hundred ml of NB was also added to each enrichment flask along with 10 ml of an 18 h NB shake culture of the isolate Br-1. The

enrichment flasks were capped with aluminum foil and incubated in a stationary position at room temperature for 48 h. Following incubation, the liquid portion of each enrichment was collected, and centrifuged at 2000 g for 10 min. Each of the clear supernatants was then filtered through a Millipore filter with 0.45 μ diam pore size.

The presence of bacteriophage in the enrichment was determined by spotting the sterile enrichment on double-layer plates. Lawns on the isolate, Br-1, were prepared by a double-layer technique (2). One ml of an 18 h NB culture of the bacterium was added to 4 ml of molten water agar (WA) at 45 C. This WA-bacterial mixture was poured over 15 ml of solidified NA in a petri dish.

Drops of each of the enrichment filtrates were spotted on the surface of the lawns. After 24 h incubation at 30 C the plates were examined for lytic areas. Of five enrichments tested, only one, derived from soil from around diseased gladiolus and the isolate, Br-1, caused lysis. The lysis was not well defined and consisted of an area of slight lessening of bacterial growth.

The bacteriophage suspension was concentrated by further enrichment. A small amount of agar from the first lawn region was removed and placed in tubes containing 6 h cultures of Br-1 in NB. After 48 h of incubation on a shaker, the cultures were centrifuged and the supernatant was filter sterilized. This filtrate was then spotted on lawns of Br-1. Clear lytic areas appeared on the Br-1 lawn where this filtrate was spotted.

The bacteriophage was purified by serially selecting single placques from lytic areas containing phage particles. Various dilutions of the phage suspension were added to the WA-Br-1 mixture used to prepare

the lawn. Small placques developed with the homologous host. After a series of three increases and dilutions from single placques, a phage stock suspension was prepared.

Bacteriophage stock suspension

In this procedure a single placque from the last purification was removed and suspended in a 6 h NB culture of Br-1. After incubation for 48 h a filtrate was prepared and used to spot large areas on several lawns of Br-1. After development of large placques, the softer WA overlayer containing the phage particles was removed and suspended in 100 ml of BS. The agar was triturated and the suspension was centrifuged. Two-ml aliquots of the supernatant were then dispensed into the screw-cap tubes over 0.5 ml of chloroform and stored at 10 C.

Bacteriophage propagation and sensitivity tests

The bacteriophage stock was tested for lysis of two pathogenic isolates of P. marginata, F-1, and S-D. Lawns of these two isolates were spotted with phage and lysis occurred on lawns of both bacteria. The lytic areas in lawns of isolate S-D were non-confluent, however, indicating a difference in concentration of phage particles causing lysis of the two bacteria. Thus, the possibility of two bacteriophages in the stock was considered. Two placque types were separated. Agar pieces containing placques, or lytic areas, of lawns of Br-1 and F-1 were removed and aseptically placed into 6 h NB cultures of their respective hosts. After 48 h of shaker incubation, each of the cultures was centrifuged and filtered. The filtrates were diluted in a tenfold dilution series. Upon plating and incubation for 24 h at 30 C, placques were observed on each of the two bacterial lawns. A very small, fine, pinpoint type placque was observed on Br-1 lawns, while on the F-1 lawns

a small number of large, turbid type placques were observed. Single placques of each type were selectively removed and placed into 6 h NB cultures of their respective homologous isolates. The small placque type was serially isolated, propagated, and tested for lytic activity on isolate Br-1. The large placque type was also serially isolated, propagated, and tested for lytic activity on isolate F-1. Ultimately, stock suspensions of each of the two phage isolates were prepared by suspending lytic areas of lawns of each of the two bacteria, Br-1 and F-1, in BS. These suspensions were shaker agitated for 48 h, centrifuged, and then filter sterilized. Each of the phage filtrates was suspended in 100 ml BS over 10 ml of chloroform and stored at 10 C.

To determine the number of virulent phage particles in each phage stock suspension, serial dilutions were made as described previously. Each dilution was mixed with 0.3 ml of homologous bacterial suspension in 4 ml of WA and poured over NA. After an incubation of 48 h at 30 C, the number of individual placques were counted and the data converted to number of phage particles per ml of stock suspension. The concentration of the Br-1 phage stock suspension was determined to be ca. 4×10^{9} particles per ml. The concentration of F-1 phage stock suspension was ca. 1.6×10^{5} particles per ml.

Subsequently, all bacterial isolates from gladioli and the authentic isolates were typed with each of the two phage stocks, labeled Br-1 and F-1, which indicated the host bacterium used to select the phage.

Bacteriophage observations

Bacteriophage from both placque types were observed with the aid of an electron microscope (EM). Droplets of bacteriophage stock

suspensions were placed on 200 mesh formvar and carbon coated copper grids. After blotting, each sample was negative stained with drops of 2% uranyl acetate plus a wetting agent. Samples were stained for 30 sec. and then blotted dry before microscopic examination.

Placques from F-1, S-D, and Br-1 bacterial lawns were removed and placed in 1 ml of distilled water. After agitation, droplet samples were spotted on the grids and negative stained as previously described. This method allowed for EM examination of both bacterium and bacteriophage at the same time.

Results

Determination of Bacterial Concentrations

Concentrations in cells/ml of bacteria in suspensions standardized to four light transmittance values are listed below. For isolate Br-1: 25%, 3.09×10^8; 40%, 2.24×10^8; 51%, 1.32×10^8; and 75%, 6.02×10^7. For isolate F-1: 25%, 2.55×10^8; 40%, 1.67×10^8; 50%, 1.36×10^8; and 75%, 6.07×10^7. For isolate S-D: 25%, 2.51×10^8; 40%, 1.33×10^8; 50%, 1.29×10^8; and 75%, 5.07×10^7.

For all these isolates 50% transmittance corresponded to slightly more than 1×10^8 cells/ml. Therefore that value was used in standardizing inoculum.

Determination of Optimum Growth Temperature

In one experiment growth rates of the bacterial isolates Br-1 and F-1 were greatest at 35 C. The next highest growth rates were recorded at 30 C, and the lowest at 40 C. This was based on the highest absorbance values measured during the logarithmic phase of bacterial growth. Of the three incubation temperatures tested in a second experiment, growth rates of Br-1 and F-1 were greatest at 35 C.

The next highest growth rate occurred at 32 C, and the lowest was at 38 C (Fig. 6 and 7). In the last experiment highest growth rates of both isolates occurred at 35 C, with secondary rates at 33 and 31 C.

Pathogenicity Tests

Gladiolus pathogenicity tests

In the early stages of this investigation bacterial isolates were tested for pathogenicity by infiltrating leaves of gladiolus plants growing in greenhouse benches (Table 1). With inoculum of 10^6 cells/ml, most isolates did not induce lesions. Exceptions were isolates Br-1 and F-1048B. With inoculum of 10^8 cells/ml, a greater number of isolates incited lesions. Of 27 test isolates from gladioli, 17 were interpreted as being pathogenic. Of the four authentic cultures, only PA-7 gave a pathogenic reaction. Isolate FTCC-2284 was negative for pathogenicity at both inoculum concentrations.

Observations were made that presence of free water enhanced the pathogenic reaction, so the remaining pathogenicity tests were with potted plants placed in a moist chamber after inoculation. Of 44 isolates tested with inoculum of 10^8 cells/ml (Table 1), 35 incited lesions. Among the non-pathogenic isolates were the authentic isolates PM-106, PM-107, and ATCC-10247. In addition P. marginalis 71-2, 71-3, P. fluorescens 65-1, E. carotovora 70-1, P. lachrymans 69-1, and FTCC-2284 gave negative results in this pathogenicity test. All of the 33 test isolates were pathogenic, but isolates S-8, Y-71-1, C-71-2, and W-7 possessed a low degree of virulence. Isolate FTCC-1869, classified as P. alboprecipitans, also gave a positive pathogenic reaction.

Disease development was most extensive on gladiolus plants held in a moist chamber. Inoculated gladiolus plants placed in a

Fig. 6. Growth rates of P. marginata Br-1 at three incubation temperatures. Growth rates determined by measuring light absorbance at 600 nm wavelength being passed through bacterial suspension.

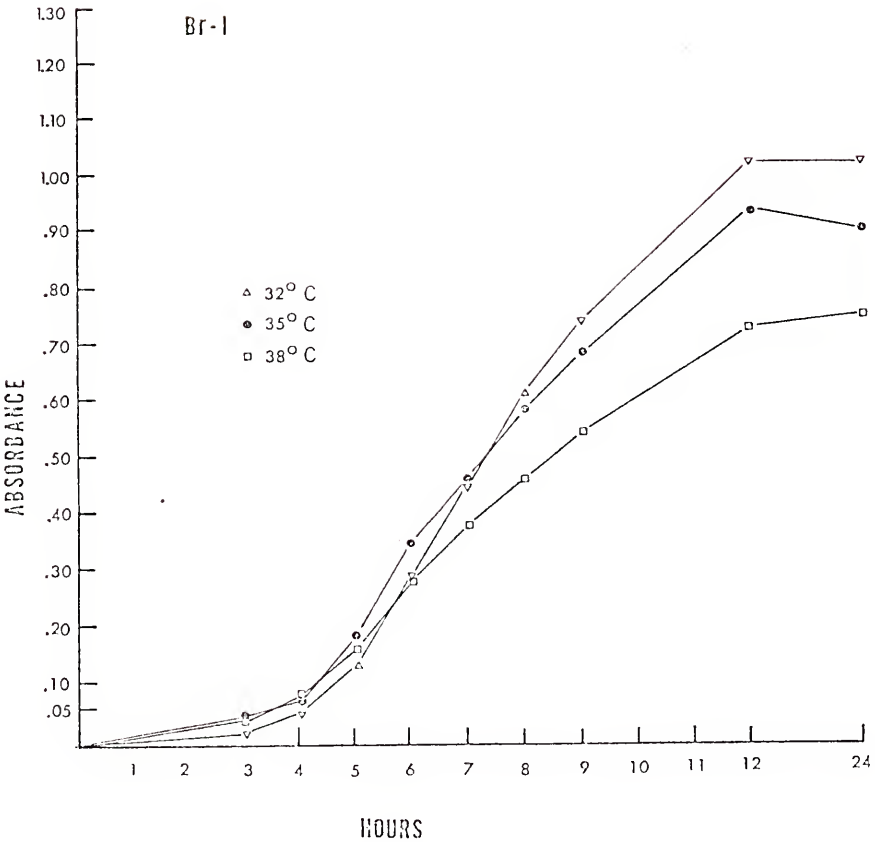
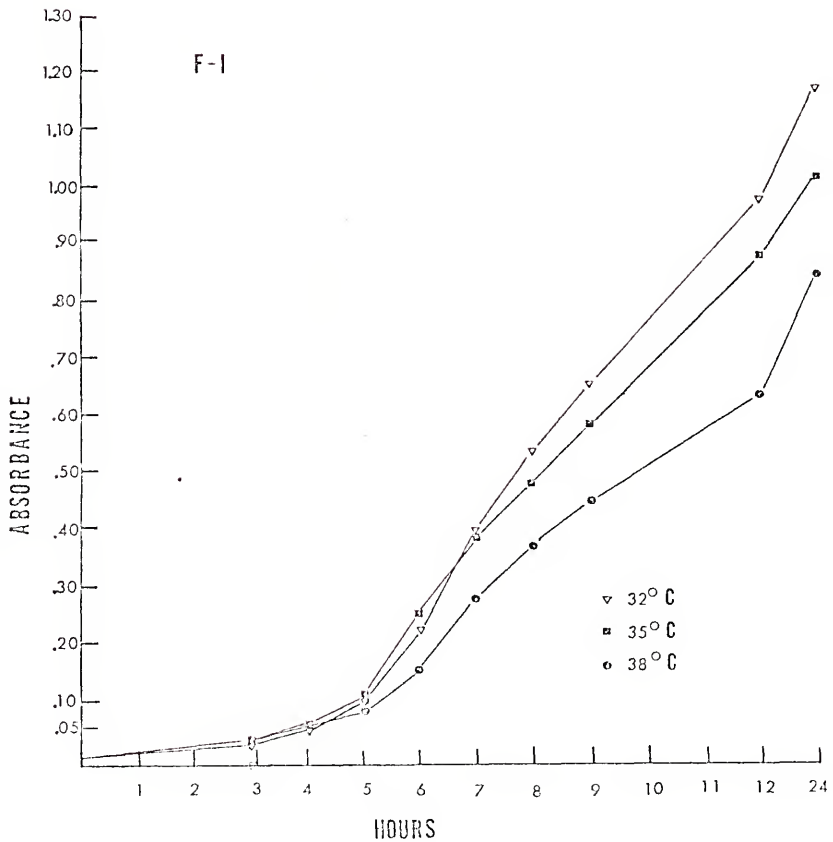

Br-1
1.30
1.20
1.10
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
.05
ABSORBANCE
32° C
35° C
38° C
1
2
3
4
5
6
7
8
9
10
11
12
24
HOURS

Fig. 7. Growth rates of P. marginata F-1 at three incubation temperatures. Growth rates determined by measuring light absorbance at 600 nm wavelength being passed through bacterial suspension.


F-1
ABSORBANCE
1.30
1.20
1.10
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
.05
1
2
3
4
5
6
7
8
9
10
11
12
24
HOURS
32° C
35° C
38° C

moist chamber zero and 2 h required the longest periods (3.33 and 3 days respectively) for symptoms to appear (Table 2). Symptom development was most rapid (1 day) in gladiolus plants placed in a moist chamber for 10 or 12 h. Four, 6 and 8 h of moisture were almost as effective, as 1.33 to 1.67 days were required for symptom development. Disease development always arrested after the inoculated plants were removed from the moist chamber and placed on the greenhouse bench.

Concentrations of bacterial inoculum influenced the rate of symptom development. Gladiolus plants inoculated with 10^7 and 10^8 cells/ml developed symptoms most rapidly as indicated by the high disease rating indices (Table 3). The highest inoculum concentration resulted in significantly greater disease ratings than for either 10^6, 10^5, or 10^4 cells/ml of inoculum. The 10^8 and 10^7 concentrations were not signficantly different, as were the 10^7 and 10^6 concentrations.

Onion pathogenicity tests

Forty bacterial isolates were tested for pathogenicity to onion, and 35 were positive when inoculated into the lacunar cavity of young onion shoots (Table 1). Five isolates, P. marginalis 71-2, 71-3, P. fluorescens 65-1, E. carotovora 70-1, and FTCC-2284 were negative for pathogenicity to onion. The test isolates S-8, C-71-3, and W-7 were weakly virulent.

Tobacco Hypersensitivity Tests

The first tobacco hypersensitivity (HR) tests were undertaken in a greenhouse environment with bacterial concentrations of 10^8 cells/ml (Table 1). Of 33 test isolates, 15 gave positive

Table 1. Reaction of bacterial isolates to pathogenicity, tobacco hypersensitivity and bacteriophage sensitivity tests

Isolate	Pathogenicity Tests				Tobacco Hypersensitivity			Bacteriophage Sensitivity	
	Gladiolus			Onion					
	Greenhouse Bench		Moist Chamber						
	10^{6}[a]	10^{8}	10^{8}	10^{8}	10^{8}	2×10^{8}	10^{9}	BR-1	F-1
Br-1	+[b]	+	+	+	±	±	+	+C	+C
Br-1SR	X	X	+	+	-	±	+	+C	+C
Br-2	X	+	+	+	±	±	+	+C	+C
Br-4	X	+W	+	+	±	X	+	+C	+C
Br-4A	X	+	+	+	±	X	+	+T	+C
Br-4B	X	+	+	+	±	X	+	+T	+T
G-3	X	X	+	+	+	+	+	+TW	+T
G-9	X	X	+	+	+	+	+	+T	+T
G-14	X	X	+	+	±	+	+	+TW	+T
G-15	X	X	+	+	±	+	+	+TW	+T
S-D	X	X	+	+	±	±	+	+C	+C
S-72-1	X	X	+	+	-	±	+	+C	+C

Table 1 - continued.

Isolate	Pathogenicity Tests: Gladiolus: Greenhouse Bench 10^{6}[a]	Gladiolus: Greenhouse Bench 10^{8}	Gladiolus: Moist Chamber 10^{8}	Onion 10^{8}	Tobacco Hypersensitivity 10^{8}	Tobacco Hypersensitivity 2×10^{8}	Tobacco Hypersensitivity 10^{9}	Bacteriophage Sensitivity: BR-1	Bacteriophage Sensitivity: F-1
G-8	X	X	+W	+W	+	+	+	+TW	+T
F-1	-	+	+	+	±	+	+	+C	+C
F-1048C	-	+	+	+	-	+	+	+C	+C
W-3	-	+	+	+	±	+	+	+T	+T
W-4	-	+	+	+	+	+	+	+TW	+TW
W-4A	-	+	+	+	+	+	+	+T	+T
W-6	-	-	+	+	+	+	+	-	-
G-1	-	+	+	+	+	+	+	+C	+C
Y-71-1	-	-	+W	+	-	-	+	-	-
L-71-2	-	+	+	+	+	+	+	+TW	+T
C-71-3	-	+	+	+W	+	+	+	+T	+T
PA-7	-	±	+	+	±	±	+	+TW	+C
PM 106	-	-	-	X	-	±	+	+T	+T

Table 1 - continued.

Isolate	Pathogenicity Tests: Gladiolus: Greenhouse Bench 10^{6}[a]	Pathogenicity Tests: Gladiolus: Greenhouse Bench 10^{8}	Pathogenicity Tests: Gladiolus: Moist Chamber 10^{8}	Pathogenicity Tests: Onion 10^{8}	Tobacco Hypersensitivity 10^{8}	Tobacco Hypersensitivity 2×10^{8}	Tobacco Hypersensitivity 10^{9}	Bacteriophage Sensitivity: BR-1	Bacteriophage Sensitivity: F-1
PM 107	-	-	-	X	±	±	+	+C	+C
ATCC 10247	-	-	-	X	-	±	+	+T	+T
Br-4C	X	X	+	+	-	±	+	+C	+C
1048 FB	+	+	+	+	±	+	+	+C	+C
H-72-1	X	+	+	+	+	+	+	+T	+T
W-1	-	+	+	+	+	+	+	+C	+C
G-2	-	+	+	+	+	+	+	+TW	+T
R-A-4	X	X	+	+	±	+	+	+C	+C
P. marginalis 71-2	X	-	-	-	-	-	-	-	-
P. marginalis 71-3	X	-	-	-	-	-	-	-	-
P. fluorescens 65-1	X	-	-	-	-	-	-	-	-

Table 1. - continued.

Isolate	Patnogenicity Tests: Gladiolus: Greenhouse Bench 10^6[a]	Patnogenicity Tests: Gladiolus: Greenhouse Bench 10^8	Patnogenicity Tests: Gladiolus: Moist Chamber 10^8	Patnogenicity Tests: Onion 10^8	Tobacco Hypersensitivity 10^8	Tobacco Hypersensitivity 2×10^8	Tobacco Hypersensitivity 10^9	Bacteriophage Sensitivity: BR-1	Bacteriophage Sensitivity: F-1
E. carotovora 70-1	X	-	-	-	-	-	-	-	-
P. lachrymans 69-1	X	-	-	X	+	+	+	-	-
FTCC-2284	-	-	-	-	-	-	-	-	-
R-A-1	X	X	+	+	±	X	+	+C	+C
G-71-2	-	-	+W	+	+	X	+	+T	+T
H-72-2	X	X	+	+	+	+	+	+C	+C
W-7	-	-	+W	+W	+	+	+	-	-
FTCC-1869	X	X	+	+	+	+	+	-	-

[a] 10^6, 10^8, 2×10^8, and 10^9 refers to inoculum concentration in cells per ml injected into leaves

[b] + = Positive reaction
- = Negative reaction
± = Variable reaction
W = Weak reaction
X = Not tested
C = Clear placque
T = Turbid placque

Table 2. Influence of hours in moist chamber upon development of disease symptoms of gladioli inoculated with P. marginata Br-1

Hours inoculated plants in moist chamber	Mean number of days for inoculated tissue to discolor
0	3.33[a]
2	3.00
4	1.33
6	1.67
8	1.67
10	1.00
12	1.00

[a] Mean number of days is average of three separate experiments. Inoculum concentration was 10^8 cells/ml.

Table 3. Rating index for spread of neck-rot symptoms as influenced by inoculum concentration of P. marginata F-1

Inoculum concentration[x]	Rating index mean[y]	
10^8	3.99	a*
10^7	3.22	a
10^6	1.77	b
10^5	2.22	b
10^4	1.66	b
BS (control)	0.00	c

x Inoculum concentrations in cells/ml injected into leaf tissue of gladioli.

y Index value of 6.0 = 1 day and index value of 0.0 = 7 days

* Means followed by the same letter are not significantly different.

reactions, 5 negative, and 13 incomplete HR. Of the authentic isolates tested, PM-106 and ATCC-10247 gave negative results, while PA-7 and PM-107 gave incomplete HR. At inoculum concentrations of 2 X 10^8 cells/ml, 21 of 27 test isolates induced HR reactions, none gave negative reactions, and six induced incomplete HR.

The suspected isolates of P. marginata, Br-1, F-1, and W-4 induced HR in tobacco inoculated with 10^8 cells/ml and incubated in a 30 C growth chamber (Table 4). With inoculum of 10^7 cells/ml, HR was not induced except by isolate W-4, which gave an incomplete HR. None of these isolates induced HR in tobacco when incubated in the greenhouse with maximum temperatures of 29-31 C.

P. lachrymans 69-3, a known HR inducer in tobacco, gave HR in tobacco incubated at 36 C when inoculum levels of 10^8 cells/ml were used. No HR was induced at that temperature with inoculum of 10^7 cells/ml. However, in the greenhouse environment, P. lachrymans induced HR with the high inoculum, and a delayed HR with 10^7 cells/ml concentration. The 0.42% BS injections were negative for HR under both temperature regimes.

Hypersensitive reactions induced by suspected isolates of P. marginata were facilitated by the high temperature regime of 37 C, rather than by the low temperature regime of 30 C (Fig. 3 and 4) (Table 5). Isolates Br-1, S-D, and PM-107 induced positive HR only when inoculum of 10^8 cells/ml and an incubation temperature of 37 C were used.

P. lachrymans 69-3 was HR positive at both 10^7 and 10^8 cells/ml at 30 C, but negative at both levels at 37 C. Check inoculations of P. fluorescens 65-1 and 0.42% BS were negative for HR regardless

Table 4. Influence of temperature on development of hypersensitive reaction after injection of two concentrations of various bacterial suspensions into F_2C_1 tobacco leaves

Isolate	Bacteria number/ml	Temperature Regime	
		Growth Chamber 36 C	Greenhouse 29-31 C
P. lachrymans 69-3	10^8	++[a]	++
	10^7	-	+
P. marginata Br-1	10^8	+	-
	10^7	-	-
P. marginata F-1	10^8	+	-
	10^7	-	-
P. marginata W-4	10^8	+	-
	10^7	±	-
BS (control)	0	-	-

[a] ++ = Complete necrosis of infiltrated tobacco leaf tissue within 24 h after inoculation.

+ = Complete necrosis by 48 h after inoculation.

± = Incomplete necrosis 48-72 h after inoculation.

- = No necrosis 72 h after inoculation.

Table 5. Influence of two growth chamber temperatures upon development of hypersensitive reaction after injection of two concentrations of various bacterial suspensions into F_2C_1 tobacco leaves

Isolate	Bacteria number/ml	Growth Chamber Temperature 30 C	37 C
P. lachrymans 69-3	10^8	++[a]	-
	10^7	+	-
P. fluorescens 65-1	10^8	-	-
	10^7	-	-
P. marginata Br-1	10^8	-	+
	10^7	-	-
P. marginata S-D	10^8	-	+
	10^7	-	-
P. marginata 107	10^8	-	+
	10^7	-	-
BS (control)	0	-	-

[a] ++ = Complete necrosis of infiltrated tobacco leaf tissue within 24 h after inoculation.

+ = Complete necrossis by 48 h after inoculation.

- = No necrosis 72 h after inoculation.

of concentration or temperature.

At 28 C P. marginata Br-1 and P. lachrymans 69-1 induced HR in tobacco only when inoculum of 10^9 cells/ml were used (Table 6). At 36 C HR was positive with both 10^8 and 10^9 cells/ml. The 10^9 inoculum induced the more rapid HR, 24 h rather than 48 h. P. lachrymans 69-1 was HR-positive under both temperature regimes with all inoculum levels except for 10^6 cells/ml. However, HR was more rapid at 10^9 and 10^8 than at 10^7 cells/ml.

Using the Br-1 strain of P. marginata at four inoculum levels, visible HR reactions were expressed in tobacco leaves held at 30 C for 24 h with 10^9 and 10^8 cells/ml (Table 7). These HR reactions were correlated with the highest electrolyte readings. The second highest electrolyte readings were from tobacco leaves inoculated with 10^9 and 10^8 concentrations incubated at 28 C. At this temperature a visible HR reaction occurred only at the highest inoculum level (10^9 cells/ml) and this occurred within 48 h rather than 24 h.

Leaves which were completely necrotic as a result of HR contained no viable bacteria (Table 7). The low temperature (28 C) treatments with inoculum of 10^9 and 10^8 cells/ml, while having high electrolyte readings, had bacterial populations of 1.5×10^6 and 2.1×10^5 cells/ml respectively, after 24 h. The HR reaction was not complete, however, at this time. At 48 h, the 28 C treatment of 10^9 cells/ml was complete, as evidenced by visible necrosis. The 10^8 inoculum continued to show an incomplete HR reaction and slowly increased in electrolyte readings and maintained a bacterial population of ca. 10^5 cells/ml up to 96 h after inoculation.

All other inoculum concentrations, at either temperature were negative for HR, and gave lower electrolyte readings, and generally

Table 6. Influence of two temperature regimes and four inoculum levels of two bacterial isolates upon development of the hypersensitive reaction in tobacco leaves

Isolate	Bacteria number/ml	Growth Chamber Temperature Regime	
		28 C	36 C
P. marginata Br-1	10^9	++[a]	++
	10^8	-	+
	10^7	-	-
	10^6	-	-
P. lachrymans 69-1	10^9	++	++
	10^8	++	++
	10^7	+	+
	10^6	-	-
BS (control)	0	-	-

[a] ++ = Complete necrosis of infiltrated tobacco leaf tissue within 24 h after inoculation.

\+ = Complete necrosis by 48 h after inoculation.

\- = No necrosis 72 h after inoculation.

Table 7. Influence of P. marginata Br-1 induced tobacco HR on bacterial populations and electrolyte leakage

Incubation Temperature	Initial Bacterial Concentration	Hours Incubation: 0 — Electrolytes µmhos	0 — Bacterial Population cells/ml	24 — Electrolytes µmhos	24 — Bacterial Population cells/ml	48 — Electrolytes µmhos	48 — Bacterial Population cells/ml	96 — Electrolytes µmhos	96 — Bacterial Population cells/ml
36 C	10^9	66	9.8×10^6	671	0	-	-	-	-
	10^8	64	3.0×10^6	646	0	-	-	-	-
	10^7	64	1.9×10^5	57	8.5×10^4	40	1.6×10^4	78	2.6×10^3
	10^6	61	1.4×10^4	53	2.9×10^4	79	5.4×10^3	170	1.5×10^5
28 C	10^9	74	7.2×10^6	417	1.5×10^6	-	-	-	-
	10^8	59	9.4×10^5	409	2.1×10^5	253	1.3×10^5	492	1.8×10^5
	10^7	47	1.4×10^5	83	3.6×10^4	65	3.4×10^4	62	5.6×10^4
	10^6	72	1.7×10^4	63	4.8×10^3	87	2.1×10^3	79	6.3×10^4

maintained a low level of bacterial population up to 96 h after inoculation. Electrolyte leakage data are presented graphically in Figs. 8 and 9 and bacterial multiplications are presented graphically in Figs. 10 and 11.

Bacterial populations monitored throughout this experiment indicated that the Br-1 isolate did not significantly increase in tobacco. Bacterial populations of near 1×10^6 cells/ml or greater were necessary for induction of HR. Bacterial populations below this level did not induce visible HR, nor did they increase to levels evidently necessary for induction.

With the above information about the effect of concentrations and temperature on expression of visible HR in tobacco, all test isolates were inoculated into tobacco leaves at 10^9 cells/ml and held at 36 C. All 33 test isolates gave positive HR reactions under these conditions, as did each of the four authentic culture isolates (Table 1). Isolates of P. marginalis 71-2, 71-3, P. fluorescens 65-1, E. carotovora 70-1, and FTCC-2284 always gave negative HR results.

Bacteria Size and Flagellar Arrangement

Bacterial isolates Br-1, F-1, S-D, PM-106, and PA-7 were generally observed to possess one to two polar flagella. However, numerous bacteria were observed that possessed three or four polar flagella.

The following range of size measurements were obtained with samples of cells. The average of ten large cells of isolate S-D was 2.3 μ long by 0.87 μ wide. The average of ten small cells was 1.8 μ by 0.88 μ. A single cell of S-D, possessing four polar flagella, measured 1.7 x 0.71 μ (Fig. 12). The average of two large cells of

Fig. 8. Rates of electrolyte leakage from tobacco leaf tissue held at 36 C after inoculation with four conc of P. marginata Br-1.

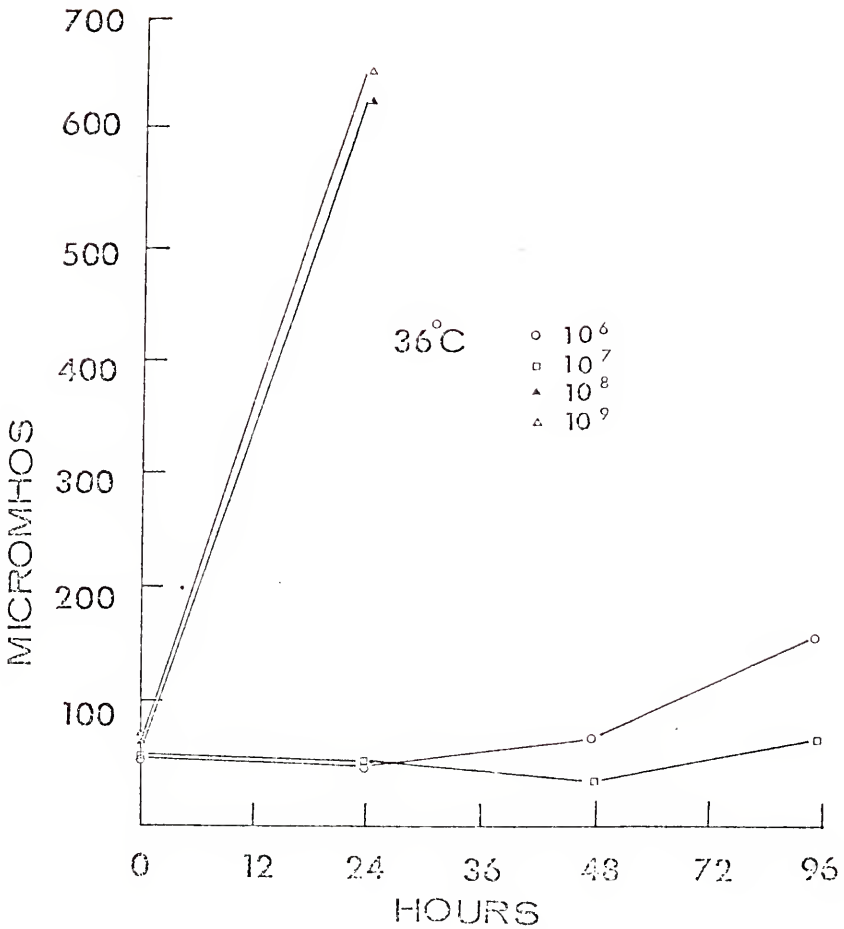

700
600
500
400
300
200
100
MICROMHOS
36°C
10 6
10 7
10 8
10 9
0
12
24
36
48
72
96
HOURS

Fig. 9. Rates of electrolyte leakage from tobacco leaf tissue held at 28 C after inoculation with four conc of P. marginata Br-1.

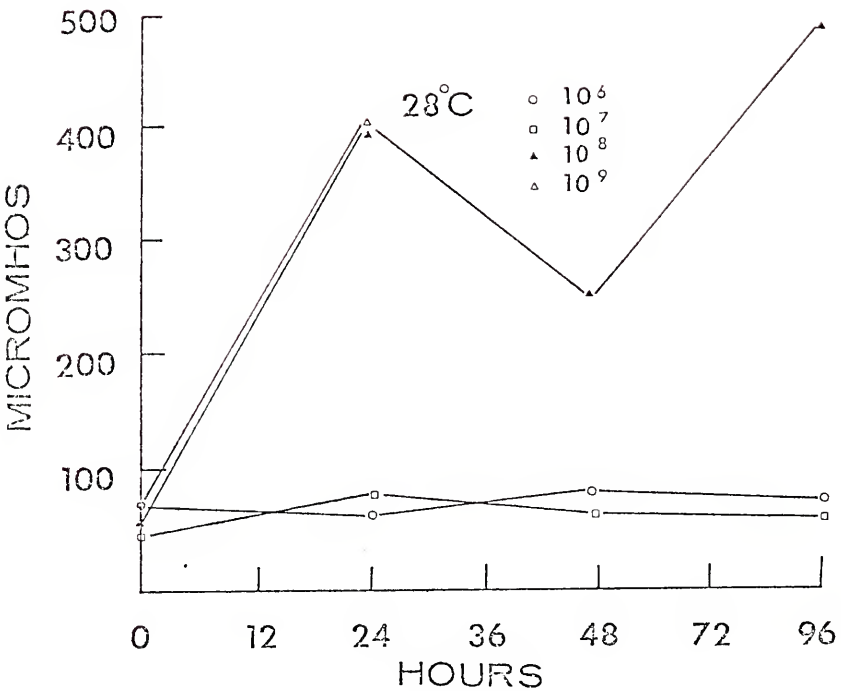

500
400
300
200
100
MICROMHOS
28°C
10 6
10 7
10 8
10 9
0
12
24
36
48
72
96
HOURS

Fig. 10. Number of P. marginata Br-1 cells retrieved from tobacco leaf tissue incubated at 36 C for 0, 24, 48, and 96 h after inoculation of four separate bacterial conc in cells/ml.

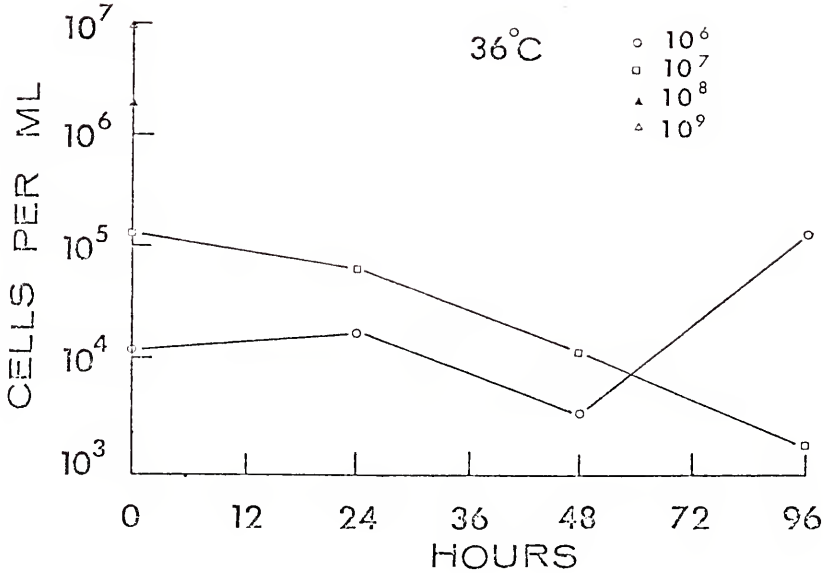

36°C
10⁶
10⁷
10⁸
10⁹
CELLS PER ML
10⁷
10⁶
10⁵
10⁴
10³
0
12
24
36
48
72
96
HOURS

Fig. 11. Number of P. marginata Br-1 cells retrieved from tobacco leaf tissue incubated at 28 C for 0, 24, 48, and 96 h after inoculation of four separate bacterial conc in cells/ml.

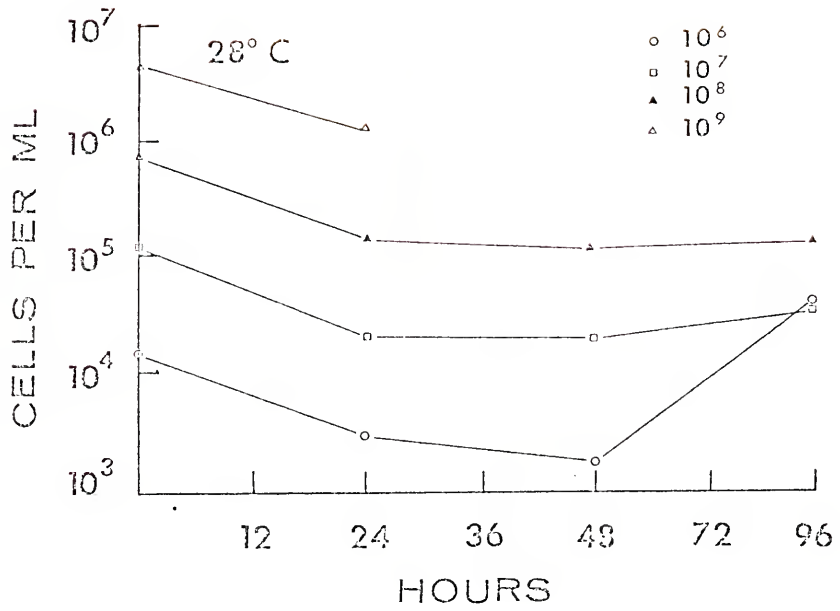
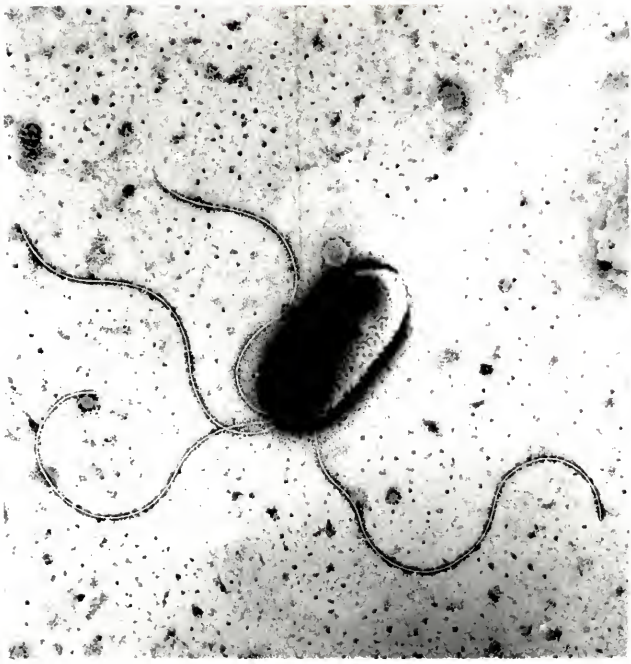

28° C
10^6
10^7
10^8
10^9
10^7
10^6
10^5
10^4
10^3
CELLS PER ML
12
24
36
48
72
96
HOURS

Fig. 12. Electron micrograph of negative stained cell of P. marginata S-D possessing four polar flagella. Size of cell is 1.7 x 0.71 μ. Magnification X 19,750.



isolate F-1 was 2.3 μ x 0.78 μ and the average of six small cells was 1.4 x 0.77μ. The average of five large cells of Br-1 was 2.1 μ x 0.76 μ, while four small cells average 1.4 μ x 0.74 μ. Ten cells of isolate PM-106 averaged 1.8 μ x 0.72 μ. Five large cells of PA-7 averaged 2.3 μ x 0.79 μ, while two small cells averaged 1.5 μ x 0.80 μ.

Physiological Tests

The results of each physiological test are summarized below. The complete results for all physiological tests are presented in Table 8.

Hugh-Liefson test

Of 44 bacterial isolates tested, only E. carotovora 70-1, a bacterium used as a control, gave a positive result within 48 h. All isolates of P. marginata were classified as aerobic bacteria.

Production of fluorescent pigment

The bacterial isolates P. marginalis 71-2, 71-3, P. fluorescens 65-1, P. lachrymans 69-1, and FTCC-2284 produced yellow-green fluorescent pigment when grown on King's medium B. Pigmentation was evident without benefit of longwave ultra-violet (LUV) radiation. However, fluorescence was visible only under LUV. None of the four authentic isolates were fluorescent, and of the test isolates only Br-2 exhibited fluorescence and this was a weak reaction.

Production of white precipitate in nutrient agar media

Thirty of 33 test isolates formed a white precipitate (WP) around colonies when cultured on NA (Fig. 5). The exceptions were isolates Y-71-1, G-71-2, and W-7. Of the four authentic isolates, only PA-7 produced WP and this was weak compared to the other positive isolates. None of the other bacterial species tested produced this WP.

Table 8. Reaction of bacterial isolates to various physiological tests

Isolate	Hugh-Liefson	Fluorescence	White precipitate in NA	Levan Production	Oxidase Reaction	Potato Soft Rot	Arginine Dihydrolase	Tobacco Hypersensitivity	Nitrate Reduction	Growth in dL-B-hydroxybutyrate after: 48 h	Growth in dL-B-hydroxybutyrate after: 2 wk	Poly-B-hydroxybutyrate accumulation
Br-1	-[a]	-	+	-	+	-	-	+	+R	+	++W	+
Br-1SR	-	-	+	-	+	-	-	+	+R	+	++	?
Br-2	-	+W	+	-	+	-	-	+	+R	+	++W	?
Br-4	-	-	+	-	+	-	-	+	+R	+	++	+
Br-4A	-	-	+	-	+	-	-	+	+P	+	++	?
Br-4B	-	-	+	-	+	-	-	+	+P	+	++	+
G-3	-	-	+	-	+	-	-	+	-	+	++	+
G-9	-	-	+	-	+[10]	-	-	+	+P	+W	++W	+
G-14	-	-	+	-	+[10]	-	-	+	-	+	++	+
G-15	-	-	+	-	+	-	-	+	-	+W	++	+
S-D	-	-	+	-	+	-	-	+	+R	+	++	?

Table 8 - continued.

Isolate	Hugh-Liefson	Fluorescence	White precipitate in NA	Levan Production	Oxidase Reaction	Potato Soft Rot	Arginine Dihydrolase	Tobacco Hypersensitivity	Nitrate Reduction	Growth in dL-B-hydroxybutyrate after: 48 h	Growth in dL-B-hydroxybutyrate after: 2 wk	Poly-B-hydroxybutyrate accumulation
S-72-1	-	-	+	-	+	-	-	+	+B	+	++	+
S-8	-	-	+	-	+	-	-	+	-	-	-	-NB
F-1	-	-	+	-	+	-	-	+	+B	+W	++	+
F-1048C	-	-	+	-	+	-	-	+	+B	+W	++	-
W-3	-	-	+	-	+	-	-	+	+R	+	++	+
W-4	-	-	+	-	+	-	-	+	+WP	+	++	+
W-4A	-	-	+	-	+10	-	-	+	+P	+	++	?
W-6	-	-	+	-	+10	-	-	+	+WP	+	-	+
G-1	-	-	+	-	+	-	-	+	+P	+	++	+
Y-71-1	-	-	-	+	+10	-	-	+	+B	+	++	+
L-71-2	-	-	+	-	+	-	-	+	-	+	++	+

Table 8 - continued.

Isolate	Hugh-Liefson	Fluorescence	White precipitate in NA	Levan Production	Oxidase Reaction	Potato Soft Rot	Arginine Dihydrolase	Tobacco Hypersensitivity	Nitrate Reduction	Growth in dL-B-hydroxybutyrate after: 48 h	Growth in dL-B-hydroxybutyrate after: 2 wk	Poly-B-hydroxybutyrate accumulation
C-71-3	-	-	+	-	+[20]	-	-	+	+P	+	-	+
PA-7	-	-	+W	-	+[20]	-	-	+	+B	+	++	+
PM-106	-	-	-	-	+[20]	-	-	+	-	+	++	-
PM-107	-	-	-	-	+[20]	-	-	+	-	+	++W	?
ATCC-10247	-	-	-	-	+[20]	-	-	+	-	+	++	-
Br-4C	-	-	+	-	+	-	-	+	+P	+	++	+
F-1048B	-	-	+	-	+	-	-	+	+B	+	++W	+
H-72-1	-	-	+	-	+	-	-	+	-	+	++	+
W-1	-	-	+	-	+	-	-	+	+R	+	++	?
G-2	-	-	+	-	+	-	-	+	-	+	++	?
R-A-4	-	-	+	-	+	-	-	+	+B	+	++	+

Table 8 - continued.

Isolate	Hugh-Liefson	Fluorescence	White precipitate in NA	Levan Production	Oxidase Reaction	Potato Soft Rot	Arginine Dihydrolase	Tobacco Hypersensitivity	Nitrate Reduction	Growth in dL-B-hydroxybutyrate after: 48 h	Growth in dL-B-hydroxybutyrate after: 2 wk	Poly-B-hydroxybutyrate accumulation
P. marginalis 71-2	-	+	-	-	+	+	+	-	-	-	-	-NB
P. marginalis 71-3	-	+	-	+	+	+	+	-	-	+	-	-NB
P. fluorescens 65-1	-	+	-	-	+	-	+	-	+MP	-	-	-NB
E. carotovora 70-1	+	-	-	-	-	+	-	-	+B	-	-	-NB
P. lachrymans 69-1	-	+	-	+	-	-	-	+	-	-	-	-NB
FTCC-2284	-	+	-	-	+	+	+	-	-	+	++W	-
R-A-1	-	-	+	-	+	-	-	+	+B	+	++W	+
G-71-2	-	-	-	-	+	-	-	+	-	+	++	+

20

Table 8 - continued.

Isolate	Hugh-Liefson	Fluorescence	White precipitate in NA	Levan Production	Oxidase Reaction	Potato Soft Rot	Arginine Dihydrolase	Tobacco Hypersensitivity	Nitrate Reduction	Growth in dL-B-hydroxybutyrate after: 48 h	Growth in dL-B-hydroxybutyrate after: 2 wk	Poly-B-hydroxybutyrate accumulation
H-72-2	-	-	+	-	+	-	-	+	-	+	++	+
W-7	-	-	-	-	+	-	-	+	+B	+	++	-
FTCC-1869	-	-	-	-	+	-	-	+	+B	+	++	+

[a]
+ = positive reaction within 48 h
- = negative reaction after 48 h
10 = time in seconds for positive oxidase reaction
20 = time in seconds for positive oxidase reaction
W = weak reaction
B = brown-red reaction for nitrate reduction test
R = red reaction for nitrate reduction test
P = pink reaction for nitrate reduction test
? = inconclusive results
NB = nutrient broth culture

Levan production

Three of 44 bacterial isolates tested, Y-71-1, P. marginalis 71-3, and P. lachrymans 69-1, were positive for levan. None of the test or authentic isolates produced any growth resembling the smooth, domed, white, mucoid colonies of the positive check P. lachrymans 69-1.

Oxidase reaction

All bacterial isolates tested, with the exception of negative checks E. carotovora 70-1, and P. lachrymans 69-1, were oxidase-positive. However some of the test and authentic isolates were slow, requiring periods greater than 10 and 20 sec to show a positive reaction.

Potato soft rot

Of 44 isolates tested only P. marginalis 71-2, 71-3, E. carotovora 70-1, and FTCC-2284 soft-rotted sliced potato tubers. While some of the test isolates did cause browning and cratering of potato discs, none could be considered a soft-rot reaction.

Potato activity test

Table 9 denotes bacterial isolates which when inoculated on potato discs caused cratering, pitting, or browning of the potato discs. Twenty-four of 32 test isolates showed some activity on potato discs. The majority of these isolates were most active when incubated at 30 C. None of the authentic isolates showed any activity. These results are to be distinguished from the potato soft-rot reaction caused by isolates P. marginalis 71-2, 71-3, E. carotovora 70-1, and FTCC-2284, included in this test.

Pectolytic activity

Results of this test (Table 9) generally coincided with results obtained by Hildebrand (34). Of 33 test isolates, 30 gave positive results by exhibiting greatest pitting activity on low pH

Table 9. Results of potato and sodium polypectate pitting tests

Isolate	Potato test activity Incubation temperature			Pitting of sodium polypectate media		
	20 C	25 C	30 C	pH5.0	pH7.0	pH8.4
Br-1	-[a]	-	+	9[b]	5	S
Br-1 SR	-	-	-	9	2	S
Br-2	+	+	+	10	2	-
Br-4	+	+	+	11	3	-
Br-4A	-	-	+	7	3	-
Br-4B	-	-	-	8	S	-
G-3	-	-	+	8	-	-
G-9	-	-	+	6	-	-
G-14	-	-	+	12	S	-
G-15	-	-	+	10	-	-
S-D	-	-	+	11	5	-
S-72-1	-	-	+	11	3	-
S-8	-	-	+	-	-	-
F-1	+	+	+	11	3	-
F-1048C	-	-	+	10	6	-
W-3	+	+	+	11	4	-
W-4	-	-	-	8	S	-
W-4A	-	-	+	8	S	-
W-6	-	-	+	-	-	-

Table 9 - continued.

Isolate	Potato test activity Incubation temperature			Pitting of sodium polypectate media		
	20 C	25 C	30 C	pH5.0	pH7.0	pH8.4
G-1	+	-	+	7	S	-
Y-71-1	-	-	-	11	5	S
L-71-2	-	-	+	-	-	-
C-71-3	-	-	+	7	S	-
PA-7	-	-	-	8	2	-
PM-106	-	-	-	10	3	-
PM-107	-	-	-	10	S	-
ATCC-10247	-	-	-	10	3	-
Br-4C	-	-	+	9	7	-
F-1048B	-	-	-	11	S	-
H-72-1	+	+	+	10	6	-
W-1	-	-	-	10	S	-
G-2	-	-	-	12	S	-
R-A-4	-	-	+	12	2	-
P. marginalis 71-2	-	+SR	+SR	-	-	-
P. marginalis 71-3	-	+SR	+SR	-	S	S
P. fluorescens 65-1	-	-	-	-	-	-
E. carotovora 70-1	+SR	+SR	+SR	2	4	5
P. lachrymans 69-1	-	-	-	1	3	6

Table 9 - continued.

Isolate	Potato test activity Incubation temperature			Pitting of sodium polypectate media		
	20 C	25 C	30 C	pH5.0	pH7.0	pH8.4
FTCC-2284	+SR	+SR	+SR	-	-	8
R-A-1	X	X	X	12	-	-
G-71-2	-	-	-	12	3	-
H-72-2	-	-	+	12	S	-
W-7	-	-	+	7	S	-
FTCC-1869	X	X	X	-	S	-

[a]
- = no activity on potato
+ = browning and/or cratering of potato disc but no soft rot reaction
SR = soft rot reaction
X = not tested

[b]
number indicates the radius of pit from colony margin in mm
- = no pitting
S = slight pitting

media; 26 of these 30 exhibited various degrees of pitting on neutral media, and only three of the 30 positive isolates showed slight signs of pitting on the high pH media. Isolates S-8, W-6, and L-71-2 did not pit any of the sodium polypectate media. All four of the authentic isolates exhibited pitting on the low and neutral pH media, but none of the high pH media. Isolate FTCC-2284 showed pitting activity only on the high pH media.

Arginine dihydrolase

This test was positive only for isolates of P. marginalis 71-2, 71-3, and P. fluorescens 65-1, and FTCC-2284. All of the test and authentic isolates gave negative results for presence of this enzyme.

Nitrate reduction

Results of this test of isolates were quite variable. Of 33 test isolates, nine gave negative reactions and 24 positive reactions. Of the 24 positive isolates, nine gave a pink reaction, seven a red reaction, and eight a dark brown reaction. Of the four authentic isolates only PA-7 gave a positive reaction. Isolates of P. marginalis 71-2, 71-3, P. lachrymans 69-1, and FTCC-2284 gave negative nitrate reduction tests.

Growth in dL-B-hydroxybutyrate as sole carbon and energy source

Of 33 suspected isolates of P. marginata tested, only isolate S-8 was negative for growth after 48 h and 2 weeks incubation. Isolates W-6 and C-71-3 exhibited positive signs of growth after 48 h, but none after 2 weeks incubation. All other isolates, including the authentic isolates gave evidence of growth in this single carbon and energy source, although some isolates exhibited weaker signs of growth after 2 weeks

incubation. Isolates which did not grow after 48 h or 2 weeks were P. marginalis 71-2, P. fluorescens 65-1, E. carotovora 70-1, and P. lachrymans 69-1. P. marginalis 71-3 showed signs of growth after 48 h, but none after 2 weeks.

Accumulation of poly-B-hydroxybutyrate (PHB)

The bacterial isolate FTCC-1869 was a positive check for this test. Of 33 test isolates 23 were positive, three negative, and seven gave inconclusive results. Of the four authentic isolates tested, PA-7 was positive, PM-106 and ATCC-10247 were negative, and PM-107 gave an inconclusive result. Isolates which would not grow in dL-B-hydroxybutyrate were grown in NB and tested for accumulation of PHB. All of these isolates tested negative. Isolate FTCC-2284, while growing in dL-B-hydroxybutyrate, did not accumulate PHB.

Phenol cleavage test

All six test isolates were positive for ortho cleavage (Table 10). Each bacterial isolate tested produced a shade of purple, including the isolates of P. cichorii and P. lachrymans included as positive checks. Toluene and distilled water, included as negative checks, did not produce a color and gave negative results.

Nutritional tests

Ballard et al. (8) found that certain nutritional tests were critical in distinguishing isolates of P. marginata from three other closely related plant pathogenic, non-fluorescent bacterial species. Thus, all 33 isolates from gladiolus plants were tested for utilization and growth on each of six substrates as sole carbon and energy sources. Results are included and compared with those reported by Ballard et al. in Table 11.

Table 10. Results of phenol cleavage test

Isolate	Color reaction after 12 h	Ortho cleavage
Br-1	Light purple	+[a]
Br-4B	Purple	+
G-3	Purple	+
W-4A	Light brown	+
F-1	Purple	+
S-D	Purple	+
P. cichorii 72-8	Purple	+
P. lachrymans 69-1	Purple	+
Toluene H_2O	Clear	-
H_2O	Clear	-

[a] + = positive test

- = negative test

Table 11. Utilization by bacterial isolates of selected substrates as sole carbon, nitrogen, and energy source

Isolate	D-(-)-Tartrate	Glycollate	Levulinate	Mesaconate	Nicotinate	Trigonelline
Br-1	+[b]	-	-	+	+	-
Br-1SR	+	-	-	+	+	-
Br-2	+	-	-	+	+	-
Br-4	+	-	-	+	+	-
Br-4A	+W	-	-	+	+	-
Br-4B	+	-	+	+	+	-
G-3	+	-	-	+	+	-
G-9	+	-	?	+	?	-
G-14	+	-	-	+	-	-
G-15	+	-	-	+	+	-
S-D	+	-	-	+	+	-
S-72-1	+	-	-	+	+	-
S-8	+	-	-	+	+	-
F-1	+	-	-	+	+	-
F-1048C	+	-	-	+	+	+
W-3	+	-	-	+	+	+
W-4	+	-	-	+	+	-

Table 11 - continued.

Isolate	D-(-)-Tartrate	Glycollate	Levulinate	Mesaconate	Nicotinate	Trigonelline
W-4A	+	-	-	+	+	-
W-6	+	-	-	+	+	-
G-1	+	-	-	-	-	-
Y-71-1	+	+	+	-	-	-
L-71-2	+	-	-	+	+	-
C-71-3	+	-	-	+	+	-
PA-7	+	-	+	+	+	-
PM-106	+	-	+	+	-	-
PM-107	+	-	+	+	-	-
ATCC-10247	+	-	+	+	-	-
Br-4C	+	-	-	-	+	-
F-1048B	+	-	-	+	+	-
H-72-1	+	-	-	+	+	-
W-1	+	-	-	+	+	-
G-2	+	-	+	-	+	-
R-A-4	+	-	-	+	+	-
P. marginalis 71-2	-	-	+	+	+	-
P. marginalis 71-3	-	-	+	+	+	-
P. fluorescens 65-1	+	-	-	-	+	+
E. carotovora 70-1	-	-	-	-	-	-

Table 11 - continued.

Isolate	D-(-)-Tartrate	Glycollate	Levulinate	Mesaconate	Nicotinate	Trigonelline
P. lachrymans 69-1	-	-	-	-	-	-
FTCC-2284	-	-	+	+	-	-
R-A-1	+	-	+	+	+	-
C-71-2	+	-	+	+	+	-
H-72-2	+	-	-	+	+	-
W-7	+	-	-	-	-	-
FTCC-1869	X	X	+	-	+	-
Ballard et al.[a]	+	-	-	+	+	+

[a] Results reported by Ballard et al. for *P. marginata*

[b]
+ = Positive growth
? = Inconclusive results
- = No growth
X = Not tested
W = Weak growth

D-(-)-tartrate. All 33 test isolates and the four authentic isolates were positive for growth on this substrate, agreeing with results of Ballard et al. Isolate Br-4A was weakly positive. Of the other isolates tested, P. marginalis 71-2, 71-3, E. carotovora 70-1, P. lachrymans 69-1, and FTCC-2284 were negative for growth, while P. fluorescens 65-1 was positive.

Glycollate. Only Y-71-1 of the 33 isolates tested grew on this substrate. All of the authentic isolates, as well as all the other test isolates were negative, again agreeing with results of Ballard et al.

Levulinate. Six of 33 isolates were positive, one inconclusive, and 16 negative for growth on this substrate. All four authentic isolates were positive, contrary to negative results reported by Ballard et al. Other isolates positive for growth included P. marginalis 71-2, 71-3, FTCC-2284, and FTCC-1869. Negative isolates included P. fluorescens 65-1, E. carotovora 70-1, and P. lachrymans 69-1.

Mesaconate. Twenty-eight of 33 isolates were positive for growth on this substrate. Negative test isolates were G-1, Y-71-1, Br-4C, G-2, and W-7. All four authentic isolates of P. marginata were positive, agreeing with results of Ballard et al. Other isolates positive for growth included P. marginalis 71-2, 71-3, and FTCC-2284. Negative isolates included P. fluorescens 65-1, E. carotovora 70-1, P. lachrymans 69-1, and FTCC-1869.

Nicotinate. Twenty-eight of 33 isolates were positive, one inconclusive, and four negative. Negative isolates included G-14, G-1, Y-71-1, and W-7. Of the four authentic isolates of P. marginata,

only PA-7 was positive for growth. The negative results for the other three authentic isolates did not agree with results of Ballard et al. Other isolates positive for growth included P. marginalis 71-2, 71-3, P. fluorescens 65-1, and FTCC-1869. Negative isolates included E. carotovora 70-1, P. lachrymans 69-1, and FTCC-2284.

Trigonelline. Three of 44 isolates were positive for growth on this substrate. These included the test isolates of F-1048C, W-3, and also the isolate of P. fluorescens 65-1. All other test isolates, and also the authentic isolates were negative. These did not agree with the positive results reported by Ballard et al.

Antibiotic sensitivity

Sensitivity to 23 antibiotics are summarized in Table 12. Both isolates tested, Br-1 and F-1048B, were sensitive to the following antibiotics: Methenamine Mandelate, Nalidixic Acid, Chlortetracycline, Novobiocin, Chloramphenicol, Erythromycin, Dihydrostreptomycin, Oxytetracycline, Tetracycline, Neomycin, and Kanamycin. The gram-negative characteristic of these isolates was confirmed by their sensitivity to Nalidixic Acid, an antibiotic active against gram-negative bacteria.

Bacteriophage Studies

Bacteriophage sensitivity tests

Of the 33 test isolates, 30 were sensitive to the two bacteriophage isolates Br-1 and F-1 (Table 1). Positive sensitivity was determined by formation of lytic zones in bacterial lawns of the test isolates. Several of these isolates, G-3, G-14, G-15, S-8, W-4, L-71-2, and G-2 when tested with Br-1 phage, gave positive but weak phage reactions. When tested with F-1 phage, only isolates W-4 gave a weak phage reaction. Negative bacterial isolates were W-6, Y-71-1, and W-7.

Table 12. Antibiotic sensitivity of two isolates of Pseudomonas marginata

Antibiotic	Concentration	Bacterial Isolate	
		Br-1	F-1048B
11-178E			
Sulfisomidine (Elkosin)	50 MCG	-[a]	-
Nitrofurantoin (Furadantin)	50 MCG	-	-
Sulfisoxazole (Gantrisin)	50 MCG	-	-
Methenamine Mandelate (Mandelamine)	1.0 Mg	1.2	1.2
Sulfadimethoxine (Madribon)	50 MCG	-	-
Nalidixic Acid (Neg Gram)	50 MCG	2.8	2.6
Triple Sulfa	50 MCG	-	-
Sulfamethythiadiazole (Thiosulfil)	50 MCG	-	-
11-160B			
Chlortetracycline	30 MCG	1.5	1.4
Novobiocin	30 MCG	1.2	1.1
Chloramphenicol	30 MCG	2.3	1.5
Erythromycin	15 MCG	1.5	1.3
Penicillin G	10 units	-	-
Dihydrostreptomycin	10 MCG	1.3	1.0
Oxytetracycline	30 MCG	1.2	1.3
Tetracycline	30 MCG	1.2	1.3
7-58F			
Chlortetracycline	30 MCG	1.5	1.3
Bacitracin	10 units	-	-
Cephalothin (Keflin)	30 MCG	-	-
Neomycin	30 MCG	1.4	1.1
7-58H			
Ampicillin (Polycillin)	10 MCG	-	-
Colistin	10 MCG	-	-
Polymixin B	300 units	-	-
Kanamycin	30 MCG	2.0	2.0

[a] - = No inhibition
Numbers indicate diameter size in centimeters of inhibition zone.

All of the four authentic isolates tested were sensitive to both phage isolates, although PA-7 reacted weakly to Br-1 phage.

The bacterial isolates P. marginalis 71-2, 71-3, P. fluorescens 65-1, E. carotovora 70-1, P. lachrymans 69-1, FTCC-2284, and FTCC-1869 gave no evidence of sensitivity to either of the two phage isolates.

Bacteriophage observations

Numerous bacteriophage particles were observed in all preparations. Several bacterial cells were observed with phage particles attached to the cell surface (Fig. 13). The majority of bacteriophage observed had very short tails. However two bacteriophage were observed with clearly discernable tails (Fig. 14). These two phages were examined closely and their heads measured about 52 nm in diam with a hexagonal configuration as viewed in two dimensions. The tail measured about 28 nm long by 8 nm wide. The majority of phages, however, had tails somewhat shorter than the ones actually measured.

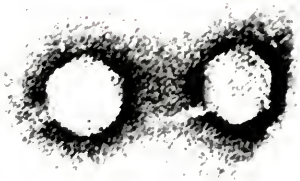
Discussion

The results with 33 test and four authentic isolates of P. marginata allows one to more fully characterize this bacterial species than has been reported to date. One character, heretofore ignored in past comparative tests of pseudomonads (8,62,71,77), including isolates labeled as P. marginata, has been that of pathogenicity on gladiolus.

All 33 test isolates were selected on the basis of causing neck-rot symptoms on gladiolus. The necessity for pathogenicity tests was dramatically illustrated in the investigation of isolate FTCC-2284, labeled P. marginata. This isolate, while fitting the taxonomic characteristics of P. marginata listed in the 7th edition of Bergey's Manual

Fig. 13. Electron micrograph of negative stained cell of P. marginata Br-1 with bacteriophage particles attached to cell surface. Magnification X 82,500.

Fig. 14. Electron micrograph of two negative stained bacteriophage particles from Br-1 phage stock. Head diam equals 52 nm. Tail equals 28 nm long by 8 nm wide. Magnification X 250,000.



of Determinative Bacteriology (9), had not been tested for pathogenicity on gladiolus. This isolate was catalogued as a Florida Type Culture of P. marginata and was used in comparative studies with other bacteria isolated from gladioli. Therefore, the method by which large numbers of bacterial isolates could be rapidly evaluated for pathogenicity developed in this study should be helpful in studying this organism in the future.

The hypodermic infiltration method of inoculation was adopted in which bacterial suspensions were injected into gladiolus leaf tissue. However, when inoculated gladioli were not placed in a moist chamber, inoculum concentrations of 10^6 cells/ml usually gave negative results, while 10^8 inoculum gave variable and inconclusive results with many of the isolates. Potted gladioli that were hypodermically inoculated and incubated in the moist chamber for up to 10 days gave disease reactions which were more pronounced and conclusive. The requirement for free water deposition correlates with field observations of neck-rot symptoms related to periods of high moisture, or water-logged fields. The presence of free water may be a requirement for bacterial multiplication and spread through the parenchyma tissue of gladiolus leaves as has been shown for onion (43,44). One hypothesis proposed has been that water-congestion reduced the osmotic potential so that bacteria can multiply and cause disease (72). Use of the moist chamber environment allowed for development of neck-rot disease from initial inoculum concentrations as low as 10^4 cells/ml. Under similar conditions, inoculations of 10^8 cells/ml of P. fluorescens, P. marginalis, P. lachrymans, E. carotovora, and FTCC-2284 resulted in negative pathogenicity.

However, isolate FTCC-1869, labeled as P. alboprecipitans, gave a pathogenic reaction when inoculated with 10^8 cells/ml on gladiolus.

Tests of pathogenicity to onion were undertaken to check if P. marginata isolates were capable of inducing disease of onion. Ballard et al. (8) stated that P. allicola, a pathogen of onion, and P. marginata should be considered as one species. While all of the test and authentic isolates of P. marginata caused lesions, all inoculations were with 10^8 cells/ml concentration, a high concentration of inoculum. These reactions could possibly be HR in nature, but the possible interrelationship between this group of bacteria and monocotyledenous plants is an interesting subject worthy of further investigation.

All 33 test isolates were pathogenic on gladioli, but isolates S-8, Y-71-1, G-71-2, and W-7 gave weak reactions in comparison to the other isolates. Isolate Y-71-1 is most probably not an isolate of P. marginata. This isolate was distinguished from the rest in being positive for levan production and glycollate utilization; negative for white precipitate formation (WP) and phage sensitivity; and producing non-water-soluble yellow pigmentation.

Isolate S-8 fit all tests for P. marginata with exception of growth in dL-B-Hydroxybutyrate, PHB accumulation, and sodium polypectate pitting tests. This isolate produced a rough colony type on NA, and is possibly a rough colony variant. Most rough isolates have been correlated with no or low pathogenicity (1).

The G-71-2 isolate fit all tests except for a negative WP formation and a very slow (20 sec) oxidase test.

An interesting group within the test isolates were six pathogenic isolates obtained from gladiolus meristem cultures (W series).

Two of these isolates gave variable results in some of the tests. Isolate W-6 was negative for phage sensitivity and pitting of sodium polypectate. Isolate W-7 was negative for WP formation, PHB accumulation, phage sensitivity, and was weak in pitting sodium polypectate and pathogenicity. However, these meristem isolates indicate the possibility of P. marginata, or closely related bacteria, as being resident flora of gladioli. The asexual methods of propagating gladioli may be a means by which this causal bacterium is being perpetuated in gladiolus planting stock. These bacteria may persist in such low numbers so as not to be pathogenic until environmental conditions develop (high temperatures and extended periods of free water) which allow for a rapid increase in bacterial populations necessary for pathogenesis. This is an area which demands further study.

Three of the four authentic isolates of P. marginata obtained for this study were non-pathogenic. Only isolate P. allicola PA-7 was pathogenic to gladioli. However, with the exception of WP formation, all of these isolates fit the tests for P. marginata. It is possible that the pathogenicity and WP characteristics were lost during the maintenance of these authentic isolates, since in all probability, no pathogenicity tests were made since the original isolation.

In reviewing the results of the many tests completed during this investigation, several require further discussion. Results of tests for the production of fluorescin on KMB confirmed the non-fluorescence property of P. marginata as reported by Ballard et al. (8) and by Schroth and Hildebrand (74). This dictates that results reported by Misaghi and Grogan (62) for P. marginata should not be considered valid as they tested fluorescent bacterial isolates labeled as P. marginata

without testing for pathogenicity on gladiolus. However, the present edition (7th) of Bergey's Manual of Determinative Bacteriology (12) reports P. marginata as producing a green fluorescent pigment in culture. Hopefully, this error will be changed in the 8th edition.

One might speculate that the confusion as to the presence of fluorescent pigments may have been due to the diffusable yellow pigment produced by many of the test isolates when cultured on NA, or more particularly KMB. However, this pigmentation does not fluoresce when viewed under LUV light. This pigment is possibly similar to the greenish-yellow pigments, partly cellular and partly diffusable, reported b Morris and Roberts (63) for strains of P. multivorans. Since then Ballard et al. (8) have designated P. multivorans and P. cepacia as one species, and P. marginata and P. allicola as another species. They have also stated that while these two complexes, plus P. caryophylli, represent three pheno-typically distinct clusters of strains, they do share many common properties. The yellow, non-fluorescent pigment may be one of these properties.

The formation of a white precipitate in nutrient agar by the majority of pathogenic test isolates was a unique characteristic revealed in this investigation. McCulloch (53) did not report this characteristic for isolates of P. marginata on peptone-beef agar, but did report "an area of increased translucency bordering the brown growth" on potato agar with dextrose (54). Another phytopathogenic bacterium described as producing a WP is P. alboprecipitans (70). Rosen isolated this bacterium from foxtail (Chaetochloa lutescens L.) and named it from its production of WP. He characterized this precipitate as being an inorganic salt, probably phosphatic in nature,

which optimally formed in beef-peptone agar of pH6.0. An isolate FTCC-1869, labeled as P. alboprecipitans, and highly pathogenic on corn (Zea mays L.) was included in this investigation of P. marginata isolates. This isolate was also pathogenic on gladioli, and fit most of the tests for P. marginata. However, this isolate did not form WP, was negative for phage sensitivity, and gave a rapid HR in tobacco with inoculum of only 10^8 cells/ml.

The chemical nature of this WP, and the possible use of the WP as a taxonomic or diagnostic character demands further study. However, for purposes of this investigation, the WP characteristic was utilized as a means of selecting pathogenic isolates from diseased gladioli, and as a marker in mite transmission studies. Of all the isolations made from diseased gladioli tissue, all WP forming isolates were pathogenic on gladioli, while only two non-WP forming isolates gave pathogenic results.

Results of the LOPAT tests of Lelliott et al. (51) were generally expected. The levan tests definitely established that P. marginata is negative for this character. Only one of the test isolates, Y-71-1 was positive for levan production, and this isolate is not considered a P. marginata isolate.

Oxidase test results agreed with those described by Ballard et al. (8) in that P. marginata is oxidase positive, but that some isolates give a slow reaction.

The potato soft-rot test gave some variable results as far as test and authentic isolates of P. marginata were concerned. Some isolates produced cratering, pitting, or browning of potato tissue, but never a true soft-rot reaction like E. carotovora or P. marginalis. While the negative soft-rot capability of P. marginata was reported by

McCulloch (53), the capacity of several of the test isolates to damage potato tissue may possibly be related to the scab symptom produced on gladiolus corms. Regardless, the determinations that P. marginata is negative for soft-rot and positive for tobacco HR fits two of the characteristics contributing to the usefulness of the LOPAT test for separating pseudomonads, although originally prescribed for only fluorescent pseudomonads.

The arginine dihydrolase test was one of the most consistent physiological tests conducted. All 33 test and four authentic isolates were negative for the constitutive presence of the enzyme arginine dihydrolase. The fluorescent pseudomonad erroneously identified as P. marginata, was positive for arginine dihydrolase, as were the isolates of P. fluorescens 65-1, and P. marginalis 71-2 and 71-3.

The tobacco hypersensitivity test as developed by Klement et al. (47) has been used as a diagnostic tool to screen pseudomonad bacterial isolates for pathogenicity. Klement et al. (47) and Lelliot et al. (51) reported that all pathogenic pseudomonads, except P. marginata, induce HR in foreign hosts (74). Misaghi and Grogan (62) reported two isolates of P. marginata as being negative for HR at inoculum concentrations as high as 10^{10} and 10^{12} cells/ml. However, they were testing fluorescent bacterial isolates labeled as P. marginata. No other references pertaining to P. marginata HR have been noted.

The results of this investigation indicates that P. marginata is capable of inducing HR in tobacco. This is a fairly significant test regarding pseudomonad taxonomy, and the realization that this characteristic may be used as a diagnostic tool for identification of P. marginata is significant. However, cognizance that high inoculum levels (10^9 cells/ml)

are necessary for reliable HR induction with this bacterium is critical (11).

An influence of temperature on HR induction seems to agree with results of Hildebrand and Riddle (35). They observed that rate and development of HR was related to optimum growth temperatures of bacterial species being tested. While the optimum temperature for growth of P. marginata is listed as between 30-32 C (20, 53, 78), results of this investigation indicate that during the logarithmic phase of bacterial growth the greatest rate of growth occurs ca. 32-35 C. Tobacco HR tests with P. marginata inoculum of 10^8 cells/ml were positive at incubation temperatures of 36-37 C, but slow, incomplete, or negative at 30 C and 28 C.

It is possible that the "weak" HR capacity of P. marginata is related to its relatively low level of virulence or aggressiveness. One might speculate that population levels necessary for HR induction, may be necessary for a pathogenic reaction in gladiolus, and that only under optimal environmental conditions of free-moisture and high temperature are bacteria able to grow to levels necessary for a pathogenic reaction.

The realization that P. marginata is HR positive should further strengthen its recent classification as a group III, non-fluorescent, arginine-dihydrolase negative, oxidase positive (74), foliar phytopathogenic pseudomonad.

Results of the sodium polypectate pitting test were surprisingly consistent with results published by Hildebrand (34). While Hildebrand reported results of seven isolates each of P. marginata and P. allicola, all but three of the 33 test isolates and all four authentic isolates gave the same results, that of greatest pitting on low pH (5.0) media.

Of the three negative isolates, W-6 was also negative for phage sensitivity, while isolate S-8, also negative for pitting, was positive for phage sensitivity, but was weakly pathogenic on gladiolus. Only isolate L-71-2 was not correlated with any other negative character. This isolate was pathogenic, produced WP on NA, and was sensitive to phage, but was negative for the sodium polypectate pitting test. On the other hand, only one isolate, Y-71-1, which was positive for the pitting test, was not considered a P. marginata isolate based on yellow pigmentation, a positive levan test, and a negative phage test.

Results of nitrate reduction tests, while constant for each isolate, were variable between isolates. A negative, plus three color types of positive reaction were determined for the test and authentic isolates. The history of this test has been one of variability with other bacterial species as well as results reported for P. marginata and P. marginalis (20,51,62,78). Confidence in this test is very suspect, and until an improved method, or interpretation becomes available, this test may be useful only for differentiation between isolates of P. marginata.

When the limited number of bacterial isolates were tested for growth in dL-B-Hydroxybutyrate, as a sole carbon and energy source, several interesting results were evident. With the exception of two test isolates, all P. marginata isolates were positive for growth, while such isolates as P. marginalis, P. fluorescens, P. lachrymans, and E. carotovora were negative. This property may have some potential as a diagnostic test for P. marginata and related bacteria. With one exception, all fluorescent isolates tested were negative or grew weakly in this defined medium.

Accumulation of PHB was not as consistent as reported by Ballard et al. (8). Since more isolates were tested in this investigation (33) than in Ballard et al.'s tests (17), these few exceptions may occur in nature. Few isolates were negative, with more giving an inconclusive result. However, all of the reported negative pseudomonad isolates included in this test were negative.

The nutritional test results, while in general agreement with, were not as uniform for all isolates tested as reported by Ballard et al. (8). Again results of 33 test isolates may be a more valid assessment of natural variation of P. marginata than was shown by the lesser number of isolates tested by Ballard et al. However, interpretation of positive or negative growth on several substrates was difficult for some of the isolates tested. The mechanics and interpretation of these tests are such that sole reliance on nutritional utilization for taxonomic purposes would be uncertain.

The isolation of bacteriophage(s) with the Br-1 isolate of P. marginata provided a useful tool for identifying isolates of P. marginata. With the exception of four test isolates, all of the test and authentic isolates were positive in phage-typing tests. However, caution needs to be exerted as several workers (9,10,79) have indicated that pseudomonad phage-typing has not been specific. Exceptions occur most frequently when larger numbers of bacterial isolates are used.

The bacteriophage specific for P. marginata in this study fits the criteria for the group III phage type as determined by Tikhonenko (81). This group is characterized by having head diameters between 40-64 nm, and very short tails having a maximum length of 30-35 nm. Tikhonenko has divided this group into two subgroups on the basis of structural

features of the short tail. The detailed structural features of this P. marginata phage require more detailed electron microscopy before placement into a subgroup. Modification of preparation might further elucidate the fine structural detail of this phage.

ATTRACTANCY TESTS OF *Anoetus feroniarum*, *Rhizoglyphus robini*, AND *Tyrophagus putresentiae* TO VARIOUS FUNGI AND BACTERIA

The following experiments were designed to determine (1) if any of several fungi and bacteria isolated from gladiolus provided suitable substrates for mite populations of *Anoetus*, *Rhizoglyphus*, or *Tyrophagus*; and (2) if attractancy and feeding preferences to specific microorganism cultures could be ascertained.

Materials and Methods

Attractancy Tests

In the first experiment potato dextrose agar (PDA) cultures in petri dishes of a *Fusarium sp.*, *Stromatinia sp.*, a fluorescent bacterium, designated as *Pseudomonas marginata* (McCulloch) Stapp FTCC-2284 (see second experiment), and a non-inoculated PDA plate were tested for their attractiveness to mite populations of *Anoetus feroniarum* DuFour and *Rhizoglyphus robini* Claparede. Covered petri-dish cultures were stacked in a closed desiccator, with separately covered PDA source plates of *A. feroniarum* and *R. robini*. The desiccator was held at 21 C under normal room lighting. Numbers of mites of each species in each test culture dish were counted after 7, 17, 27, and 57 days exposure to source plates. Counts were made by examination at 10 X magnification with a binocular dissecting microscope.

In a second experiment PDA cultures of selected fungal and bacterial isolates were tested for their attractiveness and ability to

support mite populations of A. feroniarum and Tyrophagus putresentiae (Schrank) mites. Those organisms and source tested for attractiveness are listed below. (1) Fusarium oxysporum Schlecht, f. sp. lycopersici Race 1 (Saccardo) Snyder and Hansen, FTCC-159, of Bureau of Plant Pathology, Division of Plant Industry and Consumer Services, Florida Department of Agriculture, Gainesville, Florida. (2) Stromatinia gladioli (Drayton) Whetzel, FTCC-14. (3) P. marginalis 71-3 from collection of Dr. R. E. Stall. (4) Pseudomonas marginata FTCC-2284. This isolate is believed to be misnamed. Although isolated from gladiolus, this bacterium was found to be fluorescent, and non-pathogenic when inoculated on greenhouse grown gladiolus. Results of the following LOPAT determinative tests of Lelliott et al. (51): Levan-positive; Oxidase-positive; Potato rot-positive; Arginine-positive; and Tobacco hypersensitivity-negative, indicated the probability that this bacterium was an isolate of P. marginalis.

Hyphal tips from the fungal cultures were transferred onto PDA plates. A loop from NB cultures of single colonies of the bacteria was transferred to PDA plates. Cultures were incubated under light for 72 h at 28 C before being used. An open water-agar (WA) petri plate inoculated with 100 adult mites of A. feroniarum and five mites of T. putresentiae was placed in the center of a glass desiccator. Open culture plates of the four organisms plus two non-inoculated PDA check plates were placed in circumference around the mite source plate. The desiccator was closed and placed in darkness at room temperature (21-24 C). Culture plates were examined microscopically after exposure of 1 and 6 days to the mite source plate. Numbers of mites of each species were recorded.

In a third experiment the attractiveness of the same cultures used in the second experiment to hypopi of A. feroniarum was tested. This experiment was similar to the second, except for inclusion of a non-inoculated WA check in place of a PDA check.

In a fourth experiment the relative attractancy of a fungal and two bacterial isolates for the hypopal stage of A. feroniarum was tested. PDA cultures of the following organisms were used:

(1) Fusarium oxysporum Schlecht. f. sp. gladioli (Massey) Snyder and Hansen. A pathogenic, single-spored isolate from a diseased gladiolus corm.

(2) Pseudomonas marginata Br-1. A non-fluroescent pathogenic bacterium isolated from diseased gladiolus growing at Bradenton, Florida.

(3) Pseudomonas marginalis 71-10. A fluorescent bacterium pathogenic to lettuce from collection of Dr. R. E. Stall.

Three-day old PDA cultures of the three above organisms plus a non-inoculated PDA check were arranged equidistantly around a PDA source plate containing hypopi of A. feroniarum. Cultures were placed in a closed desiccator in darkness at room temperature (21-24 C). Culture plates were removed after 24 h exposure to the mites and replaced with fresh PDA cultures. Source plates of mites were replaced when hypopi were depleted. Mean number of mites per 24 h period was calculated for each of the four test substrates. The means of each of the three cultures were compared statistically to the check treatment by means of the "student" t test (88). Significance at the 2% level was accepted. Percent attractancy of the three isolates to mites was calculated based on a method described by Yoshizawa et al. (86).

Behavior of R. robini and A. feroniarum Mites on Diseased and Clean Gladiolus Corms

Three clean (no lesions) and three diseased (numerous brown

lesions) gladiolus corms, cultivar "White Friendship", were freed of husks and extraneous tissue, washed in tap water, soaked for 10 min in 0.26% sodium hypochlorite, and then rinsed in sterile distilled water for 10 min. Each corm was dried and then placed on moistened filter paper in separate sterile 100 X 80 mm deep culture dishes. Fifteen mites each of A. feroniarum and R. robini were placed on each corm. The culture dishes were enclosed in plastic sacks and placed in darkness at 22 C. After 6 days each corm was removed and examined with a binocular dissecting microscope for the presence and location of the two mite species.

Results

Numbers of A. feroniarum and R. robini mites attracted to culture plates of the four substrates are listed in Table 13.

Apparently A. feroniarum preferred P. marginata and R. robini preferred Fusarium. Increases of A. feroniarum were also noted on Stromatinia and non-inoculated PDA check plates after 57 days exposure. However, bacterial contamination was noted and results were considered influenced by the affinity of A. feroniarum for bacterial substrates.

After exposure of mixed adult populations of A. feroniarum and T. putresentiae to selected test cultures, mites began to invade them after 1 day (Table 14). However, T. putresentiae appeared in higher numbers than A. feroniarum. After 6 days exposure, A. feroniarum preferred the two bacterial isolates, as well as the check plates after they became contaminated. T. putresentiae counts were variable on all substrates, and these data seem to confirm previous characterization of this mite being a promiscuous feeder on many substrates (75,85). The difference in rate of movement of the two mite species is probably due to movement of the hypopi of A. feroniarum. No hypopal stage is

Table 13. Numbers of mites in culture plates after exposure to separate populations of Anoetus feroniarum and Rhizoglyphus robini

Organism	Anoetus Days exposure[a]				Rhizoglyphus Days exposure[a]			
	7	17	27	57	7	17	27	57
Fusarium	0	6	10	26	13	27	40	100
Stromatinia	0	0	0	100*	0	0	0	34
Pseudomonas marginata 2284[b]	0	100	100	100	0	0	1	2
PDA check	0	0	15	42*	0	0	0	10

[a] Number of mites in culture plates. 100 = maximum number of mites counted.

[b] Tests indicate this isolate is probably P. marginalis.

* Denotes bacterial contaminated culture.

Table 14. Numbers of mites on selected cultures after exposure to mixed populations of adult Anoetus feroniarum and Tyrophagus putresentiae

Organism	Anoetus Days exposure 1	Anoetus Days exposure 6	Tyrophagus Days exposure 1	Tyrophagus Days exposure 6
Fusarium oxysporum f. sp. lycopersici	0[a]	48	40	20
Stromatinia gladioli	0	17	0	16
Pseudomonas marginata 2284	5	100	1	9
Pseudomonas marginalis 71-3	1	60	15	51
PDA Check 1	4	100[b]	0	13
PDA Check 2	0	45	4	5

[a] Number of mites in culture plates.

[b] Denotes bacterial contaminated culture.

formed by T. putresentiae, but adults are capable of immediate movement to more nutritious substrates (75). Since A. feroniarum is more mobile as a hypopus, several days were required for production of hypopal population, which then migrated to the test cultures.

Mites of A. feroniarum from predominantly hypopal populations migrated to bacterial rather than fungal, or check plates (Table 15). Sufficient numbers of mites were observed after 1 day exposure to confirm the initial observations that hypopal populations of A. feroniarum were the most migratory, and the best stage to test attractancy of cultures. Consistent with the data from the previous two experiments, A. feroniarum populations increased to highest levels on bacteria cultures.

A significant attractancy to the pathogenic isolate of P. marginata Br-1 by A. feroniarum occurred also. Mean numbers of mites per 24 h exposure to hypopal populations of A. feroniarum were significantly higher on P. marginata Br-1 plates than on either F. oxysporum f. sp. gladioli or non-inoculated PDA check plates (Table 16). The second highest number of mites was in the P. marginalis 71-10 plates. The attractancy to bacteria cultures by A. feroniarum was consistent with previous tests.

Behavior of A. feroniarum and R. robini Mites on Diseased and Clean Gladiolus Corms

No A. feroniarum mites were observed on any of the gladiolus corms. Rhizoglyphus robini and T. putresentiae mites were observed in significant numbers on all corms. The diseased corms had approximately twice as many R. robini mites as clean corms. In both corm treatments R. robini mites were observed only in areas of breakdown or decay. On the corms which were clean, R. robini and T. putresentiae mites were congregated mainly underneath the corms in areas where fungi were growing on the corm surface.

Table 15. Numbers of mites on selected cultures after exposure to hypopal populations of Anoetus feroniarum

Organism	Days exposure 1	4	7
Fusarium oxysporum f. sp. lycopersici Race 1	1[a]	56	92
Stromatinia gladioli	10	28	17
Pseudomonas marginata 2284	72	137	500
Pseudomonas marginalis 71-3	23	74	200
PDA Check	4	17	9
WA Check	6	23	42

[a] Number of mites on culture plates. 500 = maximum number counted.

Table 16. Attractancy of four substrates to hypopal populations of Anoetus feroniarum

Organism	Mean number of mites per plate per 24 hours exposure	% Attractancy
Fusarium oxysporum f. sp. gladioli	16.0[a]	49.8
Pseudomonas marginata Br-1	46.5*	74.2
Pseudomonas marginalis 71-10	34.5	68.1
PDA Check	16.1	-

[a] Numbers are means of 10 experiments.

* Denotes significant mean difference at 2% level of "student" t test.

Discussion

The soil-inhabiting, astigmatid mites A. feroniarum, R. robini and T. putresentiae can survive and reproduce on several fungi and bacteria commonly associated with gladiolus. Anoetus feroniarum is strongly attracted to bacterial cultures of P. marginata and P. marginalis. Rhizoglyphus robini prefers F. oxysporum.

The fact that mites are attracted to selected phytopathogens may be important in elucidating possible disease-vector relationships of gladiolus in Florida. The demonstrated affinity by A. feroniarum for bacterial cultures, in particular the pathogenic isolate P. marginata Br-1, suggest that the vector capabilities of this mite should be investigated. Conversely, the demonstrated affinity by R. robini for Fusarium cultures, and not bacterial cultures, does not readily relate to published reports implicating these mites as vectors of the causal organism of bacterial scab, P. marginata. It should be pointed out that the species of Rhizoglyphus used in these experiments is not the same as reported by Forsberg (28,29) to be a vector of P. marginata. Species, or even clonal, variation in preferences for certain disease-causing organisms should be investigated.

Hypopal populations of A. feroniarum were the most migratory and best stage by which to test attractancy of cultures. The hypopus is a non-feeding migratory form resistant to, and apparently formed as a result of environmental stress. They are transported, or will migrate, to areas of higher nutritional levels, and lower environmental stress before completion of life cycle (67,82,85). Since both A. feroniarum and R. robini have a hypopal stage in their life cycle, the importance of this stage relative to their suggested vector roles demands further study.

ACQUISITION AND RETENTION OF *Pseudomonas marginata* BY *Anoetus feroniarum* AND *Rhizoglyphus robini*

After discovery that *A. feroniarum* preferentially moved to bacterial cultures, particularly *P. marginata* Br-1, a series of experiments were designed to determine (1) if mites ingested bacteria; (2) whether viable bacteria passed through the mite; and (3) how long a mite retained the bacteria, whether on its body surface, or within its alimentary tract.

Materials and Methods

In these experiments two isolates of *P. marginata*, Br-1SR (streptomycin-resistant mutant) and F-1 (an isolate which formed a white precipitate (WP) on NA) were used as bacterial substrates for *A. feroniarum* and *R. robini* mites.

Detection of P. marginata from Surface Sterilized Mites

An experiment was designed to test the efficacy of surface sterilization techniques for mites, and to determine if *P. marginata* Br-1SR cells could be retrieved from within mites. Individual mites were removed from bacterial lawns and dipped into 0.78% sodium hypochlorite, or 95% ethyl alcohol, for periods of 3-5 min. After dipping, the mites were allowed to dry and placed on NA for 5 min. Each mite was then aseptically transferred to NB and placed on a rotary shaker at room temperature. Each NB tube with a mite was observed for the first turbidity, indicative of bacterial growth. After turbidity occurred samples were streaked on media of NA and NA containing 200 ppm

streptomycin and 100 ppm penicillin (NASP). Data recorded were number of days required for visible growth in NB, and whether any colonies with white precipitate (WP) developed on NA after streaking from the culture. The latter was used to identify P. marginata.

Retrieval of WP-forming Bacteria from Within Mites Exposed to Cultures of P. marginata

Separate populations of A. feroniarum and R. robini were placed on lawn cultures of Br-1SR and F-1 for 48 h. For each test, three mites observed feeding or probing the bacterial lawn were removed and surface sterilized (SS) by dipping into solutions of 95% ethyl alcohol or 0.78% sodium hypochlorite for 5 min. Mites were then removed, drained, dried for 10 min, and placed on NA. Three mites per plate were rolled over the NA surface to areas visually clear of any possible SS residue. Two of the three mites were crushed with sterile probes and separately streaked over the NA medium. The third mite was left intact, but rolled, or dragged over the NA surface. The NA plates were then incubated at 30 C for up to 72 h with daily observation for bacterial growth. Colonies forming WP were assumed to be P. marginata.

Dilution Plate Assay of Bacteria from Mites Exposed to P. marginata Br-1SR

Separate populations of A. feroniarum and R. robini were placed on 7 and 8-day old lawn cultures of P. marginata Br-1SR for 48 h. Mites observed feeding or probing the bacterial lawn were removed and SS. After mites were drained and dried, separate mites were aseptically transferred to dilution tubes containing 0.45 ml of BS, crushed, and a dilution series run. The contents were plated on NASP, and the plates incubated at 30 C for up to 72 h. Bacterial counts were made and colonies forming WP assumed to be P. marginata.

Mite-Bacteria Retention Time Studies

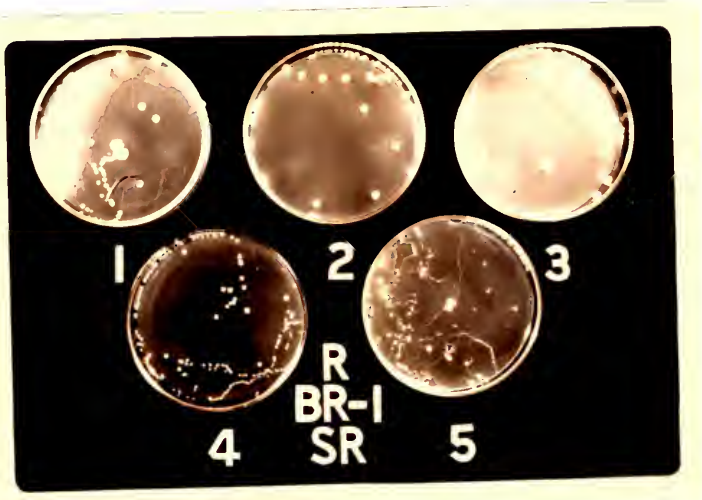
Lawns of P. marginata Br-1SR, growing on NASP, and F-1 growing on NA were prepared and infested with separate populations of 20 A. feroniarum and 20 R. robini from stock colonies. The mite-infested bacterial cultures were taped tightly closed and the mites were allowed access to the cultures for at least 24 h. Mites observed in contact with bacteria lawns were individually removed and placed either on separate NA or NASP plates. At 24 h intervals, each mite was aseptically removed and transferred to another plate of similar medium. This sequence was repeated until either the mite died, or escaped the culture plate. The agar plates from which the individual mites were removed were incubated for 24 h at 30 C. They were then visually examined for colonies of bacteria forming a white precipitate (WP) on NA, which were considered P. marginata (Fig. 15). Data recorded were number of days in which at least one colony of P. marginata was observed on a NA plate. All tests were pooled and an average number of days which a mite would transmit a bacterium forming a WP was determined for each species of mite and bacterial isolate.

A similar experiment was conducted with both species of mites after they were exposed to NA cultures of E. carotovora 70-1. Individual mites were aseptically removed and transferred to clean plates of Cuppel's (15) sodium polypectate medium. Retention of E. carotovora 70-1 by A. feroniarum and R. robini mites was determined by the presence of pit formations.

Dissemination of Bacteria by A. feroniarum Obtained from Diseased Gladioli Inoculated with P. marginata

Gladiolus plants, cv "Beverly Ann", inoculated by hypodermic

Fig. 15. Results of 5-day test for retention of Pseudomonas marginata Br-1SR by Rhizoglyphus robini. Note on plates 1-4 the pattern of discrete bacterial colonies forming a white precipitate in media surrounding the colony. On plate 5 note the formation of continuous trails of non WP-forming bacteria.


1
2
3
R
BR-1
SR
4
5

injection of a concentration of 10^8 cells/ml of P. marginata F-1, were placed in a moist chamber and allowed to develop meck-rot symptoms. As disease progressed the presence of A. feroniarum mites were observed on tissue that was rapidly deteriorating. Twenty-five mites were individually removed from diseased tissue and placed in the center of separate NA plates. The plates were incubated at 30 C for 24 h at which time the mites were removed from the plates. The NA plates were then incubated and observed daily for 7 days for signs of WP forming colonies.

Dissemination of P. marginata by R. robini Obtained from Diseased Gladioli

Gladiolus plants inoculated with Br-1, S-D, and F-1 isolates of P. marginata and with neck-rot symptoms were removed from field plots and examined for mites. Rhizoglyphus robini mites were removed from diseased tissue and individually plated on NA plates. The mites were allowed on NA plates for two successive 48 h periods at 30 C. The plates were observed daily for 10 days for signs of WP forming colonies.

Results

Detection of P. marginata from Surface Sterilized Mites

Surface sterilization techniques employed in this test were effective. Evidence for this was the fact that 2 and 3 days were required for visible growth in NB, while non-surface sterilized mites placed in NB tubes produced turbidity within 24 h.

Colonies with a WP in NA were obtained only from NB cultures containing R. robini mites exposed to bacterial cultures 7 days old, or less. A. feroniarum mites exposed to 7 and 11-day old cultures were negative for presence of Br-1SR (Table 17).

Table 17. Detection of P. marginata Br-1SR from mites after exposure to bacterial lawns

Mite species	Mite No.	Age of Br-1SR lawn in days	Days before growth in NB tubes	Presence of cells forming WP colonies from NB tubes	
				NASP[a]	NA[a]
Anoetus feroniarum	1	7	2	-	-
	2	11	2	-	-
Rhizoglyphus robini	1	3	3	+	+
	2	7	2	+	+
	3	10	2	-	-
	4	12	2	-	-

[a] NASP = Difco nutrient agar containing 200 ppm streptomycin and 100 ppm penicillin.
NA = Difco nutrient agar

Retrieval of WP-forming Bacteria from Within Mites Exposed to Cultures of P. marginata

Of 17 mites (13 A. feroniarum and 4 R. robini) which were crushed on NA plates, WP forming colonies appeared on NA from 13 mites (Table 18). Of nine intact mites which were surface sterilized and then crushed only one was positive for WP forming bacteria, and this produced only one colony. In most tests, both species of crushed mites were positive for WP forming bacteria, while the single intact mite was negative for WP bacteria. These results were interpreted as indicating the presence of WP forming bacteria within the mite's body. Surface sterilization techniques were deemed effective to eliminate the body surface as a source of these bacteria.

Dilution Plate Assay of Bacteria from Mites Exposed to P. marginata Br-1SR

Two A. feroniarum mites, obtained from 7-day old Br-1SR cultures, revealed no colonies forming WP, and only four and two bacterial colonies each from the first dilution assay (Table 19).

Two of four R. robini mites tested contained Br-1SR bacteria within their body. These WP forming colonies were interspersed among non-WP forming bacteria which greatly outnumbered the Br-1SR bacteria. However the two positive mites were taken from 7-day old Br-1SR cultures, while the two negative mites were taken from 8-day old colonies. Here, as with the first two mites, bacterial colonies too numerous to count (TNTC) were obtained from these dilution plates.

Mite-Bacteria Retention Time Studies

The mean number of days that P. marginata Br-1SR and F-1 were retained by A. feroniarum and R. robini mites are listed in Table 20.

Table 18. Assay for presence of P. marginata within body of A. feroniarum and R. robini mites

Mite species	Bacterial isolate	Colonies producing WP in NA			
		Crushed		Intact	
		No. mites	rating[c]	No. mites	rating[c]
Anoetus feroniarum	F-1	2[a]	++	1	+
		2	++	1	-
		2[b]	--	1	-
		3	++-	2	--
	Br-1SR	2[a]	++	1	0
		2	+-	1	-
Rhizoglyphus robini	F-1	2[a]	++	1	-
		2	++	1	-
		17	13 +	9	1 +

[a] Mites surface sterilized by dipping in 95% ethyl alcohol for 5 min.

[b] Mites surface sterilized by dipping in 0.78% sodium hypochlorite for 5 min.

[c] + = Presence of bacteria producing WP in NA
- = Presence of bacteria, but none produced WP in NA
0 = No bacterial growth

Table 19. Dilution plate assay of bacteria from mites after exposure to P. marginata Br-1SR

Mite species		Age of Br-1SR bacterial culture in days	Number of bacterial colonies from crushed mites	
			Dil No. 1	Dil No. 2
Anoetus feroniarum	No. 1	7	4 -[a]	0
	2	7	2 -	0
Rhizoglyphus robini	1	7	TNTC +	TNTC +
	2	7	" +	" +
	3	8	" -	" -
	4	8	" -	" -

[a] TNTC = Too numerous to count

\+ = Presence of bacterial colonies forming WP in NASP media.

\- = Presence of bacterial colonies not forming WP in NASP media.

After exposure to F-1 bacterial lawns, A. feroniarum disseminated the bacteria for an average of 2.3 days, with a minimum of 1 day and a maximum of 5 days. With Br-1SR, A. feroniarum averaged 1.8 days, with a minimum and maximum of 1 and 3 days, respectively.

After exposure to F-1 bacterial lawns, R. robini disseminated the bacteria for an average of 1.3 days, with a minimum and maximum of 1 and 3 days, respectively. With Br-1SR, R. robini averaged 2.4 days, with a minimum and maximum of 1 and 5 days, respectively.

A. feroniarum mites exposed to lawns of E. carotovora 70-1, disseminated this bacterium for an average of 1.5 days, with a minimum of 1 and a maximum of 2 days. Rhizoglyphus robini averaged 2.8 days, with a minimum of 2 and a maximum of 3 days (Table 21).

Dissemination of Bacteria by A. feroniarum Obtained from Diseased Gladioli Inoculated with P. marginata

While bacterial colonies were disseminated by all 25 mites, none of the bacterial colonies produced the WP typical of the F-1 isolate of P. marginata. Bacterial colonies selected and tested for pathogenicity on gladiolus gave negative results.

Dissemination of P. marginata by R. robini Obtained from Diseased Gladioli

As shown in Table 22 of 25 R. robini mites tested, only three disseminated WP producing colonies. Two WP colonies were observed and isolated from two NA plates which had been exposed to one R. robini mite obtained from a S-D inoculated gladiolus. One of these was fungal in nature, but the other was a bacterium that was pathogenic on gladiolus. This isolate was labeled as test isolate R-A-1. Two WP colonies were observed in separate NA plates, each of which had been exposed to single

Table 20. Bacterial retention time of mites after exposure to *P. marginata* cultures

Number and species of mite tested	Bacterial isolate exposed to:	Mean number of days bacterial colonies producing WP in NA
15 *Anoetus feroniarum*	F-1	2.3
6 "	Br-1SR	1.8
15 *Rhizoglyphus robini*	F-1	1.3
7 "	Br-1SR	2.4

Table 21. Bacterial retention time of mites after exposure to *E. carotovora* cultures

Number and species of mite tested	Bacterial isolate exposed to:	Mean number of days bacterial colonies producing pits in pectate media
15 *Anoetus feroniarum*	70-1	1.5
6 *Rhizoglyphus robini*	"	2.8

Table 22. Assay for presence and dissemination of *P. marginata* by *R. robini* obtained from diseased gladioli

Rhizoglyphus robini mites		Plate "A" 1st 48 h exposure	Plate "B" 2nd 48 h exposure
From S-D diseased gladioli	No. 1	-[a]	-
	2	-	-
	3	-	-
	4	+ (2 colonies)	-
	5	-	-
	6	-	-
	7	-	0
	8	-	-
	9	-	-
	10	-	0
From Br-1 diseased gladioli	1	-	-
	2	-	-
	3	-	0
	4	-	-
	5	-	-
From F-1 diseased gladioli	1	-	X
	2	-	X
	3	-	X
	4	-	X

Table 22 - continued.

Rhizoglyphus robini mites	Plate "A" 1st 48 h exposure	Plate "B" 2nd 48 h exposure
5	-	X
6	-	X
7	+ (1 colony)	X
8	+ (1 colony)	X
9	-	X
10	-	X

[a]

+ = Colony producing WP in NA

- = Bacterial and/or fungal colonies, but none of which produced WP in NA

X = Mite not transferred a second time

0 = Mite either gone or dead resulting in no bacterial or fungal growth in NA

R. robini mites obtained from F-1 inoculated gladioli. Both of these isolates gave similar pathogenicity, physiological, and biochemical test results and were considered as a single isolate R-A-4.

Discussion

To determine if bacteria were ingested by A. feroniarum and R. robini mites, a method for surface sterilizing mites was necessary to eliminate the body surface as a source of bacteria. By dipping mites into various concentrations of sodium hypochlorite and ethyl alcohol for periods of 3-5 min, surface sterilization was evidently accomplished. This made possible an assay for detection of P. marginata within mite bodies.

The age of the bacteria culture that mites were exposed to was also an important factor insofar as testing for the presence of P. marginata. Growth of bacteria originating from the mites may have competively inhibited P. marginata growth since mites fed on cultures 8 days or older failed to show signs of P. marginata from within their body.

When the two species of mites were separately placed in petri-dish cultures of P. marginata, A. feroniarum exhibited an affinity for the bacteria-agar substrate, while R. robini exhibited an aversion for same. Anoetus feroniarum was generally in constant association with the substrate, while R. robini had to be continually replaced onto the bacterial substrate from the upper surface of petri-dish covers. These observations are in accord with results obtained from the attractancy tests.

After exposure to viable cultures of P. marginata, both species of mites, when SS and crushed on NA, gave positive results indicating that P. marginata was ingested and survived for at least 12 h within their gut.

However the incidence of detection or retrieval was more consistent from R. robini than A. feroniarum.

While the dilution plate assay of P. marginata from mites was too limited to make any definite conclusions, results indicated that after exposure to bacterial substrates, R. robini mites contained a greater concentration of bacteria than did A. feroniarum. With one or two dilutions, colonies TNTC were observed for all four R. robini mites tested. The two A. feroniarum mites produced only four and two bacterial colonies respectively.

These experiments indicate that both species of mites are capable of ingesting P. marginata when forcibly exposed to bacterial substrates in closed petri-dish cultures. However it appears that R. robini mites may retain viable cells of P. marginata for a longer duration, or in greater numbers than A. feroniarum.

Retention time studies with both species of mites indicated that even with P. marginata on the body surface, as well as being ingested, dissemination of P. marginata was of short-term duration, averaging no more than 3 days. Dispersal patterns indicated that bacteria were being intermittantly disseminated perhaps by excretory processes. Most often by the fourth transfer day, continuous trails of non-P. marginata bacterial growth were present indicating a transition of bacterial flora from P. marginata to non-pathogenic resident flora associated with the mites (Fig. 15). Results of these experiments tend to diminish the probability of deutonymphs or hypopi acting as mobile reservoirs of P. marginata inoculum. Retention of P. marginata is probably of too short duration to enable viable bacteria to be in the gut of transforming mites.

The low incidence (3 of 25 mites tested) of R. robini mites positive for dissemination of P. marginata from diseased gladioli to NA plates indicates that though possible, this mode of inoculum dissemination is probably not efficient. On the other-hand, the completely negative results obtained with A. feroniarum from neck-rot diseased gladioli tend to diminish the probability of these mites being capable of any long-term dissemination or vectoring of P. marginata. Of the two mite species studied, R. robini seems better suited for retaining P. marginata once ingested, and therefore more suspect as a possible vector of P. marginata. However the incidence was of such low frequency that short term dissemination is more probable than long term.

DISCUSSION

All data obtained indicate at best short-term capability for mite dissemination of Pseudomonas marginata. In Florida, while both species of mites are found, Rhizoglyphus robini is the species most commonly found in association with diseased gladiolus corms. Anoetus feroniarum was found much less frequently under field conditions, but in this study occurred in large numbers on greenhouse-grown gladioli exhibiting symptoms of bacterial neck-rot.

Anoetid mites have been characterized as slime mites, living in wet organic substrates, feeding on microorganisms strained from aqueous substrates with mouthparts consisting of highly modified palpi adapted for straining (38,39,49). That anoetid mites are attracted to and will preferentially feed on bacterial substrates was shown in this investigation. Populations were observed to increase to high numbers until either deutonymph or hypopal stage of growth. However the retention of cells of P. marginata and E. carotovora, both on or within the mite's body, was shown to be of such short duration that the possibility of viable bacteria cells being present during the hypopal transformation must be considered highly improbable. One might reason that since A. feroniarum mites are adapted for feeding on bacteria, they possess enzymatic capacity for breakdown and digestion of bacteria for nutritional purposes. Another possibility is that these mites are so adapted for bacterial flora, high populations of resident bacteria may competively inhibit ingested or acquired P. marginata. Whatever the mechanism,

indications are that A. feroniarum is incapable of retaining viable cells of P. marginata longer than 3-5 days. In addition, their mouthparts are not adapted for chewing, piercing, or puncturing tissue that would be encountered on healthy gladiolus leaf and corm tissue.

All of the above factors would tend to negate the ability or capabilities of A. feroniarum to act as passive or active vectors of P. marginata, with the possible exception of very short-term passive dissemination. More probable A. feroniarum may occupy an ecological niche in which high populations of bacterial organisms predominate. In Florida, the presence of A. feroniarum in soil or plant tissue may just indicate high populations of pathogenic or saprophytic bacteria.

The role played by R. robini found in association with diseased gladioli remains uncertain. This investigation produced no data or observations which would point to R. robini acting as an active or primary vector of P. marginata. However, several aspects of this possible relationship require further study.

Rhizoglyphus species have been characterized as being primarily saprophytic scavengers (85) with an affinity for fungi as well as organic debris of plants and animals. Rhizoglyphus robini was not attracted to bacterial substrates, but upon forced exposure to P. marginata cultures this mite was found capable of ingesting and disseminating viable bacteria for up to 3-5 days. This mite species when taken from neck-rot diseased gladioli was also positive for dissemination of P. marginata colonies, although of very low incidence (3 out of 25 mites).

Since R. robini is neither attracted to, nor requires, bacteria as a nutritional substrate, it is conceivable that this mite species

does not possess the enzymatic capacity for breakdown or digestion of bacteria. Therefore this mite may provide a more suitable internal environment for the survival of passively acquired P. marginata than that of A. feroniarum.

Another factor possibly influencing R. robini retention of P. marginata is that of competition by other bacterial flora. This species of mite may not possess as high levels or diverse bacterial flora as that of anoetid mites which could competively inhibit the growth and survival of acquired P. marginata.

The role of mouthparts of Rhizoglyphus species relative to infection sites must also be further investigated. Several reports exist describing various Rhizoglyphus species causing mechanical damage to roots, bulbs, and storage organ tissue (6,28,36,73). In this investigation no indication of R. robini injuring or damaging gladiolus leaf or corm tissue was observed. However the possibility of their mouthparts being capable of causing small wounds in husk and adjacent corm tissue must be considered. Rhizoglyphus robini mouthparts, unlike those of A. feroniarum, are not adapted for straining, but instead possess chelate-dentate chelicerae adapted for grasping or grinding, which along with the pedipalps, comprise the organs of food acquisition (49). The stage of corm development or maturity may also be a factor critical to R. robini ability to cause mechanical damage.

Rhizoglyphus robini could possibly passively disseminate P. marginata from areas of bacterial infection to nearby areas of gladiolus corms while searching for and feeding on diseased or degrading plant tissue. P. marginata diseased husk tissue, the adjacent development of soft, succulent, immature corm tissue, the presence of free water and high temperature, and the movement and probing of R. robini may be the necessary

combination required for initiation of bacterial scab of gladioli. This hypothetical series of events and factors seem to be the only way one could relate or explain the results obtained by Forsberg (28,29) in his mite-scab symptom studies. Further studies are required to properly evaluate the importance of mite damage, stages of development and susceptibility of corms, and the competitive ability of P. marginata, within or on, R. robini and A. feroniarum mites.

The possibility of other soil-inhabiting organisms, such as insects and nematodes, operating in a likewise manner, needs careful investigation. The early reports of pesticides reducing scab incidence (27,87), the consideration of Phyllophaga as a possible vector (26), the ineffectiveness of Temik, the miticide, insecticide, and nematicide, in reducing scab incidence (30), and the recent work in Israel (19) indicating an association of Meloidogyne spp. (Root-knot nematode) with incidence of bacterial scab all point to the possibility of many soil-inhabiting organisms being capable of short-term passive transmission of this bacterial organism. Possibly this disease results from a necessary combination of pathogenic strains of P. marginata, proper environment of free moisture and high temperature, and the formation of areas of ingress by one or more soil-inhabiting organisms which are frequenting the rhizosphere of diseased and newly developing gladiolus corms.

SUMMARY

Thirty-three Florida isolates of Pseudomonas marginata (McCulloch) Stapp, the causal organism of bacterial neck-rot and scab of gladiolus, were collected and compared to four authentic isolates. In a series of pathogenicity, physiological, nutritional, and bacteriophage-typing tests, isolates were compared to existing literature and taxonomic schemes for P. marginata.

In pathogenicity tests a method was developed by which large numbers of isolates could be rapidly tested. Environmental factors such as presence of free-water and high temperatures were found necessary for significant disease expression.

Results of numerous tests provided a basis for the following characterization of Florida isolates of P. marginata. Bacterial cells measured 0.71-0.88 x 1.4-2.3 u. The majority possessed one to two polar flagella. Optimum growth was ca. 35 C. Cellular pigmentation was a non-fluorescent cream to yellow. The bacterium grows aerobically and does not produce levan, but is oxidase-positive. Most isolates produce a white precipitate (WP) when cultured on nutrient agar (NA). This bacterium does not soft rot potato, but some isolates are capable of pitting or cratering potato in a dry-rot manner. This bacterium is positive for pectolytic activity on substrates of pH 5.0-7.0, but negative for constitutive presence of arginine-dihydrolase. A majority of isolates are positive for nitrate reduction and growth in dL-B-hydroxybutyrate (PHB) and were positive for ortho cleavage of phenols.

P. marginata was positive for growth on D-(-)-tartrate, but negative for growth on glycollate as sole sources of carbon and energy. This bacterial species is gram negative and is tobacco hypersensitive (HR) positive when tested with inoculum of 10^9 cells/ml and at incubation temperatures of ca. 35-37 C. A bacteriophage(s) specific for P. marginata was isolated and utilized in phage-typing procedures.

Three species of soil-inhabiting, astigmatid mites were investigated relative to their potential as vectors of P. marginata to gladioli. Tyrophagus putresentiae, the culture mite, was observed primarily as a laboratory pest of diseased corms rather than in association with field diseased gladioli. This mite is a polyphagous feeder with no hypopal stage in its life cycle. Anoetus feroniarum exhibited an attractancy and feeding preference for bacterial substrates, particularly P. marginata. The hypopus was found to be the most mobile and attracted stage of this mite's life cycle, and was considered as a possible source of P. marginata under field conditions. A. feroniarum was shown to ingest cells of P. marginata but retention was of such short duration (3-5 days) that the hypopus could not be considered as a source of P. marginata inocula. This species of mite was negative for dissemination of P. marginata from neck-rot diseased gladioli, and lacks mouthparts capable of inflicting wounds in plant tissue. Indications are that this mite is probably a bacterial feeder, with an insignificant short-term, passive vector capability.

R. robini preferred fungal rather than bacterial substrates. However, this species of mite ingested cells when forcibly exposed to cultures of P. marginata, but retention was of only short-term duration

similar to A. feroniarum. The low level incidence of dissemination of P. marginata from neck-rot diseased gladioli, plus the possession of mouthparts capable of possible infliction of wounds in newly developing cormels, suggests a stronger possibility of R. robini being a significant, short-term passive vector of P. marginata.

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APPENDIX 1. Sources of Isolates Used in Study

Isolate No.	Code	Source	Host and Place of Origin
1	Br-1	Noble[a]	Gladiolus flower stalk, Bradenton, Fla. 1971
2	Br-1SR	"	Selectively induced mutant from Br-1 seeded on Streptomycin NA. 1972
3	Br-2	"	Gladiolus flower stalk, Bradenton, Fla. 1971
4	Br-4	"	Gladiolus flower stalk, Bradenton, Fla. 1971
5	Br-4A	"	Colony variant from Br-4 plate
6	Br-4B	"	Colony variant from Br-4 plate
7	G-3	"	Gladiolus corm, Bradenton, Fla. 1970
8	G-9	"	Gladiolus leaf, Bradenton, Fla. 1970
9	G-14	"	Gladiolus corm, Bradenton, Fla. 1970
10	G-15	"	Gladiolus corm, Bradenton, Fla. 1970
11	S-D	"	Gladiolus corm, Gainesville, Fla. 1972
12	S-72-1	"	Gladiolus corm, Gainesville, Fla. 1972
13	S-8	"	Gladiolus corm, Marianna, Fla. 1971

APPENDIX 1 - continued.

Isolate No.	Code	Source	Host and Place of Origin
14	F-1	Noble	Gladiolus corm scab lesion on "Big Time" corms sent from Illinois. 1971
15	F-1048C	"	Gladiolus "White Friendship" neck lesion from greenhouse experiment, Bradenton, Fla. 1972
16	W-3	"	Gladiolus meristem tissue culture, Bradenton, Fla. 1971
17	W-4	"	Gladiolus meristem tissue culture, Bradenton, Fla. 1971
18	W-4A	"	Colony variant from W-4 plate
19	W-6	"	Gladiolus meristem tissue culture, Bradenton, Fla. 1971
20	G-1	"	Gladiolus corm, Bradenton, Fla. 1971
21	Y-71-1	"	Gladiolus leaf, Marianna, Fla. 1971
22	L-71-2	"	Gladiolus leaf, Marianna, Fla. 1971
23	C-71-3	"	Gladiolus corm, Marianna, Fla. 1971
24	P. allicola 7	ICPB[b]	Received as P. allicola (NCPPB 947, ATCC 19302) isolated by W. H. Burkholder, Fall, 1939, from onion.
25	P. marginata 106	"	Received as P. marginata (ATCC 10247, NRRL B-792, NCPPB 1890) December, 1948 from W. C. Haynes, USDA Northern Regional Research Laboratory, Peoria, Illinois

APPENDIX 1 - continued.

Isolate No.	Code	Source	Host and Place of Origin
26	P. marginata 107	ICPB[b]	Received as P. marginata (ATCC 10248, NRRL B-793, NCPPB 1891) 1948, from W. C. Haynes, USDA Northern Regional Research Laboratory, Peoria, Illinois.
27	ATCC-10247	ATCC[c]	Received as P. marginata (NRRL B-792, NCPPB 1890) from W. C. Haynes, 1948, USDA Northern Regional Research Laboratory, Peoria, Illinois.
28	Br-4C	Noble	Colony variant from Br-4 plate
29	F-1048B	"	Gladiolus "White Friendship" neck lesion from greenhouse experiment, Bradenton, Fla. 1972
30	H-72-1	"	Gladiolus husk, Gainesville, Fla. 1972
31	W-1	"	Gladiolus meristem tissue culture, Bradenton, Fla. 1971
32	G-2	"	Gladiolus leaf, Bradenton, Fla. 1970
33	R-A-4	"	Turbid colony from Rhizoglyphus robini trail on NA, Bradenton, Fla. 1973
35	P. marginalis 71-2	Stall[d]	Lettuce, Belle Glade, Fla. 1971
36	P. marginalis 71-3	"	Lettuce, Belle Glade, Fla. 1971
38	P. fluorescens 65-1	"	Obtained from ICPB, 1965
39	E. carotovora 70-1	"	Lettuce, Belle Glade, Fla. 1970

APPENDIX 1 - continued.

Isolate No.	Code	Source	Host and Place of Origin
40	P. lachrymans 69-1	Stall	Watermelon, Immokolee, Fla. 1969
41	FTCC-2284	FTCC[e]	Gladiolus, Gainesville, Fla. Listed as P. marginata.
42	R-A-1	Noble	Turbid colony from Rhizoglyphus robini trail on NA, Bradenton, Fla. 1973
43	G-71-2	"	Non-turbid colony variant from L-71-2, Marianna, Fla. 1971
44	H-72-2	"	Gladiolus husk, Gainesville, Fla. 1972
45	W-7	"	Gladiolus meristem tissue culture, Bradenton, Fla. 1971
47	FTCC-1869	FTCC	Corn, Gainesville, Fla. Listed as P. alboprecipitans

[a]Noble = isolated from fresh plant material.

[b]ICPB = International Collection of Phytopathogenic Bacteria, Davis, California.

[c]ATCC = American Type Culture Collection, Rockville, Maryland.

[d]Stall = Dr. R. E. Stall, Plant Pathology Department, University of Florida, Gainesville, Florida.

[e]FTCC = Florida Type Culture Collection, at Bureau of Plant Pathology, Division of Plant Industry and Consumer Services, Florida Department of Agriculture, Gainesville, Florida.

BIOGRAPHICAL SKETCH

William Estep Noble was born on July 21, 1940 in Washington, D. C. He attended grade school and high school in the city of his birth. He was graduated from Calvin Coolidge High School in June, 1958, and entered the University of Maryland at College Park in September, 1958. He received the Bachelor of Science degree in floriculture and ornamental horticulture in January, 1964. He began graduate studies at the University of Maryland in February, 1964. Under the supervision of Dr. J. B. Shanks, he received in January, 1969, the Master of Science degree in horticulture, with minors in plant pathology and biometrics. During the period from 1967 to 1969 he was Graduate Horticulturist for Woodside Gardens, Inc. in Rockville, Maryland. He continued his graduate education at the University of Florida in September, 1969 by commencing work towards a Doctor of Philosophy degree in plant pathology. He is presently employed as an Assistant Professor in the Ornamental Horticulture Department of the California Polytechnic State University, at San Luis Obispo, California.

He is a member of the American Society for Horticultural Science, American Horticultural Society, Florida State Horticultural Society, and the American Phytopathological Society.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert E. Stall

Robert E. Stall, Chairman
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Arthur W. Engelhard Robert E. Stall

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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