DISSERTATION

FACTORS AFFECTING POTATO EARLY DYING IN THE SAN LUIS VALLEY, COLORADO

Submitted by

Robert Day Davidson

Department of Plant Pathology and Weed Science

In partial fulfillment of the requirements

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Colorado State University

Fort Collins, CO. 80523

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY ROBERT DAY DAVIDSON ENTITLED "FACTORS AFFECTING POTATO EARLY DYING IN THE SAN LUIS VALLEY, COLORADO" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Monty D. Harrison

Kenneth W. Knutson

Gary A. McIntyre

Harold F. Schwartz

Adviser

Gary A. McIntyre

Department Head

ABSTRACT OF DISSERTATION

FACTORS AFFECTING POTATO EARLY DYING IN THE SAN LUIS VALLEY, COLORADO

A three year comparison of Russet Burbank potatoes field grown in microplots containing combinations of Verticillium (V), Erwinia carotovora subsp. carotovora (Ecc) and E. c. subsp. atroseptica (Eca) inoculated into the seed and, in the case of Ecc, also applied in the irrigation water was conducted. Three levels of irrigation were used for all treatments.

Verticillium appeared to have minimal impact. Plant stand, height and tuber numbers were not significantly different among treatments. Verticillium wilt or potato early dying (PED) progress was, in general, significantly greater than normal maturity in controls. Yield was significantly depressed in only one of three years. In the two warmest years, yields increased as irrigation increased. In the coolest year, the reverse was true with yields increasing as irrigation decreased.

Greenhouse studies completed in 1989 showed that air temperature can play a major role in PED symptom development. Specialized chambers were held at three different air temperatures 15, 25 and 30°C with treatments similar to those used in the field studies. PED symptom progression was fastest under the highest temperature (30°C), but did not reach the same level of severity as found under lower temperatures. PED was greatest under the 25°C temperature, while almost non-existent at 15°C.

Soil fumigation with Busan[R] resulted in reductions in Verticillium microsclerotial counts which were maintained for at least two further growing seasons. However, unfumigated soil also showed similar reductions in microsclerotial counts during the same time period.

Ecc and Eca appeared to be the primary pathogens causing disease in the PED complex in the San Luis Valley, while V. dahliae appeared to have a secondary role. Synergistic V + *Erwinia* interactions were found. Progress of PED in V + *Erwinia* seed treatments was similar to or sometimes significantly greater than PED progress in plants exposed to either *Erwinia* or *Verticillium* alone. Yields were significantly lower than the control or either pathogen alone in three of the V + *Erwinia* seed treatments. Under pathogen combination treatments there was an obvious trend toward reduced yields and, as irrigation increased, toward increased PED severity, disease progress and yield loss.

Erwinia carotovora treatments had significant reductions in stand in two of three years with *Erwinia* seed treatments, but not when Ecc was introduced through irrigation water. Overall, as irrigation and *Erwinia* inoculum density increased, stand loss increased. Tuber numbers and yields were, in general, depressed significantly under *Erwinia* seed treatments. In 1990 the high Ecc irrigation treatment under optimum moisture also significantly decreased yields. *Erwinia* infection of daughter tubers was greatest in the two cooler seasons. Overall, the higher the inoculum level used or the greater the water stress, the higher the infection rate.

Robert Day Davidson
Department of Plant Pathology
and Weed Science
Colorado State University
Fort Collins, CO 80523
Summer, 1994

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INTRODUCTION

Colorado's potato industry has shown significant growth over the past few years, moving from ninth in total U.S. production in 1979 to third or fourth for the last three years. Currently over 31,161 ha (77,000 acres) are grown across the state with 27,520 ha (68,000 acres) comprised of 20% certified seed and the rest table stock grown in the San Luis Valley. In 1990 the potato crop in Colorado was valued in excess of $175 million. Average yields of potatoes in the San Luis Valley have climbed from 32.5 mt/ha (290 cwt/acre) in 1979 to 39.2 mt/ha (350 cwt/acre) in 1992 resulting in record level production of over 1.08 million mt (23.8 million cwt) (109). This increase in yields is a result of improved production practices, use of higher quality seed and varietal improvement. In the San Luis Valley, however, the rise in acreage has not been without sacrifices. Growers have improved many aspects of production. They utilize center pivot irrigation systems as the standard for potatoes and have constructed new, modern storage facilities while using new, modern production equipment. On the other hand, they have reduced crop rotations from an average of three or four years between potatoes to either continuous potatoes or at most a two year potato-grain-potato rotation. While major soilborne disease problems have been quite limited, the potential threat to the potato industry is high. Potato early dying (PED), caused by the soilborne fungus Verticillium dahliae Kleb., is one of the disease problems that could threaten the crop. In the San Luis Valley, PED is not commonly seen as a yield limiting factor. Even though relatively high numbers of Verticillium microsclerotia may be present in the soil and plant infection with Verticillium commonly takes place, the short growing season of 90+ days coupled with overall cool temperatures (Ave. minimum 6^0C to Ave. maximum 26^0C) may obscure the symptoms in the field (Davidson, Knutson & Harrison, personal experience).

The purpose of this research was to; 1) Study factors affecting PED under San Luis Valley conditions with emphasis on the growing environment and irrigation. 2) Examine the potential interaction of *V. dahliae* with *Erwinia* spp. commonly associated with both potato seed and irrigation water. 3) Determine base parameters necessary for the occurrence of PED in the San Luis Valley. These parameters include environmental factors necessary for expression of PED (air temperature and irrigation regime), *Verticillium* inoculum threshhold and effects of crop rotation or fumigation on inoculum densities.

LITERATURE REVIEW

Potato Early Dying

Potato Early Dying (PED), a disease associated with premature loss of plant vigor, senescence and early plant death, is a potential threat throughout most U.S. potato growing areas. This disease is one of the major factors limiting potato production and is especially important in fields or areas where long term, intensive potato production has occurred (14, 27, 49, 64, 98, 101, 112, 133, 136).

Rowe *et al.* (137) in their review of PED discussed the insidious nature of this disease. It has developed over many years in the major, established potato production areas of the Northcentral states of Ohio, Michigan and Wisconsin, the Red River Valley (North Dakota and Minnesota) and Idaho. Because of its insidious nature, when overall production practices, cultivars planted and pest control were improving, PED was not recognized by the growers. In fact, while perceptible yield losses may not have occurred, PED certainly limited yield potential. They (137) contrasted this with PED development found in the Northwest (Washington and Oregon) where the disease has moved in quite rapidly. Because of ideal environmental conditions for disease development and intensive cropping with susceptible crops like potatoes, PED has become a major threat to the potato industry in only a few years. Growers in the Northwest have substantial investments in land, production and irrigation equipment. The declining potato yields when compared with the original high yields obtained after the soils were first planted to potatoes were quite distressing and resulted in quicker recognition of PED as a problem by the growers. Colorado likely falls in-between these two situations. In Northern Colorado, PED has been recognized for many years with corresponding yield reductions of up to 25% (114). In the San Luis Valley, however, PED is rarely seen as a serious limiting factor in production (98, and Davidson, Knutson & Harrison, personal experience) and average potato yields have advanced in each of the last 20 years (109).

Causal Organism

The causal organism of PED, as it occurs in most production areas, is a soil borne fungus, Verticillium (93, 114). Verticillium dahliae (Kleb.) and Verticillium albo-atrum (Reinke & Berthold) are the two major species involved. Verticillium albo-atrum was first implicated in potato wilt by Reinke and Berthold in 1879 (130). Orton in 1914 (115) described it in the U.S., but it was not until Nielson's work in 1948 (112) that Verticillium was directly linked to potato "early dying". There was a great deal of confusion about whether or not the two species were distinct or if both were forms of V. albo-atrum. In the 1970's several authors, including Issac recognized them as distinct species (38, 78, 116).

The two species differ in several important ways. V. albo-atrum is generally considered a more virulent pathogen than V. dahliae. V. albo-atrum is not regarded as host specific and grows optimally at 21^{0}C. It forms melanized hyphae which serve as resting structures. Usually considered a poor competitor in the soil, populations tend to decrease rapidly when suitable host plants are not present. V. albo-atrum either alone, or in combination with V. dahliae, is the species involved in PED in production areas where average temperatures do not exceed 21-24^{0}C during the growing season including Maine, the Red River Valley and winter production areas in Florida.

V. dahliae, in contrast, is considered host specific in many cases. Specific strains attack specific crops including mint, potato, cotton and pepper. Optimum growth temperature, between 23-25^{0}C and up to 30^{0}C in some cases, is higher than that of V. albo-atrum. It is the more widespread of the two species, but often produces less severe symptoms than V. albo-atrum. V. dahliae is found over a wide range of areas and is particularly troublesome in the warmer production areas of the Pacific Northwest, Idaho and Colorado. *V. dahliae* produces true microsclerotia as survival structures. The organism is considered a strict root inhabiter with little or no growth in the soil. Microsclerotia survive well in the roots and rhizomes of weeds (42) and on the surface or inside of potato stems and roots (91, 146). In addition, microsclerotia are easily spread to non-infested soils by wind, equipment and/or plant debris (137). These structures can survive for several years in the soil in a dormant state (61, 104, 140). Once *V. dahliae* is established in a field, it can be present at extremely low levels until a suitable host is planted. Then, it can reach high levels quickly and become quite destructive to susceptible crops (38, 133, 137).

Infection Process, Microsclerotial Characteristics and Threshold Levels

Infection of potato plants by *Verticillium* spp. is usually from infested soil (42, 47, 53). In the case of *V. dahliae*, microsclerotia germinate when the root system of a suitable host plant is present and the physiological state of the plant is appropriate for penetration by the fungus (18). There are indications that root exudates help trigger germination (63, 104). Germ tubes from microsclerotia grow into hyphal strands which directly penetrate the root 3 to 4 mm behind the root cap and enter into the root cortex (47). After entry the fungus forms hyphae and produces conidia which can rapidly colonize and move throughout the plant (119, 120). Microsclerotia are produced as the disease progresses and under the right conditions can be found in all tissues of the plant (121). At vine death, microsclerotia are present in plant debris (140) and once incorporated into the soil can be found throughout the soil profile at depths of up to 76 cm. However, Wilhelm (150) found that the 0-30 cm layer of soil contained three to four times more microsclerotia than the deeper soil layers.

Microsclerotial germination is affected by many things. Green *et al.* (63) found that soil amended with glucose, sucrose or $NaNO_3$ caused population increases followed by rapid declines of viable propagules to levels lower than the initial populations. Farley *et al.* (45) reported that soil containing microsclerotia could be dried and re-moistened with sucrose or water repeatedly and the microsclerotia would germinate after each cycle. Even pre-germinated microsclerotia would germinate repeatedly. Finally, Ben-Yephet and Pinkas (7) established that germination rate and number of germ tubes produced were correlated positively with microsclerotial size. They also suggested that these results plus microscopic examination of soil and plant debris indicate microsclerotia may exist in clusters in the soil.

Inoculum thresholds for V. dahliae were proposed by several authors. Nnodu and Harrison (113, 114) reported that the minimum inoculum level necessary for significant potato yield reductions is 17.5-23 ms/gm of soil. Reports by Ashworth *et al.* (4) suggested that only 0.03 ms/gm of soil were necessary for cotton infection and 3.5 ms/gm of soil provided enough inoculum for 100% infection under the proper conditions. Spaulding (144) described the minimum threshold values for potatoes to range between 15 to 1000 ms/gm of soil. He stated that there were inoculum densities beyond which the addition of more inoculum would not significantly increase disease severity. However, he found that at air temperatures in the 27-30^{0}C range, 50-100 ms/gm of soil was the minimum threshold for wilt development and 100-150 ms/gm of soil was the minimum threshold to cause plant death. Other authors reported threshold ranges between 10-75 ms/gm of soil for significant yield loss to occur (27, 93).

Symptoms of Verticillium wilt or PED

Symptoms of PED are easily confused with normal senescence or other disease problems. First symptoms begin early in the season at about the time of flowering or tuber set (147) with lower leaves expressing chlorosis and occasional wilting. Not all infected plants show symptoms at the same time and often only a single stem or leaflet may express symptoms. Sometimes, leaves do not wilt, but instead develop an irregular yellowing and wither or die from the base of the plant upward. Affected areas of the plant eventually become necrotic and may die. Dead leaves often hang onto the plant through the remainder of the season. Generally chlorosis and wilt continue up the plant, stunting its growth, limiting root development (89, 90) and reducing the overall leaf area available for photosynthesis. Plant death usually occurs two weeks or more earlier than normal (17, 18, 51, 64, 101, 112, 133). Guthrie (64) found that under ideal conditions for the pathogen, even earlier plant death might occur and substantially reduce tuber yields. Issac and Harrison (66, 79) described unilateral wilting and chlorosis-necrosis of leaves as the only characteristic and permanent symptom of "early dying". They also demonstrated that stunting of infected plants plus reduction in overall leaf area available for photosynthesis were other common symptoms expressed later in the season. The combination of these resulted in overall reduction of fresh weight leaf area and contributed to subsequent reduction in tuber number and increased yield of small potatoes. Kotcon *et al.* (88, 89) studied root deterioration resulting from PED. They reported that root deterioration was associated with the premature senescence of foliar tissue independent of the colonization of the roots by Verticillium. A reddish-brown vascular discoloration or xylem stem streaking is commonly observed after foliar symptoms occur. It may extend to the highest points in the plant (79, 133). In addition, the stem end of daughter tubers from Verticillium-infected plants may show a light brown vascular discoloration easily confused with symptoms of other diseases (64, 66). Robinson *et al.* (132) described how additional spread of PED could occur by planting internally infected tubers. There are also reports of surface borne inoculum on tubers infecting sprouts directly, thus causing systemic infection, and of infection taking place by direct penetration of the leaf epidermis by the fungal hyphae (91, 146).

Some confusion exists in diagnosis of PED because primary symptoms of foliar chlorosis, necrosis and wilt in the early stages may mimic normal plant senescence. Harrison and Issac (67) showed that normal, healthy plants have lower leaves on or near the soil surface which become chlorotic, necrotic and may eventually drop off. Leaf drop usually occurs only in lower leaves of determinate cultivars such as Norgold Russet, but may occur anywhere along the stems of indeterminate cultivars like Russet Burbank when leaves are on or near the soil surface. In diseased plants, symptom expression may develop rapidly with little delay and/or progress steadily during the growing season, while in healthy plants leaves do not start to drop until normal senescence occurs. Additionally, PED symptoms can be confused with other diseases such as potato blackleg caused by Erwinia carotovora (11, 74, 125).

Yield losses from PED may be highly variable. Losses ranging from 25-50% are documented in Idaho (27), Ohio (14, 135), Oregon (85) and Colorado (114). Even with these observations, it is not certain that yield loss will be correlated with the presence of foliar symptoms. There are many other factors which may interact to either inhibit or increase plant symptom development and expression with subsequent yield reduction.

Factors Affecting PED Symptom Expression

PED symptom expression is related to numerous factors including planting date, fertility, air and soil temperatures, irrigation, virulence of Verticillium isolates and plant resistance. Guthrie (64) mentioned that late planting dates in the Upper Snake River Valley resulted in less early dying and a greater percentage of U.S. #1 potatoes than earlier planting dates. He established that heavy rates of nitrogen significantly delayed PED symptoms where nitrogen was a limiting factor in production. Walker *et al.* (149) found that greater incidence of Verticillium wilt in cotton was correlated with higher levels of nutrition. This is in direct contrast to reports by Davis (27, 29) that higher available nitrogen early in the season reduced stem colonization by V. dahliae and thus suppressed the severity of PED in Russet Burbank. Most authors concede that high available nitrogen early in the season will delay onset of PED and can suppress symptom development. Several individuals found that air and soil temperatures influence infection by Verticillium spp. and subsequent symptom development (22, 40, 51, 54, 84, 112, 144). Colhoun (22) discussed predisposition to disease by the environment. He suggested that plants can be rendered more susceptible to infection by relatively high temperatures before inoculation takes place. This agrees with Spaulding's (144) findings that as temperature increased from 24 to 30^{0}C, the inoculum threshold of Verticillium required for disease development decreased. Francl *et al.* (51) found that periods of high temperature had negative effects on tuber yield from infected potato plants. Nielson (112) stated that there is a positive relationship between high early season temperatures and early appearance of PED symptoms in Idaho. Additionally, there are reports of high temperatures masking or obscuring the influence of Verticillium populations and virulence in relation to disease severity (54). Khew and Busch (84) demonstrated that there was greater infection of potato by microsclerotial strains of Verticillium at 27^{0}C than at 16 or 22^{0}C. However, temperatures exceeding 28-30^{0}C can reduce disease development of V. dahliae (93).

Powelson *et al.* (21, 126) studied irrigation effects on PED symptom development in Oregon. She reported that disease onset was earlier and severity significantly greater when plants were grown in infested soils receiving the highest amounts of applied water when compared with plants receiving lesser amounts of water. The greatest yield depression occurred under the highest inoculum density and highest irrigation regime. In contrast, inoculum density made no difference in yield when plants were grown under deficit irrigation. She suggested that PED severity and associated yield loss are enhanced when soils are kept wet. This differs from the view of Davis (27) that moisture stress increases PED severity. This difference may be the result of temperature variation during the growing season (144).

Frank *et al.* (52) found that V. albo-atrum inoculum could be adjusted so that varying degrees of wilt would occur among cultivars with different resistance levels. They eventually broke the resistance of all cultivars tested by increasing inoculum to extremely high levels (100,000 spores/ml inoculated onto the seedpiece prior to planting). In contrast, Davis, Pavek and Corsini (24, 31) found breeding material which had stable resistance. Also, they stated that inoculum levels in the soil are highly correlated with the rate of V. dahliae colonization of potato-stem tissue and subsequent PED symptom development.

PED Control

Approaches to management and control of PED have been varied and met with mixed success. Reduction of the amount of root infection, rate of vascular colonization or both are considered keys to successful control strategies (137). Efforts to reduce soilborne inoculum concentrated on vine burning to delay inoculum buildup (39), crop rotation (27), application of chemicals such as Benomyl (10) and soil fumigation with one of several chemicals (8, 152). Neither crop rotation nor burning have long term effects on V. dahliae, since it is a soilborne fungus with long lived microsclerotia. Davis and Sorenson (32) explored the use of soil solarization using polyethylene mulching to suppress PED and V. dahliae populations. The suppressive effects of such treatments can last up to two years.

Soil fumigation has gained popularity over the past few years because of the ease in handling chemicals through center pivot irrigation systems. Davis (28) discussed the role of several chemical fumigation treatments. He found that there were significant yield increases after fumigation of highly infested soil and that this effect can carry over for an additional year after final treatment. Other authors (91, 152) reported similar results, but the expense of using fumigants on an annual basis is often prohibitive. Another role of fumigation in reducing PED is the control of populations of other organisms, such as nematodes, involved indirectly with PED (137). The reduction in nematodes, particularly Pratylenchus spp., reduces PED severity even though Verticillium spp. may be present. Currently, the reason for this particular relationship is not well understood. Other approaches to PED control include managing those factors which determine how rapidly the fungus invades and colonizes the potato stem. These include nitrogen fertility and irrigation methods (29), as well as, amounts of water applied (21, 126).

Resistant cultivars probably offer the most potential for real, long-term control. Verticillium tolerant cotton varieties have long been used in areas with high Verticillium populations (91). While potatoes have been bred extensively for Verticillium resistance, it has only been recently that the USDA Breeding program in Aberdeen, Idaho produced clones with true resistance to V. dahliae stem colonization and successfully matched resistance with the more desirable qualities of vine, tuber type and yield (23, 24).

Whatever controls are ultimately used for PED, some sort of integrated approach combining two or more of the above strategies will probably be essential (91, 137).

PED Disease Complex

"Early dying" is generally thought to be caused by more than just Verticillium spp.. Many other organisms are implicated in what is now known as the "potato early dying complex". These include nematodes, other fungi, bacteria and viruses which often do not play a direct role in "early dying". Instead, they have an interactive, indirect effect that can, in combination with Verticillium, produce additive and/or synergistic responses which affect PED symptoms and yield loss.

Several species of nematodes from different genera were studied to determine their roles in the PED complex. MacGuidwin and Rouse (94) were unable to show any interaction between Meloidogyne hapla and V. dahliae. However, Rowe *et al.* (138) demonstrated significant, synergistic interactions between V. dahliae and Pratylenchus penetrans regarding PED severity, although each individually had little or no effect on the potatoes. Much work has been done to verify the strong relationship between lesion nematodes and Verticillium wilt (50, 90, 131).

Viruses and other fungi were also implicated in the PED complex. Goodell *et al.* (57) found that plants infected with PVX had significantly higher colonization by V. dahliae than those which were PVX free, although there was no difference in incidence of fungal infection. They suggested that higher losses from PED might occur in PVX infected plants which had higher V. dahliae populations. In the same study, they failed to demonstrate an interaction between V. dahliae and Colletotrichum atramentarium as did Kotcon *et al.* (90) and Kirkland (85). Johnson *et al.* (82) showed that when potato leafhopper (Empoasca fabae), Alternaria solani and V. dahliae occurred together, significant interactions occurred in one of two years.

Finally, several authors have examined the role of Erwinia spp. alone and in combination with V. dahliae. Powelson (125) suggested that Erwinia spp. can cause PED under certain circumstances in the Pacific Northwest without the presence of Verticillium. Davis *et al.* (32) reported that inoculation with both V. dahliae and E. carotovora subsp. atroseptica and E. c. subsp. carotovora resulted in decreased Verticillium wilt. They proposed that changes in plant maturity and/or host responses could have influenced early V. dahliae stem infection. These findings contradict those of Kirkland and Powelson (85) who reported strong evidence that the severity of PED symptom expression in Russet Burbank increased when plants were inoculated with both V. dahliae and E. carotovora subspp. This interaction was additive and due to enhanced stem colonization by V. dahliae. Rahimian and Mitchell (129) also found similar results in greenhouse studies. The effects of inoculating Norgold Russet or Russet Burbank with both pathogens as compared with inoculating with each separately, were synergistic and resulted in accelerated PED symptom expression. In addition, they showed that more soft rot developed in plant stems inoculated with both pathogens. This is supported by the field work of Zink and Secor (154).

Erwinia spp.

Erwinia carotovora subsp. carotovora (Jones) Bergey et al. (Ecc) and Erwinia carotovora subsp. atroseptica (van Hall) Dye (Eca) are gram negative, soft rotting bacteria which cause a variety of stem and tuber symptoms on potatoes in the field. Both organisms are implicated in seedpiece decay, blackleg and various stem rots (11, 12, 60, 105, 111, 123). The pathogens are primarily seedpiece borne (58, 74), but may be found in the soil or rhizosphere of weeds and crop plants (16, 34, 100) and various water sources (20, 69). Eca infects potatoes and has been found on sunflower and sugarbeets (74), while Ecc is much more ubiquitous in nature, and has many host plants, including members of most families of fruits and vegetables (15, 118). The two organisms can be distinguished by biochemical and growth temperature tests (12). Also, Ecc was divided into numerous serogroups based on reaction with specific antisera (35). There is evidence of strain variation in physiological and pathological aggressiveness, and host specificity is common (141).

Disease symptoms can occur at any time during potato development. Early losses may be due to seedpiece decay. Aleck and Harrison (2) reported significant increases in pre-emergence tuber and shoot decay as inoculum density increased. Under high moisture, high temperature conditions, pre-emergence decay was prevalent, while under low inoculum, low temperature conditions, post-emergence blackleg predominated. However, conditions which favor the pathogen may reduce the post-emergence phase of the disease, but cause increased total loss because of seedpiece decay and pre-emergence death of shoots. Symptoms of post-emergence blackleg consist of inky-black decay at the base of the infected stem originating at the attachment point to the seedpiece which is usually rotted. Vascular discoloration above the rotted portion of the stem is common. Infected plants are often stunted and chlorotic early in the season, and may wilt or die prematurely as the season progresses (74, 106). Aerial infection on above ground plant parts can occur when plants are wounded, although the seedpiece may be healthy (20). Erwinia can spread to tubers during the growing season by movement of the bacteria in the soil from infected plant parts and rotted seedpieces, along the stolon, to the daughter tubers. Also, lenticel infection can take place during harvest and handling, depending upon environmental conditions and tuber maturity (1, 26). Tuber symptoms range from a tan discoloration at the stolon attachment point to a complete, wet breakdown of the entire tuber (74). Many times, however, Erwinia-infested tubers show no visible symptoms and can be an important source of inoculum the following season (71).

Approaches to control are varied, but include use of pathogen-free, tissue-culture propagated stocks (87), control of certain production and harvest practices, use of chemical seedpiece treatments such as formaldehyde, use of fungicidal seedpiece treatments to control fungi implicated in increasing Erwinia infection (i.e. Fusarium spp.) and keeping all equipment used in the potato operation clean and properly disinfested (60).

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FACTORS AFFECTING THE EXPRESSION OF POTATO EARLY DYING IN THE SAN LUIS VALLEY OF COLORADO (CHAPTERS 1-3)

Abstract:

Microplots planted with Russet Burbank were studied over a three year period, 1988-1990, to investigate the effects of Verticillium wilt or potato early dying (PED) in the San Luis Valley. Three levels of Verticillium dahliae, zero, low and high, were incorporated into fumigated soil and placed under three irrigation regimes, optimum, deficient (50% optimum) and excessive (200% optimum).

Stand and plant height were not significantly affected by treatments. Irrigation regime affected symptom expression. Plants grown under deficient moisture demonstrated a significant increase in Verticillium wilt or potato early dying in two of three years for both levels of Verticillium. PED disease progress was similar among treatments. In general, plants grown in microplots with Verticillium infested soil demonstrated higher levels of PED expression by the end of the season than senescence exhibited by the negative controls. There was no consistent effect on tuber number during the study and yield was significantly depressed in only one of the three years, and then only under deficient irrigation and at the highest Verticillium level. Significant differences in yield were observed among the three irrigation regimes in all three years. In the two warmer years, 1989 and 1990, excessive irrigation resulted in the highest tuber yields, with yields decreasing as less water was applied. In the third year, 1988, which had a cool spring coupled with overall cool air temperatures during the growing season, this relationship was reversed.

A greenhouse study completed in the summer of 1989 showed that air temperature plays a major role in symptom development under San Luis Valley conditions. Specialized chambers were held at three different air temperatures (15, 25 and 30^{0}C) and used to evaluate treatments utilized in the field studies. Stand was unaffected by temperature. Plant height increased as temperature rose, but there were no differences observed among treatments within each temperature range. Onset of PED was earliest and symptoms progressed fastest at the highest temperature. PED symptom expression and progress increased as temperature increased. PED symptoms were minimal at the lowest temperature. At 15^{0}C and 25^{0}C the final PED readings on plants in the Verticillium infected treatments were significantly higher than the control's senescence. No significant variation was obtained among treatments relating to tuber numbers. Yields were significantly reduced only at 25^{0}C between the low and high Verticillium inoculum levels.

Four fields were assayed for the effects of soil fumigation on the number of Verticillium microsclerotia present. After fumigation, a dramatic decrease in number of microsclerotia was found. The decrease was maintained for at least two growing seasons, even after a PED susceptible cultivar, Russet Norkotah, was grown. However, similar decreases in populations of microsclerotia were observed in the non-fumigated fields. Other factors such as environment and crops grown in a rotation may contribute as well to a reduction of microsclerotial populations.

Chapter 1

Role of Verticillium dahliae Inoculum Level and Irrigation Regime on Expression of Potato Early Dying

Introduction:

Verticillium wilt or potato early dying (PED) caused by Verticillium dahliae is responsible for significant yield reductions in potatoes in many production areas (4,18,23,29,30,33,37). This disease problem, however, is rarely associated with yield decreases in the San Luis Valley of Colorado. Factors such as air temperature (5,19,20,23,28,40), irrigation regime (7,11,36) and density of Verticillium inoculum (2,9,34) play an important role in disease expression. Rowe *et al.* (38) in their review of PED discussed many of these factors at length and concluded that there are production areas found in cooler regions where PED may not be a significant factor in potato production. The San Luis Valley of Colorado fits into this category. This study was designed, in part, to examine two primary factors involved in the potato early dying process, Verticillium inoculum levels and crop irrigation, under the cool growing season conditions found in the San Luis Valley. Field microplots were utilized over a three year period (1988-1990) to examine these factors.

Materials and Methods:

Plot Preparation: Microplots were established at the San Luis Valley Research Center in May of 1988, 1989 and 1990. Each year, prior to plot establishment, a composite soil sample was collected from the area to be used for the study. A soil test was completed upon this sample and fertility was modified based upon the test to levels recommended for Russet Burbank commercial production in the San Luis Valley (39).

The area where the plots were to be located was fumigated in April of each year. PicChlor 60[R] (60% chloropicrin/40% telone) was injected into the soil to a depth of 60 cm at a rate of 393 kg/ha (273 l/ha). After fumigant injection, the soil was sealed by sprinkling with water to wet the soil to a depth of approximately 2 cm. Soil samples for nematode assays were collected prior to fumigation in 1988. Nematodes commonly associated with the potato early dying complex were not detected in the soil and since the same area was used in subsequent years, the assays were not repeated.

One week prior to planting, trenches 29 cm deep and 1.2 m wide were dug in the fumigated soil using a backhoe. Soil was piled along the edge of each trench for use as the planting medium in the microplots. Pots 30 cm high and 31 cm internal diameter with a 10 cm hole drilled in the center of the base to provide for water drainage were used as the microplots. Pots were arranged on 60 cm centers within the row and 92 cm centers between rows. Five trenches 4 m apart were cut in the plot and three rows of pots were set in each trench resulting in a total of fifteen replicated rows.

A drip irrigation system was installed in the plot. Pairs of individual pressure compensating emitters were placed in each pot to insure uniform water delivery. The irrigation system was designed to deliver water at optimum, excessive and deficient amounts for potatoes based on tensiometer and gypsum block readings, as well as, potato growth models for the San Luis Valley. The tensiometers and gypsum blocks were placed at several random locations throughout the plot at depths of 20 cm. Readings were taken three times per week. Irrigation rates used were 3.8 l/hr (optimum), 7.6 l/hr (excessive) and 1.9 l/hr (deficient). Microplots were irrigated with well water known to be free from <u>Erwinia carotovora</u> on a one to four day schedule during the summer based upon readings obtained from the tensiometers, gypsum blocks and utilizing evapotranspiration (ET) tables. The amount of water applied was that required to maintain the optimum moisture regime within the desired limits. Rainfall was factored into the irrigation regimes mentioned above.

Inoculum Preparation: Verticillium dahliae inoculum was prepared from a mixture of three isolates recovered from potatoes grown in Oregon. Axenic cultures were grown on Potato Dextrose Agar in plastic 12 x 100 mm plates for 21 days in dark conditions at 21°C. The surface of twenty plates was washed with 10 ml of sterile water on each plate to remove conidia and hyphae from the cultures. The hyphae/conidia suspensions were mixed into one liter of sterile water. The resulting suspension was spread onto a modified minimal medium (7) overlayed with a layer of sterile cellophane at a rate of 0.1 ml per plate. The plates were incubated at 21°C in the dark for 21-30 days or until good microsclerotial production was evident. The cellophane disks containing the microsclerotia were stripped from the plates and placed in a Waring[R] food blender containing sterile distilled water. The suspension was blended for one minute at high speed to macerate the cellophane and liberate the microsclerotia. The mixture was then washed through a series of metal sieves beginning with a #50 sieve (0.0117" opening) then to #100 (0.0059"), #200 (0.0029"), #325 (0.0017") and finally to a catch basin. Microsclerotia (ms) were collected from the #100, #200 and #325 sieves, while the waste cellophane collected on the #50 and conidia and hyphal fragments in the catch basin were discarded. The ms were washed thoroughly after collection to remove remaining hyphal fragments and conidia. This was accomplished by spraying the ms collected in the #325 sieve repeatedly with sterile distilled water using a spray bottle. Examination of the ms under 10X magnification assured that the debris was removed from the ms preparation.

The concentration of ms, after washing and suspension in sterile water, was determined by using a haemocytometer. Appropriate amounts of ms were mixed into the three microplots-worth of fumigated plot soil by tumbling the soil in a cement mixer for 3 minutes while the ms suspensions were sprayed onto the soil. The resulting ms-infested soil was to contain population levels of approximately 15 and 50 ms/gm soil. These levels were based upon dry weight of the soil necessary to fill a microplot to within 2 cm of the pot's top. Soil for controls was mixed, but no inoculum added, for the same amount of time. Recommended preplant fertilizer was also added to the soil as it was being mixed. Mixed soil was put into the pots which were placed in appropriate locations in the trenches according to a plot diagram. After all of the pots were filled and placed, the remaining fumigated soil was used to fill in around the pots and level off the ground to within 6 cm of the top of the pots.

Experimental Design: A completely randomized block with 15 microplots (replications) per treatment at each moisture level was used for the study. A total of 15 treatments, 675 total microplots in 1988 and 21 treatments representing 945 microplots in 1989 and 1990 were included (Table 4.1, page 75). Each of the fifteen rows in the plot included a complete set of treatments and comprised one replication.

Generation 1 seed tubers (cv. Russet Burbank) from the Oregon State University Foundation Seed project in 1988 and 1989 and from the Worley Seed Company in Colorado in 1990 were cut into single eye seedpieces two weeks prior to planting with a 2.5 cm melon scoop disinfested in 10% chlorox between cuts. These were placed in chambers at 21^{0}C and high relative humidity for seven days to aid suberization and germination. The pre-sprouted seedpieces were treated (inoculated with Erwinia or not depending upon the treatment) and planted in the soil at the center of each of the appropriate microplots to a depth of 6 cm.

Beginning at planting and continuing through the growing season, air and soil temperatures were recorded at 15 to 30 minute intervals using a CR21X micrologger[1]. Soil temperatures were taken using small electrode

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[1]Campbell Scientific, Inc. P.O. Box 551, Logan, UT 84321

type sensors from one representative pot in each irrigation regime at a depth of 10 cm.

Soil in the microplots was used to determine populations of *Verticillium dahliae*. *Verticillium* populations were determined prior to and after infestation. Screening was done using the standarized procedure developed by Harrison and Livingston (24) using ethanol agar as the assay medium. Five composite samples, representing five replications of the zero, low and high levels of *Verticillium*, were air dried, thoroughly mixed and gently sieved through a series of screens (#50 - #200) to eliminate larger particles. The resultant fine soil was measured and placed onto filter paper in 0.1 gm increments. Three replicate samples of soil from each composite sample were used. These samples were individually deposited, using an Anderson Sampler (6), onto plates containing the ethanol medium. Plates were incubated for twenty one days in the dark at 21^{0}C following deposition of the soil onto the medium and *Verticillium* colonies were counted using a 40X binocular microscope. Total counts from each plate were determined and data were converted to total number of ms/gm of soil using appropriate diliution factors.

The presence and severity of PED symptoms and/or senescence in controls were monitored weekly beginning at the time of first symptoms. A modified Horsfall/Barratt scale (25) of 0 to 9 was used to estimate the percentage of foliage in each plot showing PED symptoms or senescence (Appendix-Table 4.2). At harvest, the actual number of tubers per plant plus total yield per plant were recorded.

Results:

Results from this study were divided into several categories including effects of treatments on stand, plant height, onset of PED, PED symptom progress over the growing season, tuber number and total yield. Each parameter was compared across *Verticillium* inoculum levels and irrigation regimes, as well as, among years.

There were no significant effects of treatments on stand or plant height in any of the three years. Plant height data are shown in Appendix-Table 1.1.

First symptoms of PED were seen between 50 to 55 days after planting each year. Plants were read weekly for PED symptoms and/or maturity beginning with symptom onset. Progress of PED and/or maturity is illustrated in Figures 1.1 to 1.3 and Appendix-Tables 1.4 to 1.6. Under optimum water conditions, there were no consistent significant treatment effects. Under the deficit irrigation regime, both the low and high *Verticillium* inoculum densities consistently increased PED symptom expression significantly in 1988 and 1989. Under the excessive irrigation regime, a significant increase in disease progression was found at the highest *Verticillium* level only in 1990. Overall, disease progress was similar, but symptoms were more severe under all irrigation regimes and inoculum levels in 1989 and 1990 which had relatively high early summer temperatures (Figure 1.4 and Appendix-Table 1.7) than 1988.

There was little consistent effect of treatments on the number of tubers harvested per plant (Appendix-Table 1.2). Tuber yields (Figure 1.5 and Appendix-Table 1.3) were significantly decreased in only one of three years (1988) under deficient irrigation at the highest inoculum level. Yearly variation was evident with the excessive irrigation regime producing the highest yields in the two warmer seasons. Yields increased steadily as more water was applied. In the cooler season (1988) this relationship was reversed, and the highest yields occurred under the deficit irrigation regime. Again, this reversal in the yield versus irrigation level relationship in the cool season may be the result of early spring weather patterns as shown in Figure 1.4.

Figure 1.1

Effect of Differing Levels of Verticillium dahliae Incorporated into Soil on PED Symptom Progress or Plant Senescence Under Three Irrigation Regimes.

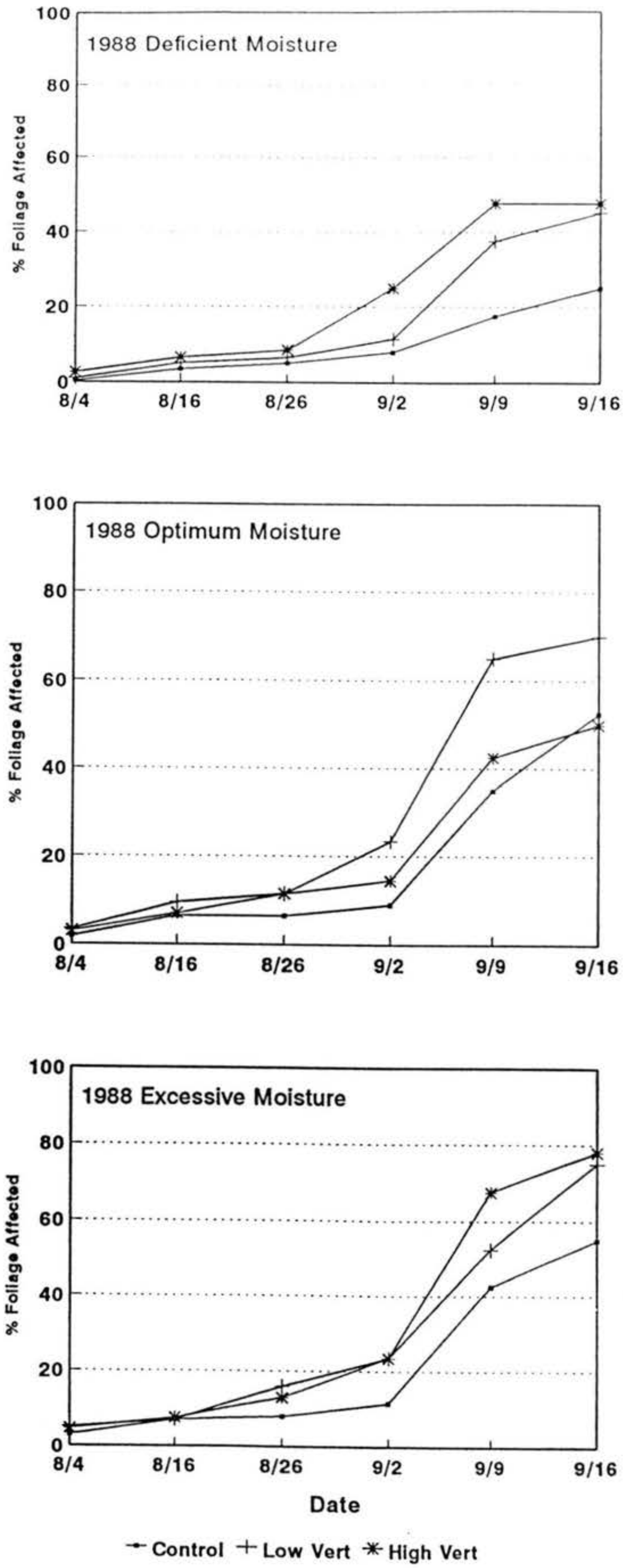


Figure 1.2
Effect of Differing Levels of Verticillium dahliae Incorporated in Soil on PED Symptom Progress or Plant Senescence Under Three Irrigation Regimes.

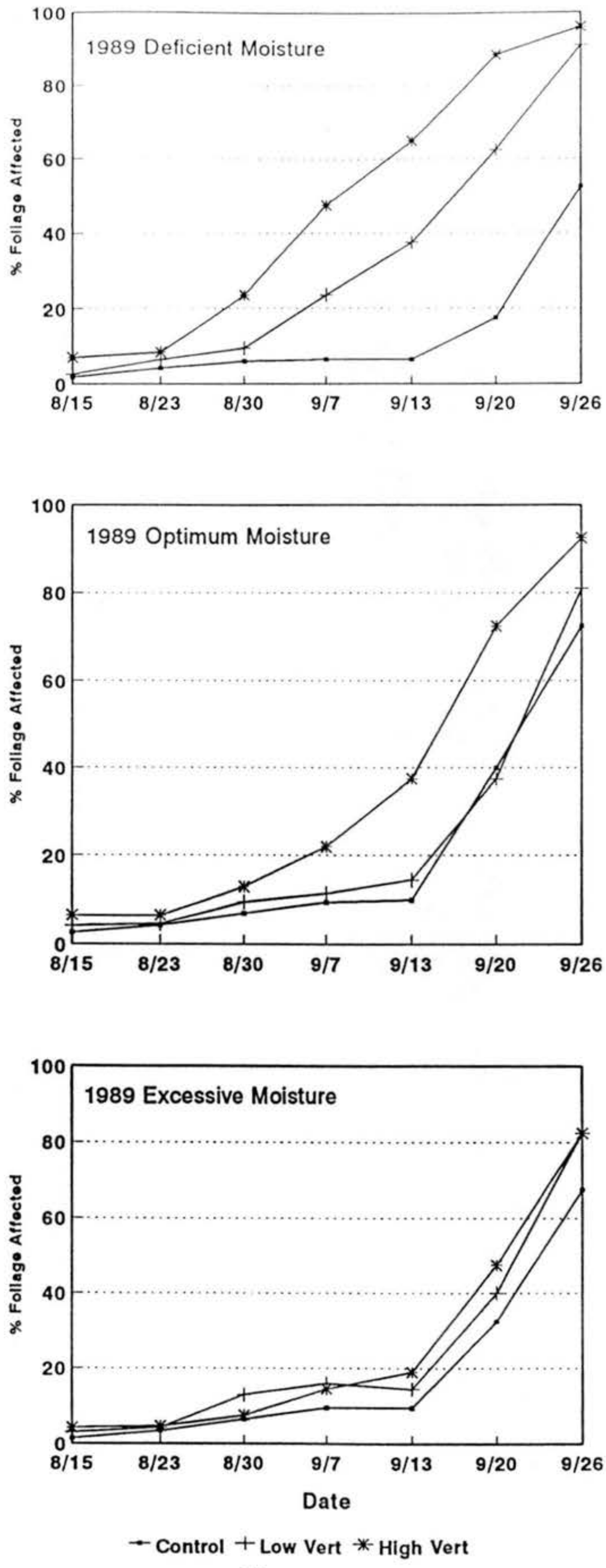
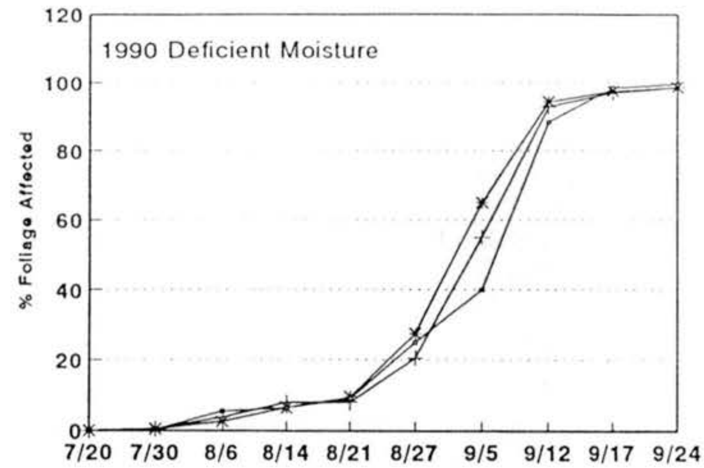
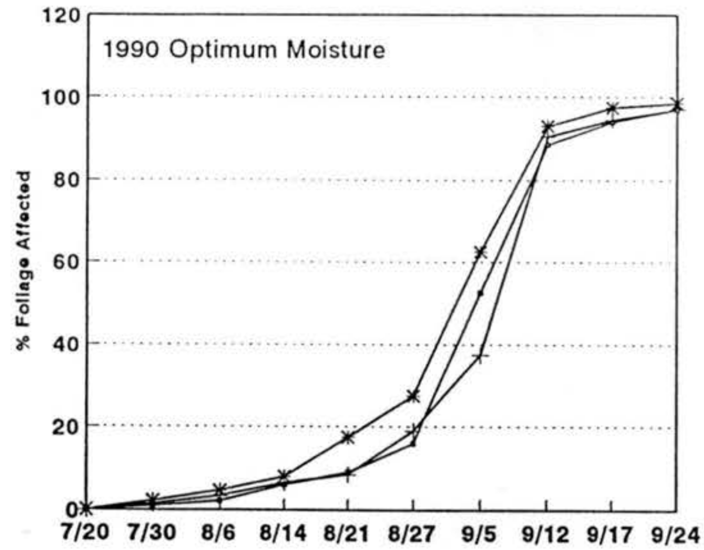


Figure 1.3
Effect of Differing Levels of Verticillium dahliae Incorporated into Soil on PED Symptom Progress or Plant Senescence Under Three Irrigation Regimes.





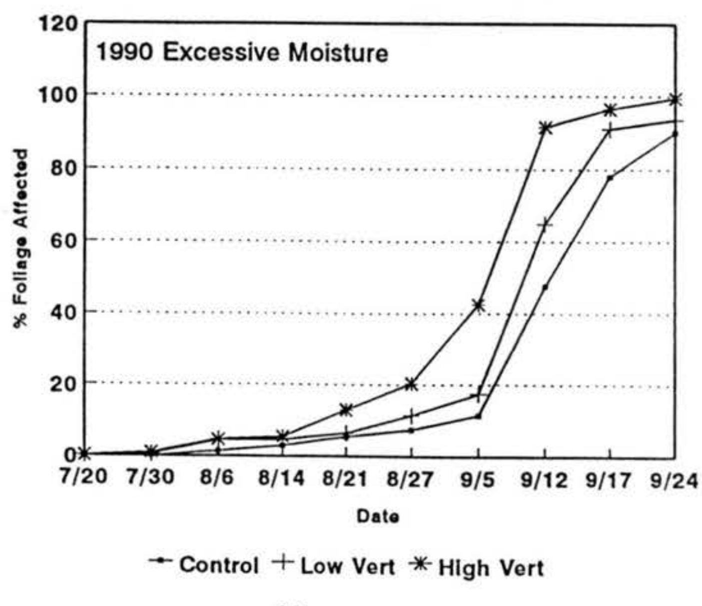


Figure 1.4
Early Season Weather Data for the San Luis Valley, Colorado

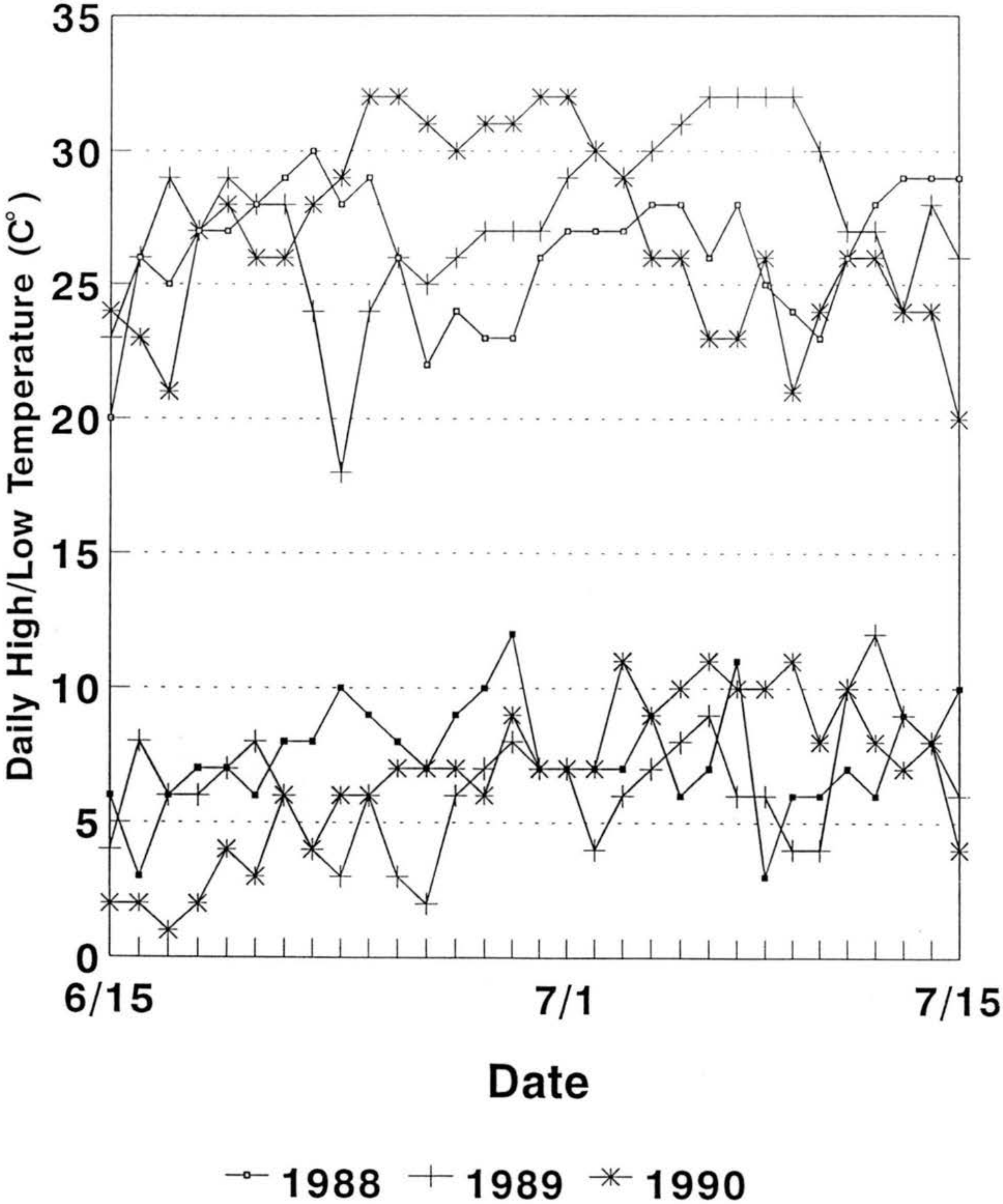
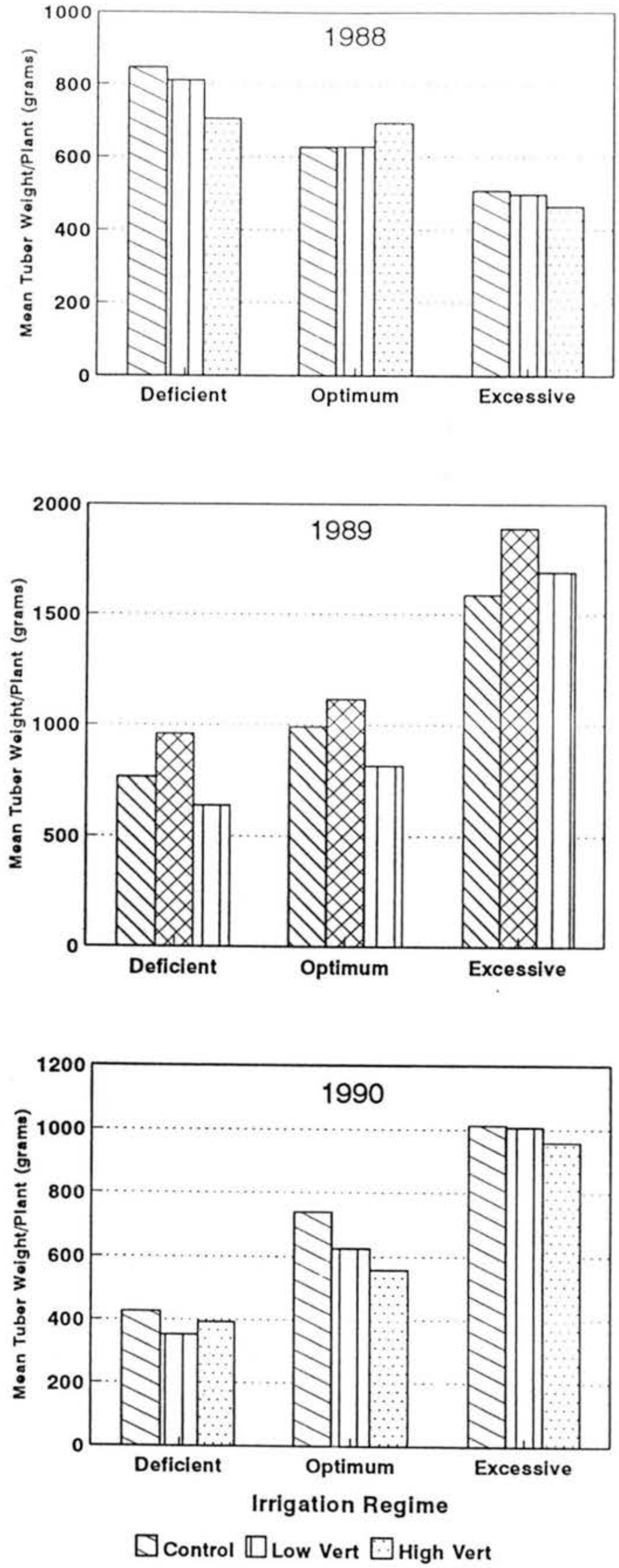


Figure 1.5
Effect of Verticillium dahliae on Yield per Plant Under Three Irrigation Regimes.



Discussion:

The Verticillium dahliae inoculum density or threshold required for infection and PED symptom development and subsequent measurable yield loss can be quite variable from one production area to another (9, 18, 23, 34, 40). It is currently recognized that production areas differ sufficiently in their environmental conditions that a rather broad range of V. dahliae inoculum densities required to produce PED symptoms exists. The range falls somewhere between 10 and 75 ms/gm of soil for significant yield loss to occur. This range of inoculum does not appear to be applicable to San Luis Valley conditions. Spaulding (40) reported that at air temperatures in the 26-30^{0}C range (80-85^{0}F), 50-100 ms/gm of soil was the minimum inoculum threshold necessary for wilt development and much higher populations were necessary for plant death to occur. This temperature range represents the highest temperatures that normally occur in the San Luis Valley during the growing season.

Results from this study agree with Spaulding's data which show the importance of the inoculum density/air temperature relationship. PED symptoms including wilt, chlorosis and eventual plant death were commonly found during the course of this study. The control plants demonstrated a fairly normal pattern of maturity which was rated as senescence using the same scale as that used for rating PED symptoms. Senescence of the controls always rated lower than PED expression found in plants grown in soil in which Verticillium was incorporated. However, while PED symptoms are readily detected under SLV conditions, they rarely translate into yield loss. There was only one year where significant yield reductions were observed and then only under one irrigation regime. Given the variation in weather patterns over the three seasons this project was carried out, this strongly indicates that the consistent minimum inoculum threshold for PED expression in the San Luis Valley is above 50 ms/gm of soil and considerable inoculum may not produce yield reductions under normal production practices. In addition, inoculum levels required to produce yield reductions may be slow to develop in the San Luis Valley. Unique environmental factors like relatively cool air temperatures during the growing season may play a prominent role. It appears to take more inoculum to produce disease symptoms, and even when symptoms appear, additional increase of the fungus in the plant may be slow.

This is important when reviewing the growers' options for reduction of soilborne diseases. Soil fumigation with Busan[R] and Vapam[R] is increasing in the San Luis Valley to help control several pests including nematodes, western leak and Verticillium wilt. This practice has effectively reduced Verticillium propagule counts in SLV soils and the microsclerotial buildup is slow, probably due to the same environmental factors which appear to limit the extent of symptom development in the SLV.

Irrigation can play a major role in the onset and severity of PED symptoms and yield loss. Under optimal irrigation conditions, there was a significant increase in PED symptoms in plants grown in soil infested with the highest Verticillium inoculum level in 1989 only. As expected, there was no significant reduction in yield during any of the three years.

Under excessive moisture, 200% the optimal rate, significant increases in PED disease progression in infested soil over the progress of the control's senscence were found only in 1990. In the other two years, only one or two disease readings during the course of the season showed the presence of significantly higher PED expression than senescence of the uninoculated control and overall the readings for all treatments were similar. PED disease progress, even though significantly higher as inoculum density increased, did not translate into yield reductions. This is in direct contrast to results obtained by Cappaert *et al.* (7) which showed a significantly higher rate of PED disease progress and subsequent yield reduction under excessive irrigation. It should be noted, however, that under cool weather conditions, similar to those found every year in the San Luis Valley, yield reductions were not nearly as dramatic and would relate more closely to the results found in this study.

The deficient water regime provided the most interesting data in two of the three years of the study. PED disease progression was significantly higher (P=0.05) when V. dahliae was present (as much as 40% higher) than the normal maturation expressed by the control. Under the highest inoculum density (50 ms/gm soil), a significant yield reduction (17%) was found in 1988. In 1989 and 1990, non-significant yield reductions of 17% and 8% respectively, were recorded. This suggested that under generally cooler conditions, like those found in 1988, plants infected with Verticillium and grown under a water deficient condition sustained greater damage from PED than plants grown under optimum or excessive water regimes. In other words, the optimum or excessive regimes appeared to give the plants sufficient growth advantage that the effects of PED were negated or reduced. The ability of the fungus to sporulate repeatedly under repeated applications of high moisture, as reported by Cappaert *et al.* (7) was confirmed, but this did not appear to have a major impact on either plant infection or disease progression during the season.

This relationship between soil moisture and disease development may be very important for growers when managing irrigation schedules. By keeping crops from deficient water conditions and avoiding excessive water applications, they may prevent or greatly reduce the effects of PED in the few years when it could be a threat in the San Luis Valley.

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Appendix

Table 1.1 **Effect of Season, Irrigation Regime and *Verticillium* Inoculum Density on Plant Height**

	1988			1989			1990		
	Irrigation Regime								
	DEF[1]	OPT	EXC	DEF	OPT	EXC	DEF	OPT	EXC
	Mean Plant Height (cm)								
Control[2]	35.1	35.0	35.3	9.1	9.5	14.3	19.7	24.5	26.3
Low Vert	34.2	36.8	35.2	8.8	11.6	14.5	18.1	22.5	26.1
High Vert	35.3	36.9	35.3	6.4	11.1	13.4	19.7	21.8	27.2

1/ DEF-deficient (1/2 optimum), OPT-optimum and EXC-excessive (2X optimum)
2/ Control-0 ms/gm soil, Low-15 ms/gm soil and High-50 ms/gm soil Vert

Plant height measurements taken approximately 60 days after planting.
No significant differences when compared with controls at P=0.05.

Table 1.2 **Effect of Season, Irrigation Regime and *Verticillium* Inoculum Density on Tuber Number per Plant.**

	1988			1989			1990		
	Irrigation Regime								
	DEF[1]	OPT	EXC	DEF	OPT	EXC	DEF	OPT	EXC
Control[2]	7.7a	6.1	6.6	5.9 b	7.6	9.0	7.2ab	10.9	10.5
Low Vert	5.9 b	6.4	6.3	9.5a	8.9	7.4	8.6a	10.7	9.4
High Vert	7.7a	7.4	5.7	5.1 b	6.3	7.2	6.1 b	9.4	9.6

1/ DEF-deficient (1/2 optimum), OPT-optimum and EXC-excessive (2X optimum)
2/ Control-0 ms/gm soil, Low-15 ms/gm soil and High-50 ms/gm soil Vert

Numbers followed by a different letter are significantly different from the control at P=0.05.

Table 1.3 **Effect of Season, Irrigation Regime and Verticillium Inoculum Density on Tuber Yield per Plant.**

	1988			1989			1990		
	Irrigation Regime								
	DEF[1]	OPT	EXC	DEF	OPT	EXC	DEF	OPT	EXC
Control[2]	847a	626	506	765	992	1588	426	739	1013
Low Vert	811ab	627	496	959	1116	1889	353	626	1007
High Vert	705 b	693	463	638	816	1691	393	559	959

1/ DEF-deficient (1/2 optimum), OPT-optimum and EXC-excessive (2X optimum)
2/ Control-0 ms/gm soil, Low-15 ms/gm soil and High-50 ms/gm soil Vert

Numbers followed by a different letter are significantly different from the control at P=0.05.

Table 1.4 **Effect of Irrigation Regime and <u>Verticillium</u> Inoculum Density on PED Symptom Progress or Senescence as Expressed by Percentage of Foliage Affected[1] During the 1988 Growing Season.**

	Days after Planting					
	57	69	79	86	93	100
			Deficient Moisture[2]			
Control[3]	0.3 b	3.4 b	5.0 b	8.0 c	17.5 b	25.0 b
Low Vert	1.0a	5.0ab	6.5ab	11.5 b	37.5a	45.0a
High Vert	2.6a	6.5a	8.5a	25.0a	47.5a	47.5a
			Optimum Moisture			
Control	1.8	6.5	6.5 b	9.0 b	45.0 b	52.5
Low Vert	3.4	9.5	11.5a	23.5a	65.0a	70.0
High Vert	3.0	7.0	11.5a	14.5ab	42.5ab	52.5
			Excessive Moisture			
Control	3.0	7.0	9.0	11.5	42.5 b	55.0
Low Vert	5.0	7.0	16.0	23.5	52.5ab	75.0
High Vert	4.6	7.5	13.0	23.5	67.5a	78.0

1/ PED foliar symptom progression measurements were made using the modified Horsfall/Barratt scale and converting to appropriate percentage.
2/ Moisture regimes - Optimum, Deficit (1/2 Optimum) and Excessive (2X Optimum)
3/ Control-0 ms/gm soil, Low-15 ms/gm soil and High-50 ms/gm soil Vert

Numbers followed by a different letter are significantly different from the control at P=0.05

Table 1.5 **Effect of Irrigation Regime and <u>Verticillium</u> Inoculum Density on PED Symptom Progress or Senescence as Expressed by Percentage of Foliage Affected[1] During the 1989 Growing Season.**

	Days after Planting						
	72	**80**	**87**	**95**	**101**	**108**	**114**
				<u>**Deficient Moisture**</u>[2]			
Control[3]	1.8 b	4.2	6.0 b	6.5 b	6.5 b	17.5 b	52.5 b
Low Vert	2.6 b	6.5	9.5ab	23.5a	32.5a	62.5a	91.0a
High Vert	6.0a	7.5	20.5a	37.5a	57.5a	81.0a	93.5a
				<u>**Optimum Moisture**</u>			
Control	3.0 b	4.2	7.0	9.5	10.0 b	40.0	72.5 b
Low Vert	4.2ab	4.6	9.5	11.5	14.5ab	37.5	81.0ab
High Vert	6.5a	6.5	13.0	22.0	37.5a	72.5	92.5a
				<u>**Excessive Moisture**</u>			
Control	1.4 b	3.4	6.5 b	9.5	9.5	32.5	67.5a
Low Vert	3.0ab	4.2	13.0a	16.0	14.5	40.0	82.5a
High Vert	4.2a	4.6	7.5ab	14.5	19.0	47.5	82.5a

1/ PED foliar symptom progression measurements were made using the modified Horsfall/Barratt scale and converting to appropriate percentage.
2/ Moisture regimes - Optimum, Deficit (1/2 Optimum) and Excessive (2X Optimum)
3/ Control-0 ms/gm soil, Low-15 ms/gm soil and High-50 ms/gm soil Vert

Numbers followed by a different letter are significantly different from the control at P=0.05

Table 1.6 Effect of Irrigation Regime and Verticillium Inoculum Density on PED Symptom Progress or Senescence as Expressed by Percentage of Foliage Affected[1] During the 1990 Growing Season.

	Days after Planting						
	64	72	79	85	94	101	106
				Deficient Moisture[2]			
Control[3]	5.5	6.5	9.0	25.0	40.0	88.5	98.5
Low Vert	3.8	8.0	8.0	20.5	55.0	93.0	97.0
High Vert	2.6	6.5	9.5	27.5	65.0	94.5	97.5
				Optimum Moisture			
Control	2.2	6.0	9.0	16.0	52.5	88.5	94.5
Low Vert	3.4	6.5	8.5	19.0	37.5	90.5	94.5
High Vert	4.6	8.0	17.5	27.5	62.5	93.0	97.5
				Excessive Moisture			
Control	1.4 b	3.0	5.5 b	7.5 b	11.5 b	47.5 b	78.0 b
Low Vert	4.6a	4.6	6.5 b	11.5ab	17.5ab	65.0ab	91.0ab
High Vert	4.6a	5.5	13.0a	20.5a	42.5a	91.5a	96.5a

1/ PED foliar symptom progression measurements were made using the modified Horsfall/Barratt scale and converting to appropriate percentage.
2/ Moisture regimes - Optimum, Deficit (1/2 Optimum) and Excessive (2X Optimum)
3/ Control-0 ms/gm soil, Low-15 ms/gm soil and High-50 ms/gm soil Vert

Numbers followed by a different letter are significantly different from the control at P=0.05

Table 1.7 Weather data (1988-90) from 6/15 to 7/15 annually.

	Daily High C^0 (F^0)			Daily Low C^0 (F^0)		
Date	1988	1989	1990	1988	1989	1990
6/15	20 (68)	23 (73)	24 (76)	6 (42)	4 (40)	2 (35)
	26 (79)	26 (79)	23 (73)	3 (37)	8 (46)	2 (36)
	25 (77)	29 (84)	21 (70)	6 (42)	6 (42)	1 (34)
	27 (80)	27 (80)	27 (81)	7 (44)	6 (43)	2 (36)
	27 (81)	29 (84)	28 (82)	7 (44)	7 (44)	4 (40)
	28 (82)	28 (83)	26 (78)	6 (42)	8 (47)	3 (38)
	29 (84)	28 (82)	26 (79)	8 (46)	6 (43)	6 (42)
	30 (86)	24 (76)	28 (82)	8 (47)	4 (40)	4 (40)
	28 (83)	18 (65)	29 (84)	10 (50)	3 (37)	6 (42)
	29 (84)	24 (76)	32 (88)	9 (49)	6 (43)	6 (42)
	26 (79)	26 (79)	32 (90)	8 (46)	3 (37)	7 (44)
	22 (72)	25 (77)	31 (88)	7 (45)	2 (35)	7 (44)
	24 (76)	26 (78)	30 (86)	9 (48)	6 (43)	7 (44)
	23 (73)	27 (81)	31 (88)	10 (50)	7 (45)	6 (42)
	23 (73)	27 (81)	31 (88)	12 (53)	8 (46)	9 (48)
	26 (78)	27 (81)	32 (89)	7 (44)	7 (45)	7 (44)
7/1	27 (80)	29 (84)	32 (91)	7 (44)	7 (44)	7 (44)
	27 (80)	30 (87)	30 (87)	7 (45)	4 (40)	7 (44)
	27 (80)	29 (85)	29 (85)	7 (45)	6 (43)	11 (52)
	28 (82)	30 (86)	26 (78)	9 (48)	7 (45)	9 (48)
	28 (82)	31 (88)	26 (79)	6 (42)	8 (46)	10 (50)
	26 (79)	32 (91)	23 (73)	7 (44)	9 (48)	11 (52)
	28 (83)	32 (91)	23 (73)	11 (51)	6 (43)	10 (50)
	25 (77)	32 (89)	26 (79)	3 (38)	6 (43)	10 (50)
	24 (75)	32 (89)	21 (70)	6 (42)	4 (40)	11 (52)
	23 (73)	30 (86)	24 (76)	6 (43)	4 (40)	8 (46)
	26 (78)	27 (81)	26 (78)	7 (44)	10 (50)	10 (50)
	28 (82)	27 (81)	26 (78)	6 (43)	12 (54)	8 (46)
	29 (85)	24 (76)	24 (76)	9 (48)	9 (48)	7 (44)
	29 (85)	28 (83)	24 (75)	8 (47)	8 (47)	8 (46)
7/15	29 (85)	26 (78)	20 (69)	10 (50)	6 (43)	4 (40)

Chapter 2

Role of Air Temperature on Expression of Potato Early Dying

Introduction:

The unique environmental conditions found in the San Luis Valley, Colorado make it an ideal potato growing area. These same conditions may suppress PED symptom expression in the field and reduce PED impact on the crop. One of the most important environmental factors which affects PED development is air temperature (7,15,18,22). A greenhouse study was initiated to compare PED expression under three different air temperatures representing a cool production area such as the San Luis Valley, a mid-range area and a relatively warm production area such as Northeastern Colorado. Tissue-culture produced Russet Burbank potatoes were grown in pots containing soil with the same three _Verticillium_ _dahliae_ inoculum levels (zero, low and high) and similar fertility and irrigation regimes as those used in the field experiments.

Materials and Methods:

Growth chamber: A greenhouse bench approximately 1.5m wide x 9.0m long was divided into three parts. Frames for 1.5m high chambers were built from wood and double layers of plastic sheeting enclosed the area. The chambers were designed to be placed on the greenhouse bench and enclose a given area. Each chamber was designated for one of the three temperatures. Thirteen cm round holes were cut into the bottom of the wooden bench so that when sixteen cm plastic pots were placed into the holes each protruded almost entirely below the level of the bench. This exposed the growth medium to uniform ambient air temperatures while the uppermost portion of the pot rested at the level of the bench. Stems and foliage of plants growing in the pots were then exposed to the three different air temperatures maintained in the chambers while the soil and roots were maintained at a uniform temperature. Pots were placed on twenty centimeter centers between pots and at least thirty centimeters from the edges of the chamber. A plastic saucer was attached to the bottom of each pot to insure uniform watering of each plant. The saucer for each pot was filled to capacity with water each time the plants were watered thus allowing each plant the chance to take up water as needed at any time. Temperatures were regulated by placing a room air conditioner in each of the two chambers maintained at 15 and 25^{0}C and allowing the greenhouse controls to maintain a temperature of 30^{0}C in the third chamber. Temperatures were decreased approximately 8^{0}C at night to more closely simulate normal field conditions and to allow for proper tuberization. The average maximum temperature in the three chambers was not allowed to exceed the upper limits of the target 15, 25 or 30^{0}C temperatures. Temperatures were constantly monitored using a Hygro-Thermograph and/or a recording thermometer in each chamber and the greenhouse. The experiment was conducted between April 24 and September 6 when day length was most satisfactory for plant growth and tuber production.

Experimental design: Temperatures were selected based on the averages measured in two different potato producing areas of the state; one, Platteville, Co., is an area where typically high levels of potato early dying damage occurs and the other, the San Luis Valley, an area where little or no potato early dying appears in most years. The 15^{0}C (59^{0}F) regime represented the average May 1 to August 30 minimum air temperature measured in Platteville over a 5 year period (1984-1988), 25^{0}C (77^{0}F) the average maximum temperature measured in the San Luis Valley over a five year period and 30^{0}C (86^{0}F) the average maximum temperature measured at Platteville during the same five years. The overall average early season temperatures for the normal planting dates (late April through June) in the San Luis Valley fall near the 15^{0}C range, while later season overall average temperatures fall in the 20 to 25^{0}C range.

Sixteen centimeter plastic pots filled with standard Metro-Mix^R were used. Prior to filling the pots, the growth medium was amended where appropriate with Verticillium dahliae, isolate #326 microsclerotia derived from potatoes produced in Oregon. Microsclerotia were grown, measured and added to the Metro-Mix^R in a manner similar to that used in the field experiments in the SLV. Verticillium inoculum levels were assayed and found to be zero, 10 and 30 ms/gm growth medium.

Rates of fertilizer applied to the growth medium were calculated using Havlin and Soltanpour's formulations for greenhouse soils (12). Each pot contained 314 gm of oven dried Metro-Mix^R, as determined by weighing the medium in seven pots and taking the average weight. Fertilizer was added to each pot at the following rates: N 300 ppm (0.0942 gm NH_4NO_3), P 400 ppm (0.1256 gm $Ca(H_2PO_4)_2$), K 400 ppm (0.1256 gm KCl), S 50 ppm (0.0157 gm $CaSO_4$), Fe 4 ppm (0.00126 gm FeEDDHA), Zn 10 ppm (0.00314 gm $ZnSO_4$) and Mn 5 ppm (0.00157 gm $MnSO_4$). In addition, 50 ppm N was added to each pot on 6/8/89, 45 DAP and 6/23/89, 60 DAP. The solution used was mixed using a liquid N with a formulation of 32-0-0. This provided the equivalent of 400 ppm N for each pot during the growing season.

Generation 2 (two generations from tissue-culture sources), Russet Burbank seed from lots with no visible blackleg in the field were obtained from Worley Seed Company, Center, Colorado. Single eyes were removed with a melon ball scoop to provide uniform seedpieces. Seed tubers were surface disinfested by soaking in 0.5% chlorox for 10 minutes prior to removing the balls. The melon ball scoop was disinfested with 1% Hyamine and Chlorox between tubers. The seedpieces were allowed to suberize for 48 hours under high humidity in a chamber and then planted. All eyes showed evidence of sprouting at the time of planting. The seedpieces were planted into pre-irrigated soil to a depth of six cm in the center of each pot on 4/24/89. All pots were irrigated to runoff one hour after planting.

A randomized complete block design with 4 replications of each treatment per block was used for the experiment. Data on emergence and plant height were collected early in the growth cycle. Readings to determine PED disease progress or plant senescence in the case of the controls were made at 2 week intervals starting from the onset of symptoms (approximately 45 DAP) and continuing throughout the growing period. The proportion of foliage affected by PED was estimated for each plant using a modified Horsfall/Barratt scale (13) and transformed to percentages. At harvest (135 DAP) data on general plant appearance, tuber number and total tuber weight in grams were collected for each pot.

Results:

Emergence occurred 10-15 DAP with the 15^{0}C treatment having the slowest emergence and the 30^{0}C treatment the fastest. Water did not appear to be a limiting factor at any time during plant growth. Overall plant appearance was normal. The following observations were noted during the course of the experiment:

37 DAP[2] 15^{0}C - plants have leaves which appear larger and darker green and overall are 20-25 cm. shorter than plants at the other two temperatures.

25^{0}C - plants are taller than the 30^{0}C treatment, but not as vigorous as plants at 15^{0}C. Some legginess evident.

30^{0}C - some heat damage evident in plants closest to walls of chamber.

53 DAP Insecticide fogged on plants for thrips control. 25^{0}C - Some phytotoxicity evident on the lower parts of the plants. Stolon growth from the pots common.

60 DAP 15^{0}C - All plants appear negative for Verticillium symptoms. Plants at all temperatures show some phytotoxicity on the bottom leaves from insecticide treatments for thrips control.

95 DAP 15^{0}C - All plants quite healthy in appearance compared to the plants in the other two chambers.

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[2]DAP=Days after planting

123 DAP 15^0C - Some of the plants near the air conditioner are dead from apparent freezing injury. All plants with 100% damage were affected in this manner and PED readings from the previous week will be used as final readings.

135 DAP At harvest, the tubers from the 15^0C treatment looked normal while those tubers from plants at higher temperatures were often misshapen with additional knobs and multiple tubers (double growth).

Stand was not significantly affected (P=0.05) by treatment. Plant heights within a given temperature regime (Table 2.1) were similar, but differences occurred among temperatures. Plants in the 15^0C chamber were more normal in appearance both in height and size of the leaves compared with the other temperature treatments. Overall the plants were 20-25 cm shorter in the 15^0C chamber than plants at the other two temperature regimes.

Table 2.2 shows data for Verticillium wilt/PED progress and senescence, tuber number and tuber yield. At the 15^0C temperature, PED symptoms were detected during the course of this study in plants grown in the infested soil, but overall symptoms were mild. Normal maturity and first PED symptoms were recorded beginning at 109 DAP. This was 49 days later than PED onset in the 25^0C chamber and 14 days later than in the 30^0C regime. Both Verticillium treatments at 15^0C had significantly more (P=0.05) disease expression than the maturity expressed by the control, but symptoms never exceeded 50% of the foliage affected, even at the highest level of Verticillium inoculum.

PED disease onset was earliest and progress most rapid at 25^0C. Again, both Verticillium treatments had significantly more (P=0.05) PED symptoms than the control's senescence. At 30^0C, disease expression was intermediate between the other two temperatures both in time of disease onset and amount of PED symptom expression. There were no significant differences in percentage of foliage affected with PED between the *Verticillium* treatments and maturity of the control at 30⁰C.

Tuber numbers per plant were similar among temperature regimes. There was a significant increase in tuber number in the high *Verticillium* inoculum level compared with the low inoculum level and the control at 25⁰C. Overall tuber yield per plant decreased as air temperature increased. Tuber yields were not significantly different among treatments at the 15⁰C or the 30⁰C regime. However, at 25⁰C, yield at the low *Verticillium* inoculum level was significantly depressed when compared with the high inoculum level.

Table 2.1 Effect of Air Temperature and *Verticillium* Inoculum Level on Mean Plant Height[1]

	Control[2]	Low Vert	High Vert
15⁰C	65.8	54.3	62.0
25⁰C	103.0	93.0	87.0
30⁰C	81.0	78.0	90.0

1/ Plant height measured in cm at 43 days after planting
2/ Control-0 ms/gm soil, Low-15 ms/gm soil and High-50 ms/gm soil Vert

No significant differences when compared between temperature regimes at P=0.05.

Table 2.2 Effect of Air Temperature and Verticillium Inoculum Levels on PED Symptom Progress or Plant Maturation, Tuber Number and Tuber Yield (grams).

	Percent Of Foliage with PED Symptoms[1] Days after Planting 60	95	109	123	Mean Tuber #/Plant	Mean Tuber Yield/Plant
			15°C			
Control[2]	0	0	17.5	3.0a	2.3	95.8
Low Vert	2.2	7.5	22.0	37.5 b	2.0	100.5
High Vert	0	0	22.0	37.5 b	2.5	90.8
			25°C			
Control	0.5	22.0	57.5	25.0a	1.8a	51.0ab
Low Vert	10.0	22.0	62.5	70.0 b	1.0a	30.3 b
High Vert	9.0	37.5	75.0	87.0 b	3.3 b	73.3a
			30°C			
Control	0.3	32.5	50.0	32.5	1.8	36.0
Low Vert	0.5	45.0	57.5	45.0	1.5	38.0
High Vert	1.0	32.5	50.0	45.0	2.0	61.0

1/ PED foliar symptom progression and plant maturation measurements were made using the modified Horsfall/Barratt scale and converting to appropriate percentage.
2/ Control-0 ms/gm soil, Low-15 ms/gm soil and High-50 ms/gm soil Vert

Numbers followed by a different letter are significantly different from the control at P=0.05

Discussion:

Air temperature has been shown to play a major role in PED expression and subsequent yield loss. In cooler growing areas such as the San Luis Valley of Colorado, PED is considered a minor problem. Results from this study provide a reasonable explanation. Plants grown in the cooler temperatures similar to those found in the San Luis Valley exhibited more normal growth habit and produced higher yields than those grown in the warmer temperature regimes. Potatoes grown in air temperatures similar to the averages for the San Luis Valley showed little effect from PED in any aspect of production including tuber numbers and yield. PED symptoms were mild and onset was later than at higher temperatures. This is consistent with observations made in the field.

At the air temperature which approximated the daily high maximum temperature for the San Luis Valley (25^{0}C), PED onset was early, 60 DAP, and symptom severity significantly higher (P=0.05) in the Verticillium treatments than maturity showed by the control. It should be noted, however, that this temperature was constant for sixteen hours per day and thus represents a temperature regime more similar to warmer areas like northern Colorado than the San Luis Valley. At most, temperatures in the San Luis Valley reach these highs for only two to four hours per day and drop to near 10^{0}C at night making average air temperatures for this area much nearer the 15^{0}C regime than the 25^{0}C regime. These findings generally agree with reports from other warm weather areas (10,14,21).

The highest temperature, 30^{0}C, produced lower overall levels of PED than the 25^{0}C regime. However, PED development was more rapid in the 30^{0}C regime peaking by 109 DAP while PED symptom development was slower, but continued to increase through 123 DAP in the other two temperature regimes. It is possible that physiological development of plants at the higher temperature was accelerated allowing a faster PED symptom development than at the other two temperature regimes. Under the conditions of this experiment, the 25^{0}C regime was more favorable for development of PED symptoms than the 30^{0}C regime. It is possible that the higher temperature regime, representing the upper limits of growth for the fungus, reduced the effectiveness of the inoculum. It is interesting to speculate what influence a constant high temperature, both day and night, might have on physiological development of the plant and ability of Verticillium to actively infect plants and cause PED symptoms. It is reasonable to deduce that the temperature range found in the 25^{0}C regime was more conducive to good plant growth and also good fungal growth resulting in more impact from PED. However, it should be noted again that even though San Luis Valley temperatures can be found in the 25^{0}C range, both the day and night mean temperatures generally fit more closely with the 15^{0}C regime.

Overall plant yields increased with decreasing temperature. Yields in the 30^{0}C regime were lower than in the other two regimes, even though higher levels of PED symptoms were seen in the 25^{0}C regime. It appears that the effect of temperature on potato growth and production was greater than the potential effect of PED.

These results indicate that the impact of PED is lessened when air temperature is cool. Onset of the disease is delayed and symptom expression is mild under such conditions when compared with temperatures found in warmer growing areas. Having ideal growing conditions for potatoes without any limiting production factors like fertility or irrigation appears to allow maximum yields from the crop even when Verticillium is present. Because PED symptoms are mild under cool conditions, impact on yield is limited. In contrast, areas with warmer growing conditions are impacted by PED. Onset of PED is early and symptoms can be severe under such conditions, acting to limit tuber yields, even when all production factors are optimum. There may be an upper temperature beyond which PED impact does not increase as temperature increases. Higher temperatures may have a greater detrimental effect on plant growth and tuber yields than does PED when it is present.

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Chapter 3

Impact of Soil Fumigation on Soil Borne *Verticillium* Inoculum

Introduction:

Soil fumigation was found by several researchers to be an effective control measure for PED by controlling soilborne *Verticillium* inoculum (1,3,5,14,21). Some potato growers in the Pacific Northwest must use soil fumigation each year to avoid major yield losses due to soilborne pathogens (5,17,18). Most fumigation research was done in warmer production areas of the country, where PED is a more chronic yield limiting factor. Little research, however, was done in areas where PED is a minimal problem such as the San Luis Valley of Colorado. This study, done in cooperation with a potato producer in the San Luis Valley, was designed to determine the effects of soil fumigation on soil borne *Verticillium* microsclerotia.

Materials and Methods:

Four fields were screened for presence of *Verticillium dahliae* microsclerotia. The cropping history for each field is shown in Table 3.1. Screening was done using the standarized procedure developed by Harrison and Livingston (12) and ethanol agar as the assay medium. Each field was sampled by selecting five 1/2 ha blocks in a diagonal pattern across an 18 ha portion of the field. Samples were collected from the four fields in the fall of 1988, prior to soil fumigation, in the spring of 1989, two months after soil fumigation and in the late summer of 1990. Ten soil samples of about two kg each were collected from each 1/2 ha block by taking random samples to a depth of 30 cm (plow layer) and combining them into a composite sample. The five composite samples, one from each 1/2 ha block, were air dried, thoroughly mixed and gently sieved through a series of screens (#50 - 200) to eliminate larger particles. The resultant fine soil was measured and placed onto filter paper in 0.1 gm increments. Three replicate samples of soil from each block were used resulting in a total of 15 assay plates per field for each sampling period. These samples were individually processed by using an Anderson Sampler (2) to deposit them uniformly onto plates containing the ethanol medium. Plates were incubated in the dark at 21^{0}C for twenty one days following deposition of the soil onto the medium and Verticillium colonies counted using a 40X binocular microscope. Total counts from each plate were determined and the data converted to total number of microsclerotia per gram (ms/gm) of air dried soil using appropriate diliution factors to make the conversion.

Two of the four fields studied were fumigated with BUSAN 1020^{R}[3] at a rate of 371 l/ha (40 gal/acre) in early March, 1989. Two crops, either potato/grain or grain/potato, were grown prior to final soil sampling in 1990. The remaining two fields served as controls and were not fumigated.

Results:

Initial populations of Verticillium microsclertia (ms) were high in fields A, B and E (62 to 132 ms/gm soil), and low in field F (19 ms/gm soil) in the fall of 1988. Potatoes were grown in fields A and F immediately prior to the initial assay, while grain was grown in B and E. Table 3.2 shows ms counts for the different sampling dates.

After fumigation, fields A and F showed a dramatic decline in ms numbers. A 70 percent reduction was found in field A and an 80 percent decrease in field F. However, the two non-fumigated fields had similar or even greater reductions in ms counts. After two crops, one grain and one potatoes, both fumigated fields showed even further declines in ms counts, even after having had PED susceptible potatoes growing just prior to sampling. Field A had an additional 80 percent decline in ms counts

[3] BUSAN 1020^{R} manufactured by Buckman Laboratories.

following the potato crop while field F showed an additional 50 percent decrease. The other two non-fumigated fields, B and E, showed a 75 percent decline and a four fold increase in ms counts respectively.

Table 3.1 Cropping History of Fields Used to Study the Effects of Soil Fumigation on *Verticillium dahliae* Populations.

	Field			
	A	B	E	F
1988	Potatoes	Grain	Grain	Potatoes
1989	Grain (Fumigation)	Potatoes	Potatoes	Grain (Fumigation)
1990	Potatoes	Grain	Grain	Potatoes

Fumigation with Busan[R] done in early spring 1989, two months prior to crop planting. Potatoes planted in the fields were either Russet Burbank or Russet Norkotah, both quite susceptible to potato early dying.

Table 3.2 Populations of *Verticillium dahliae* Microsclerotia in Field Soil Before and After Fumigation.

	Field			
	A	B	E	F
	***V. dahliae* ms/gm soil**			
FALL 1988	119	132	62	19
SPRING 1989	35	48	4	4
LATE SUMMER 1990	8	16	12	2

Fumigation occurred in the spring, 1989 in fields A and F.

Discussion:

As reported for most other potato production areas, soil fumigation is effective in reducing soilborne *Verticillium* inoculum and thus reducing the impact of PED on the potato crop. Results from this study indicate that control of PED in the San Luis Valley with soil fumigation is not so easily interpreted. Prior to fumigation ms counts in the fields were high, easily at or above minimum threshold numbers necessary to produce severe PED problems in most other production areas. After fumigation, however, ms counts were reduced dramatically and continued to decrease even through successive cropping seasons. This indicates that soil fumigation with Busan^{R} and possibly other soil fumigants may be a successful control measure in the San Luis Valley with benefits that carry through more than one year.

However, it is interesting to note that fumigation alone does not provide the entire answer to fluctuation of V. dahliae populations in soil in the San Luis Valley. Even without fumigation, ms populations were reduced and in one field continued to decline. This suggests that other factors, including shifts in soil microflora, soil amendments with organic matter (high levels of grain straw), the possibility of severe winter temperatures, high summer soil temperatures and soil solarization (4,11) may play a role in inoculum reduction. These factors should not be discounted, but may not be reliable ways to continuously reduce V. dahliae inoculum. One additional year of study on the fields would have been appropriate, resulting in the complete series of crop rotations. It does appear that fumigation is both effective in reducing V. dahliae inoculum and that its effects will carry over for at least two growing seasons. Fumigation should also reduce other problems such as nematodes, viable weed seeds, etc. in addition to reducing Verticillium ms counts in the soil. This provides reduced risk from PED if environmental conditions in a given year are favorable for symptom expression. The potential reduction of PED symptom expression associated with soil fumigation in the San Luis Valley, however, must be tempered with cost considerations ($660/ha) associated with this fumigation. At average potato prices, a yield advantage of over 2.7 mt/ha would be necessary to justify the cost of fumigation. Given the sporadic occurence of severe "PED years" and potential ms reductions available because of environmental factors, use of soil fumigation must be considered carefully!

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Chapter 4

INTERACTION BETWEEN VERTICILLIUM DAHLIAE, ERWINIA CAROTOVORA SUBSP. CAROTOVORA AND E. C. SUBSP. ATROSEPTICA AND IRRIGATION

Abstract:

During three growing seasons, 1988-1990, microplots planted with the cultivar Russet Burbank were used to study the effects of infesting soil with *Verticillium dahliae* (V) and simultaneously introducing *Erwinia carotovora* subsp. *carotovora* (Ecc) and *E. carotovora* subsp. *atroseptica* (Eca) by individually inoculating them into seed tubers (Ecc/Eca seed) and, in the case of Ecc, applying inoculum with irrigation water during the growing season (Ecc irrigation). *Verticillium* was studied alone and in combination with each of the *E. c.* subspp. under differing irrigation regimes.

Ecc and Eca appeared to be the primary pathogens involved in a potato early dying complex, while *V. dahliae* appeared to have a secondary role. Plant heights, stands and tuber numbers were reduced when seedpieces were inoculated with Ecc or Eca, but not when Ecc was applied in irrigation water. No *Verticillium* + *Erwinia* interaction was found. There was a trend toward greater stand losses and plant height reductions under the highest moisture. PED progress was greatest under excessive irrigation and least under a moisture deficient regime. Progress of PED in the V + Ecc seed or V + Eca seed treatments was similar to or sometimes significantly greater (P=0.05) than when the plants were exposed to *Verticillium* alone. Overall the trend was toward higher disease readings throughout the season in plots with pathogen combinations compared to plots with *Erwinia* or *Verticillium* alone.

Per plant tuber yields were significantly affected (P=0.05) by treatment. Significant V + Ecc seed or V + Eca seed interactions, synergistic in nature, occurred in three cases during the two warmer years of the study. Sixty four percent of the time co-infection with V + Ecc seed or V + Eca seed did not produce significantly lower yields than either pathogen alone, but there was a clear trend toward lower yields when pathogens were combined. Yields were significantly affected by irrigation regime. Excessive irrigation appeared to have the greatest effect on PED severity and disease progress during the season. The greatest yield losses occurred in treatments representing interactions between Verticillium and Ecc and Eca.

Introduction:

Verticillium dahliae Kleb., the principal causal agent of Verticillium wilt or potato early dying, is a significant component in yield loss in most production areas in North America (2,6,23,32). Recognition, diagnosis, severity and prediction of potential yield losses from this particular disease are often influenced by environmental factors, especially temperature (14,20,23,33,41). More early dying is observed in warm than in cool growing regions; disease severity is also influenced by production management factors including fertility, irrigation and frequency of potatoes in the crop rotation (11,15,23,40). In addition, this can be further complicated by the presence of other potato pathogens and/or nematodes to create a "potato early dying complex" (40).

Several pathogens interact with V. dahliae. They include the lesion nematodes; Pratylenchus penetrans and P. crenatus (19,31,41), other fungi; Alternaria solani and Colletotrichum coccodes (27,29) and bacteria; Erwinia carotovora subsp. carotovora and E. c. subsp. atroseptica (29,39,44). The relationship between V. dahliae and Erwinia carotovora subspp. was studied in the greenhouse and in large field plots, but little has been done using controlled microplots. Studies in Oregon demonstrated that early dying symptom expression was more severe when the two pathogens were co-inoculated than when either was present alone. It was suggested that this might be due to an enhanced stem colonization by V. dahliae (29). Research conducted in Wisconsin showed similar results with a synergistic increase in symptom severity when coinciding infections with both pathogens occurred. In addition to reduction of plant growth there were marked increases in leaf chlorosis and plant wilting plus enhanced development of soft rot (39). Also, when E. carotovora subspp. were used as primary pathogens and plants co-inoculated with V. dahliae in field plots in North Dakota, a significant increase in incidence of blackleg occurred (44).

None of these studies, however, addressed the effect of co-infection with both V. dahliae and E. carotovora subspp. under controlled conditions in the field. A better understanding of these relationships would be important under cool growing conditions such as those found in the San Luis Valley of Colorado where potato early dying is less frequently observed, but where blackleg can be a major problem. It is known that V. dahliae infects potatoes in the San Luis Valley and that relatively high population levels of microsclerotia (compared with other reports in the literature) can be found in stems of infected plants as well as soils where potatoes have been growing (Harrison, Davidson, personal experience). The objectives of this study were to examine major facets of potato production including stand, disease progress, symptom expression and yield associated with co-infection of V. dahliae and E. carotovora subspp. (Ecc and Eca) under environmental conditions favorable for blackleg disease development and expression, but not conducive to severe potato early dying.

Materials and Methods:

Plot Preparation: Microplots were established at the San Luis Valley Research Center in May of 1988, 1989 and 1990. Each year, prior to plot establishment, a composite soil sample was collected from the field to be used for the study. A soil test was performed and fertility was adjusted to levels appropriate for Russet Burbank commercial production in the San Luis Valley (42).

The plot field area was fumigated in April of each year. PicChlor 60^{R} (60 percent chloropicrin/40 percent telone) was injected into the soil to a depth of 60 cm at a rate of 393 kg/ha (273 l/ha). After fumigant injection, the soil was sealed by sprinkling with water to wet the soil to a depth of approximately 2 cm. Soil samples for nematode assays were collected prior to fumigation in 1988. Nematodes commonly associated with the potato early dying complex were not detected in the soil and since the same area was used in subsequent years, the assays were not repeated.

One week prior to planting, five trenches, 4 m apart, 29 cm deep and 1.2 m wide were dug in the fumigated soil using a backhoe. Soil was piled along the edge of each trench for use as the planting medium in the microplots. Pots 30 cm high and 31 cm inside diameter with a 10 cm hole drilled in the center of the base to provide for water drainage were used as the microplots. Pots were arranged on 60 cm centers within the row and 92 cm centers between rows. Three rows of pots were set in each of the five trenches resulting in a total of fifteen replicated rows.

A drip irrigation system was installed in the plot. Pairs of individual pressure compensating emitters were placed in each pot to insure uniform water delivery. The irrigation system was designed to deliver water at optimum, excessive and deficient amounts based on tensiometer, gypsum block readings and ET tables appropriate for potato production in San Luis Valley. The tensiometers and gypsum blocks were placed at several random locations throughout the plot at depths of 20 cm. Readings were taken three times per week. Irrigation rates used were 3.8 l/hr (optimum), 7.6 l/hr (excessive) and 1.9 l/hr (deficient). Microplots were irrigated with well water known to be free from <u>Erwinia carotovora</u> on a one to four day schedule; based upon readings from the tensiometers, gypsum blocks that were referenced to ET tables. The amount of water applied was that required to maintain the optimum moisture regime within the desired limits. Rainfall was factored into the irrigation regimes mentioned above.

Inoculum Preparation: <u>Verticillium</u> <u>dahliae</u> inoculum was prepared from a mixture of three isolates isolated from potatoes grown in Oregon. Axenic cultures were grown on Potato Dextrose Agar in plastic 12 x 100 mm petri plates for 21 days in dark conditions at 21^{0}C. The surface of twenty plates was washed with 10 ml of sterile water on each plate to remove conidia and hyphae from the cultures. The hyphae/conidia suspensions were mixed into one liter of sterile water. The resulting suspension was spread onto a modified minimal medium (10) overlayed with a layer of sterile cellophane at a rate of 0.1 ml per plate. The plates were incubated at 21^{0}C in the dark for 21-30 days or until good microsclerotial production was evident. The cellophane disks containing the microsclerotia were stripped from the plates and placed in a Waring^R food blender containing sterile distilled water. The suspension was blended for one minute at high speed to macerate the cellophane and liberate the microsclerotia. The mixture was then washed through a series of metal sieves beginning with a #50 sieve (0.0117" opening) then sequentially to #100 (0.0059"), #200 (0.0029"), #325 (0.0017") and finally to a catch basin. Microsclerotia (ms) were collected from the #100, #200 and #325 sieves, while the waste cellophane collected on the #50 and the conidia and hyphal fragments in the catch basin were discarded. The microsclerotia were washed thoroughly after collection to remove remaining hyphal fragments and conidia. This was accomplished by spraying the ms collated in the #325 sieve repeatedly with sterile distilled water using a spray bottle. Examination of the ms under 10X magnification assured that the debris was removed from the ms.

The concentration of microsclerotia after washing and suspension in sterile water was determined by using a haemocytometer and a compound microscope. Appropriate amounts of inoculum were mixed into fumigated plot soil representing three microplots by tumbling the soil in a cement mixer for 3 minutes while the ms were sprayed onto the soil. The target was to reach population levels of approximately 15 and 50 ms/gm soil. These levels were based upon oven dry weight of the soil necessary to fill a microplot to within two cm of the pot's top. Soil for controls was mixed, but no inoculum was added, for the same amount of time. Recommended preplant fertilizer was also added to the soil as it was being mixed. Mixed soil was put into the pots which were placed in appropriate locations in the trenches according to a plot diagram. After all of the pots were filled and placed, the remaining fumigated soil removed from the trenches was used to fill in around the pots and level off the ground to within 6 cm. of the top of the pots.

Erwinia carotovora inoculum consisted of the type strain of Erwinia carotovora subsp. atroseptica (Eca) and two strains of E. c. subsp. carotovora (Ecc) serogroup XVIII. All strains were isolated from the San Luis Valley from infected potato stems in 1987. Serogroup XVIII represents one of the predominant serogroups found in the San Luis Valley (10). Isolates were stored on CVP medium at $4^{0}C$. Inoculum was increased for the study by rinsing cells off the CVP medium, spread plating on nutrient agar and incubating for 48 hours prior to seedpiece inoculation or application in the irrigation water. Inoculum dilutions were made by rinsing bacteria from the nutrient agar with sterile distilled water, centrifuging the suspension and re-suspending in sterile distilled water. A spectrophotometer was used at $O.D._{640}$ to measure the absorption of the suspension and the bacterial population was adjusted to approximate a population x 10^8 cfu/ml as derived from a standard curve developed by Aleck (1). After preparing appropriate dilutions, populations were confirmed by spread plating to nutrient agar at appropriate rates and the plates incubated at $21^{0}C$ for 72 hours before reading.

Seedpieces were inoculated by placing 0.01 ml of the appropriate dilution of either Eca or Ecc in an eye next to a sprout. A sterile toothpick was used to prick the tuber and allow the bacteria to enter near the base of the sprout. The wound was sealed with vaseline. Uninoculated control tubers were handled similarly except sterile distilled water was allowed to enter the tuber instead of inoculum. Inoculum concentrations of 10^2 and 10^6 cfu per seedpiece were used for each of the subspecies.

Application of Ecc inoculum in irrigation water was accomplished by dispensing 1 ml of the appropriate dilution of Ecc into 250 ml of sterile water for the deficient, 500 ml for the optimum and 1000 ml for the excessive water regime, and applying the suspension to the soil surface in the pots designated for these treatments. The amount of water applied represented what would be applied during a normal irrigation. For example, this resulted in the application of approximately 1.3 cm/ha

(0.2 in/ac) of water for the optimum moisture regime. Inoculum concentrations of 10^2 and 10^6 cfu were used for each irrigation regime, but applied in a different amount of water. Uninoculated controls received the equivalent amount of water as inoculated plots, but without Ecc present. Ecc infested irrigation water was applied two or three times during the season beginning 40 days after planting (DAP) and continuing at two week intervals until 75 DAP.

Experimental Design: A completely randomized block with 15 microplots (replications) per treatment at each moisture level was used for the study. A total of 15 treatments, 675 total microplots in 1988 and 21 treatments representing 945 microplots in 1989 and 1990 were included (Table 4.1). Each of the fifteen rows in the plot included a complete set of treatments and comprised one replication.

Generation 1 seed tubers (cv. Russet Burbank) from the Oregon State University Foundation Seed project in 1988 and 1989 and from the Worley Seed Company in Colorado in 1990 were cut into single eye seedpieces two weeks prior to planting with a 2.5 cm melon scoop disinfested in 10 percent chlorox between cuts. These were placed in chambers at 21°C and high relative humidity for seven days to aid suberization and germination. The pre-sprouted seedpieces were treated (inoculated or not depending upon the treatment) and planted in the soil at the center of each of the appropriate microplots to a depth of 6 cm.

Beginning at planting and continuing through the growing season, air and soil temperatures were recorded at 15 to 30 minute intervals using a CR21X micrologger[4]. Soil temperatures were taken using small thermocouple, "electrode type", sensors from one representative pot in each irrigation regime at a depth of 10 cm.

Soil samples collected from the microplots were used to determine populations of _Verticillium dahliae_ and _Erwinia carotovora_. _Verticillium_

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[4]Ibid

populations were determined prior to and after infestation. Assays were performed using the standarized procedure developed by Harrison and Livingston (24) using ethanol agar as the assay medium. Five composite samples, representing five replications of the zero, low and high levels of <u>Verticillium</u>, were air dried, thoroughly mixed and gently sieved through a series of screens (#50 to #200) to eliminate larger particles. The resultant fine soil was measured and placed onto filter paper in 0.1 gm increments. Three replicate samples of soil from each composite sample were used. These samples were individually deposited onto plates containing the ethanol medium using an Anderson Sampler (9). Plates were placed for 21 days in dark conditions at 21^{0}C following deposition of soil onto the medium, and <u>Verticillium</u> colonies were counted using a 40X binocular microscope. Total counts from each plate were determined and data were converted to total number of ms/gm of soil using appropriate dilution factors. Assays of <u>Erwinia</u> populations in soil and irrigation water were completed prior to planting in 1988. Soil samples were obtained by removing the top 15 cm of soil at several random locations within the plot area and collating into six sets representing twenty gm of air dried soil each. Each twenty gm mixed sample was suspended in PT medium (a pectate based enrichment medium (8)) and incubated for 72 hours at 21^{0}C. Duplicate samples were plated on CVP medium and plates examined for the presence of <u>Erwinia</u> spp.. Water samples were assayed using CVP medium (12) and the techniques described by Jorge and Harrison (28).

Symptoms of PED were observed and recorded regularly over the season. The presence and severity of PED symptoms were monitored weekly beginning at the time first symptoms appeared. A modified Horsfall/Barratt scale of 0 to 9 was used to estimate the percentage of foliage affected by PED or plant maturity (Appendix-Table 4.2). In addition, plants were monitored for the presence of blackleg symptoms throughout the season.

At harvest, the actual number of tubers per plant plus total yield per plant were recorded. Two randomly selected tubers per treatment from each of three replications in the plot were collected at harvest time and assayed for Erwinia contamination using a toothpick puncture/wrap test. This consisted of puncturing each tuber up to 50 times with a sterile toothpick, wrapping the wounded tuber in a wet paper towel and covering it with Saran wrap to make the sample air tight. Samples were incubated for 72 hours at 21^{0}C and presence or absence of soft rot symptoms was recorded. Tissue from rotting areas was suspended in sterile distilled water and streaked onto CVP medium which was incubated anaerobically at 21^{0}C for 72 hours to verify the presence of Erwinia carotovora.

Table 4.1 Pathogens and Pathogen Combinations Used in the Study.

Verticillium dahliae	**Combination Treatments**
Control (C) - Zero ms/gm soil	
Low (LV) - 10-15 ms/gm soil	
High (HV) - 35-50 ms/gm soil	
Erwinia carotovora subsp. carotovora	
Control (C) - Zero cfu/seedpiece	Low V + Low Ecc (LVLEC)
Low (LEC) - 10^2 cfu/seedpiece	Low V + High Ecc (LVHEC)
High (HEC) - 10^6 cfu/seedpiece	High V + Low Ecc (HVLEC)
	High V + High Ecc (HVHEC)
Erwinia carotovora subsp. atroseptica	
Control (C) - Zero cfu/seedpiece	Low V + Low Eca (LVLEA)
Low (LEA) - 10^2 cfu/seedpiece	Low V + High Eca (LVHEA)
High (HEA) - 10^6 cfu/seedpiece	High V + Low Eca (HVLEA)
	High V + High Eca (HVHEA)
Erwinia carotovora subsp. carotovora in Irrigation water	
Control (C) - Zero cfu	Low V + Low Ecc (LVLEW)
Low (LEW) - 10^2 cfu	Low V + High Ecc (LVHEW)
High (HEW) - 10^6 cfu	High V + Low Ecc (HVLEW)
	High V + High Ecc (HVHEW)

All treatments were included in 1989 and 1990. In 1988, E. carotovora subsp. atroseptica treatments were not included. All treatments were studied under each of the three irrigation regimes.

Results:

Plot data were statistically analyzed using two way ANOV comparisons in a split-split plot design with four factors; Verticillium, E. carotovora inoculum density, E. carotovora subspecies/inoculation method and irrigation regime. Comparisons among inoculum levels, irrigation regime and year were also made. Early season air temperatures were cooler in 1988 than in either 1989 or 1990. In general, 1988 was cooler throughout the growing season than either of the other years (Appendix-Figure 4.25). Fertility (all major and minor nutrients) was not limiting in any of the treatments during any year or at any time during mid to late season based upon analysis of petiole samples compared against standard curves for Russet Burbank potatoes grown under San Luis Valley conditions.

No significant differences in plant height were found when analysis was conducted among individual treatments. However, when the effect of E. carotovora was considered by averaging plant height for each Erwinia inoculum level across all Verticillium treatments some significant differences emerged. No differences in plant height were observed in any of the three years when Ecc was applied in irrigation water. Significant differences (P=0.05) were found between the controls and Erwinia seedpiece inoculation treatments in 1989 and 1990, but not in 1988. In 1989 plant height was significantly reduced in: a) the high Eca seed treatment under deficient moisture, b) all E. carotovora seed treatments under optimum moisture and c) in the high Ecc seed treatment and both Eca seed inoculation concentrations under excessive moisture. Also, significant height reductions were recorded in 1990 in: a) the high Ecc seed and both Eca seed inoculation levels under deficient moisture and b) all E. carotovora treatments under optimum and excessive moisture (Table 4.2).

Plant height was also significantly affected by level of Erwinia inoculum and subspecies used when effects of Verticillium were ignored and the Erwinia data averaged across both V. dahliae treatments (Table 4.3). No reductions were seen in 1988. Plant heights from treatments where Ecc was applied in the irrigation water were similar to the control in all three years. In 1989, plants were significantly shorter than the control when seedpieces were inoculated with 10^2 cfu and grown under optimum irrigation. Both the low and high inoculum levels reduced plant height significantly under excessive irrigation. Results were similar in 1990 except the high Erwinia inoculum level reduced plant height under the optimum irrigation regime while it did not do so in 1989. There was only one instance in 1990, under excessive irrigation when plant height under the high inoculum level was significantly shorter than height under the low inoculum level. Also, in only one instance, under optimum irrigation in 1990, did the Ecc seed treatments cause significant reductions in plant height compared with the Eca seed treatments.

Plant stands were unaffected by any treatment in 1988. Neither Verticillium inoculum alone nor any Ecc inoculum concentration applied in irrigation water reduced stands in any of the three years. In 1989 and 1990 plant stands were significantly lower (P=0.05) than the control when Ecc or Eca were inoculated into the seedpiece at both inoculum levels. This appeared to be a function of Erwinia inoculation and the environment rather than an interaction between Erwinia and V. dahliae.

A summary of weekly PED symptom progress or plant maturation in the case of the controls is presented in Appendix-Tables 4.2 to 4.10. Disease progress curves are shown in Appendix-Figures 4.1 to 4.24. Disease onset was similar among years with first symptoms appearing between 50 to 55 days after planting. Symptoms early in the season were more severe under excessive irrigation than under the moisture deficient regime. In general, PED disease progress was most rapid under the excessive irrigation regime and slowed progressively as irrigation was decreased. In all years the Ecc irrigation water application treatment alone followed closely the normal maturation rate of the controls while the V + Ecc inoculation treatments mirrored PED progress in plots inoculated with Verticillium alone. Disease progress throughout the cooler 1988 growing season was quite consistent among all treatments. There were no significant differences (P=0.05). Seedpiece inoculations with Ecc alone produced PED progress curves which closely followed normal maturation in the controls while all of the combined V + Erwinia inoculum treatments followed the Verticillium PED progress curve. By the end of the season, the control and plots receiving Ecc inoculum alone had PED readings similar to the other treatments. There were a few cases in which the V + Ecc PED disease progress was significantly greater than when the pathogens were present alone. In the warmer seasons of 1989 and 1990 disease progress showed greater variation among treatments than in 1988. While PED disease progress was seldom significantly increased by combining V + Erwinia treatments versus Verticillium alone, the large majority of the readings of the V + Ecc seed or Eca seed treatments were higher than plots where the single pathogens were present or senescence in the uninoculated control. PED disease development during specific time periods within the season was greater in 1990 than in 1989 and disease progress in both 1989 and 1990 was greater than 1988.

No significant differences (P=0.05) were found among treatments with regard to tuber numbers produced per plant. However, when the effects of Verticillium were removed by calculating tuber numbers across both Verticillium treatments as was done with the plant height data, some significant differences were found (Table 4.4). No differences in tuber numbers were found when Ecc was applied in the irrigation water, but there was a significant reduction in numbers when the seedpieces were inoculated with either Ecc or Eca. Level of Erwinia inoculum did not seem to be critical since significantly lower tuber numbers occured under the low inoculum level, the high inoculum level, or both when compared with the control. A trend toward reduced tuber numbers as irrigation decreased was also evident. In addition, there were no significant differences between subspecies Ecc and Eca or between the low and high inoculum levels.

Tuber yields per plant are shown in Table 4.5. Yields were quite variable, but followed closely the differences found among treatments observed in the disease progress curves. There was little yield difference among treatments in 1988 or in any of the three years under any irrigation regime when Ecc was applied in the irrigation water. In 1989 and 1990 plants inoculated with V + Ecc seed or V + Eca seed produced lower yields than plants inoculated with single pathogens. However, in only three of the forty eight dual inoculation treatments over the two years were yield reductions significant (P=0.05) when compared with the control or single pathogen inoculations. In the other forty five cases, yields of plants inoculated with combined pathogens were not different from the Verticillium only treatments in 3 cases, from the plants inoculated with Erwinia only in 15 cases and both the Verticillium alone and Erwinia alone treatments in 27 cases. In 1988, the coolest growing season, yields decreased as amount of irrigation water increased. The yield depressing effects of Verticillium in that year may have been greater than the potential benefits of increased availability of water. In 1989 and 1990 this relationship was reversed and yields increased as amount of water applied increased.

Significant yield reductions due to Erwinia inoculation were found when the effects of Verticillium were removed by averaging across the Verticillium treatments. Significant reductions occurred in 1989 and 1990 when Erwinia was inoculated into seedpieces. In 1989 there were further significant yield reductions between the low and high level of Ecc inoculum under deficient and excessive irrigation. No other significant differences were found between the low and high Erwinia inoculations or between subspecies Ecc and Eca (Table 4.6).

Table 4.2 Effect of Season, Irrigation Regime and Erwinia carotovora Inoculum Density on Plant Height.

	Irrigation Regime		
	DEF 1/	OPT	EXC
Erwinia Levels 2/	Mean Plant Height (cm) 3/		
1988			
Control	34.9 a	36.3	35.3
LEW	36.7 b	36.4	34.6
HEW	35.5 ab	36.4	35.0
LEC	36.3 ab	36.3	34.7
HEC	35.2 ab	36.3	34.2
LEA	----	----	----
HEA	----	----	----
1989			
Control	8.1 a	10.7 a	14.1 a
LEW	8.4 a	10.2 ab	14.2 a
HEW	8.5 a	10.8 a	14.3 a
LEC	8.0 ab	8.3 bc	11.9 ab
HEC	8.2 ab	8.5 bc	9.8 b
LEA	7.9 ab	8.2 c	9.7 b
HEA	6.0 b	8.3 c	10.5 b
1990			
Control	19.2 ab	23.0 a	26.5 a
LEW	20.0 a	23.3 a	26.7 a
HEW	20.3 a	22.0 ab	26.0 a
LEC	15.4 ab	17.2 cd	22.4 b
HEC	15.2 b	15.7 d	17.8 c
LEA	16.9 b	19.8 bc	21.2 bc
HEA	11.4 c	19.4 bc	19.2 bc

1/ DEF - deficient, OPT - Optimum and EXC - Excessive

2/ Control; LEW and HEW - Low and High Ecc Irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Eca seed.

3/ Plant height data averaged across all replications having plants present. Measurements were taken 57 to 60 days after planting. Effect of Verticillium dahliae was not considered.

Numbers followed by a different letter are significantly different from the controls at P=0.05.

Table 4.3 Effect of Season, Irrigation Regime and _Erwinia carotovora_ Inoculum Levels and Subspecies on Plant Height.

	Irrigation Regime		
	DEF 1/	OPT	EXC
	Mean Plant Height (cm) 2/		
Inoculum Level 3/		1988	
Control	34.9 a	36.3	35.3
Low	36.5 b	36.4	34.6
High	35.4 ab	36.3	34.7
Subspecies 4/			
Control	34.9	36.3	35.3
EW	36.1	36.4	34.8
EC	35.8	36.3	34.5
EA	----	----	----
Inoculum Level		1989	
Control	8.1	10.7 a	14.1 a
Low	8.2	9.2 b	12.5 b
High	7.5	9.4 ab	12.5 b
Subspecies			
Control	8.1 ab	10.7 a	14.1 a
EW	8.5 a	10.5 a	14.2 a
EC	8.1 ab	8.4 b	11.0 b
EA	6.9 b	8.3 b	10.1 b
Inoculum Level		1990	
Control	19.2	23.0 a	26.5 a
Low	17.9	20.9 b	24.3 b
High	17.3	19.5 b	22.3 c
Subspecies			
Control	19.2 a	23.0 a	26.5 a
EW	20.1 a	22.7 a	26.3 a
EC	15.3 b	16.4 c	20.0 b
EA	15.1 b	19.6 b	20.2 b

1/ DEF - Deficient, OPT - Optimum and EXC - Excessive

2/ Plant height data averaged across all treatments having plants present. Measurements taken 57-60 days after planting. Effect of _Verticillium dahliae_ not considered.

3/ Control (zero cfu); Low (10 cfu/ml or seedpiece); and High (10 cfu/ml or seedpiece)

4/ Control (no inoculum), EW (Ecc applied with irrigation water), EC (Ecc seedpiece) and EA (Eca seedpiece)

Numbers followed by a different letter are significantly different from the controls at P=0.05.

Table 4.4 Overall Effect of Season, Irrigation Regime and *Erwinia carotovora* on Number of Tubers Produced per Plant.

Inoculum Levels 2/	Irrigation Regime DEF 1/	OPT	EXC
	Mean Number of Tubers per Plant 3/		
1988			
Control	7.1	6.7	6.2 a
LEW	6.8	6.9	5.7 ab
HEW	6.5	7.5	6.2 a
LEC	5.8	6.9	5.3 b
HEC	6.4	7.2	5.6 ab
LEA	---	---	---
HEA	---	---	---
1989			
Control	6.8 a	7.6 a	7.9 ab
LEW	6.9 a	6.8 ab	8.7 a
HEW	6.7 ab	7.2 a	8.7 a
LEC	5.4 c	6.4 abc	8.4 ab
HEC	4.8 c	4.6 c	5.5 c
LEA	5.5 bc	5.5 bc	6.5 bc
HEA	4.9 c	5.1 c	6.1 bc
1990			
Control	7.3 bc	10.3 a	9.8 ab
LEW	8.6 ab	9.5 ab	10.5 ab
HEW	8.9 a	9.4 ab	11.4 a
LEC	8.1 ab	7.0 c	9.7 ab
HEC	7.4 abc	7.4 c	8.8 b
LEA	6.1 c	7.6 bc	9.3 ab
HEA	6.5 bc	8.2 abc	8.6 b

1/ DEF - Deficient, OPT - Optimum and EXC - Excessive

2/ Control; LEW and HEW - Low and High Ecc irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Eca seed.

3/ Tuber numbers averaged across all treatments having plants present. Effect of *Verticillium dahliae* not considered.

Numbers followed by a different letter are significantly different from the controls at P=0.05.

Table 4.5 Effect of Season, Irrigation Regime and Verticillium dahliae and Erwinia carotovora Alone and in Combination on Yield per Plant.

	Irrigation Regime/Year								
	DEF 1/			OPT			EXC		
	1988	1989	1990	1988	1989	1990	1988	1989	1990
Treatment 2/				Yield per Plant (gm) 3/					
Control	847 a	765 ab	426 ab	626	992 a	739 a	506	1588	1013
LV	811 ab	959 a	353 b	627	1116 a	626 ab	496	1889	1007
HV	705 b	638 ab	393 ab	693	816 ab	559 ab	463	1691	959
LEW	893 a	680 ab	420 ab	684	874 ab	632 ab	505	1775	1016
HEW	829 ab	753 ab	345 b	660	868 ab	523 b	524	1654	1158
LVLEW	787 ab	878 ab	351 b	669	898 ab	644 ab	433	1669	989
LVHEW	756 ab	599 b	523 a	639	1115 a	562 ab	502	1830	959
HVLEW	817 ab	565 b	411 ab	602	620 b	656 ab	533	1551	1056
HVHEW	745 b	797 ab	478 ab	623	826 ab	620 ab	533	1461	1034
Control	847 a	765 ab	426 b	626	992 ab	739 a	506	1588 ab	1013 a
LV	811 ab	959 a	353 bc	627	1116 a	626 a	496	1889 a	1007 a
HV	705 b	638 abc	393 b	693	816 abc	559 ab	463	1691 a	959 ab
LEC	766 ab	873 ab	789 a	649	386 c	399 b	451	1867 a	822 abc
HEC	768 ab	454 bc	227 bc	704	608 abc	481 b	399	1270 ab	692 abc
LVLEC	841 a	959 a	355 bc	651	836 abc	549 ab	454	476 c	907 abc
LVHEC	757 ab	318 c	295 bc	717	473 c	383 b	477	851 b	612 c
HVLEC	800 ab	408 c	181 c	672	363 c	374 b	519	1157 a	693 bc
HVHEC	723 ab	454 bc	323 bc	625	559 bc	421 b	408	748 c	737 bc
Control	847 a	765 ab	426 a	626	992 ab	739 a	506	1588 ab	1013 a
LV	811 ab	959 a	353 ab	627	1116 a	626 ab	496	1889 a	1007 a
HV	705 b	638 ab	393 ab	693	816 abc	559 abc	463	1691 ab	959 a
LEA	---	462 bc	408 ab	---	680 bc	620 a	---	1191 bc	850 ab
HEA	---	505 bc	242 ab	---	627 bc	363 bc	---	983 c	754 ab
LVLEA	---	363 bc	221 b	---	520 c	539 abc	---	953 c	741 ab
LVHEA	---	383 c	204 b	---	610 bc	669 a	---	1315 ab	686 b
HVLEA	---	462 bc	413 ab	---	631 bc	388 c	---	1080 c	890 ab
HVHEA	---	532 bc	378 ab	---	693 bc	382 c	---	737 c	794 ab

1/ DEF - Deficient, OPT - Optimum and EXC - Excessive

2/ Control; LV and HV - Low and High levels of Verticillium; LEW and HEW - Low and High Ecc irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Eca seed.

3/ Yield averaged across replications having plants present.

Numbers followed by a different letter are significantly different from the controls at P=0.05. Control, LV and HV are included with each set of Erwinia treatments only to provide a reference for analysis and ease of interpretation. Data for each of the three treatments are identical.

Table 4.6 Effect of Season, Irrigation Regime and *Erwinia carotovora* Inoculum Density on Yield per Plant.

	Irrigation Regime		
	DEF 1/	OPT	EXC
Erwinia Levels 2/	Mean Yield per Plant (gm) 3/		
1988			
Control	788	649	488
LEW	832	651	490
HEW	777	641	520
LEC	802	657	474
HEC	749	684	428
LEA	---	---	---
HEA	---	---	---
1989			
Control	787 a	975 a	1719 a
LEW	705 a	797 ab	1665 ab
HEW	714 a	934 a	1648 ab
LEC	711 ab	590 b	1267 b
HEC	408 c	539 b	956 c
LEA	433 bc	610 b	1075 c
HEA	477 c	649 b	1011 c
1990			
Control	392 ab	639 a	993 ab
LEW	394 ab	644 a	1020 a
HEW	449 a	569 ab	1050 a
LEC	387 ab	452 bc	798 c
HEC	290 b	429 c	678 c
LEA	358 ab	503 bc	835 bc
HEA	293 b	460 bc	745 c

1/ DEF - deficient, OPT - Optimum and EXC - Excessive

2/ Control; LEW and HEW - Low and High Ecc irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Eca seed.

3/ Yield averaged across all treatments having plants present. Effect of *Verticillium dahliae* was not considered.

Numbers followed by a different letter are significantly different from the controls at P=0.05.

Discussion:

Ecc or Eca alone appear to act as primary pathogens under San Luis Valley conditions while Verticillium alone acts as a secondary pathogen with little or no effect on potato productivity in most instances. When the two pathogens co-infect plants, an interaction is possible, if environmental factors (air temperature, planting date, emergence, etc.) and certain production conditions such as crop irrigation are favorable.

It is apparent that infection with V. dahliae alone is not a significant factor in potato production during most years in the San Luis Valley. This is demonstrated by the fact that there was little or no effect on plant stand, plant height, tuber numbers or tuber yield, except in the one year when combined with Ecc seedpiece inoculum. While PED disease progress was fastest at the highest inoculum levels, this did not seem to create much potential for yield losses in the San Luis Valley based upon data presented. Infection with Ecc or Eca alone was, however, a different matter. Both Ecc and Eca produced severe disease reactions during the warmer production years. They reduced stands, plant height, tuber numbers and most importantly, tuber yields. Method of inoculation was important. Introduction of Ecc in irrigation water produced only mild symptoms at best, while seedpiece inoculations with Ecc or Eca produced considerable symptom expression.

Co-inoculation with the two pathogens (V and Ecc or Eca) resulted in a significant disease interaction in some cases, producing disease reactions more severe than when either pathogen was present alone.
V + Ecc applied in irrigation water treatments produced no significant PED increase compared to senescence in controls or inoculations with V or Erwinia alone. V + Ecc seed or V + Eca seed produced some significant interactions (P=0.05). Stand and plant height were similar to those measured when Ecc or Eca was present alone. Tuber numbers per plant were not significantly affected by combining Verticillium and Erwinia inoculations.

PED disease progress in plants inoculated with both pathogens paralleled disease progress found when Verticillium was present alone, but was often significantly higher (P=0.05), especially later in the season. In cases where differences were not significant, co-inoculated plants usually had higher PED readings than those inoculated with Verticillium alone. Several authors (29,39,44) have shown that PED symptoms are more severe when Verticillium and Erwinia are both involved in infection. A synergistic response was observed by Rahimian and Mitchell (39). Less plant growth, more chlorosis and increased soft rot in the stems was found in co-infected plants. Zink and Secor (44) also found increased incidence of soft rot when pathogens were combined in field studies.

During this research, plant height was unaffected by combined treatments compared with the control or inoculation with single pathogens. However, general observations of overall plant canopy growth suggested that the plant canopy was reduced by combined treatments in all three years compared to the control or inoculation with single pathogens. In addition, PED symptom expression was generally more severe in the combined treatments. However, in contrast to results reported by other workers, there were no cases where there was increased stem soft rot compared to controls or inoculations with single pathogens. Kirkland (29) found that interactions between Verticillium and Erwinia increased PED symptom expression in an additive rather than a synergistic manner. Increases in severity of PED in combined treatments enhanced vascular colonization by V. dahliae in the basal stem region of co-infected plants. While stem colonization by V. dahliae was not examined in this study, it is possible that vascular colonization was indeed enhanced because PED progression was faster in plants infected with both V. dahliae and E. carotovora than in those infected with either pathogen alone. Disease progression in plants inoculated with Ecc or Eca via the seedpiece or Ecc applied in the irrigation water paralleled the normal maturation of the control. PED symptom expression and severity in the presence of V. dahliae alone, while greater than the normal senescence in the control, was significantly lower than the combined pathogen treatments. This suggests that increased vascular invasion by V. dahliae in combination treatments might be the cause of increased severity of PED symptoms during the season.

Tuber yields on a per plant basis were variable. There were no significant differences among treatments in 1988. There were, however, signficant yield reductions (P=0.05) resulting from pathogen interactions in three of the forty eight treatments in the other two years. These included: a) the low V + low Ecc seed and the high V + high Ecc seed in 1989 under excessive irrigation and b) the high V + low Ecc seed under deficient irrigation in 1990. Yields in these cases ranged from 48 to 75 percent lower than the controls or treatments involving single pathogens. The interaction appeared to be synergistic rather than additive in nature. In the other forty five treatments involving pathogen combinations, yields, while not significantly reduced compared with the control or the pathogens inoculated alone, were lower than those in the control or inoculations with either pathogen alone in 26 (58%) cases. The yield reduction average for the forty five statistically non-significant pathogen combination treatments when compared with the control or either pathogen inoculated alone was: V + Ecc; one percent, no change and 12 percent and V + Eca; six percent, one percent and five percent, in the deficient, optimum and excessive irrigation regimes, respectively.

It appears that several factors play a role in the severity of pathogen interactions. These include air temperature, soil moisture and level of inoculum. Francl *et al.* (20) found that air temperatures, especially high temperatures early in the plant growth cycle (11-20 days after planting) appeared to be very important in determining the amount of infection by Verticillium, subsequent disease progression and potential yield loss. In addition, high temperatures 65 days or longer after planting can significantly increase PED disease progression and subsequent yield loss. These findings agree well with data collected during this study. In the coolest season, 1988, average air temperatures during the two time frames mentioned above were low and perhaps acted to decrease the overall infection of Verticillium and subsequent symptom development and yield reduction. In the two warmer years, 1989 and 1990, temperatures were unusually high during the early time frame (11-20 days after planting) and continued warm through the growing season thus providing conditions favorable for both increased Verticillium infection and overall high rate of PED disease progression throughout the season resulting in higher yield reductions. This temperature/disease severity relationship also applies to infection and damage by Ecc and Eca, but with even more potential yield impact due to effects on stand, plant height and tuber numbers. Thus, it is to be expected that under environmental conditions which favor both pathogens, potential for interactions between the two would be increased. This might explain why no interactions were apparent in this study in the cool 1988 season, but some were found in the warmer 1989 and 1990 seasons. Areas where other authors reported interactions (29, 39, 44) typically have much warmer growing seasons than the San Luis Valley. This further supports this hypothesis. Soil moisture can also play a significant role in severity of PED symptom development and subsequent yield loss. Cappaert *et al.* (11) found that PED severity was greatest when excessive moisture was applied compared with conditions of moisture stress and that increasing inoculum density under excessive moisture conditions resulted in a significant reduction of tuber yield. Data from this study agreed with these results in only one of three years, the cool season of 1988. During that season, excessive irrigation produced the fastest PED progression and greater severity compared with the deficient irrigation regime. In the two warmer seasons (1989 and 1990), however, this relationship was reversed. In addition, effects on yield losses did not consistently correspond with the data of Cappaert *et al.* (11). Relative yield reductions were fairly consistent among all irrigation regimes, yet, under excessive moisture the highest significant interactions between pathogens with respect to yield reductions were found. In the cool 1988 season overall yields were highest under deficient moisture and lowest under the excessive regime. This relationship was reversed in the two warmer years with overall yields higher in 1989 than in 1990. There was little effect on the relative yield reduction among irrigation regimes when combinations of pathogens were involved. This suggests that this effect may be more closely related to temperature during the growing season than to disease expression or moisture level.

Finally, no apparent relationship between low and high inoculum levels and pathogen interactions was found. Significant interactions occurred with the high level of V. dahliae inoculum in two of three cases and with the low level in the other. The low level of Ecc seedpiece inoculum was involved in the interaction with Verticillium in two of three cases and the high level in the other (Appendix-Table 4.15). This indicates that the most significant yield reductions occur when the right combination of Verticillium and Erwinia inoculum levels exist, rather than only under the highest inoculum levels. Presently, there is insufficient published research to explain how environmental and production factors interact with co-infection by Verticillium and Erwinia. Consequently, it is difficult to explain why a more logical relationship between increasing inoculum and increased disease severity did not occur in this study. It may be that interactions between V. dahliae and Ecc and Eca occur only when environmental and other factors favorable for disease expression by both pathogens occur. In addition, specific pathogen to plant relationships, which are again not well understood, would have to occur together.

Both Verticillium wilt or potato early dying and blackleg are significant problems in potato production. It is apparent that co-infection by the two causal agents can be interactive and can increase overall disease progress and severity and decrease yields. This apparently occurs only occasionally in a relatively cool growing region such as the

San Luis Valley, but may be much more common and significant in warmer regions. A greater emphasis should be placed on techniques to eliminate E. carotovora subspp. prior to planting in the field and to limit exposure to these pathogens in all other phases of production. Ecc and Eca appear to be the primary pathogens involved in the PED complex interaction with V. dahliae in the San Luis Valley. Their elimination should provide the greatest chance for effective control of both PED and blackleg.

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Appendix

Table 4.1 Modified Horsfall/Barratt Scale (25)

Rating	Percent of plant affected
0	None
1	0 - 1
2	+1 - 5
3	+5 - 10
4	+10 - 25
5	+25 - 50
6	+50 - 75
7	+75 - 90
8	+90 - 95
9	100% or death

Disease assessments were based on visual *Verticillium* related leaf and plant symptoms such as leaf wilt (unilateral), plant chlorosis, stem wilt and early plant death.

Table 4.2 Effect of *Verticillium dahliae* and *Erwinia carotovora* Alone and in Combination on Potato Early Dying Progress in 1988 Under Deficient Moisture Conditions.

	PED Severity 1/						
	Days After Planting						
Treatment 2/	57	69	79	86	93	100	
Control	0.3 d	1.6 c	2.0 d	2.6 cd	3.5 b	4.0 b	:
LV	1.0 ab	2.0 abc	2.3 abcd	3.1 b	4.5 a	4.8 a	:
HV	1.4 a	2.3 a	2.7 ab	4.0 a	4.9 a	4.9 a	:
LEW	0.5 cd	1.5 bc	2.2 bcd	2.4 d	4.1 ab	4.5 ab	:
HEW	0.4 cd	1.5 bc	2.2 bcd	2.9 bc	4.1 ab	4.3 ab	:
LVLEW	0.9 bc	1.9 abc	2.7 ab	3.2 bc	4.4 a	4.9 a	:
LVHEW	1.2 ab	2.4 a	2.9 a	3.6 ab	4.8 a	4.7 ab	:
HVLEW	1.4 a	2.3 a	2.6 ab	3.1 b	4.5 a	4.6 ab	:
HVHEW	1.3 ab	2.2 a	2.7 ab	3.3 abc	4.5 a	4.7 ab	:
Control	0.3 c	1.6 d	2.0 d	2.6 c	3.5 c	4.0 b	:
LV	1.0 ab	2.0 bcd	2.3 bcd	3.1 bc	4.5 ab	4.8 a	:
HV	1.4 a	2.3 abc	2.7 abc	4.0 a	4.9 a	4.9 a	:
LEC	0.5 bc	1.5 d	2.2 cd	2.7 c	4.4 ab	4.5 ab	:
HEC	0.6 bc	1.8 cd	1.8 d	2.6 c	4.1 bc	4.4 ab	:
LVLEC	1.3 a	2.3 ab	3.1 a	3.8 ab	4.7 ab	4.6 ab	:
LVHEC	1.2 a	2.3 abc	2.8 ab	3.5 ab	4.3 ab	4.6 ab	:
HVLEC	1.2 a	2.6 a	2.8 ab	3.9 a	4.5 ab	4.8 a	:
HVHEC	1.3 a	2.7 a	2.9 a	3.8 ab	4.5 ab	4.7 ab	:

1/ Disease severity measurements derived by examining plant foliage for symptoms and scoring on a 0-9 scale using a modified Horsfall/Barratt system.

2/ Control; LV and HV - Low and High levels of *Verticillium*; LEW and HEW - Low and High Ecc irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Eca seed.

Numbers followed by a different letter are significantly different from the controls at P=0.05.

Control, LV and HV are included with each set of *Erwinia* treatments only to provide a reference for analysis and ease of interpretation. Data for each of the three treatments are identical.

Table 4.3 Effect of <u>Verticillium dahliae</u> and <u>Erwinia carotovora</u> Alone and in Combination on Potato Early Dying Progress in 1988 Under Optimum Moisture Conditions.

	PED Severity 1/						
	Days After Planting						
Treatment 2/	57	69	79	86	93	100	
Control	1.2 bc	2.3 ab	2.3 c	2.8 bc	4.8 bc	5.1 bc	:
LV	1.6 ab	2.9 a	3.1 ab	3.9 a	5.6 a	5.8 ab	:
HV	1.5 ab	2.4 a	3.1 ab	3.3 ab	4.7 bc	5.1 bc	:
LEW	0.9 c	1.6 c	2.0 c	2.4 c	4.2 c	4.6 c	:
HEW	0.9 c	2.1 bc	2.5 bc	3.1 b	4.9 bc	5.3 abc	:
LVLEW	1.7 a	2.7 a	3.4 a	4.0 a	5.7 a	6.1 a	:
LVHEW	1.7 ab	2.5 ab	3.1 ab	4.1 a	5.2 ab	5.3 abc	:
HVLEW	1.6 ab	2.8 a	3.3 a	4.1 a	5.6 a	5.8 ab	:
HVHEW	1.6 ab	2.4 ab	3.3 a	3.5 ab	5.0 ab	5.3 abc	:
Control	1.2 bc	2.3 bc	2.3 d	2.8 c	4.8 cd	5.1 ab	:
LV	1.6 ab	2.9 ab	3.1 bc	3.9 ab	5.6 a	5.8 a	:
HV	1.5 abc	2.4 bc	3.1 bc	3.3 bc	4.7 d	5.1 ab	:
LEC	1.1 c	2.1 c	2.6 cd	3.6 bc	4.9 bcd	5.3 ab	:
HEC	1.5 abc	2.3 bc	2.5 cd	3.0 c	4.6 cd	4.7 b	:
LVLEC	1.8 a	2.9 ab	3.3 b	4.1 ab	5.4 ab	5.9 a	:
LVHEC	1.7 a	3.0 a	3.6 ab	4.2 ab	5.5 ab	5.9 a	:
HVLEC	1.9 a	3.2 a	3.5 ab	4.1 ab	5.1 abcd	5.5 ab	:
HVHEC	2.0 a	3.5 a	4.1 a	4.5 a	5.8 a	6.0 a	:

1/ Disease severity measurements derived by examining plant foliage for symptoms and scoring on a 0-9 scale using a modified Horsfall/Barratt system.

2/ Control; LV and HV - Low and High levels of <u>Verticillium</u>; LEW and HEW - Low and High Ecc irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Eca seed.

Numbers followed by a different letter are significantly different from the control at P=0.05.

Control, LV and HV are included with each set of <u>Erwinia</u> treatments only to provide a reference for analysis and ease of interpretation. Data for each of the three treatments are identical.

Table 4.4 Effect of Verticillium dahliae and Erwinia carotovora Alone and in Combination on Potato Early Dying Progress in 1988 Under Excessive Moisture Conditions.

	PED Severity 1/						
	Days After Planting						
Treatment 2/	57	69	79	86	93	100	
Control	1.5	2.4	2.8 b	3.1 bc	4.7 b	5.2 bc	:
LV	2.0	2.4	3.4 ab	3.9 ab	5.1 ab	6.0 abc	:
HV	1.9	2.5	3.2 abc	3.9 ab	5.7 a	6.2 ab	:
LEW	1.5	2.3	2.6 c	2.8 c	4.5 b	5.1 c	:
HEW	1.5	2.5	2.7 c	2.9 c	4.7 b	5.4 abc	:
LVLEW	1.7	2.3	2.9 abc	3.5 abc	5.2 ab	5.9 abc	:
LVHEW	1.9	2.5	3.1 abc	3.7 ab	5.1 ab	5.9 abc	:
HVLEW	1.9	2.6	3.5 ab	4.0 a	5.7 a	6.3 a	:
HVHEW	1.8	2.7	3.5 a	4.2 a	5.6 a	5.7 abc	:
Control	1.5 bc	2.4 cd	2.8 d	3.1 c	4.7 cd	5.2 bc	:
LV	2.0 ab	2.4 cd	3.4 bc	3.9 b	5.1 bcd	6.0 abc	:
HV	1.9 ab	2.5 bcd	3.2 bc	3.9 b	5.7 ab	6.2 ab	:
LEC	1.5 bc	2.4 cd	2.9 cd	3.9 b	5.5 abc	6.5 ab	:
HEC	1.3 c	2.1 d	2.4 d	2.5 c	4.5 d	5.1 c	:
LVLEC	2.1 a	2.5 bcd	3.6 abc	4.3 ab	5.4 bc	5.5 bc	:
LVHEC	2.4 a	3.1 ab	3.6 ab	4.4 ab	6.1 a	6.4 ab	:
HVLEC	2.4 a	2.9 abc	3.6 ab	4.2 a	5.4 bc	5.9 abc	:
HVHEC	2.3 a	3.3 a	4.1 a	4.9 a	6.2 a	6.6 a	:

1/ Disease severity measurements derived by examining plant foliage for symptoms and scoring on a 0-9 scale using a modified Horsfall/Barratt system.

2/ Control; LV and HV - Low and High levels of Verticillium; LEW and HEW - Low and High Ecc irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Eca seed.

Numbers followed by a different letter are significantly different from the control at P=0.05.
Control, LV and HV are included with each set of Erwinia treatments only to provide a reference for analysis and ease of interpretation. Data for each of the three treatments are identical.

Table 4.5 Effect of Verticillium dahliae and Erwinia carotovora Alone and in Combination on Potato Early Dying Progress in 1989 Under Deficient Moisture Conditions.

	PED Severity 1/							
	Days after Planting							
Treatment 2/	72	80	87	95	101	108	114	
Control	1.2 d	1.8 b	2.2 d	2.3 d	2.3 d	3.5 d	5.1 d	:
LV	1.4 cd	2.3 ab	2.9 bcd	3.9 abc	4.3 ab	5.5 bc	7.2 abc	:
HV	2.2 a	2.5 ab	3.7 ab	4.5 ab	5.3 a	6.4 ab	7.7 a	:
LEW	1.6 abcd	2.1 ab	2.2 d	2.8 cd	3.0 cd	4.3 cd	5.7 cd	:
HEW	1.5 bcd	1.9 b	2.4 cd	2.7 cd	2.6 d	4.5 cd	6.3 bcd	:
LVLEW	1.9 abc	2.4 ab	3.3 abc	4.2 ab	4.6 ab	6.8 a	7.8 a	:
LVHEW	1.7 abcd	2.2 ab	3.1 abcd	3.5 bcd	3.6 bcd	5.6 bc	7.5 ab	:
HVLEW	2.2 ab	2.9 a	4.1 a	5.0 a	5.5 a	6.7 ab	8.4 a	:
HVHEW	2.3 a	2.4 ab	3.7 ab	5.0 a	5.2 a	7.1 a	7.9 a	:
Control	1.2 c	1.8 bc	2.2 c	2.3 e	2.3 b	3.5 c	5.1 d	:
LV	1.4 c	2.3 bc	2.9 bc	3.9 bcd	4.3 c	5.5 b	7.2 bc	:
HV	2.2 ab	2.5 ab	3.7 ab	4.5 bc	5.3 bc	6.4 ab	7.7 ab	:
LEC	1.4 bc	1.4 c	2.0 c	2.6 de	3.1 cd	4.6 bc	6.0 cd	:
HEC	1.8 abc	1.5 bc	3.3 bc	3.3 cde	3.8 cd	5.0 bc	6.3 bcd	:
LVLEC	2.6 a	2.6 ab	3.4 b	4.6 bc	4.4 c	5.9 b	6.3 bcd	:
LVHEC	2.0 abc	2.0 abc	4.3 ab	4.8 bc	5.0 bc	6.5 ab	8.5 ab	:
HVLEC	2.7 a	3.2 a	4.9 a	6.9 a	7.7 a	8.0 a	8.9 a	:
HVHEC	1.7 abc	3.0 ab	4.3 ab	6.0 ab	6.7 abc	7.3 ab	8.3 ab	:
Control	1.2 bc	1.8 b	2.2 d	2.3 d	2.3 c	3.5 d	5.1 e	:
LV	1.4 bc	2.3 a	2.9 bcd	3.9 bc	4.3 ab	5.5 bc	7.2 bc	:
HV	2.2 a	2.5 a	3.7 abc	4.5 ab	5.3 a	6.4 ab	7.7 abc	:
LEA	1.9 ab	2.3 ab	2.9 cd	3.0 cd	3.0 bc	4.8 cd	6.6 cd	:
HEA	1.0 c	2.0 ab	2.3 d	3.1 cd	3.2 bc	4.6 d	6.2 de	:
LVLEA	1.8 abc	3.0 a	3.2 abc	3.8 abcd	4.6 ab	6.0 abc	8.6 ab	:
LVHEA	2.0 ab	2.1 ab	3.5 abc	3.8 bcd	4.8 ab	6.5 abc	8.4 ab	:
HVLEA	1.1 bc	2.4 ab	3.3 abc	5.0 ab	5.8 a	7.6 a	8.8 ab	:
HVHEA	2.2 a	2.9 a	4.4 a	5.7 a	5.6 a	7.0 ab	8.8 a	:

1/ Disease severity derived by examining plant foliage for symptoms and scoring on a 0-9 scale using a modified Horsfall/Barratt system.

2/ Control; LV and HV - Low and High levels of Verticillium; LEW and HEW - Low and High Ecc irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Eca seed.

Numbers followed by a different letter are significantly different from the controls at P=0.05.
Control, LV and HV are included with each set of Erwinia treatments only to provide a reference for analysis and ease of interpretation. Data for each of the three treatments are identical.

Table 4.6 Effect of *Verticillium dahliae* and *Erwinia carotovora* Alone and in Combination on Potato Early Dying Progress in 1989 Under Optimum Moisture Conditions.

	PED Severity 1/							
	Days after Planting							
Treatment 2/	72	80	87	95	101	108	114	
Control	1.5 cd	1.8 c	2.4 c	2.9 b	3.0 c	4.6 bc	5.9 c	:
LV	1.8 abcd	1.9 c	2.9 bc	3.1 b	3.3 bc	4.5 bc	6.4 bc	:
HV	2.3 a	2.3 bc	3.2 bc	3.8 ab	4.5 ab	5.9 ab	7.5 ab	:
LEW	1.4 d	1.9 c	2.4 c	3.0 b	3.5 bc	4.6 bc	5.8 c	:
HEW	1.4 cd	2.1 bc	2.6 c	2.8 b	2.6 c	4.4 c	6.8 abc	:
LVLEW	2.1 abc	2.3 bc	3.2 bc	3.9 ab	4.6 ab	5.8 abc	7.7 ab	:
LVHEW	1.6 bcd	2.0 c	3.0 bc	3.6 b	3.6 bc	4.8 bc	5.8 c	:
HVLEW	2.3 ab	2.8 ab	3.9 ab	4.8 a	5.3 a	6.7 a	7.7 ab	:
HVHEW	2.2 ab	3.2 a	4.5 a	4.8 a	5.4 a	6.7 a	8.0 a	:
Control	1.5 c	1.8 c	2.4 c	2.9 c	3.0 b	4.6 a	5.9 b	:
LV	1.8 bc	1.9 c	2.9 bc	3.1 bc	3.3 b	4.5 a	6.4 ab	:
HV	2.3 ab	2.3 bc	3.2 bc	3.8 bc	4.5 ab	5.9 a	7.5 ab	:
LEC	2.0 abc	1.5 bc	3.0 abc	3.0 bc	3.0 ab	5.0 a	6.5 ab	:
HEC	1.4 c	1.8 bc	2.6 bc	2.4 c	2.8 b	4.6 a	6.2 ab	:
LVLEC	2.2 abc	3.0 ab	4.0 ab	4.7 ab	4.3 ab	6.2 a	8.2 a	:
LVHEC	1.4 c	2.2 bc	2.8 bc	4.0 abc	4.2 ab	5.4 a	6.4 ab	:
HVLEC	3.5 a	4.5 a	5.5 a	6.5 a	6.5 a	7.0 a	7.0 ab	:
HVHEC	2.0 bc	1.8 bc	3.5 abc	4.3 abc	4.5 ab	6.5 a	8.3 a	:
Control	1.5 c	1.8 cd	2.4 c	2.9 b	3.0 c	4.6 cd	5.9 d	:
LV	1.8 bc	1.9 cd	2.9 bc	3.1 b	3.3 bc	4.5 cd	6.4 cd	:
HV	2.3 ab	2.3 cd	3.2 bc	3.8 b	4.5 b	5.9 c	7.5 abc	:
LEA	1.3 cd	2.3 cd	2.6 bc	2.4 b	2.9 c	3.9 d	5.3 e	:
HEA	0.8 d	1.6 d	3.1 bc	2.8 b	3.1 bc	5.0 cd	5.9 d	:
LVLEA	1.7 bc	1.7 cd	3.0 bc	3.6 b	3.9 bc	5.8 bcd	7.6 abcd	:
LVHEA	2.2 abc	2.6 bc	3.8 ab	4.0 b	4.1 bc	5.6 bcd	7.1 bcd	:
HVLEA	1.7 bc	3.5 ab	4.5 a	5.9 a	6.3 a	7.9 a	9.0 a	:
HVHEA	2.5 a	3.7 a	4.8 a	5.6 a	6.1 a	7.2 ab	7.9 ab	:

1/ Disease severity derived by examining plant foliage for symptoms and scoring on a 0-9 scale using a modified Horsfall/Barratt system.

2/ Control; LV and HV - Low and High levels of *Verticillium*; LEW and HEW - Low and High Ecc irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Eca seed.

Numbers followed by a different letter are significantly different from the controls at P=0.05.

Control, LV and HV are included with each set of *Erwinia* treatments only to provide a reference for analysis and ease of interpretation. Data for each of the three treatments are identical.

Table 4.7 Effect of Verticillium dahliae and Erwinia carotovora Alone and in Combination on Potato Early Dying Progress in 1989 Under Excessive Moisture Conditions.

	PED Severity 1/							
	Days after Planting							
Treatment 2/	72	80	87	95	101	108	114	
Control	1.1 b	1.6	2.3 bc	2.9 abc	2.9 b	4.3 ab	5.7 ab	:
LV	1.5 ab	1.8	3.2 a	3.4 abc	3.3 ab	4.6 ab	6.5 ab	:
HV	1.8 a	1.9	2.5 abc	3.3 abc	3.6 abc	4.9 ab	6.5 ab	:
LEW	1.3 ab	1.7	2.3 bc	3.0 abc	2.8 b	4.3 ab	5.3 b	:
HEW	1.4 ab	1.6	2.2 c	2.6 c	2.7 c	4.1 b	5.5 ab	:
LVLEW	1.6 ab	1.9	2.7 abc	2.8 bc	2.7 b	4.3 ab	5.8 ab	:
LVHEW	1.5 ab	1.5	2.9 abc	3.7 a	3.2 b	4.5 ab	6.9 a	:
HVLEW	1.7 a	1.9	3.0 ab	3.7 ab	4.2 ab	5.3 a	6.4 ab	:
HVHEW	1.6 ab	1.7	2.5 abc	3.2 abc	3.1 bc	4.9 ab	6.0 ab	:
Control	1.1 c	1.6 ab	2.3 bc	2.9 ab	2.9 ab	4.3 cd	5.7 bc	:
LV	1.5 abc	1.8 ab	3.2 a	3.4 a	3.3 a	4.6 bcd	6.5 ab	:
HV	1.8 ab	1.9 a	2.5 abc	3.3 a	3.6 a	4.9 bcd	6.5 ab	:
LEC	1.0 bc	1.0 b	1.5 c	2.0 b	2.0 b	3.3 d	4.5 c	:
HEC	1.5 abc	2.0 ab	2.5 abc	3.5 ab	3.3 ab	6.3 abc	6.0 abc	:
LVLEC	2.5 a	1.5 ab	3.5 ab	3.5 ab	4.0 ab	7.5 a	9.0 a	:
LVHEC	1.5 abc	2.0 ab	4.0 a	4.0 ab	4.0 ab	7.0 ab	9.0 a	:
HVLEC	2.3 a	1.8 ab	3.5 ab	4.0 a	4.3 a	5.8 abc	6.0 abc	:
HVHEC	1.5 abc	1.5 ab	2.0 abc	2.5 ab	3.0 ab	4.0 cd	8.0 a	:
Control	1.1 cd	1.6 c	2.3 c	2.9 bc	2.9 b	4.3 b	5.7 c	:
LV	1.5 bc	1.8 bc	3.2 ab	3.4 abc	3.3 b	4.6 ab	6.5 abc	:
HV	1.8 ab	1.9 abc	2.5 bc	3.3 abc	3.6 ab	4.9 ab	6.5 abc	:
LEA	0.7 d	1.7 abc	2.6 abc	2.4 c	2.9 b	3.9 b	5.6 bc	:
HEA	1.1 bcd	2.0 abc	2.4 bc	2.5 c	2.5 b	4.4 ab	5.9 abc	:
LVLEA	1.6 abcd	2.4 ab	3.6 a	4.3 a	4.7 a	6.0 a	7.9 a	:
LVHEA	1.5 bcd	1.3 c	2.8 abc	2.9 bc	2.9 b	3.8 b	5.6 c	:
HVLEA	2.4 a	1.9 abc	3.0 abc	3.9 ab	3.8 ab	5.9 a	7.6 ab	:
HVHEA	1.7 abc	2.7 a	3.7 a	3.2 abc	3.8 ab	5.3 ab	7.0 abc	:

1/ Disease severity derived by examining plant foliage for symptoms and scoring on a 0-9 scale using a modified Horsfall/Barratt system.

2/ Control; LV and HV - Low and High levels of Verticillium; LEW and HEW - Low and High Ecc irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Eca seed.

Numbers followed by a different letter are significantly different from the controls at P=0.05.

Control, LV and HV are included with each set of Erwinia treatments only to provide a reference for analysis and ease of interpretation. Data for each of the three treatments are identical.

Table 4.8 Effect of Verticillium dahliae and Erwinia carotovora Alone and in Combination on Potato Early Dying Progress in 1990 Under Deficient Moisture Conditions.

	PED Severity 1/							
	Days after Planting							
Treatment 2/	72	79	85	94	101	106	113	
Control	2.3 ab	2.8 b	4.0 ab	4.6 b	6.9	8.7	8.9	:
LV	2.6 a	2.6 b	3.7 ab	5.2 ab	7.6	8.4	8.7	:
HV	2.3 ab	2.9 b	4.1 ab	5.6 ab	7.9	8.5	8.7	:
LEW	1.6 b	2.7 b	3.5 b	4.5 b	7.7	8.1	8.7	:
HEW	2.1 ab	2.7 b	3.2 b	4.9 ab	7.4	7.9	8.3	:
LVLEW	2.3 ab	2.5 b	3.5 b	5.0 ab	7.7	8.6	8.9	:
LVHEW	2.0 ab	2.7 b	3.4 b	4.5 b	7.3	7.9	8.7	:
HVLEW	2.6 a	3.3 ab	4.1 ab	5.6 ab	7.9	8.5	8.9	:
HVHEW	2.6 a	4.0 a	4.7 a	6.1 a	8.1	8.9	9.0	:
Control	2.3 ab	2.8 ab	4.0	4.6	6.9	8.7 a	8.9 a	:
LV	2.6 a	2.6 b	3.7	5.2	7.6	8.4 a	8.7 ab	:
HV	2.3 ab	2.9 ab	4.1	5.6	7.9	8.5 a	8.7 ab	:
LEC	1.4 b	3.0 ab	3.6	5.6	6.2	7.2 ab	8.0 ab	:
HEC	1.8 ab	2.2 b	3.2	4.0	7.2	8.2 ab	9.0 ab	:
LVLEC	2.2 ab	3.4 ab	4.2	5.5	7.5	8.7 a	9.0 a	:
LVHEC	1.9 ab	2.5 b	3.4	4.5	6.6	7.2 b	7.8 b	:
HVLEC	2.7 ab	3.8 a	4.0	5.4	7.6	8.0 ab	8.2 ab	:
HVHEC	2.6 ab	3.1 ab	4.1	6.1	7.6	8.8 a	9.0 a	:
Control	2.3 ab	2.8 a	4.0 ab	4.6 abc	6.9 a	8.7 a	8.9 a	:
LV	2.6 a	2.6 ab	3.7 ab	5.2 ab	7.6 a	8.4 a	8.7 a	:
HV	2.3 ab	2.9 a	4.1 a	5.6 a	7.9 a	8.5 a	8.8 a	:
LEA	1.6 b	2.4 ab	2.9 b	3.9 bc	6.5 ab	8.5 a	8.8 a	:
HEA	1.3 ab	1.0 b	2.3 ab	2.3 c	4.0 b	6.0 b	6.3 b	:
LVLEA	2.7 ab	2.6 ab	3.9 ab	5.3 ab	7.6 a	8.3 a	8.3 a	:
LVHEA	2.0 ab	2.3 ab	2.5 ab	4.0 abc	8.5 a	8.8 a	9.0 a	:
HVLEA	2.6 a	3.1 a	4.0 ab	5.6 ab	7.8 a	8.3 a	8.6 a	:
HVHEA	2.0 ab	2.5 ab	3.7 ab	4.8 abc	6.8 a	7.7 ab	8.0 a	:

1/ Disease severity derived by examining plant foliage for symptoms and scoring on a 0-9 scale using a modified Horsfall/Barratt system.

2/ Control; LV and HV - Low and High levels of Verticillium; LEW and HEW - Low and High Ecc irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Ecc seed.

Numbers followed by a different letter are significantly different from the controls at P=0.05.
Control, LV and HV are included with each set of Erwinia treatments only to provide a reference for analysis and ease of interpretation. Data for each of the three treatments are identical.

Table 4.9 Effect of Verticillium dahliae and Erwinia carotovora Alone and in Combination on Potato Early Dying Progress in 1990 Under Optimum Moisture Conditions.

	PED Severity 1/						
	Days after Planting						
Treatment 2/	72	79	85	94	101	106	113
Control	2.2 abc	2.8 bcd	3.4 bc	5.1 b	6.9 b	7.9	8.4
LV	2.3 abc	2.7 bcd	3.6 bc	4.5 b	7.1 b	7.9	8.4
HV	2.6 ab	3.5 ab	4.1 ab	5.5 ab	7.6 ab	8.5	8.7
LEW	1.7 c	2.1 d	2.9 c	4.2 b	6.8 b	8.1	8.8
HEW	1.9 bc	2.5 cd	3.3 bc	4.3 b	6.8 b	7.9	8.5
LVLEW	2.1 bc	3.0 abc	3.7 abc	5.1 b	7.4 ab	8.3	8.5
LVHEW	1.8 bc	2.9 abcd	4.1 ab	5.1 b	7.7 ab	8.4	8.9
HVLEW	3.0 a	3.5 ab	4.5 a	6.6 a	7.8 ab	8.1	8.7
HVHEW	3.0 a	3.7 a	4.5 a	6.9 a	8.5 a	8.6	8.6
Control	2.2 abc	2.8 ab	3.4 bc	5.1 b	6.9 ab	7.9 ab	8.4 ab
LV	2.3 abc	2.7 abc	3.6 bc	4.5 bc	7.1 ab	7.9 ab	8.4 ab
HV	2.6 ab	3.5 a	4.1 ab	5.5 ab	7.6 ab	8.5 ab	8.7 ab
LEC	1.6 bc	2.4 abc	2.8 bcd	3.0 cd	6.0 bc	7.0 bc	7.8 abc
HEC	1.4 c	1.8 c	2.3 d	3.0 d	5.1 c	6.4 c	7.8 bc
LVLEC	1.4 c	1.9 bc	2.7 cd	4.1 bcd	6.1 bc	6.4 c	7.0 c
LVHEC	2.1 abc	2.4 bc	3.3 bcd	4.1 bcd	6.9 ab	8.2 ab	8.6 ab
HVLEC	3.1 a	3.7 a	4.9 a	6.9 a	8.4 a	8.9 a	9.0 a
HVHEC	1.9 bc	2.9 ab	3.8 abc	4.4 bcd	7.0 ab	8.2 ab	8.9 a
Control	2.2 ab	2.8 bc	3.4 bc	5.1 a	6.9 ab	7.9	8.4
LV	2.3 ab	2.7 bc	3.6 bc	4.5 a	7.1 ab	7.9	8.4
HV	2.6 a	3.5 ab	4.1 ab	5.5 a	7.6 a	8.5	8.7
LEA	1.3 b	1.5 d	2.7 c	2.5 b	5.3 b	7.7	8.3
HEA	2.0 ab	2.0 cd	3.3 abc	6.0 a	7.3 ab	8.3	9.0
LVLEA	2.3 ab	3.2 abc	4.2 ab	5.6 a	7.8 a	8.6	8.8
LVHEA	3.0 a	4.5 a	4.0 abc	6.0 a	7.3 ab	7.8	8.5
HVLEA	2.9 a	3.9 a	5.0 a	6.0 a	8.0 a	9.0	9.0
HVHEA	2.7 a	3.3 abc	3.9 abc	5.3 a	8.0 a	8.0	8.1

1/ Disease severity derived by examining plant foliage for symptoms and scoring on a 0-9 scale using a modified Horsfall/Barratt system.

2/ Control; LV and HV - Low and High levels of Verticillium; LEW and HEW - Low and High Ecc irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Ecc seed.

Numbers followed by a different letter are significantly different from the controls at P=0.05.

Control, LV and HV are included with each set of Erwinia treatments only to provide a reference for analysis and ease of interpretation. Data for each of the three treatments are identical.

Table 4.10 Effect of Verticillium dahliae and Erwinia carotovora Alone and in Combination on Potato Early Dying Progress in 1990 Under Excessive Moisture Conditions.

	PED Severity 1/							
	Days after Planting							
Treatment 2/	72	79	85	94	101	106	113	
Control	1.5 b	2.1 b	2.5 b	3.1 c	4.9 c	6.2 d	7.0 b	:
LV	1.9 ab	2.3 b	3.1 ab	3.5 bc	5.6 bc	7.2 abcd	7.7 ab	:
HV	2.1 ab	3.2 a	3.7 a	4.7 a	7.3 a	8.3 a	8.9 a	:
LEW	1.7 ab	2.0 b	2.6 b	3.7 abc	5.5 bc	6.4 cd	7.6 ab	:
HEW	1.8 ab	2.1 b	2.7 b	3.4 bc	5.6 bc	6.8 bcd	7.9 ab	:
LVLEW	1.8 ab	2.7 ab	3.6 a	4.8 a	6.8 ab	8.2 a	8.7 a	:
LVHEW	2.3 a	2.8 ab	3.1 ab	3.9 abc	5.8 bc	6.9 abcd	7.8 ab	:
HVLEW	2.1 ab	2.8 ab	3.6 a	4.4 ab	6.7 ab	7.6 abc	8.1 ab	:
HVHEW	2.3 a	2.7 ab	3.2 ab	4.1 abc	6.1 abc	7.9 ab	8.6 a	:
Control	1.5 ab	2.1 bc	2.5 b	3.1 c	4.9 bc	6.2 bc	7.0 bc	:
LV	1.9 ab	2.3 bc	3.1 ab	3.5 bc	5.6 bc	7.2 ab	7.7 ab	:
HV	2.1 a	3.2 a	3.7 a	4.7 a	7.3 a	8.3 a	8.9 a	:
LEC	1.5 ab	2.0 bc	2.8 ab	4.9 ab	6.4 ab	7.5 ab	8.0 ab	:
HEC	1.5 ab	1.5 c	2.3 b	3.0 abc	5.0 bc	6.0 bc	6.5 bc	:
LVLEC	1.8 ab	1.6 c	2.8 ab	3.4 abc	5.0 bc	6.4 abc	7.4 abc	:
LVHEC	1.5 ab	1.9 c	2.4 b	2.3 bc	4.0 c	5.2 c	6.0 c	:
HVLEC	2.2 ab	3.2 ab	3.3 ab	3.8 abc	6.2 ab	7.7 ab	8.7 ab	:
HVHEC	1.1 b	2.0 bc	3.0 ab	3.1 bc	5.3 bc	7.1 ab	8.3 ab	:
Control	1.5 bcd	2.1 bcd	2.5 bcd	3.1 c	4.9 bc	6.2 bc	7.0 c	:
LV	1.9 abc	2.3 bc	3.1 abc	3.5 bc	5.6 bc	7.2 ab	7.7 abc	:
HV	2.1 ab	3.2 a	3.7 a	4.7 a	7.3 a	8.3 a	8.9 a	:
LEA	1.1 cd	1.3 d	2.1 cd	3.1 bc	5.8 abc	6.5 bc	7.3 bc	:
HEA	1.0 cd	1.4 cd	2.0 d	2.7 c	4.4 c	5.3 c	6.1 c	:
LVLEA	1.5 abcd	2.3 abcd	2.7 abcd	4.0 abc	5.8 abc	6.5 abc	6.5 c	:
LVHEA	0.9 d	1.3 d	1.9 d	2.8 c	4.4 c	5.6 bc	6.4 c	:
HVLEA	2.5 a	3.0 ab	3.8 a	4.6 ab	6.0 abc	6.8 abc	6.9 c	:
HVHEA	2.4 ab	2.8 ab	3.5 ab	4.0 abc	6.5 ab	8.1 a	8.8 ab	:

1/ Disease severity derived by examining plant foliage for symptoms and scoring on a 0-9 scale using a modified Horsfall/Barratt system.

2/ Control; LV and HV - Low and High levels of Verticillium; LEW and HEW - Low and High Ecc irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Ecc seed.

Numbers followed by a different letter are significantly different from the controls at P=0.05.
Control, LV and HV are included with each set of Erwinia treatments only to provide a reference for analysis and ease of interpretation. Data for each of the three treatments are identical.

Figures 4.1 - 4.24 **Effect of Differing Levels of Verticillium dahliae Incorporated into the Soil, Erwinia carotovora subspp. Inoculated into the Seedpiece and Applied in Irrigation Water and Combination Treatments on PED Symptom Progress or Plant Senescence Under Three Moisture Regimes.**

Control; LV and HV - Low and High levels of Verticillium; LEW and HEW - Low and High Ecc irrigation; LEC and HEC - Low and High Ecc seed; LEA and HEA - Low and High Eca seed.

Disease severity derived by examining plant foliage for symptoms and/or senescence and scoring on a 0-9 scale using a modified Horsfall/Barratt system.

Figure 4.1A Deficient Moisture (LV + LEC)
1988

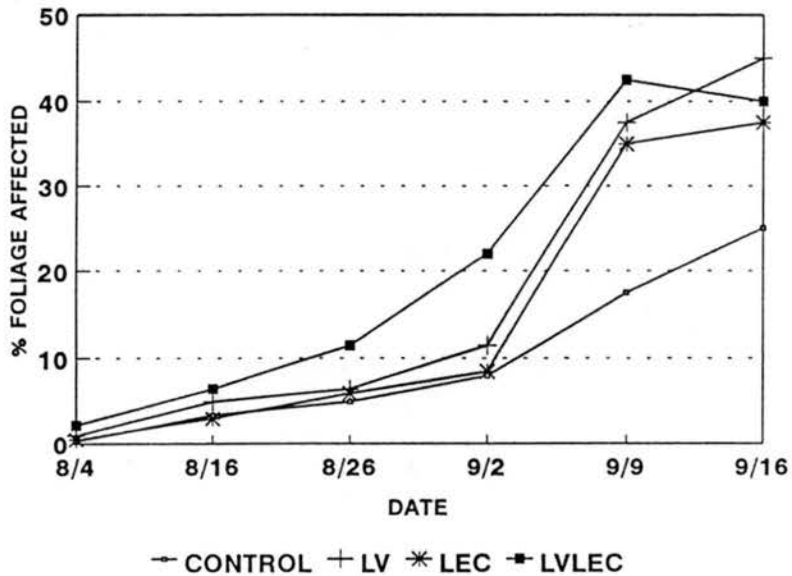

Figure 4.1B Deficient Moisture (LV + HEC)
1988

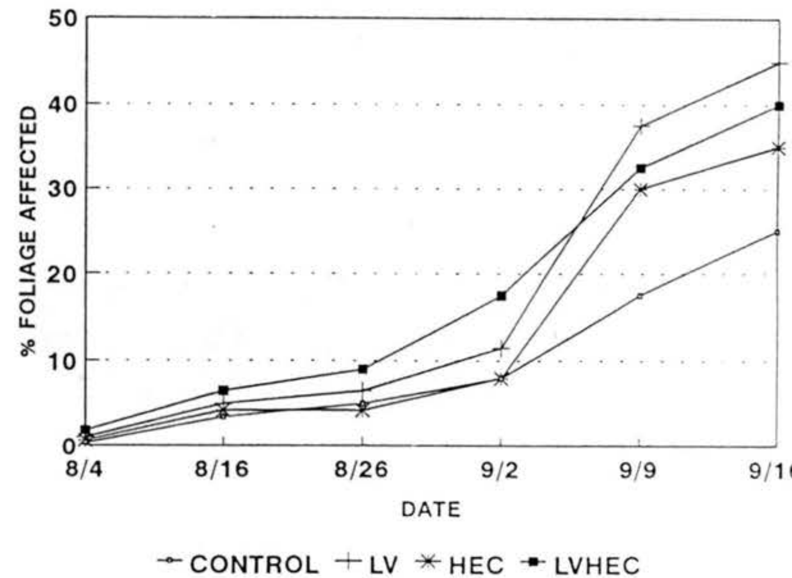

Figure 4.1C Deficient Moisture (HV + LEC)
1988

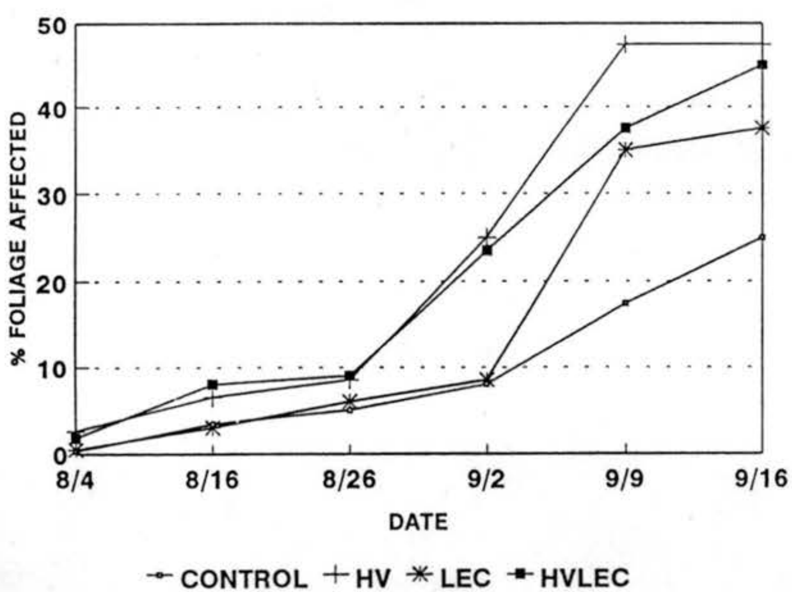

Figure 4.1D Deficient Moisture (HV + HEC)
1988

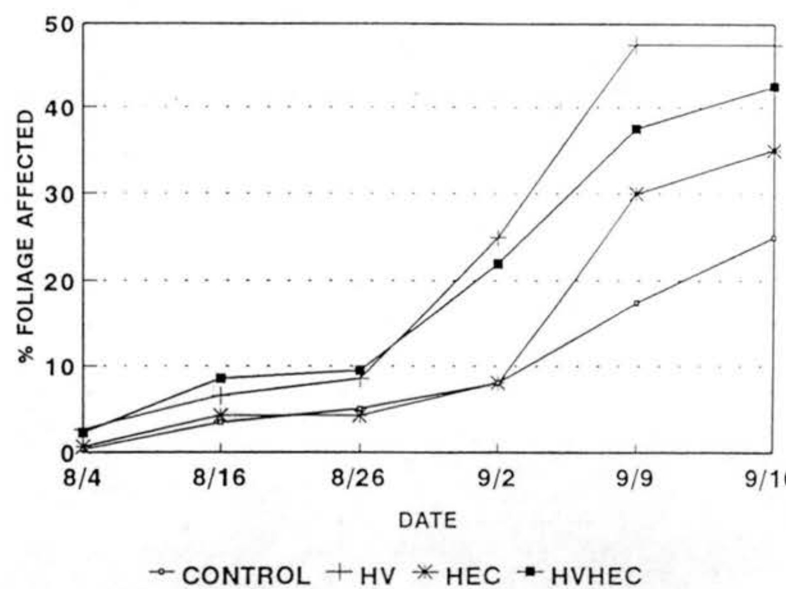

Figure 4.2A Optimum Moisture (LV + LEC)
1988

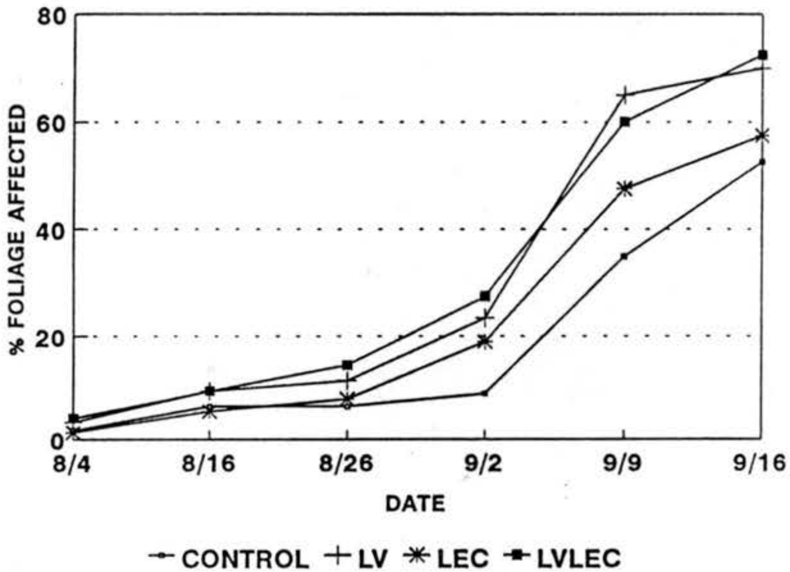

Figure 4.2B Optimum Moisture (LV + HEC)
1988

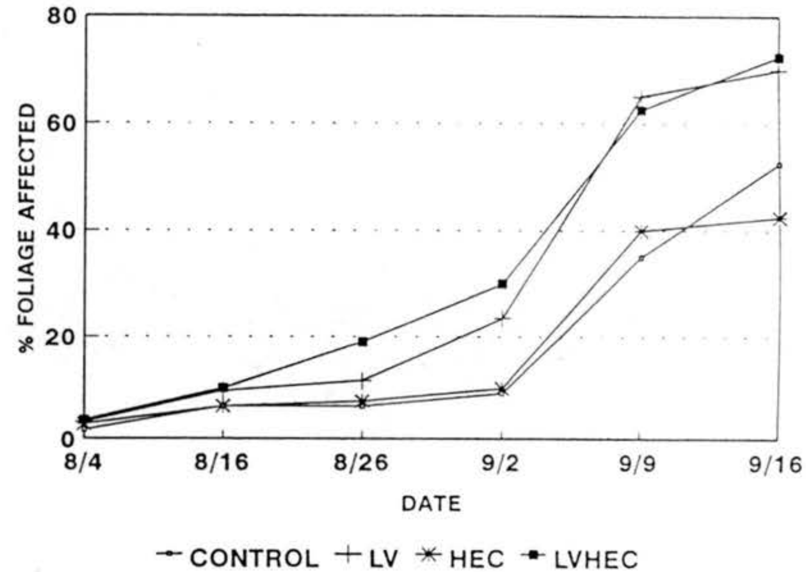

Figure 4.2C Optimum Moisture (HV + LEC)
1988

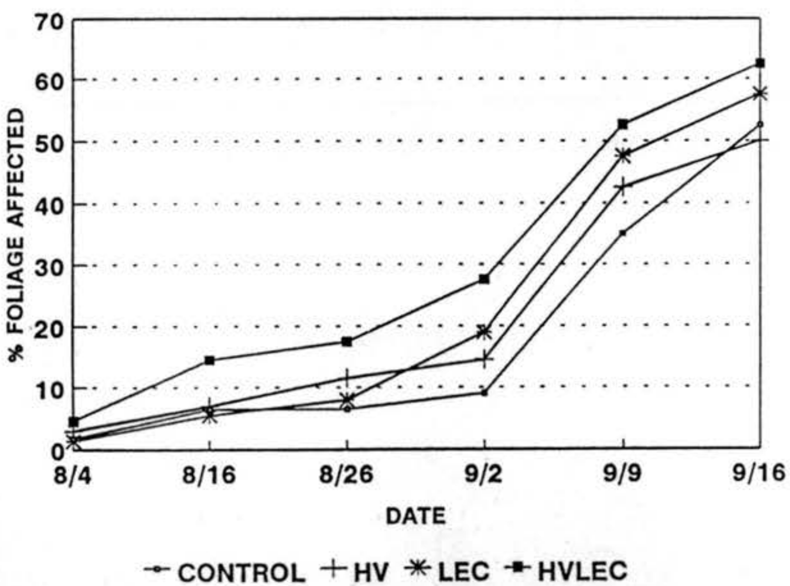

Figure 4.2D Optimum Moisture (HV + HEC)
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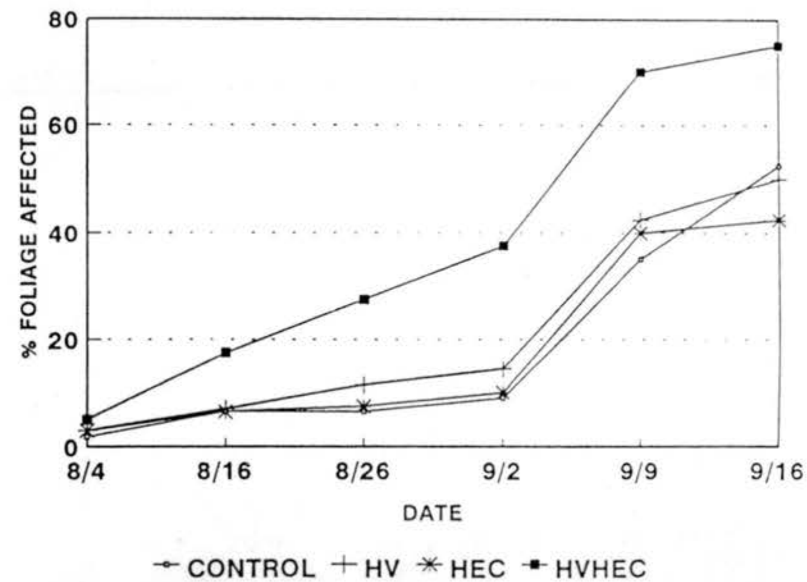

Figure 4.3A Excessive Moisture (LV + LEC)
1988

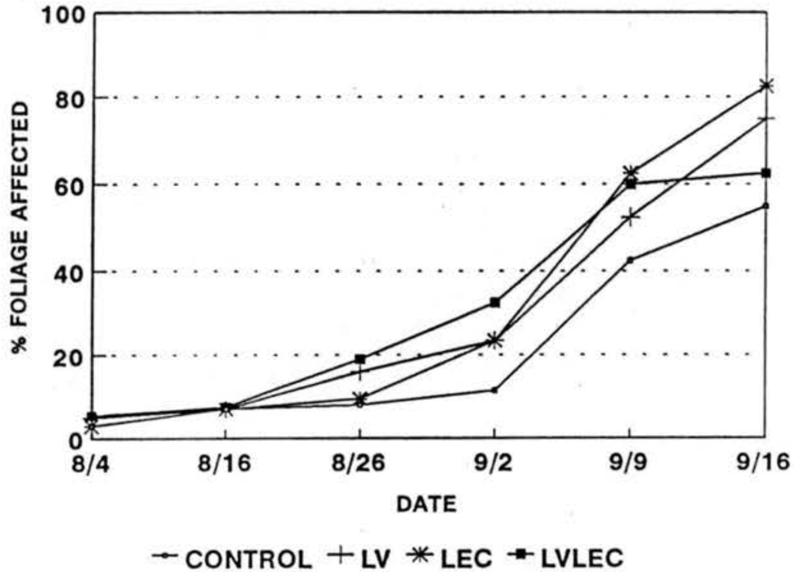

Figure 4.3B Excessive Moisture (LV + HEC)
1988

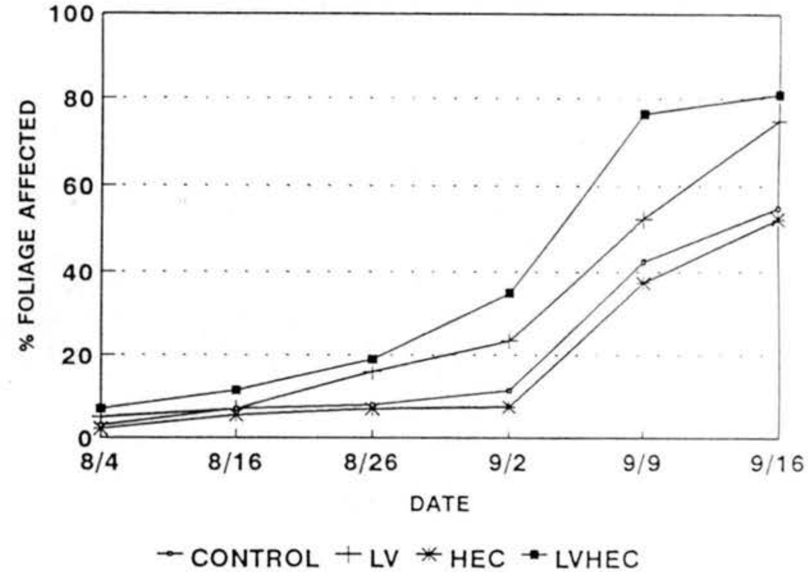

Figure 4.3C Excessive Moisture (HV + LEC)
1988

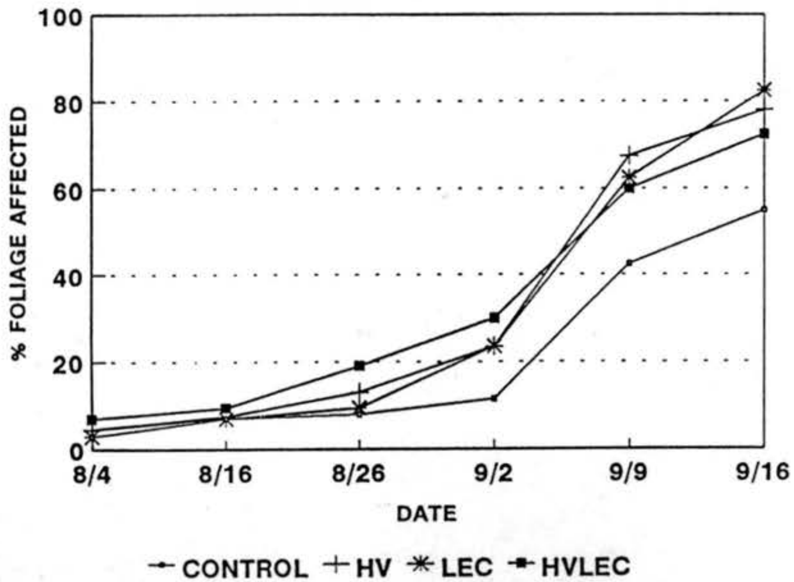

Figure 4.3D Excessive Moisture (HV + HEC)
1988

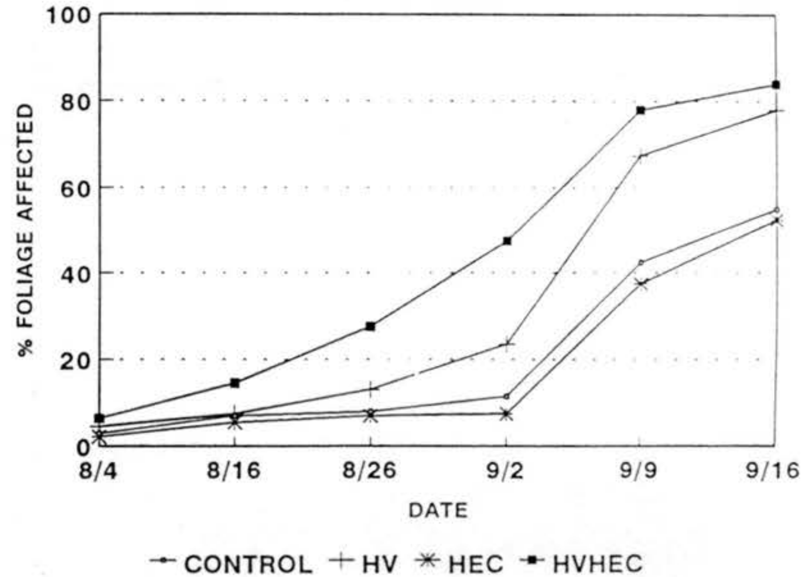

Figure 4.4A Deficient Moisture (LV + LEW)
1988

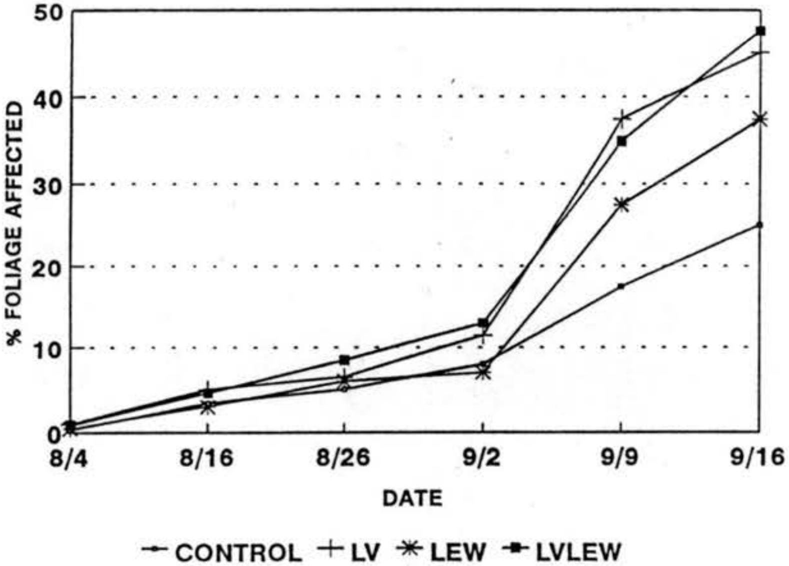

Figure 4.4B Deficient Moisture (LV + HEW)
1988

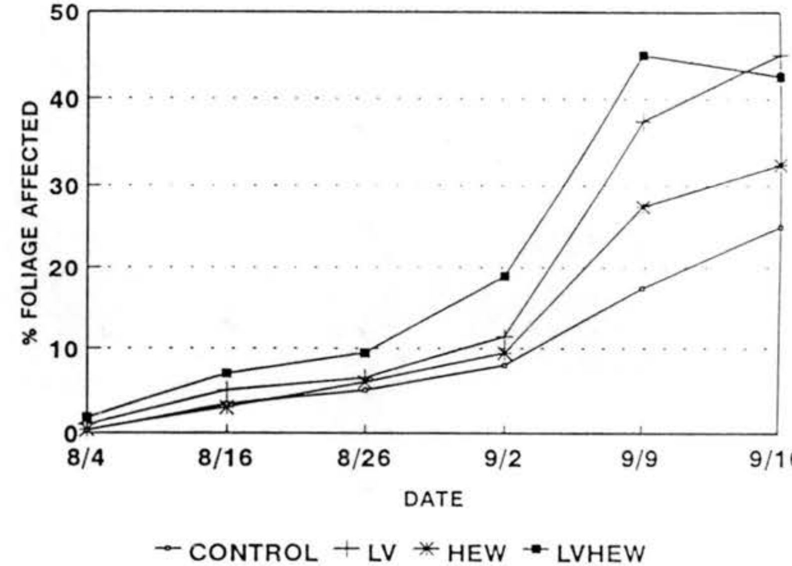

Figure 4.4C Deficient Moisture (HV + LEW)
1988

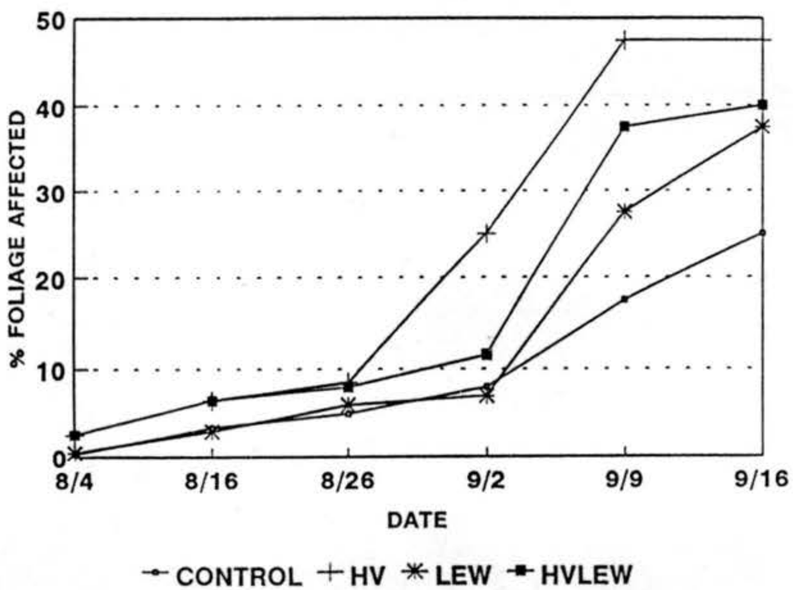

Figure 4.4D Deficient Moisture (HV + HEW)
1988

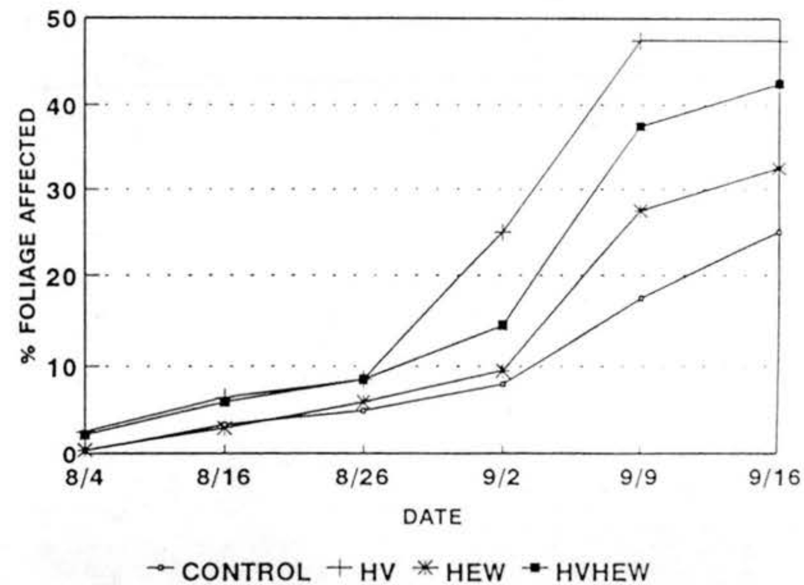

Figure 4.5A Optimum Moisture (LV + LEW)
1988

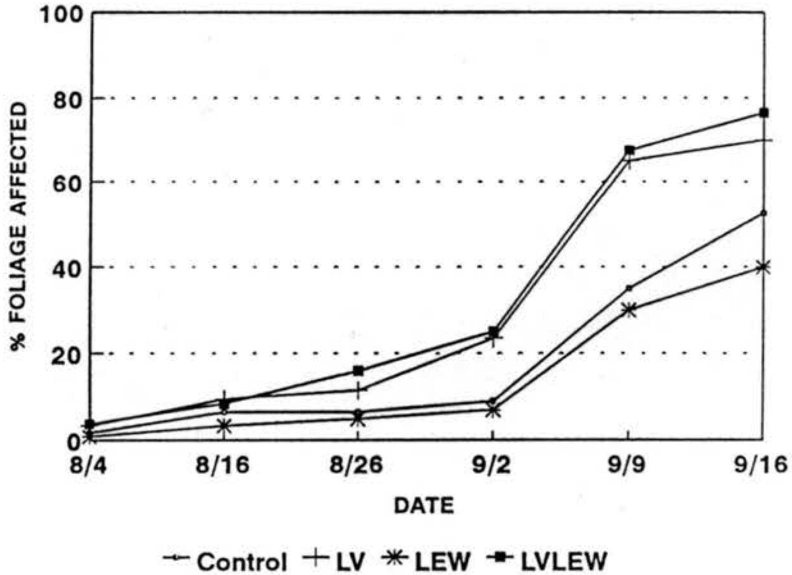

Figure 4.5B Optimum Moisture (LV + HEW)
1988

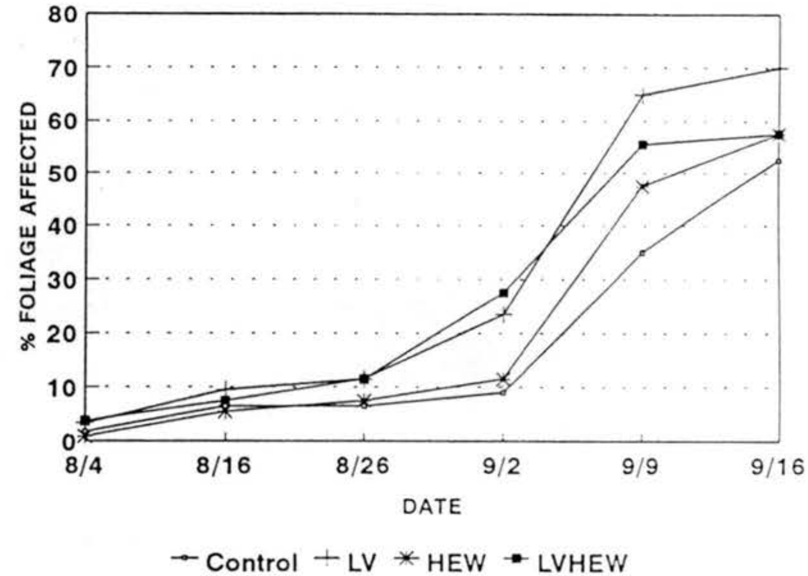

Figure 4.5C Optimum Moisture (HV + LEW)
1988

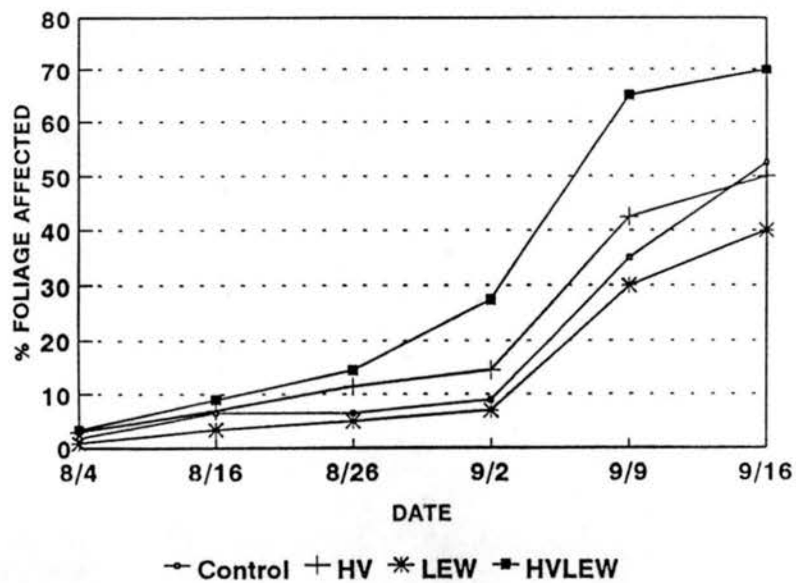

Figure 4.5D Optimum Moisture (HV + HEW)
1988

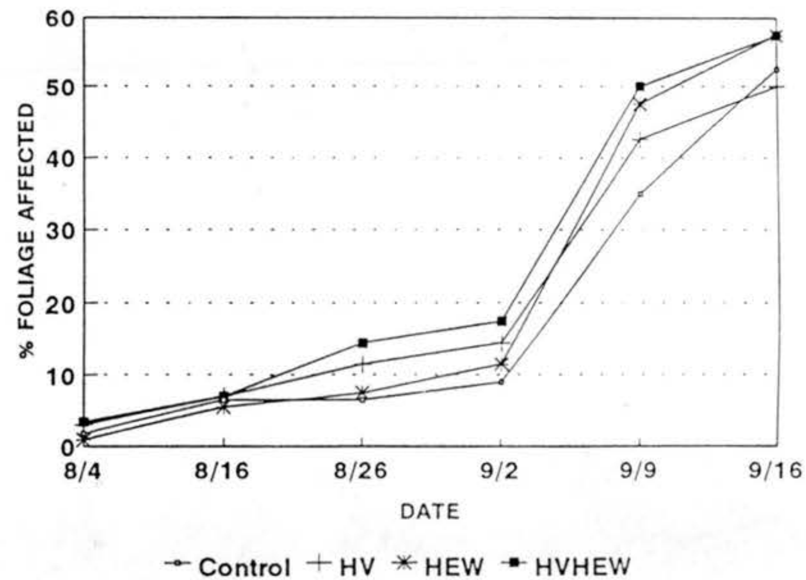

Figure 4.6A Excessive Moisture (LV + LEW)
1988

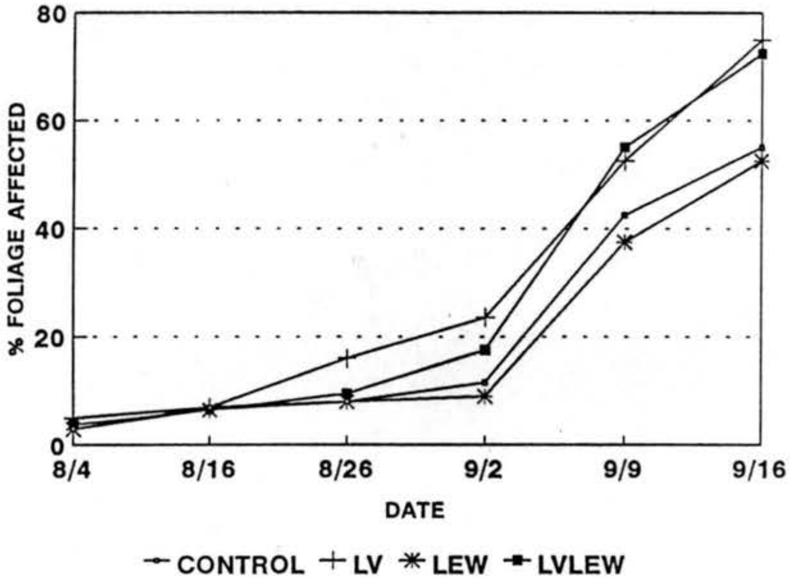

Figure 4.6B Excessive Moisture (LV + HEW)
1988

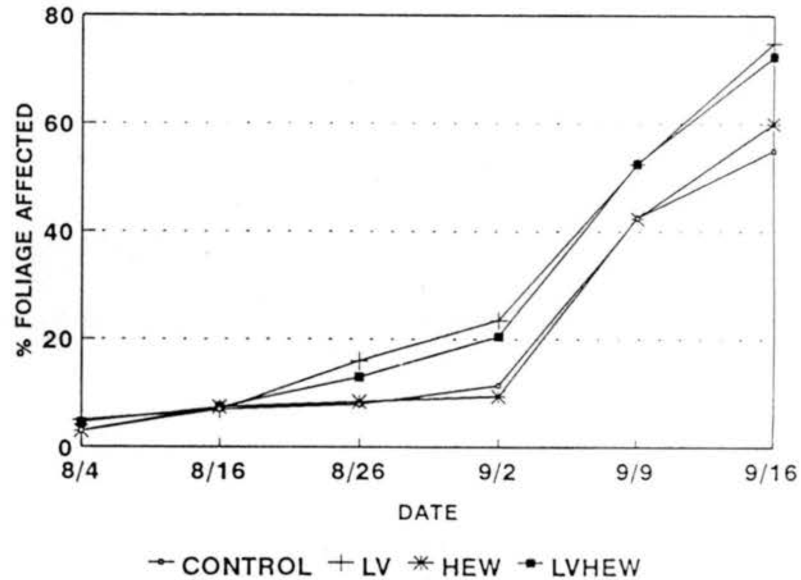

Figure 4.6C Excessive Moisture (HV + LEW)
1988

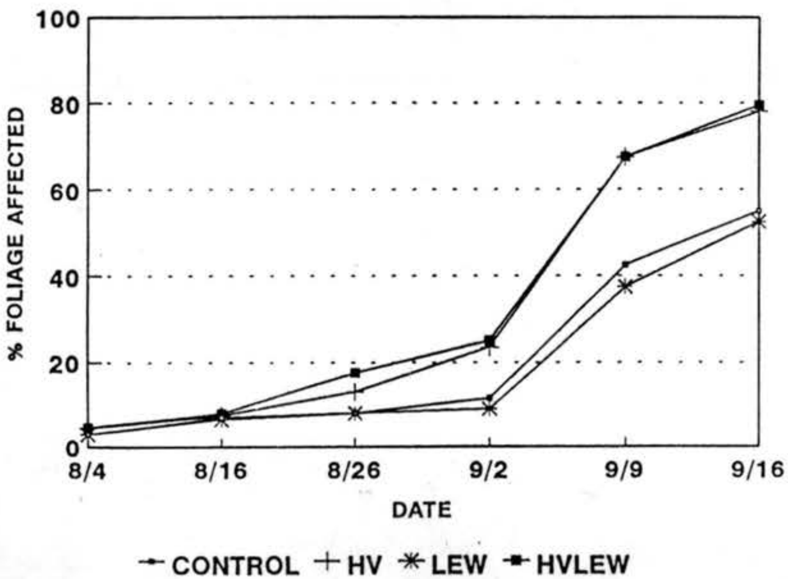

Figure 4.6D Excessive Moisture (HV + HEW)
1988

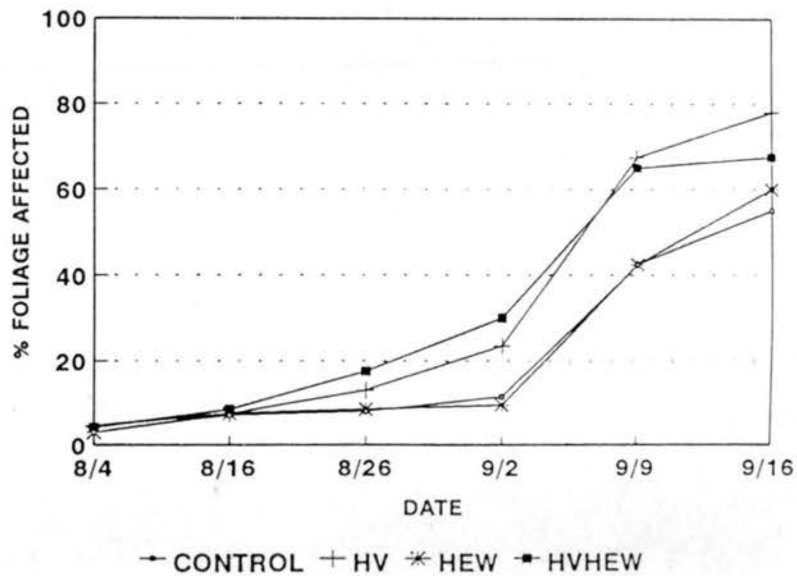

Figure 4.7A Deficient Moisture (LV + LEC)
1989

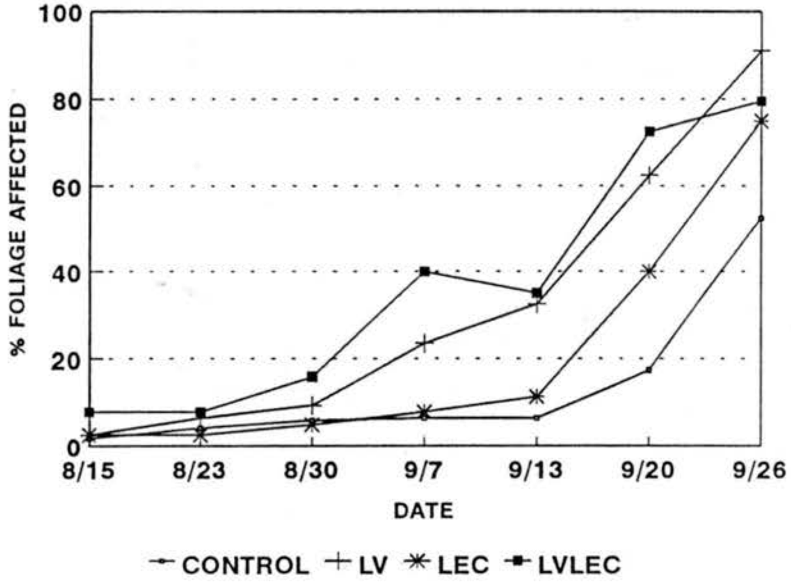

Figure 4.7B Deficient Moisture (LV + HEC)
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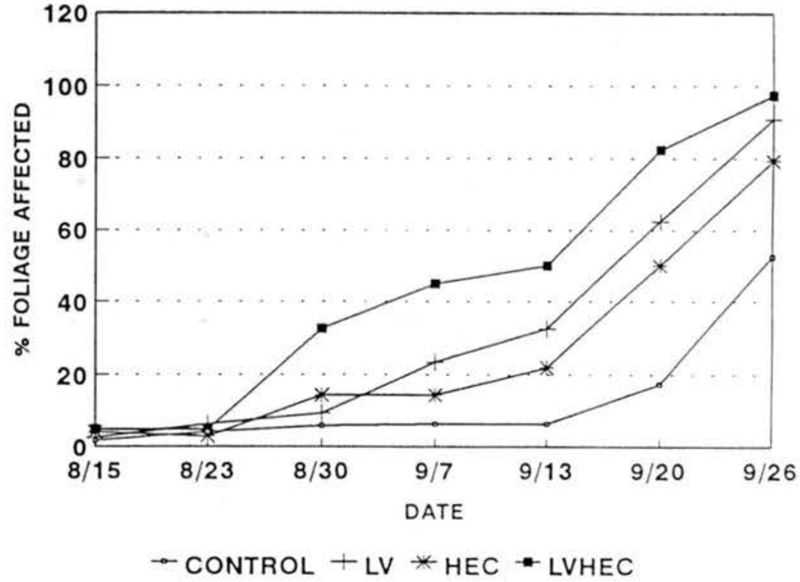

Figure 4.7C Deficient Moisture (HV + LEC)
1989

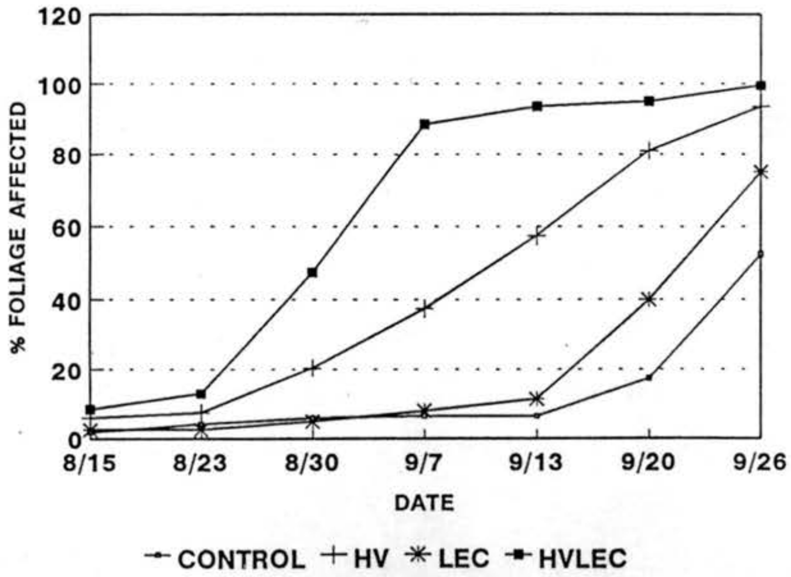

Figure 4.7D Deficient Moisture (HV + HEC)
1989

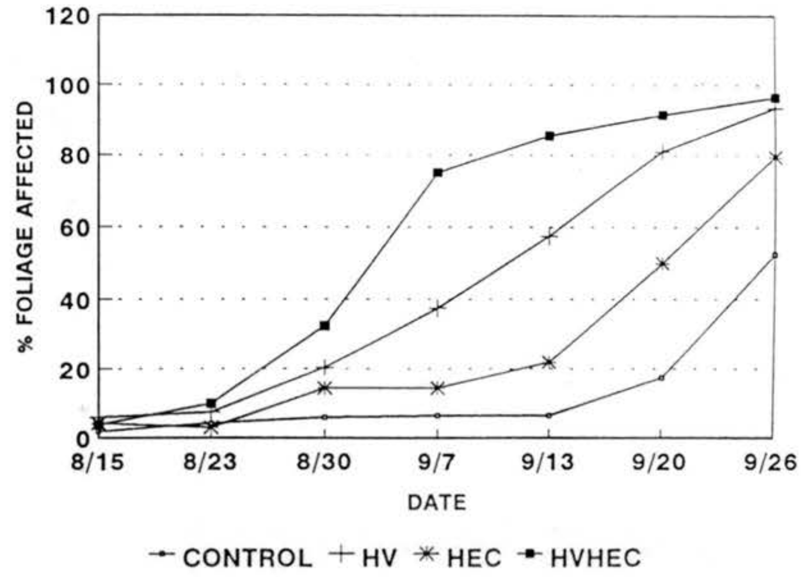

Figure 4.8A Optimum Moisture (LV + LEC)
1989

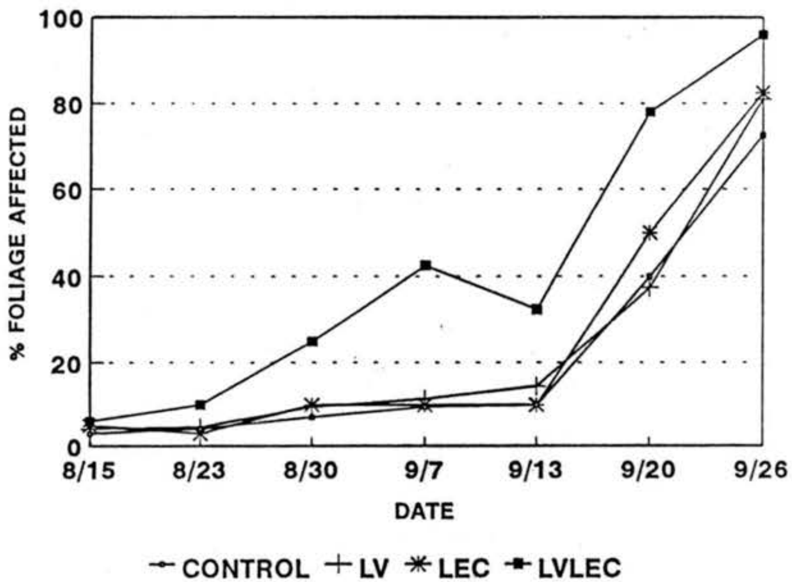

Figure 4.8B Optimum Moisture (LV + HEC)
1989

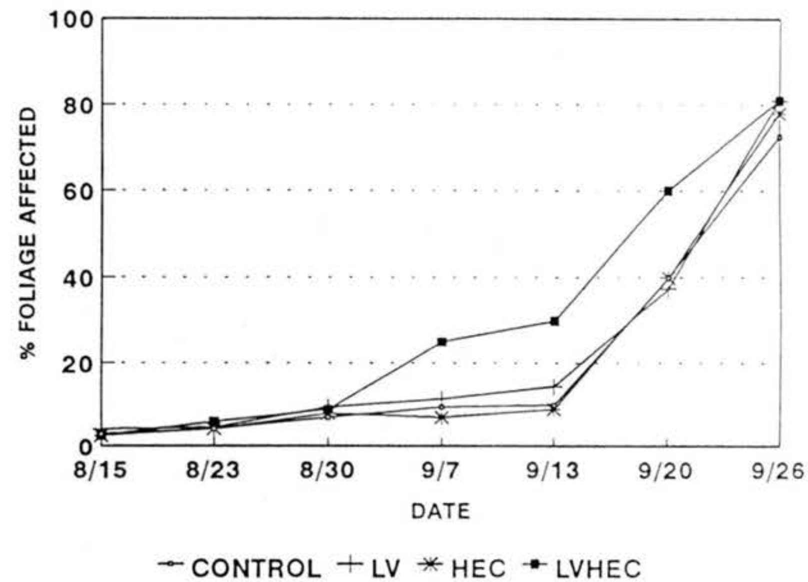

Figure 4.8C Optimum Moisture (HV + LEC)
1989

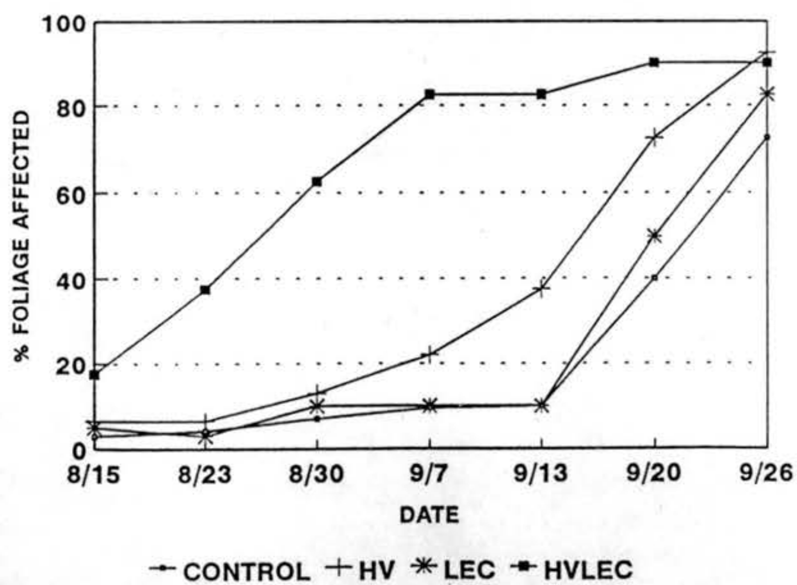

Figure 4.8D Optimum Moisture (HV +HEC)
1989

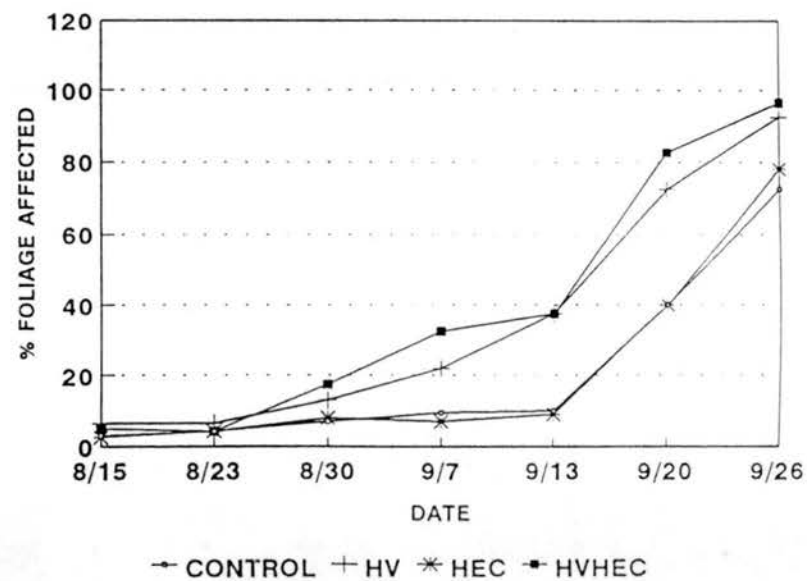

Figure 4.9A Excessive Moisture (LV + LEC)
1989

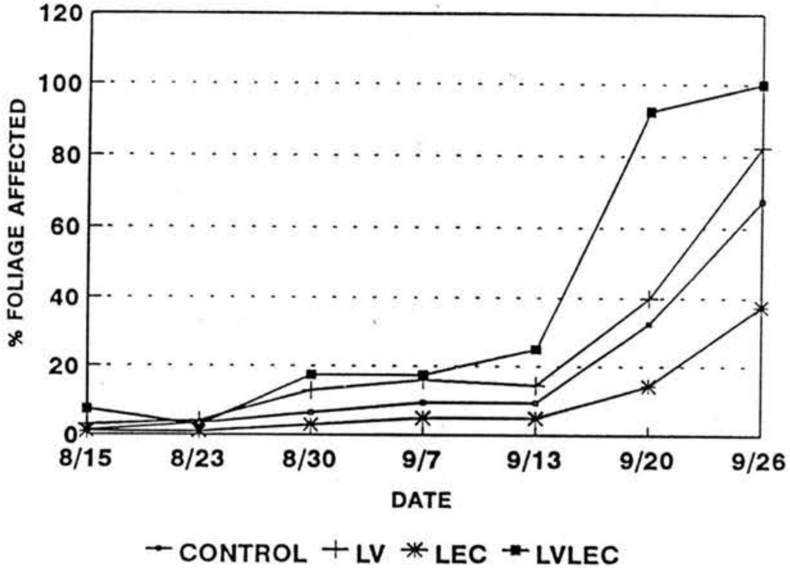

Figure 4.9B Excessive Moisture (LV + HEC)
1989

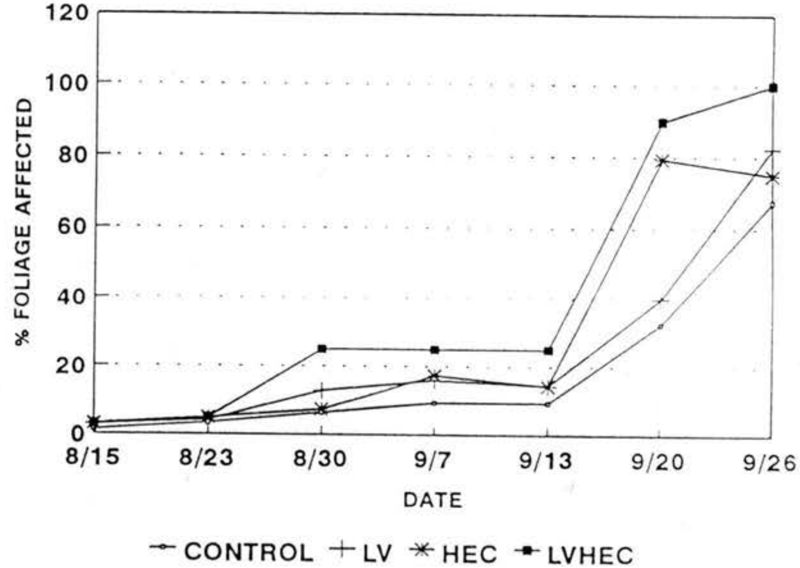

Figure 4.9C Excessive Moisture (HV + LEC)
1989

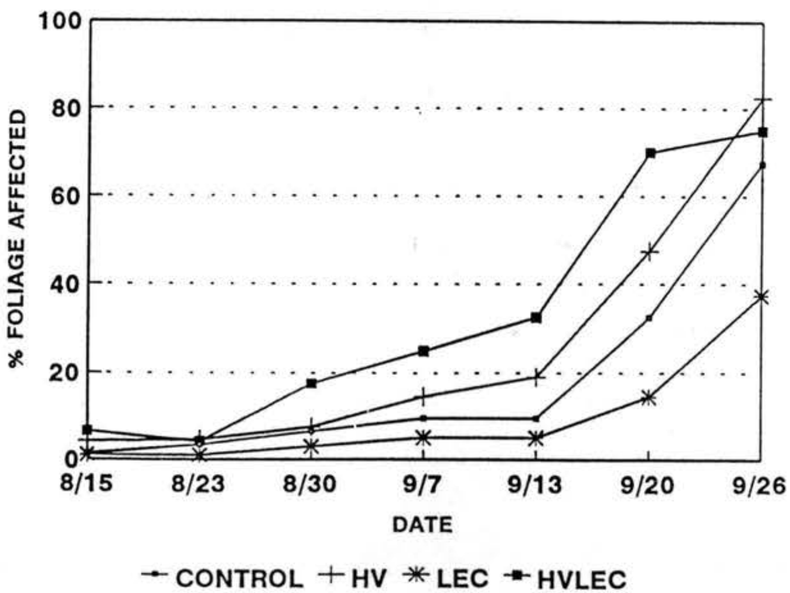

Figure 4.9D Excessive Moisture (HV + HEC)
1989

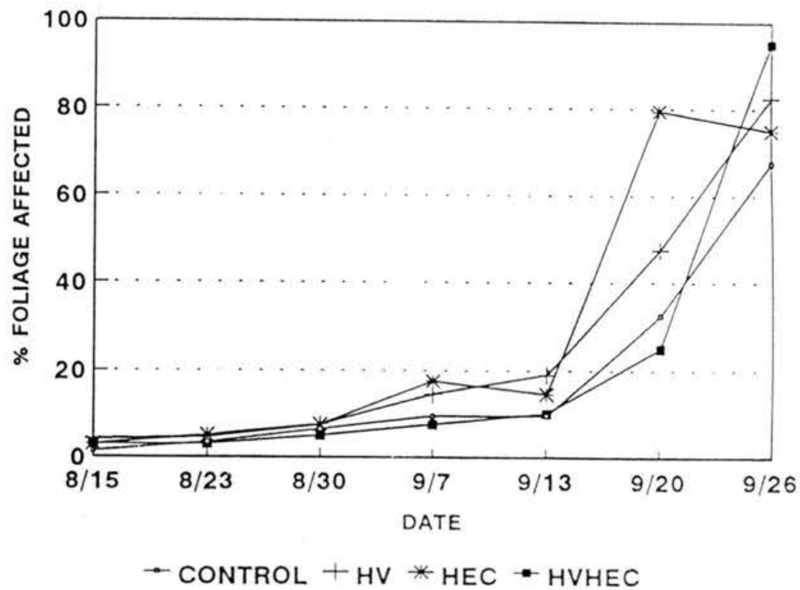

Figure 4.10A Deficient Moisture (LV + LEA)
1989

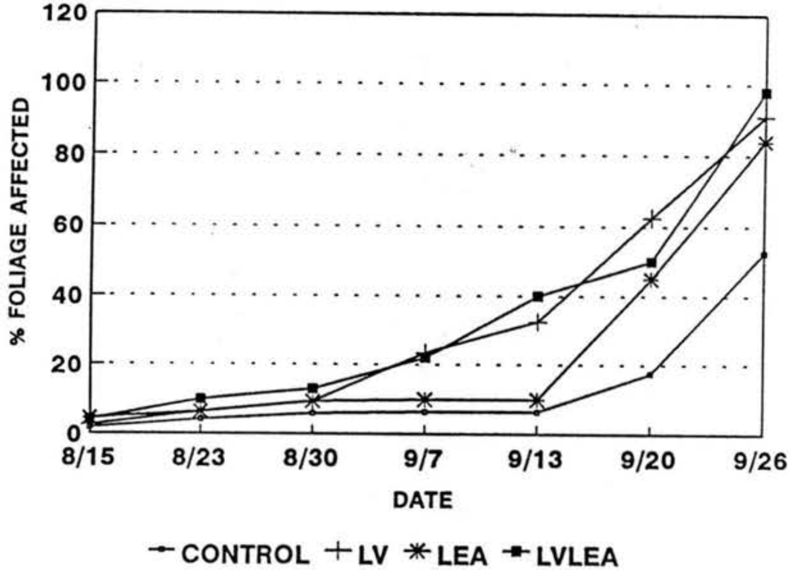

Figure 4.10B Deficient Moisture (LV + HEA)
1989

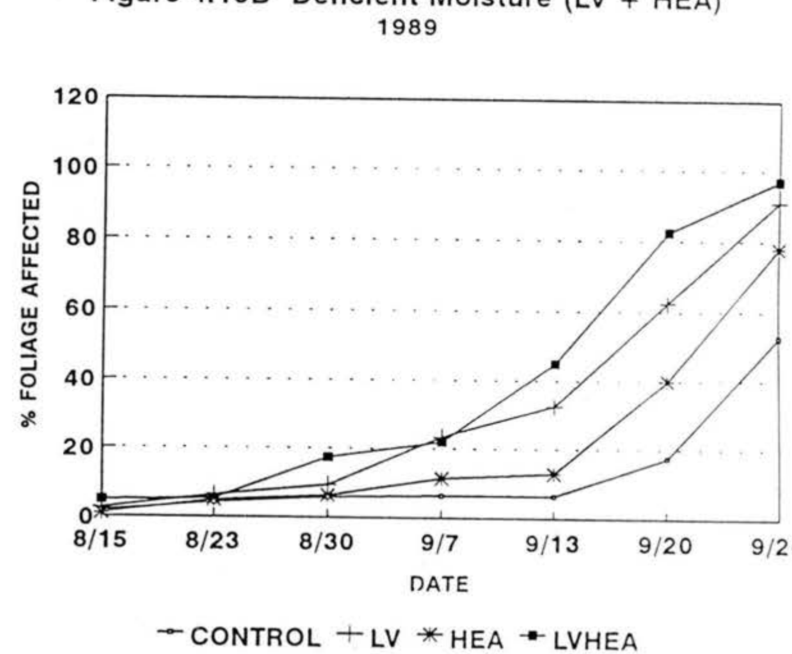

Figure 4.10C Deficient Moisture (HV + LEA)
1989

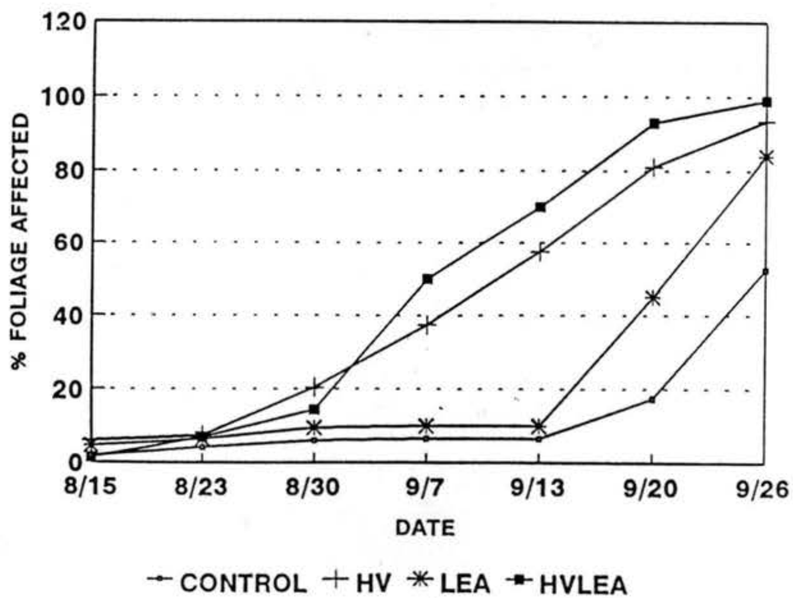

Figure 4.10D Deficient Moisture (HV + HEA)
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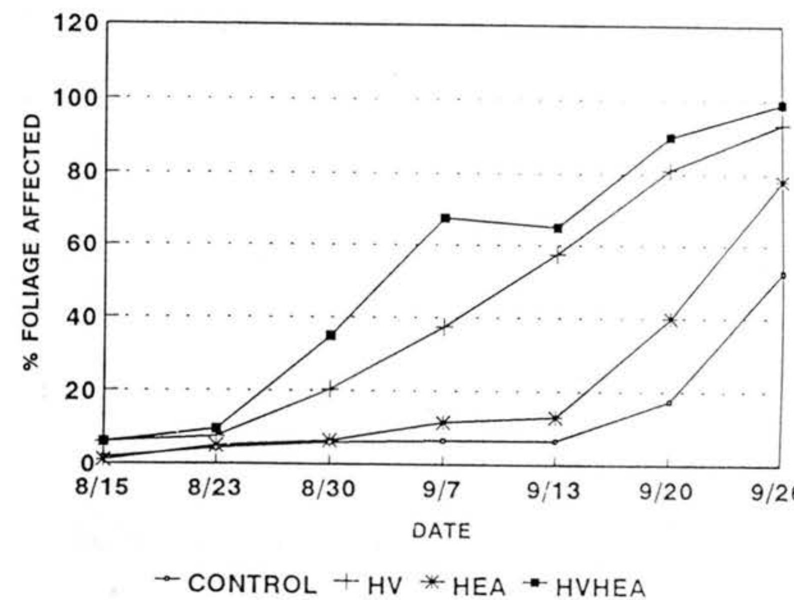

Figure 4.11A Optimum Moisture (LV + LEA)
1989

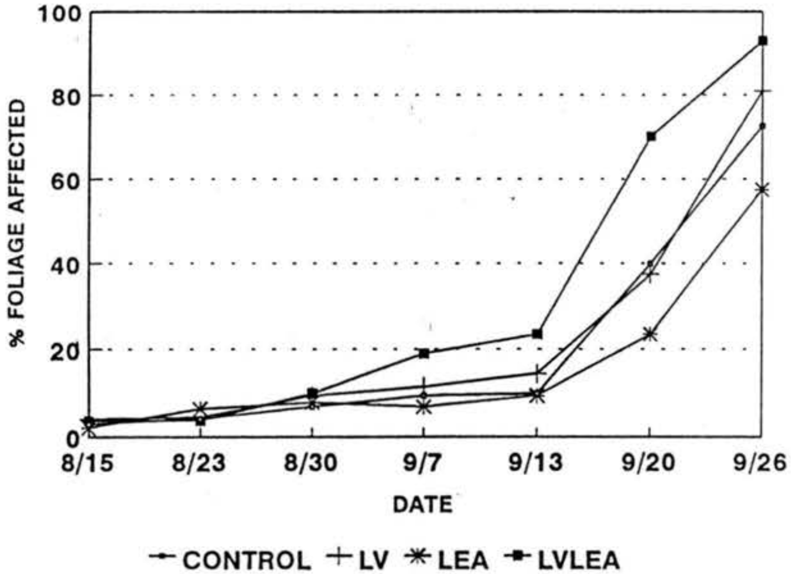

Figure 4.11B Optimum Moisture (LV + HEA)
1989

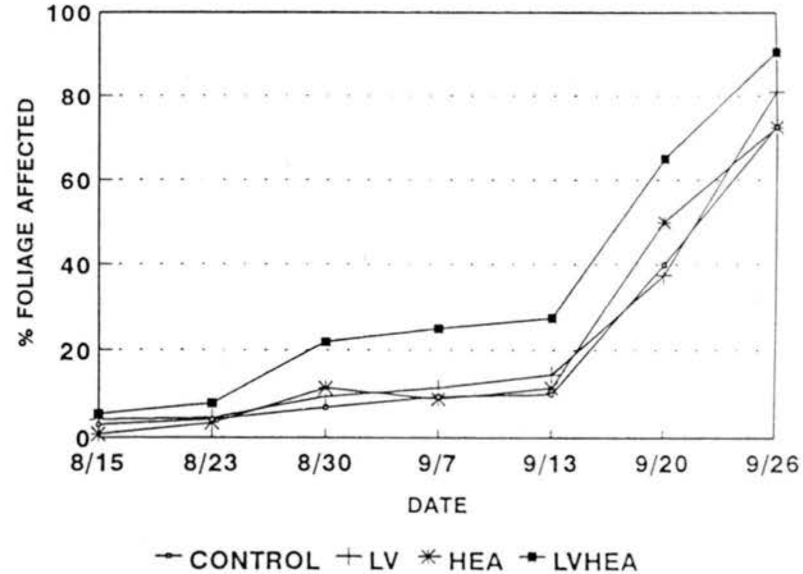

Figure 4.11C Optimum Moisture (LV + LEA)
1989

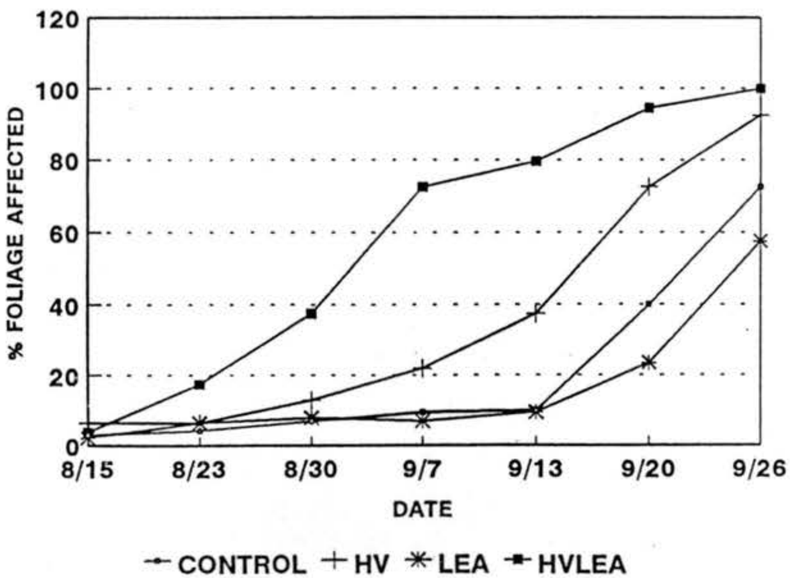

Figure 4.11D Optimum Moisture (HV + HEA)
1989

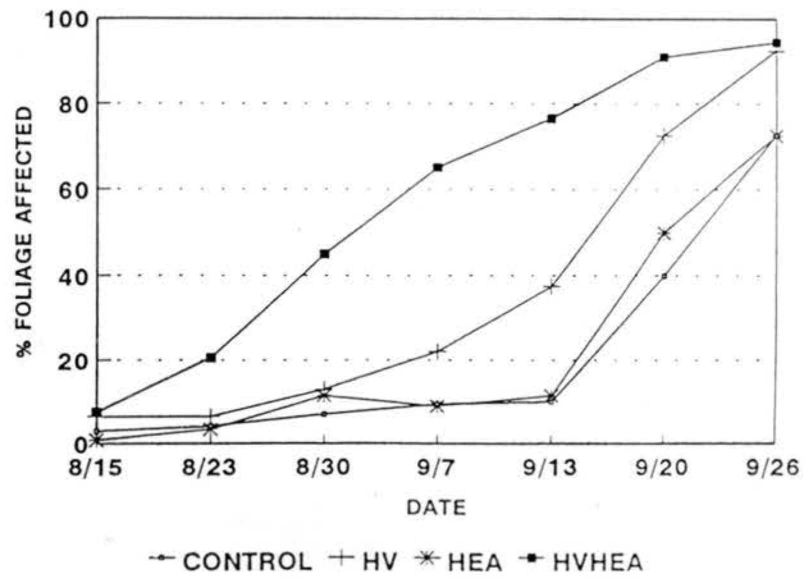

Figure 4.12A Excessive Moisture (LV + LEA)
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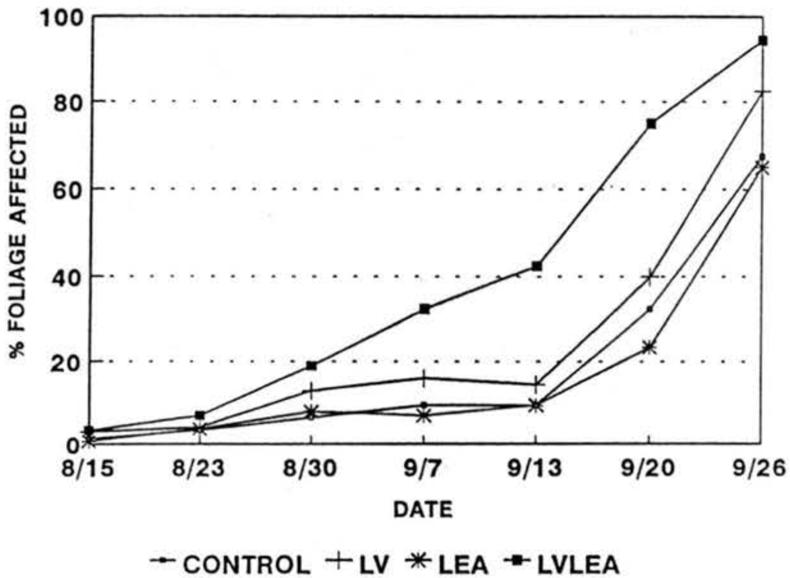


Figure 4.12B Excessive Moisture (LV + HEA)
1989

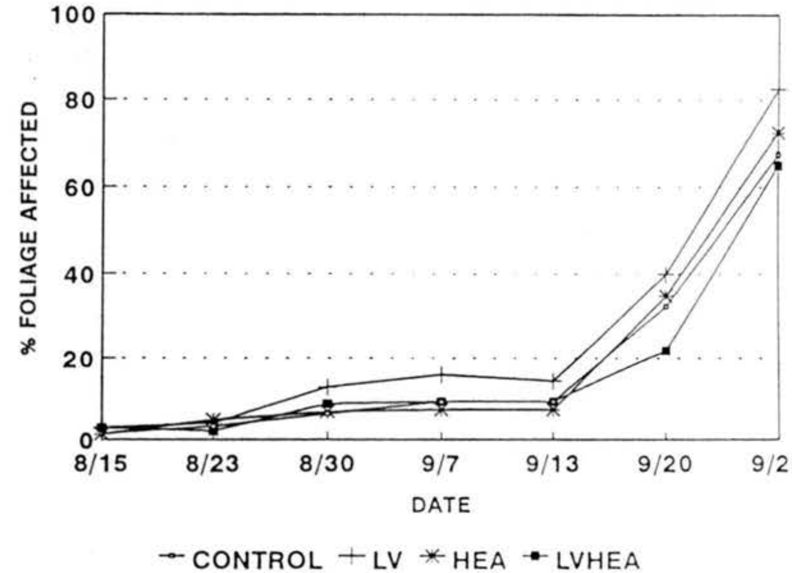


Figure 4.12C Excessive Moisture (HV + LEA)
1989

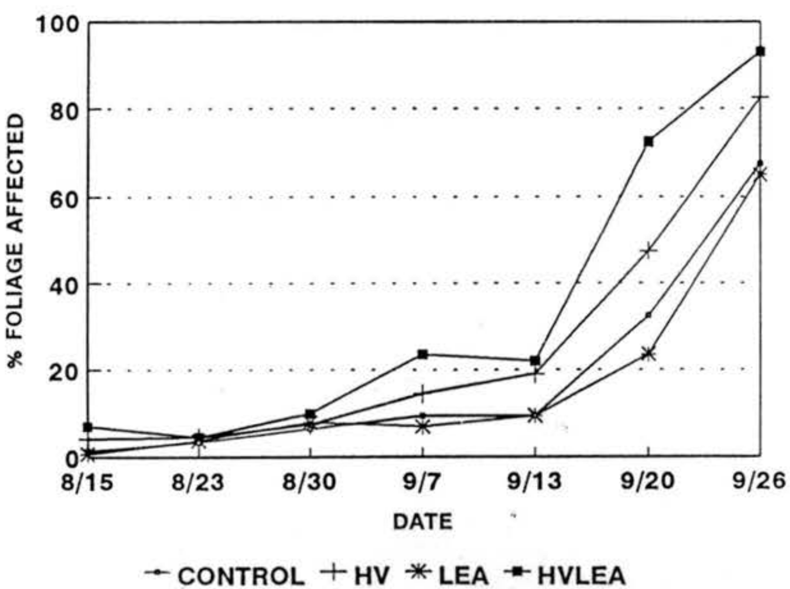


Figure 4.12D Excessive Moisture (HV + HEA)
1989

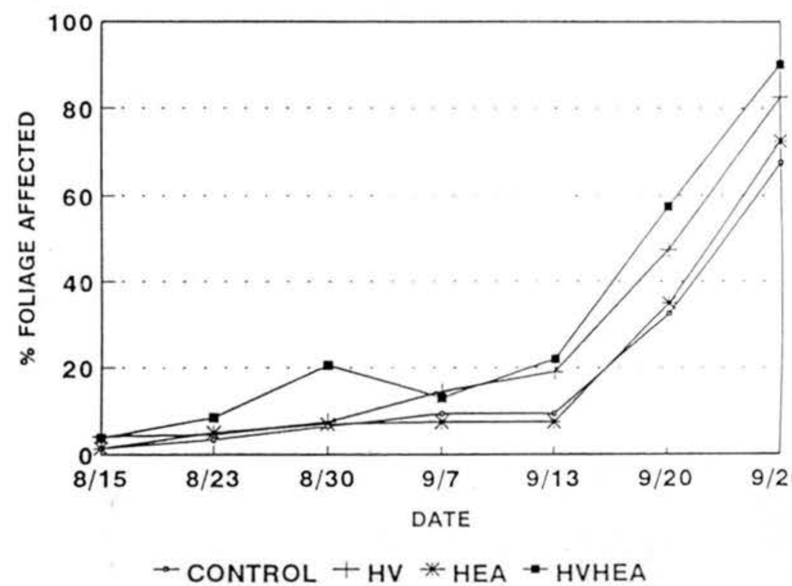


Figure 4.13A Deficient Moisture (LV + LEW)
1989

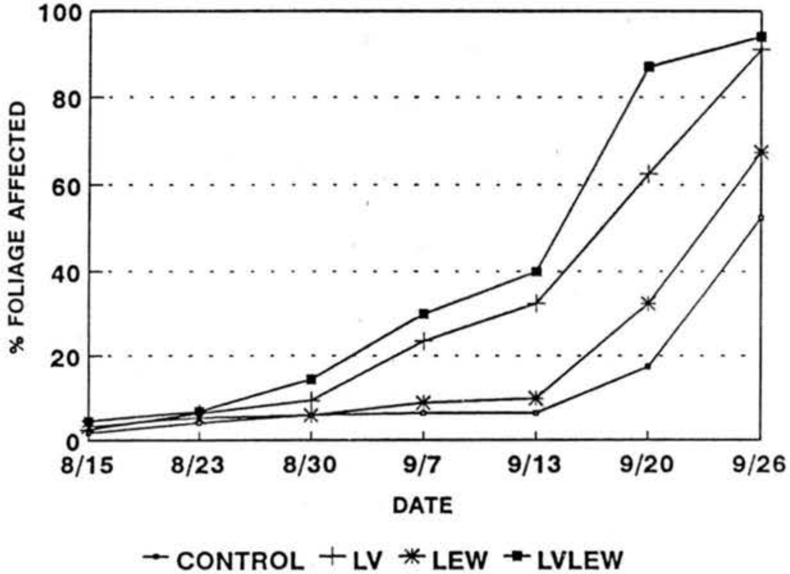


Figure 4.13B Deficient Moisture (LV + HEW)
1989

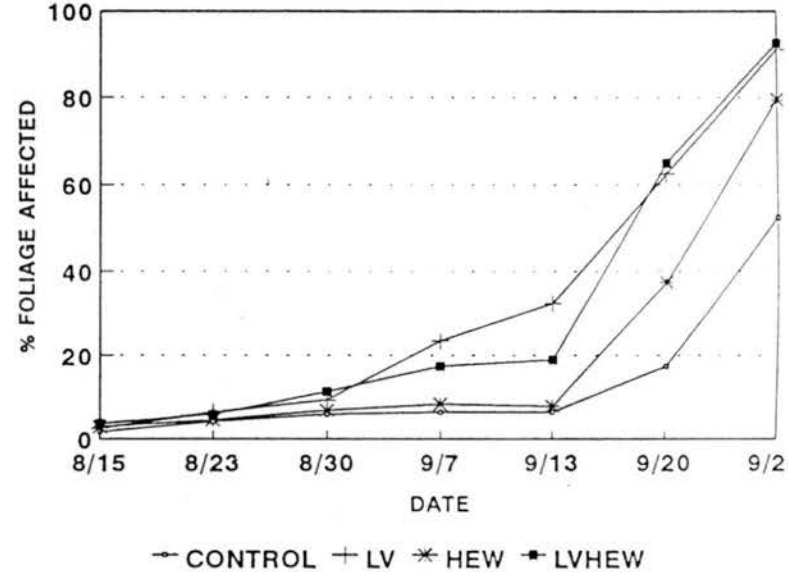


Figure 4.13C Deficient Moisture (HV + LEW)
1989

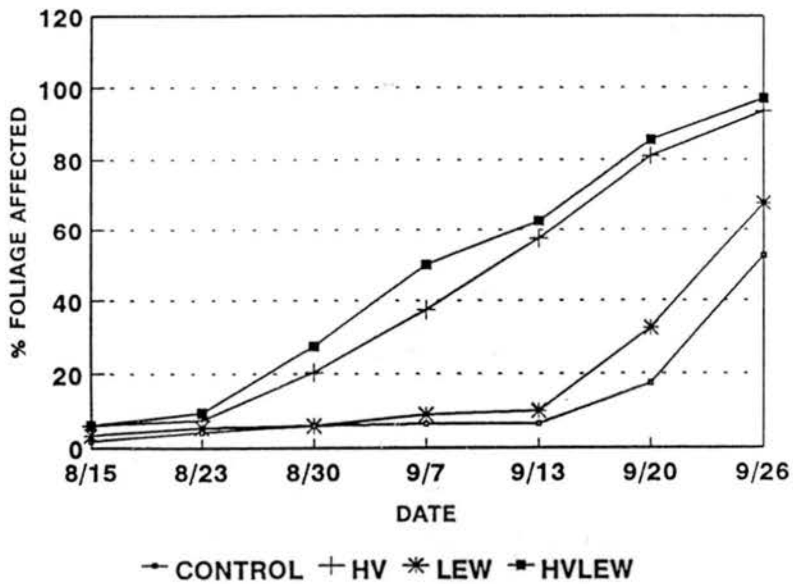


Figure 4.13D Deficient Moisture (HV + HEW)
1989

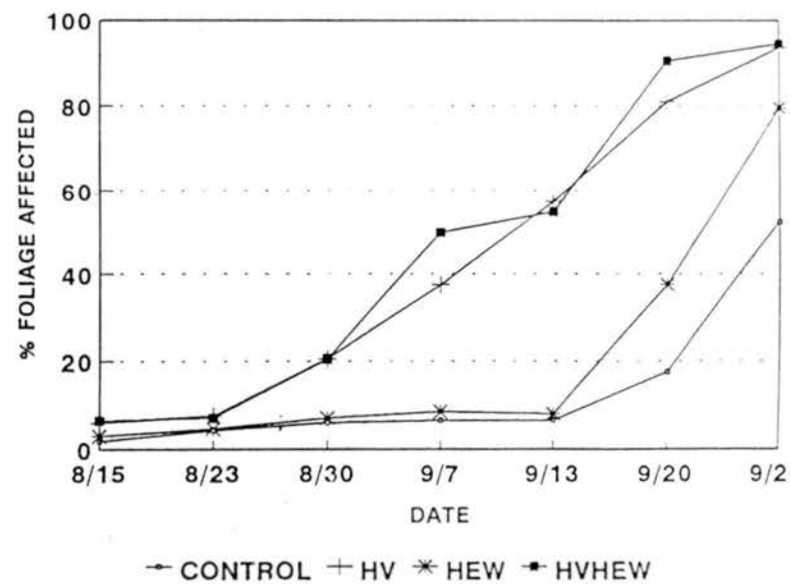


Figure 4.14A Optimum Moisture (LV + LEW)
1989

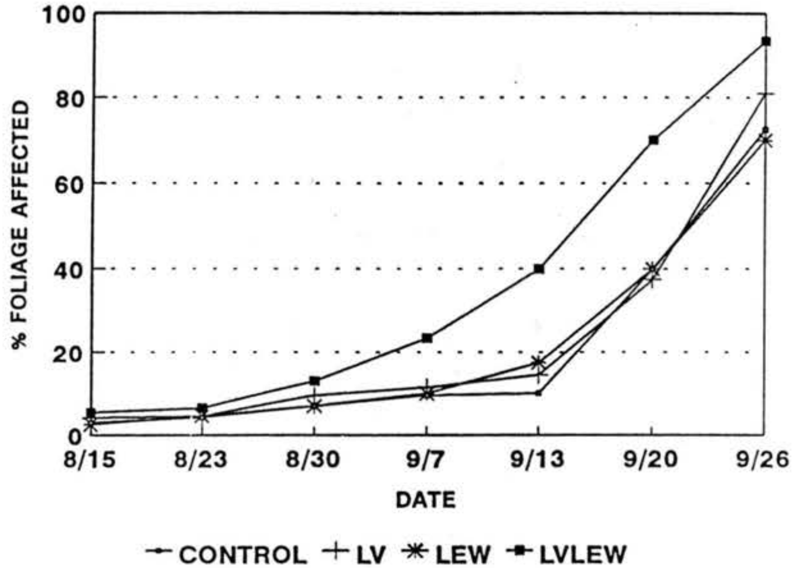


Figure 4.14B Optimum Moisture (LV + HEW)
1989

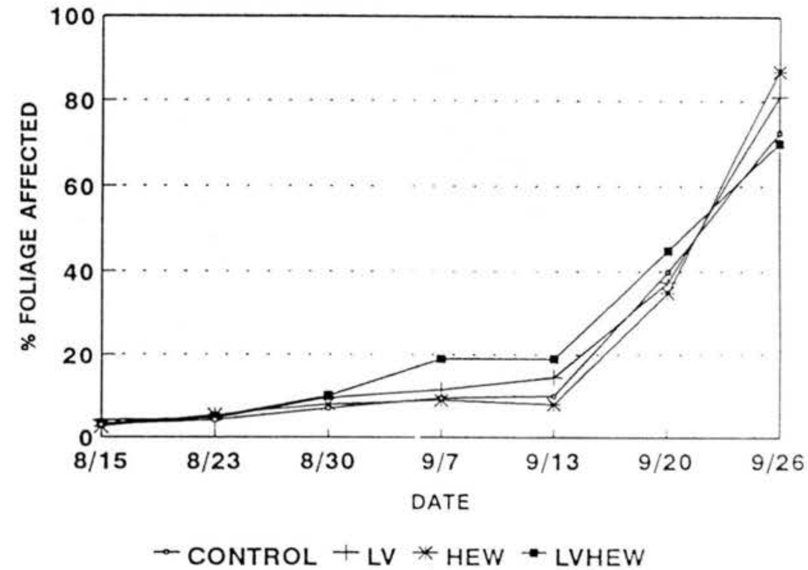


Figure 4.14C Optimum Moisture (HV + LEW)
1989

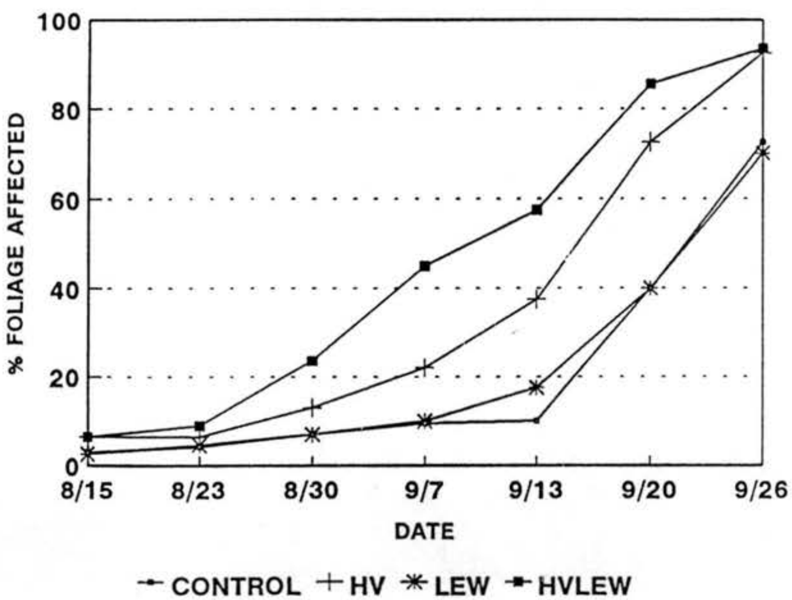


Figure 4.14D Optimum Moisture (HV + HEW)
1989

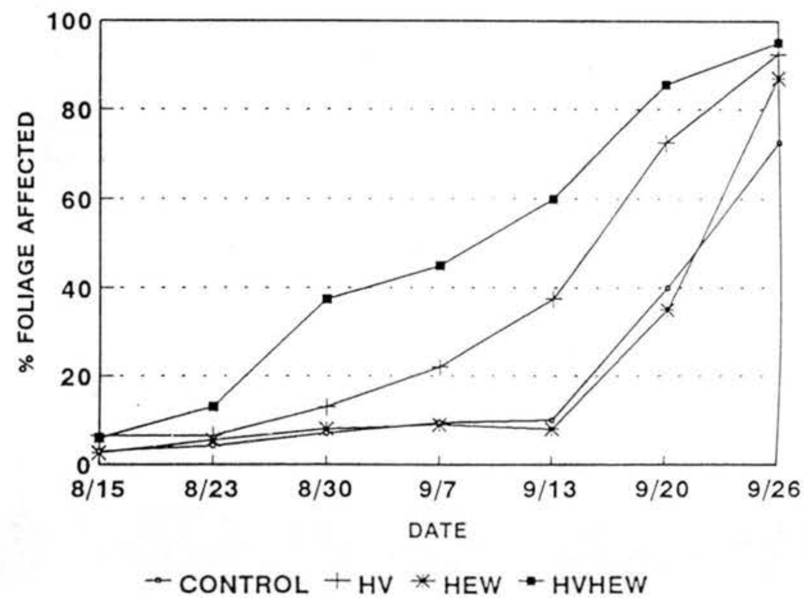


Figure 4.15A Excessive Moisture (LV + LEW)
1989

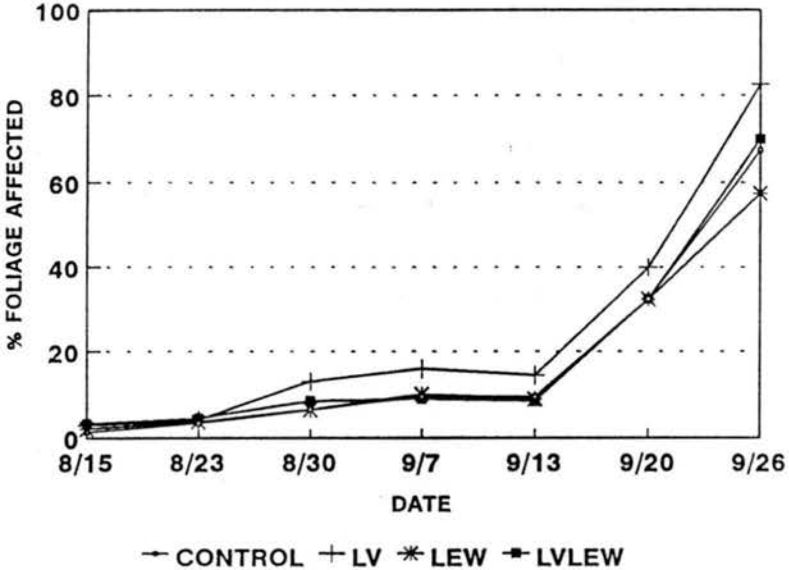


Figure 4.15B Excessive Moisture (LV + HEW)
1989

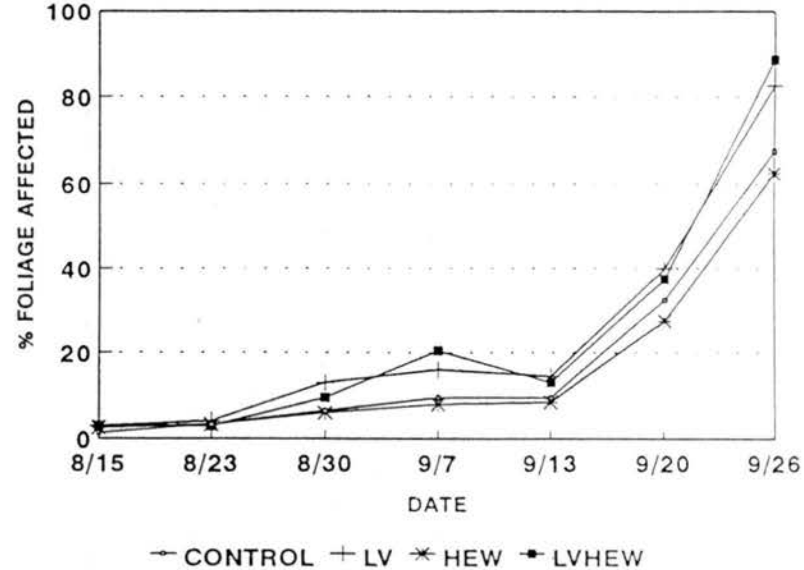


Figure 4.15C Excessive Moisture (HV + LEW)
1989

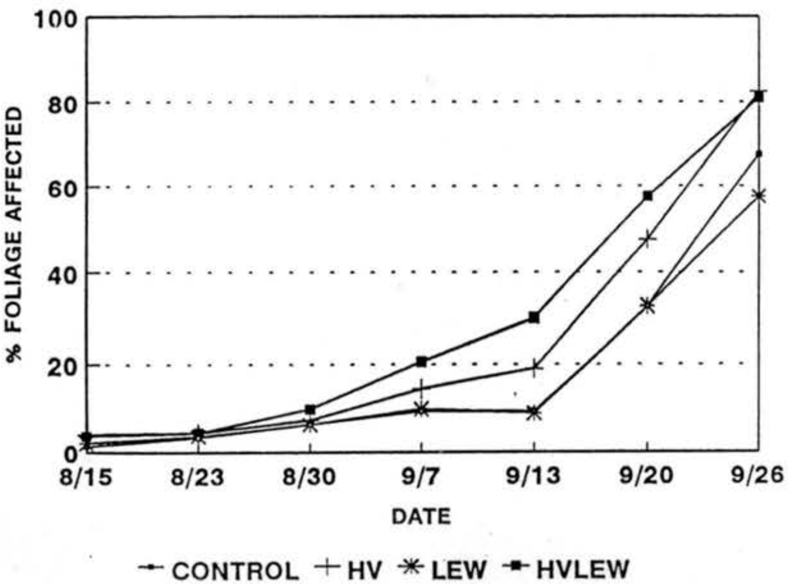


Figure 4.15D Excessive Moisture (HV + HEW)
1989

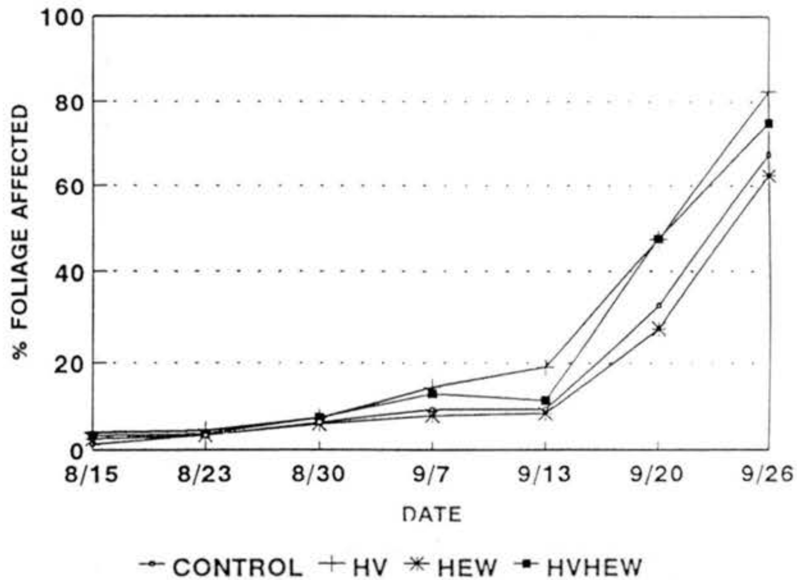


Figure 4.16A Deficient Moisture (LV + LEC)
1990

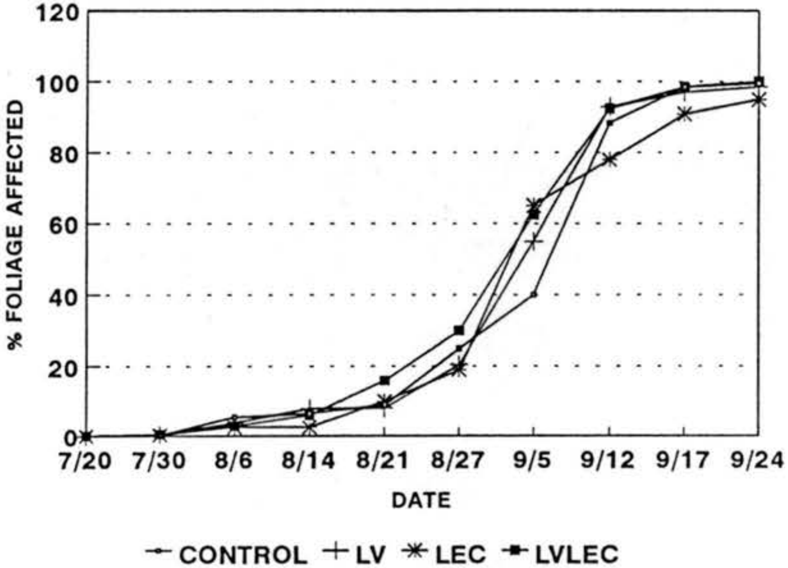


Figure 4.16B Deficient Moisture (LV + HEC)
1990

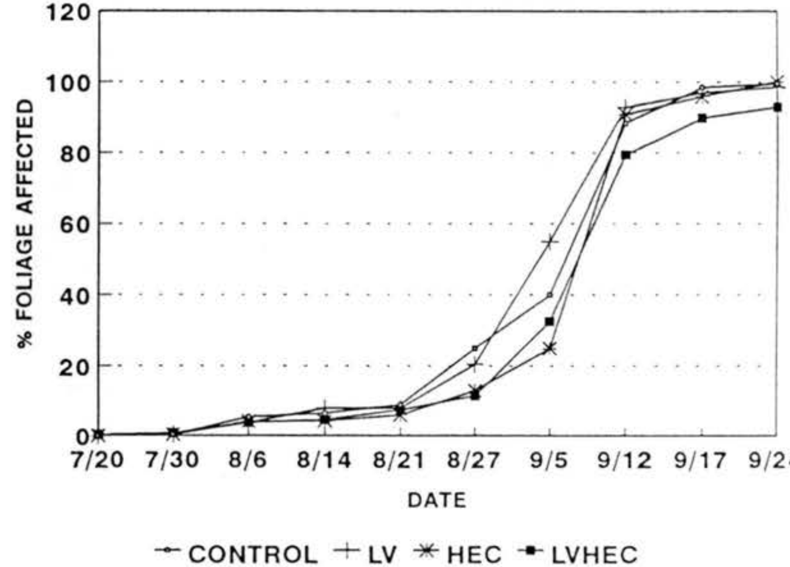


Figure 4.16C Deficient Moisture (HV + LEC)
1990

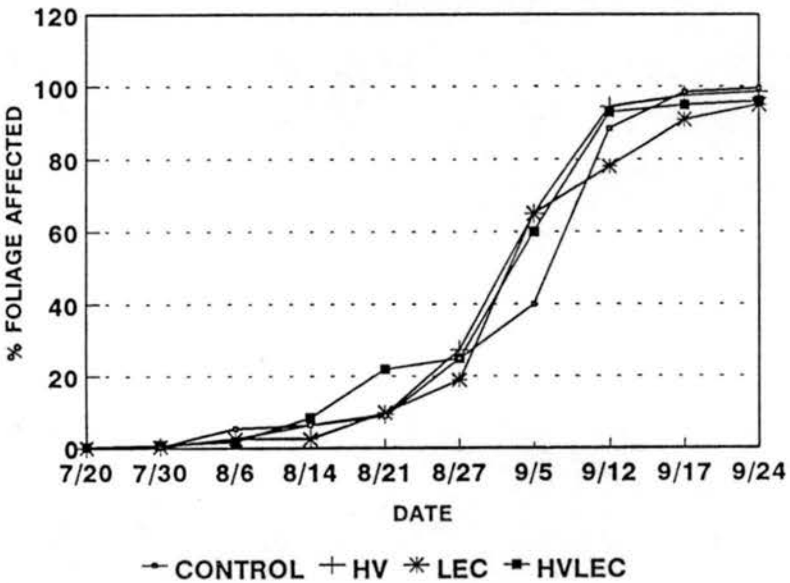


Figure 4.16D Deficient Moisture (HV + HEC)
1990

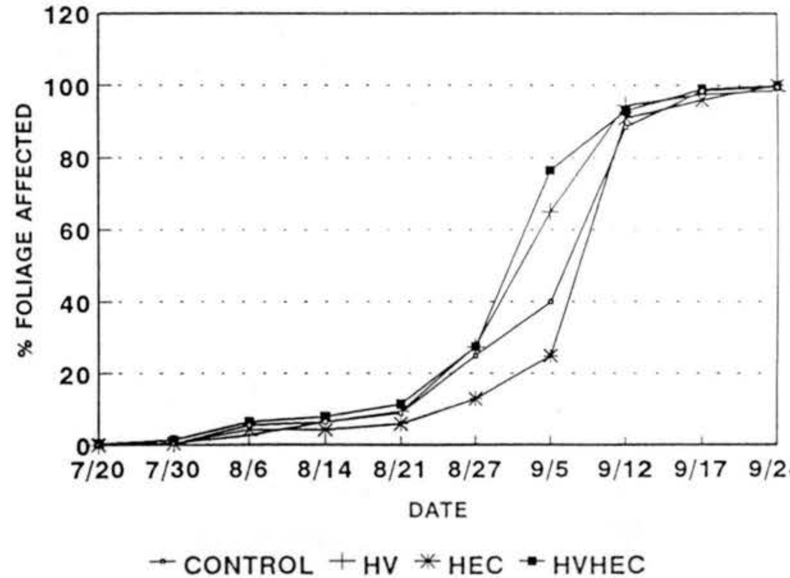


Figure 4.17A Optimum Moisture (LV + LEC)
1990

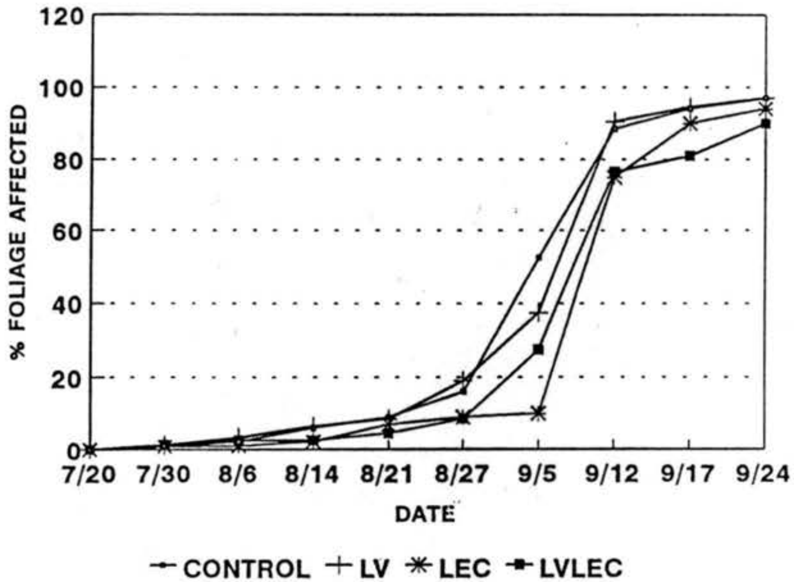


Figure 4.17B Optimum Moisture (LV + HEC)
1990

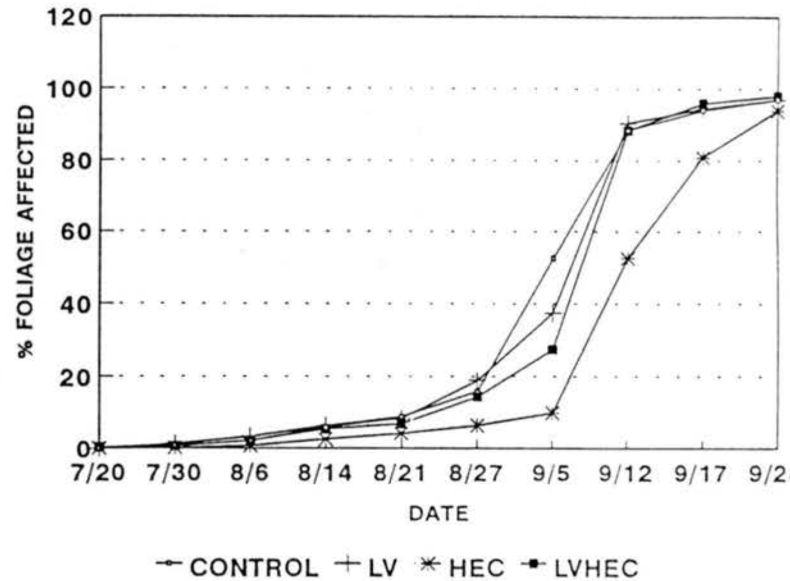


Figure 4.17C Optimum Moisture (HV + LEC)
1990

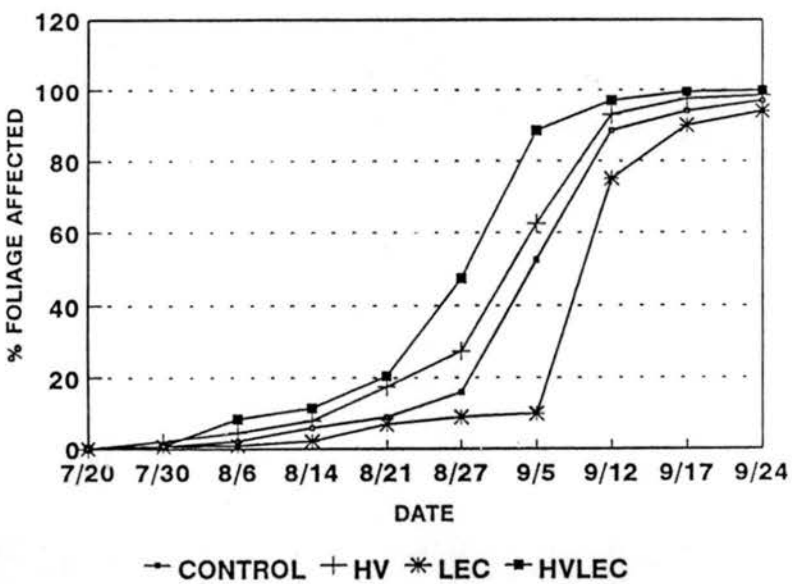


Figure 4.17D Optimum Moisture (HV + HEC)
1990

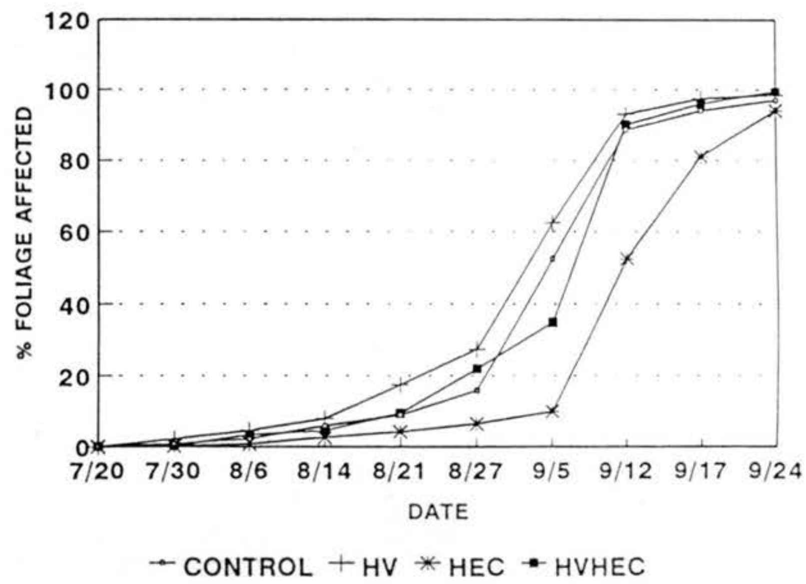


Figure 4.18A Excessive Moisture (LV + LEC)
1990

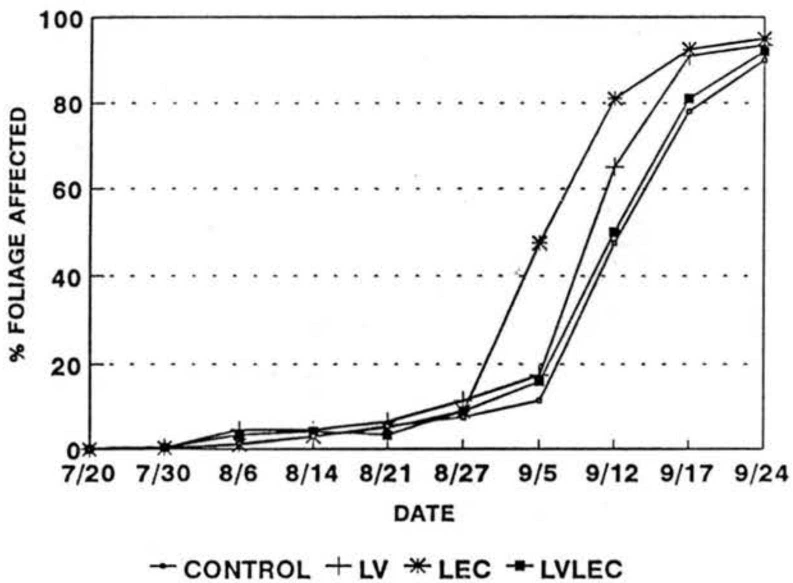


Figure 4.18B Excessive Moisture (LV + HEC)
1990

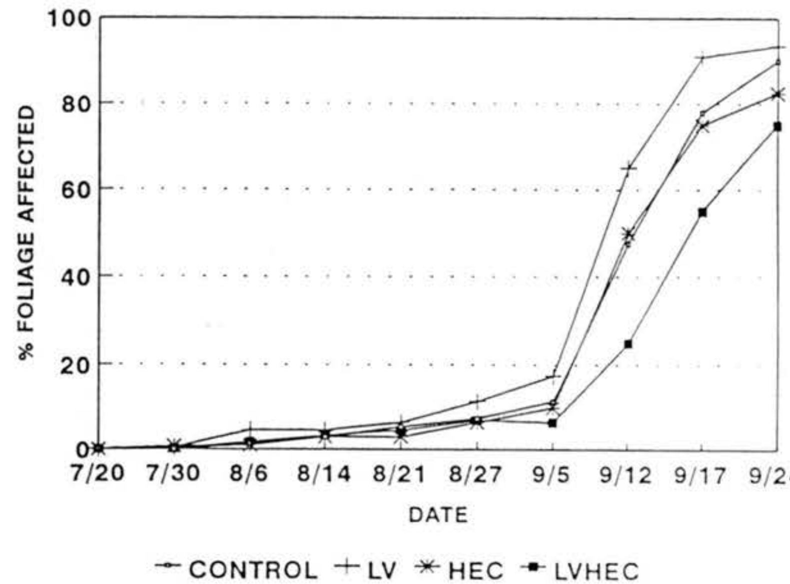


Figure 4.18C Excessive Moisture (HV + LEC)
1990

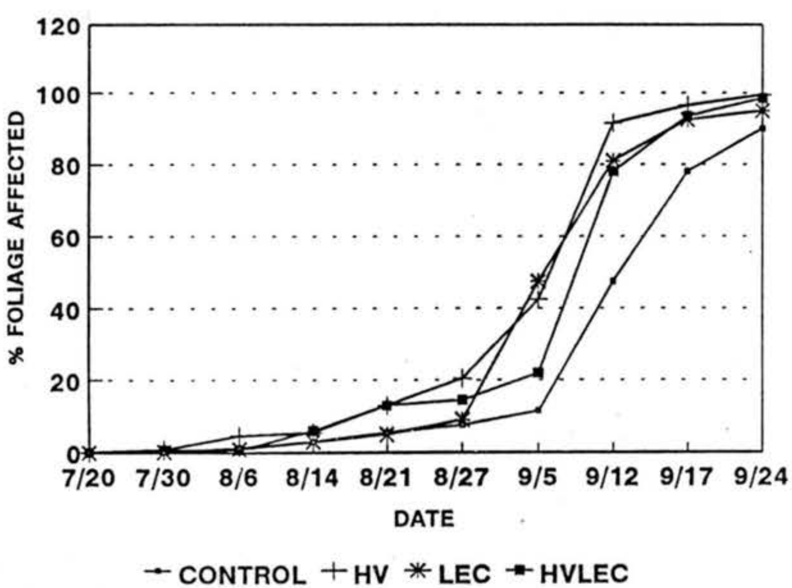


Figure 4.18D Excessive Moisture (HV + HEC)
1990

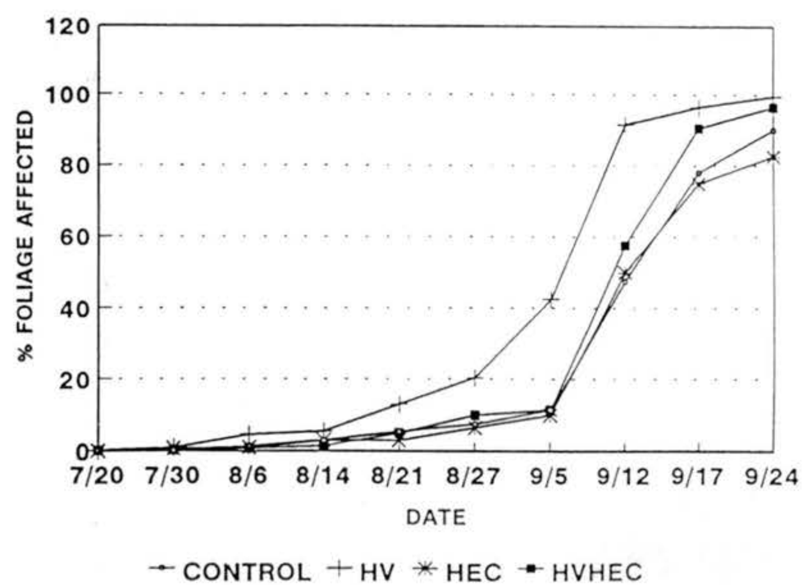


Figure 4.19A Deficient Moisture (LV + LEA)
1990

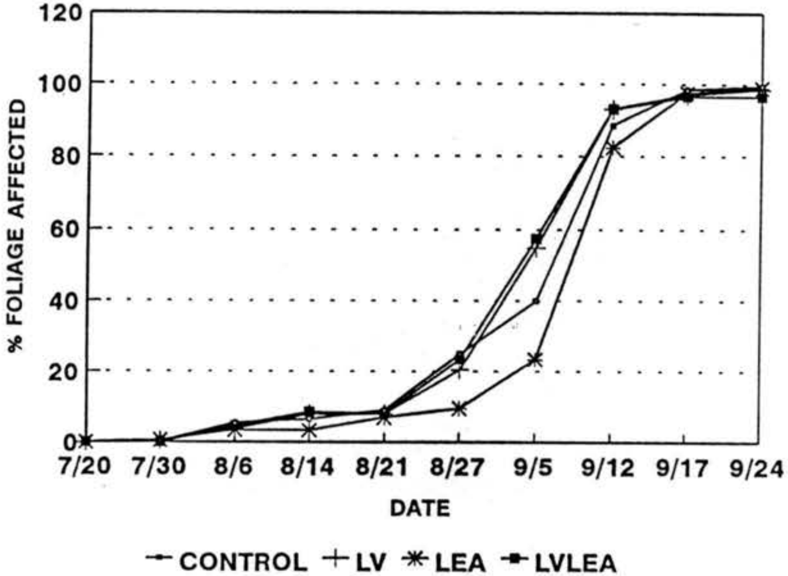


Figure 4.19B Deficient Moisture (LV + HEA)
1990

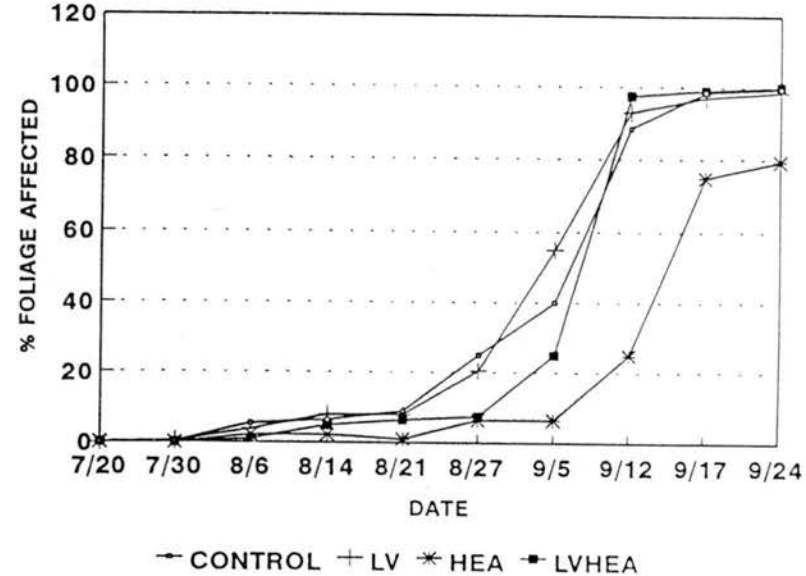


Figure 4.19C Deficient Moisture (HV + LEA)
1990

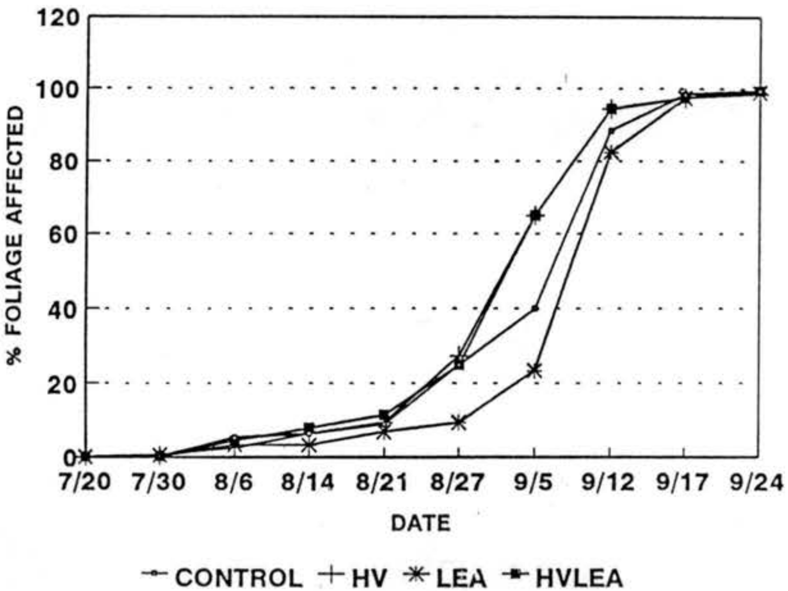


Figure 4.19D Deficient Moisture (HV + HEA)
1990

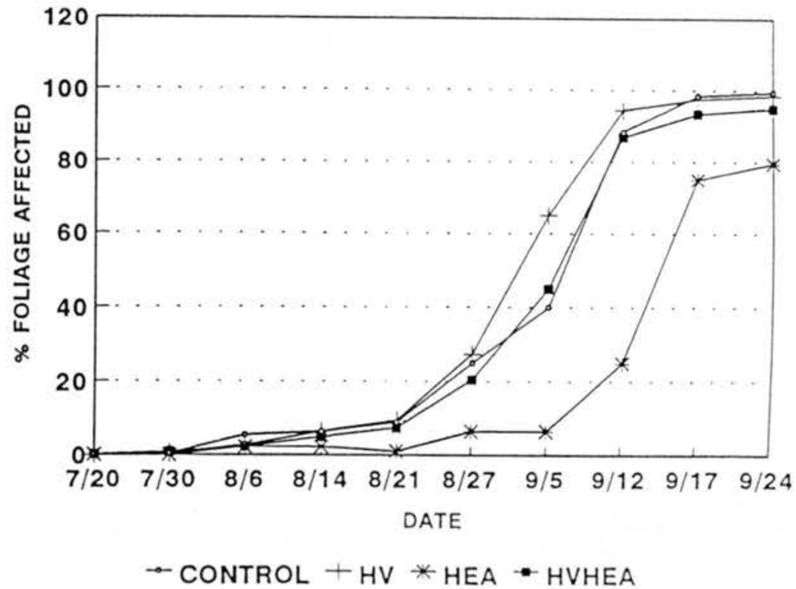


Figure 4.20A Optimum Moisture (LV + LEA)
1990

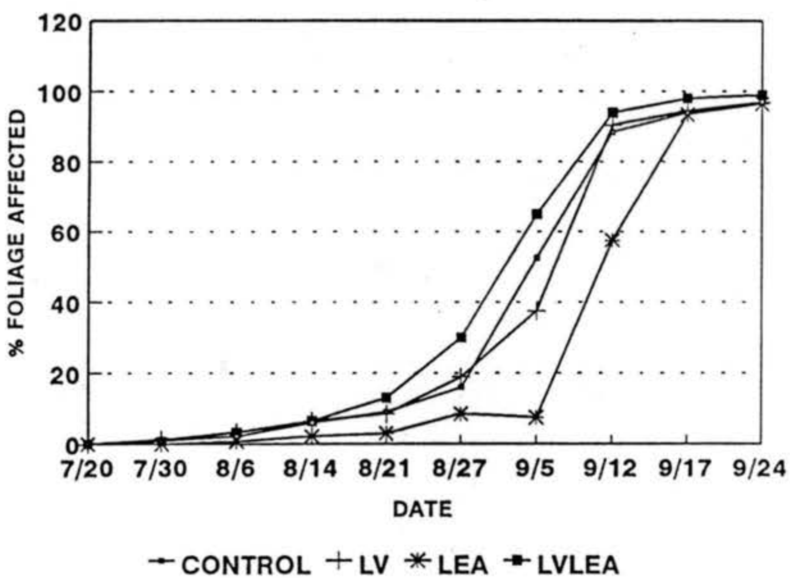


Figure 4.20B Optimum Moisture (LV + HEA)
1990

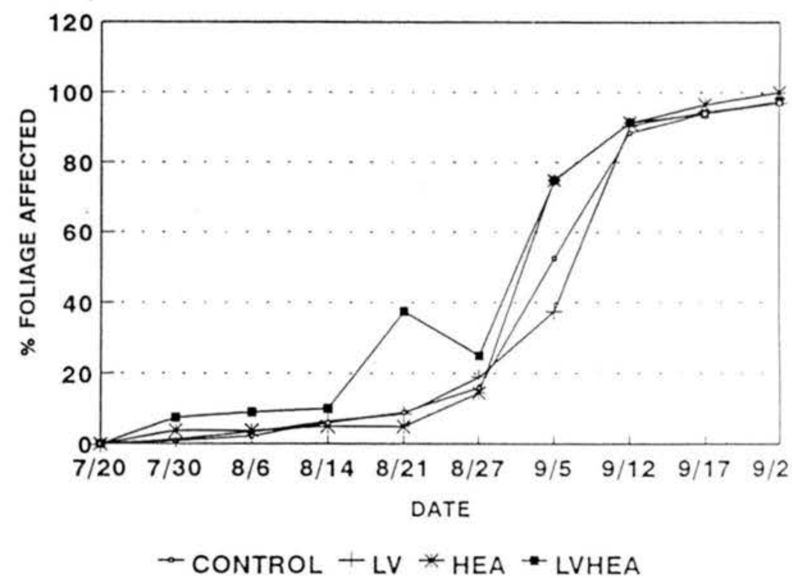


Figure 4.20C Optimum Moisture (HV + LEA)
1990

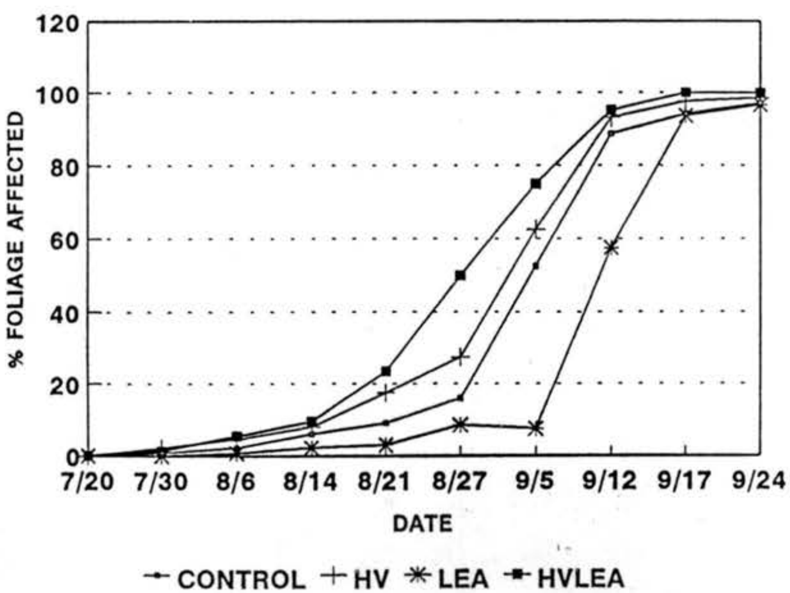


Figure 4.20D Optimum Moisture (HV + HEA)
1990

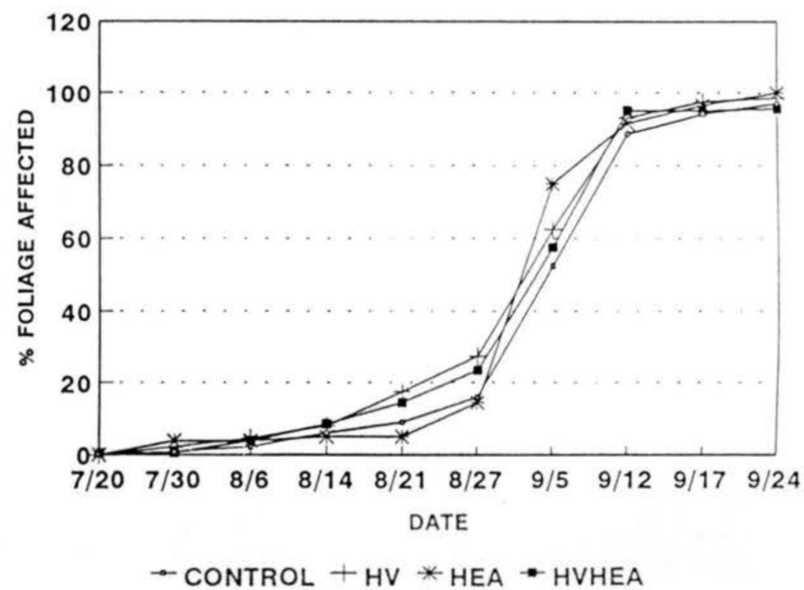


Figure 4.21A Excessive Moisture (LV + LEA)
1990

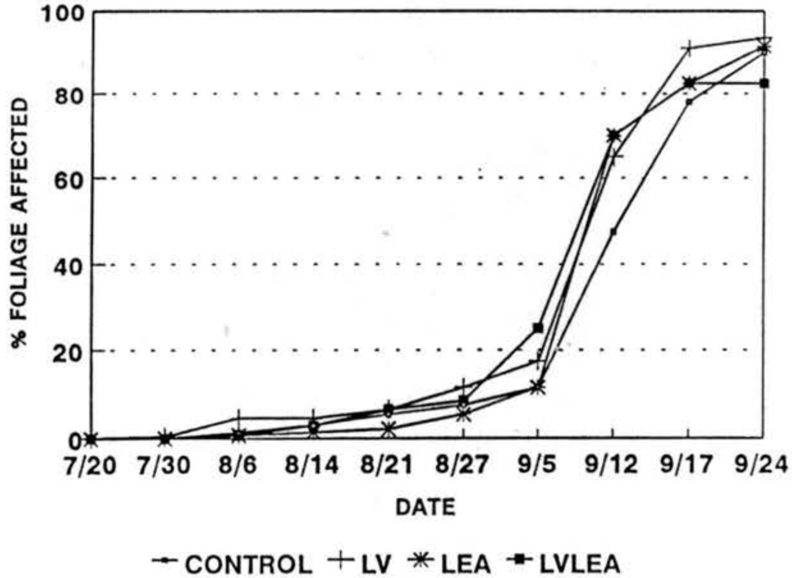


Figure 4.21B Excessive Moisture (LV + HEA)
1990

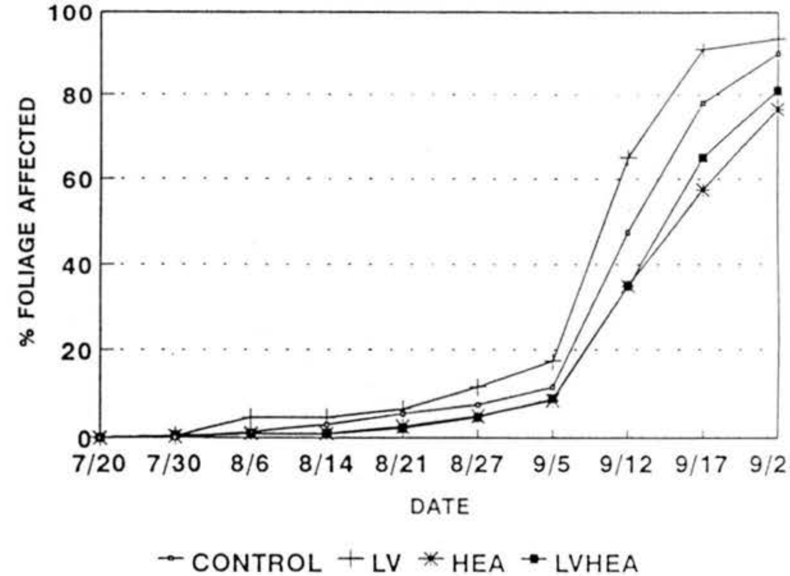


Figure 4.21C Excessive Moisture (HV + LEA)
1990

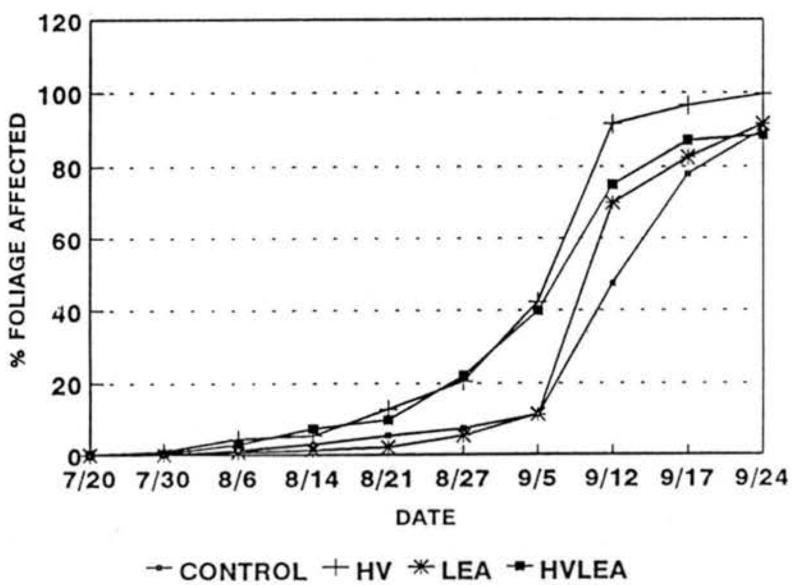


Figure 4.21D Excessive Moisture (HV + HEA)
1990

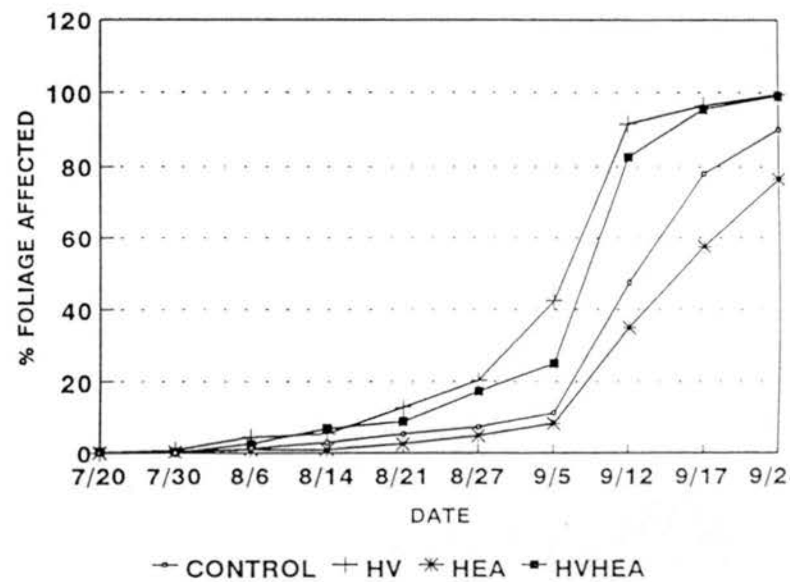


Figure 4.22A Deficient Moisture (LV + LEW)
1990

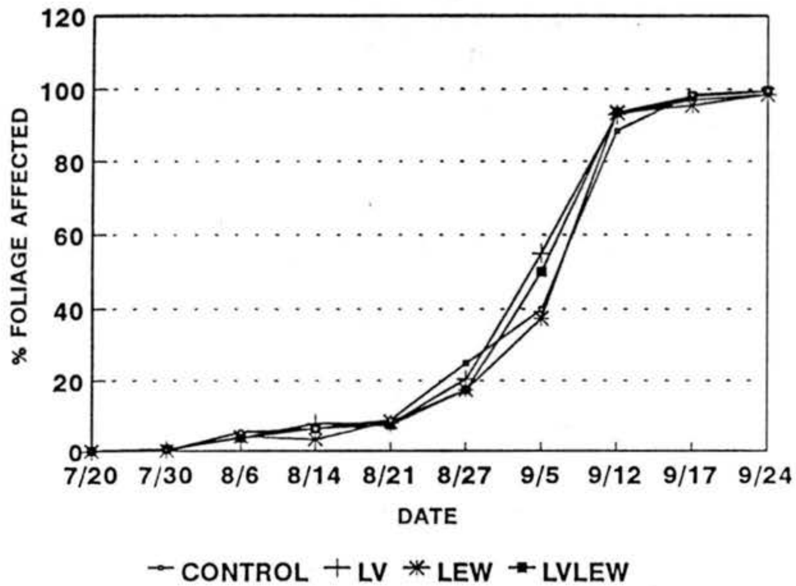


Figure 4.22B Deficient Moisture (LV + HEW)
1990

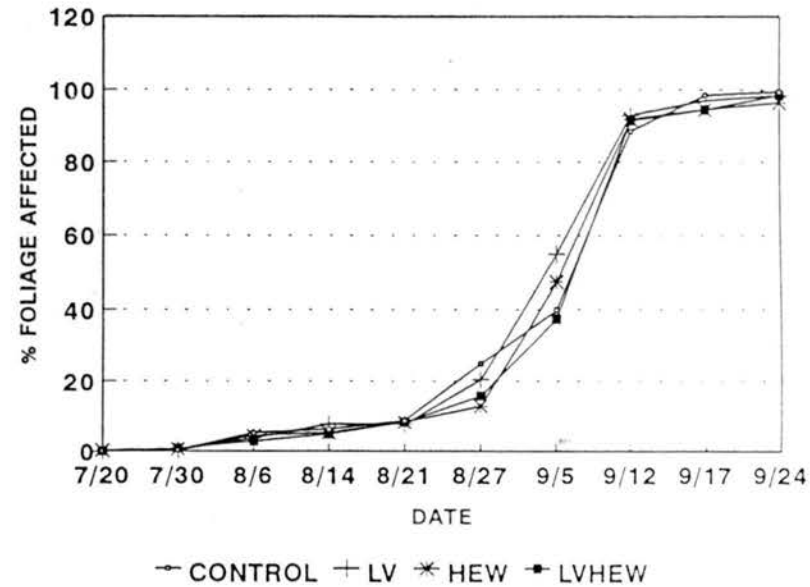


Figure 4.22C Deficient Moisture (HV + LEW)
1990

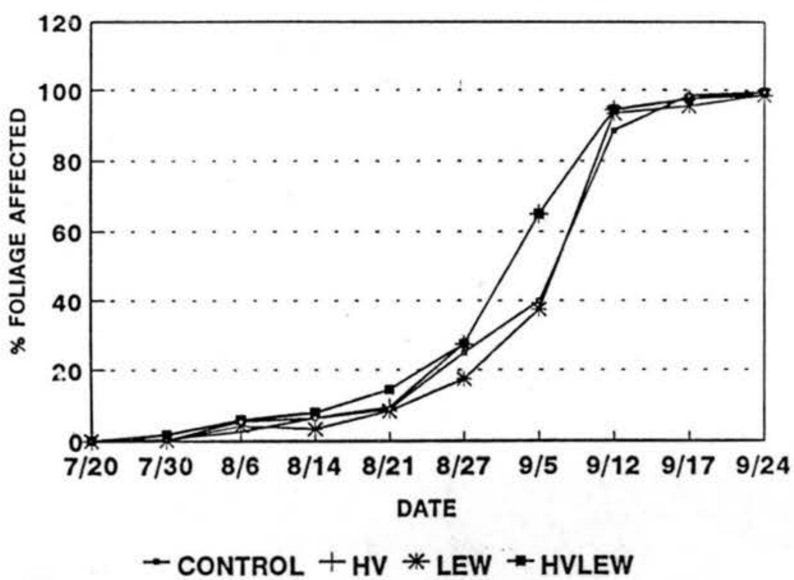


Figure 4.22D Deficient Moisture (HV + HEW)
1990

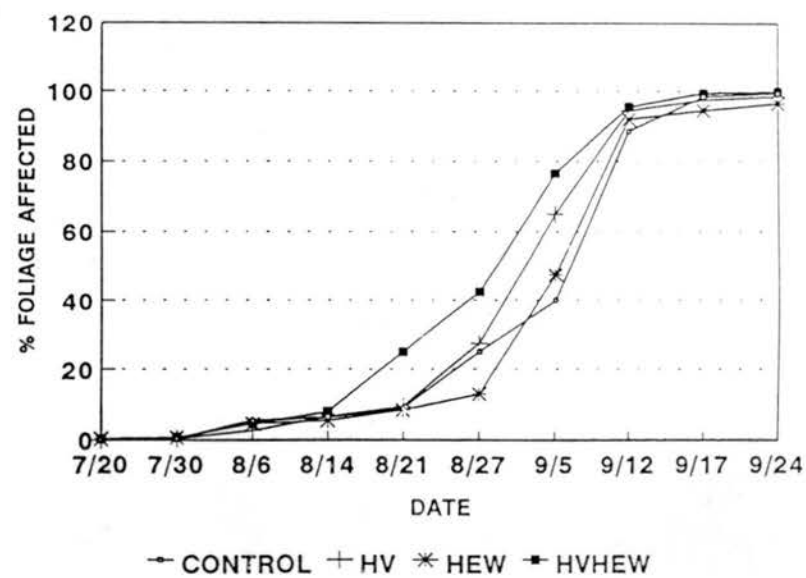


Figure 4.23A Optimum Moisture (LV + LEW)
1990

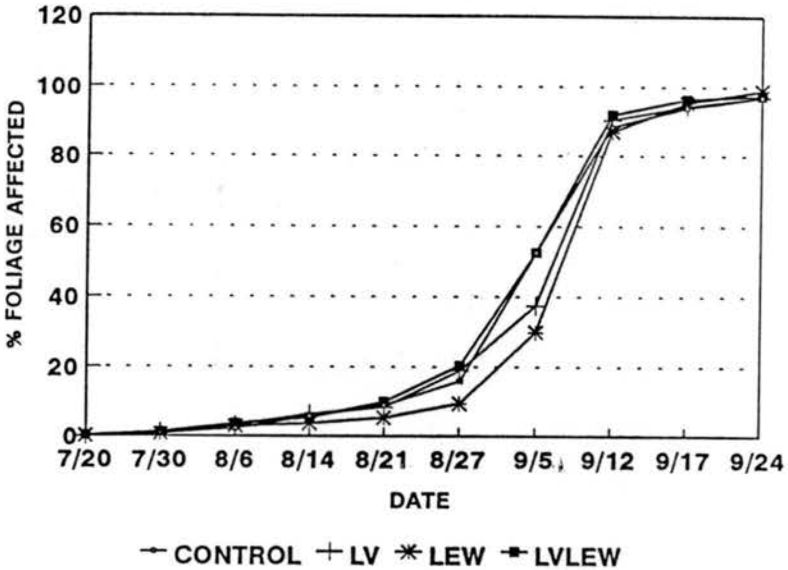


Figure 4.23B Optimum Moisture (LV + HEW)
1990

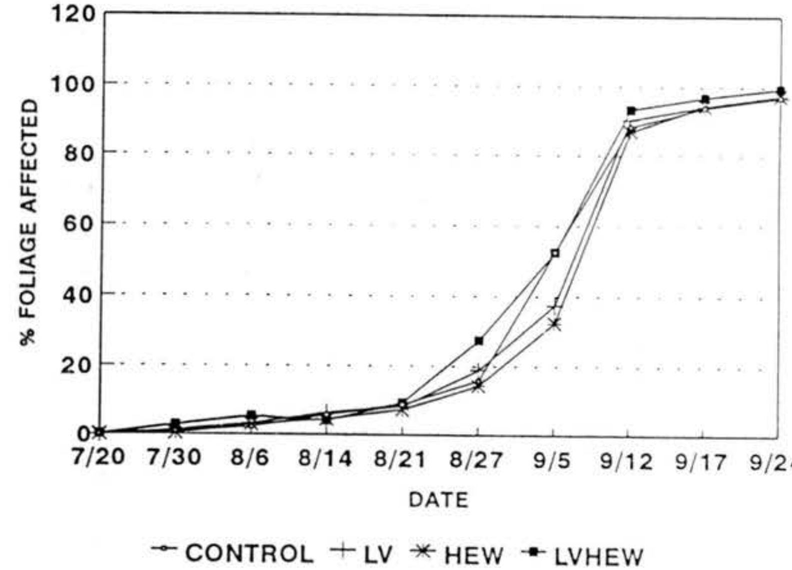


Figure 4.23C Optimum Moisture (HV + LEW)
1990

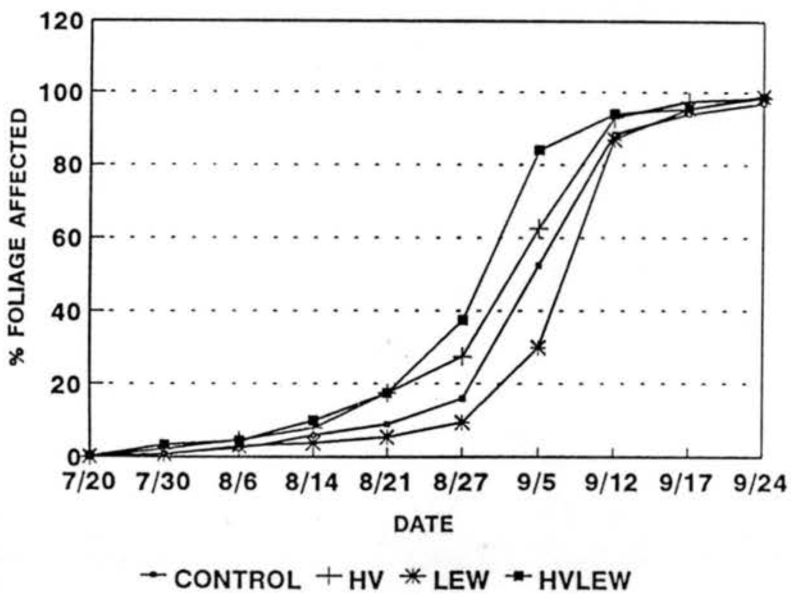


Figure 4.23D Optimum Moisture (HV + HEW)
1990

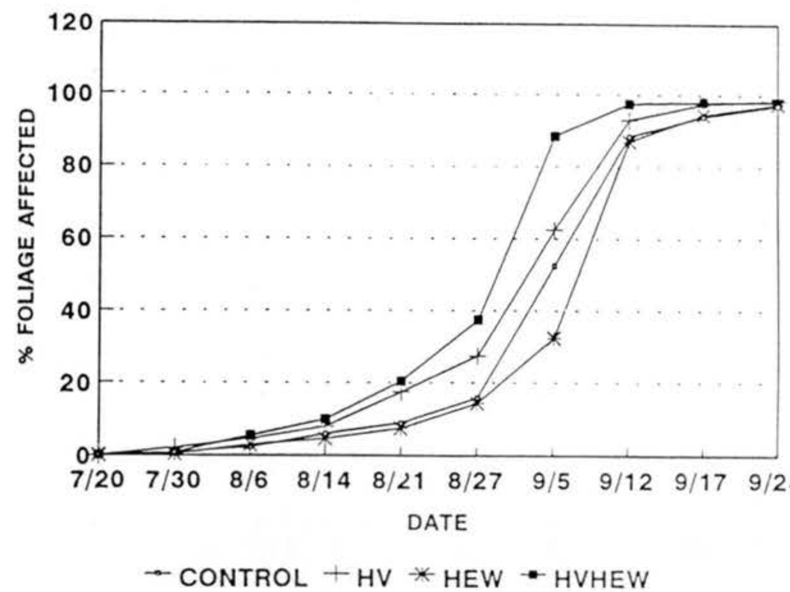


Figure 4.24A Excessive Moisture (LV + LEW)
1990

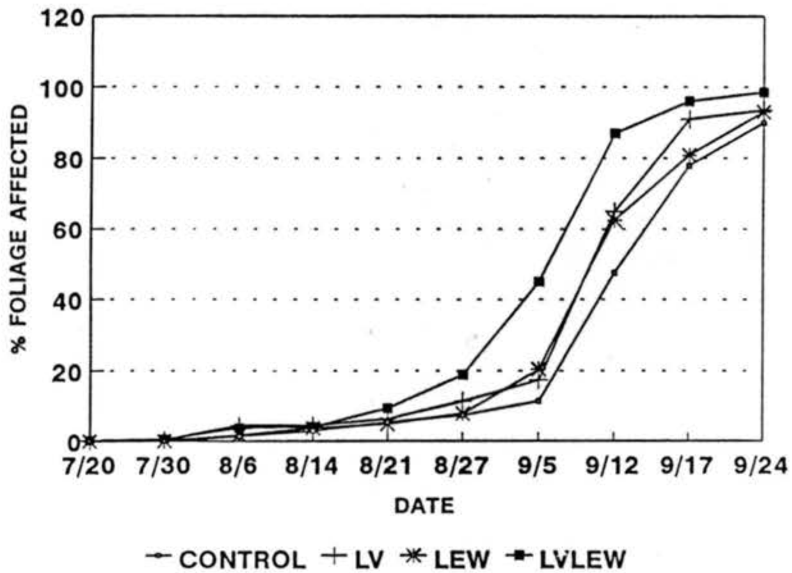


Figure 4.24B Excessive Moisture (LV + HEW)
1990

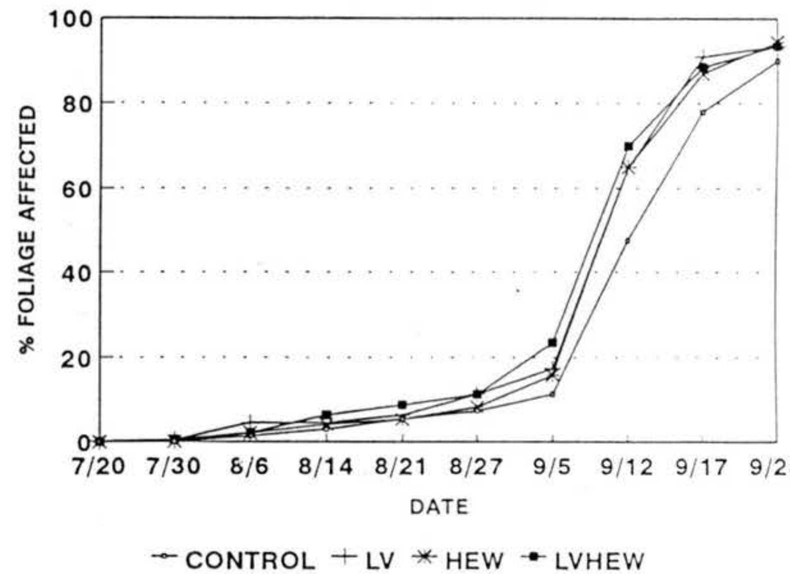


Figure 4.24C Excessive Moisture (HV + LEW)
1990

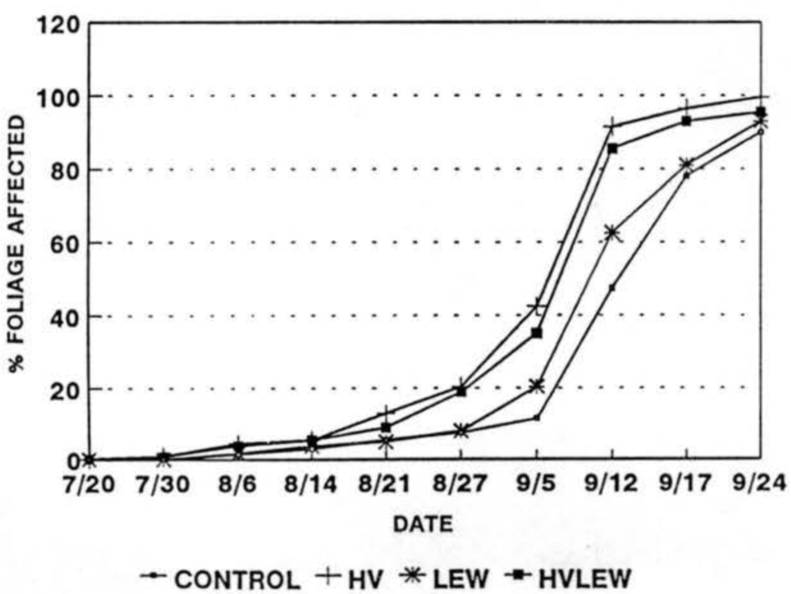


Figure 4.24D Excessive Moisture (HV + HEW)
1990

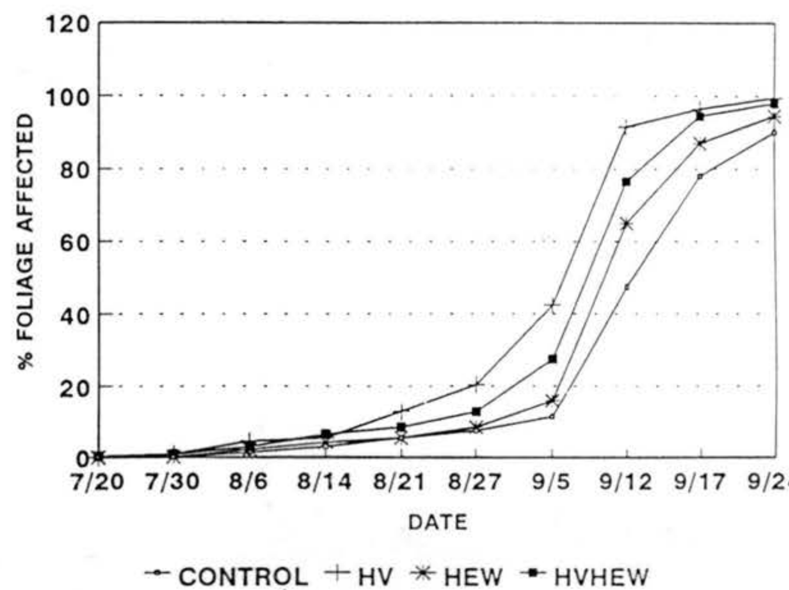
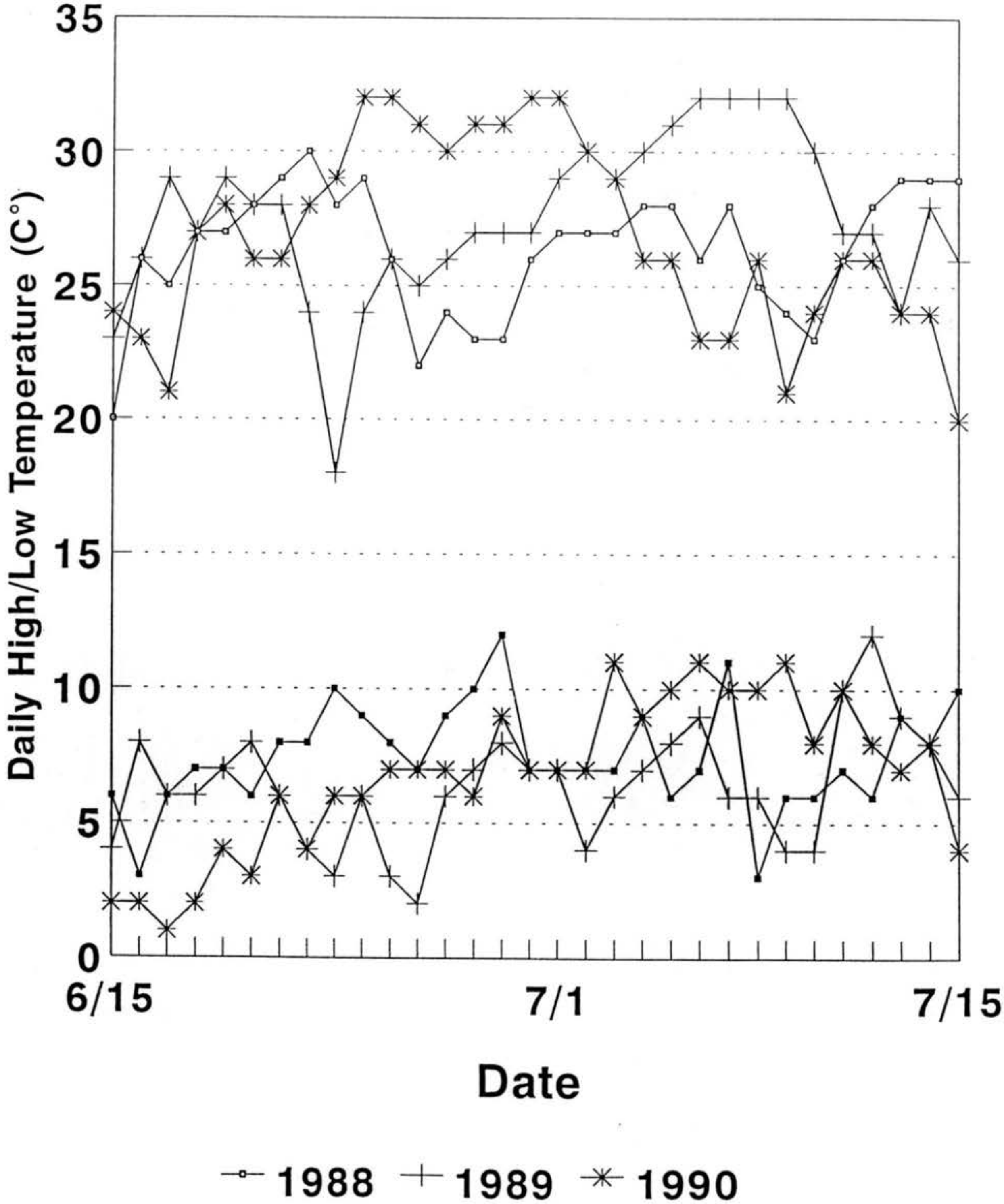


Figure 4.25
Early Season Weather Data for the San Luis Valley, Colorado



CHAPTER 5

EFFECT OF SUB-SYMPTOMATIC LEVELS OF ERWINIA CAROTOVORA SUBSP. CAROTOVORA AND E. C. SUBSP. ATROSEPTICA ON PRODUCTION OF RUSSET BURBANK POTATOES

Abstract:

Microplots planted with the cultivar Russet Burbank were utilized over a three year period, 1988-1990, to examine the effects of *Erwinia carotovora* subspp. on potato production in the San Luis Valley. Three levels of *Erwinia carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica* (Ecc and Eca, respectively) inoculum, zero, low and high were inoculated into pathogen-free seedpieces or introduced in irrigation water (Ecc only). Potatoes were planted into fumigated soil and grown under three irrigation regimes, optimum, deficient (1/2 optimum) and excessive (2X optimum).

Stands were significantly reduced in two of three years by both inoculum levels of Ecc and Eca when seedpieces were inoculated, but not in any of the years when Ecc was introduced in irrigation water. Plant heights were not affected by the treatments. Stand reduction was considered a symptom of blackleg, but foliar symptoms of potato blackleg were not seen in any of the three years. However, plants grown from inoculated seedpieces showed delayed maturity because of late emergence.

Both reductions and increases in tuber numbers per plant due to treatment were observed. There were no consistent trends from year to year. *Erwinia* seedpiece inoculations both increased and reduced per plant yields in two of three years and in one of the years the application of Ecc in irrigation water significantly decreased yield. There was a strong trend toward reduced per plant yields resulting from inoculation with *E.*

carotovora. Even more striking was the significant yield reduction evident with Ecc and Eca seedpiece inoculation treatments when overall total yields were compared. Yield reductions significantly different from the controls were not found in microplots which had Ecc applied in irrigation water.

Infection of daughter tubers by *Erwinia* was greatest under excessive and deficient moisture. Overall, the higher the inoculum level used, the higher the amount of daughter tuber infection. Daughter tuber contamination was greatest in the cooler seasons of 1988 and 1990. Infection by Ecc applied in irrigation water was very consistent over the three years of the project.

Introduction:

Potato blackleg caused by Erwinia carotovora subsp. carotovora (Ecc) and E. carotovora subsp. atroseptica (Eca) is a significant factor in production wherever potatoes are grown. This disease is among the most studied potato problems in modern plant pathology (17,32). Ecc, in particular, affects a wide range of crops and can be destructive. Worldwide the potato industry has taken major steps to control the effects of these pathogens by restructuring seed potato programs to utilize limited generation, tissue-culture based seed production with full disease testing of stocks prior to field production (23). This approach insures that basic seed stocks are Erwinia free. However, as stocks are grown in the field, re-infection by Erwinia carotovora subspp. can occur at any of several stages of production. Typically, infection of early generation stocks is latent until they have been grown one or more years in the field (personal experience). The inoculum source for recontamination may be soil, weed or other plant hosts (7,26), irrigation water (18,22,25), insects (17), infected seed potatoes (34) or other currently undefined sources.

The effect of sub-symptomatic levels of Erwinia carotovora subspp. on potatoes produced in the field is unclear. This research was designed to determine how low levels of Erwinia inoculated directly into seedpieces or applied to plants via irrigation water affect production of potatoes under various irrigation regimes.

Materials and Methods:

This study was done in conjunction with other research examining the role of Verticillium dahliae and its potential interaction with Erwinia carotovora, thus, materials and methods are similar to those described in Chapter 4 of this dissertation.

Plot Preparation: Microplots were established at the San Luis Valley Research Center in May of 1988, 1989 and 1990. Each year, prior to plot establishment, a composite soil sample was collected, a soil test completed upon this sample and fertilizer applications were modified based upon levels recommended for Russet Burbank commercial production in the San Luis Valley (35).

The field plot area was fumigated in April of each year with PicChlor 60[R] (60% chloropicrin/40% telone) injected into the soil to a depth of 60 cm at a rate of 393 kg/ha (273 l/ha). After fumigation, the soil was sealed by sprinkling with water to wet the soil to a depth of approximately two cm.

One week prior to planting, trenches 29 cm deep and 1.2 m wide were dug in the fumigated soil using a backhoe. Soil was piled along the edge of each trench for use as the planting medium in the microplots. Pots 30 cm high and 31 cm inside diameter with a 10 cm hole drilled in the center of the base to provide for water drainage were used as the microplots. Pots were arranged on 60 cm centers within the row and 92 cm centers between rows. Five trenches four m apart were cut in the plot and 3 rows of pots were set in each trench resulting in a total of fifteen replicated rows.

A drip irrigation system was installed in the plot. Pairs of individual pressure compensating emitters were placed in each pot to insure uniform water delivery. The irrigation system was designed to deliver water at optimum, excessive and deficient amounts for potatoes based on tensiometer and gypsum block readings, as well as, potato growth models for the San Luis Valley. The tensiometers and gypsum blocks were placed at several random locations throughout the plot at depths of 20 cm. Readings were taken three times per week. Irrigation rates used were 3.8 l/hr (optimum), 7.6 l/hr (excessive) and 1.9 l/hr (deficient). Microplots were irrigated with well water, tested previously and found to be free from <u>Erwinia carotovora</u>, on a one to four day schedule during the summer based upon readings obtained from the tensiometers, gypsum blocks and ET tables. The amount of water applied was that required to maintain the optimum moisture regime within the desired limits. Rainfall was factored into the irrigation regimes mentioned above. Fumigated soil sufficient to fill three microplots was mixed with preplant fertilizer by tumbling the soil in a cement mixer after addition of the fertilizer for 3 minutes. Mixed soil was put into the pots which were placed in appropriate locations in the trenches according to a plot diagram. After all pots were filled and placed, the remaining fumigated soil was used to fill in around the pots and level off the ground to within 6 cm of the top of the pots.

Inoculum Preparation: Erwinia carotovora inoculum consisted of the type strain of Erwinia carotovora subsp. atroseptica (Eca) and two strains of E. c. subsp. carotovora (Ecc) serogroup XVIII. All strains were isolated from the San Luis Valley from infected potato stems in 1987. Serogroup XVIII represents one of the predominant serogroups found in the San Luis Valley (8). Isolates were stored on CVP medium at 4^{0}C. Inoculum was increased by rinsing cells off the CVP medium, spread plating on nutrient agar and incubating 48 hours at 21^{0}C prior to seedpiece inoculation or application in the irrigation water. Inoculum dilutions were made by rinsing bacteria from the nutrient agar with sterile distilled water, centrifuging the suspension and resuspending the pellet in sterile distilled water. A spectrophotometer was used at $O.D._{640}$ to measure the absorption of the suspension and the bacterial population was adjusted to approximate a population x 10^{8} cfu/ml as derived from a standard curve developed by Aleck (2). After preparing appropriate dilutions, bacterial populations were confirmed by spread plating to nutrient agar at appropriate rates and the plates incubated at 21^{0}C for 72 hours before reading.

Both seedpiece inoculation and inoculation by applying Erwinia to plants in irrigation water were used to simulate known common re-introduction methods. Seedpieces were inoculated by placing 0.01 ml of an appropriate dilution of either Eca or Ecc in the eye next to a sprout and with a sterile toothpick pricking the tuber to allow bacteria to enter the tuber near the base of the sprout. The wound was sealed with vaseline. Uninoculated control tubers were handled in the same manner except sterile distilled water was allowed to enter the tuber instead of inoculum. Inoculum concentrations of 10^2 and 10^6 cfu per seedpiece were used for each subspecies.

Application of Ecc inoculum in irrigation water was accomplished by dispensing 1 ml of the appropriate dilution of Ecc into 250 ml of sterile water for the deficient water regime, 500 ml for the optimum regime and 1000 ml for the excessive regime and applying the suspension to the soil surface in the pots designated for these treatments. The amount of water applied represented what would be applied during a normal irrigation. For example, this resulted in the application of approximately 1.3 cm/ha (0.2 in/ac) of water for the optimum moisture regime. Inoculum concentrations of 10^2 and 10^6 cfu were used for each irrigation regime, but applied in a different amount of water. Uninoculated controls received the equivalent amount of water as inoculated plots, but without Ecc present. Ecc infested irrigation water was applied two or three times during the season beginning 40 days after planting (DAP) and continuing at two week intervals until 75 DAP.

Experimental Design: A completely randomized block with 15 microplots (replications) per treatment at each moisture level was used for the study. A total of seven treatments; Control, Low and High Ecc water inoculation, Low and High Ecc seed inoculation and Low and High Eca seed inoculation (1989 and 1990 only) were included. Each of the fifteen rows in the plot included a complete set of treatments and comprised one replication.

Generation 1 seed tubers (cv. Russet Burbank) from the Oregon State University Foundation Seed project in 1988 and 1989 and from the Worley Seed Company in Colorado in 1990 were cut into single eye seedpieces two weeks prior to planting with a 2.5 cm melon ball scoop disinfested in 10% chlorox between each cut. These were placed in chambers exposed to light at 21^0C and high relative humidity for seven days to aid suberization and germination. The pre-sprouted seedpieces were treated (inoculated or not depending upon the treatment) and planted in the soil at the center of each of the appropriate microplots to a depth of 6 cm.

Beginning at planting and continuing through the growing season, air and soil temperatures were recorded at 15 to 30 minute intervals using a CR21X micrologger[5]. Soil temperatures were taken using small thermocoupler, "electrode type", sensors from one representative pot chosen at random in each irrigation regime at a depth of 10 cm.

Soil in the microplots was used to determine populations of Erwinia carotovora. Assays of Erwinia populations in soil and irrigation water were done prior to planting in 1988. For the soil assay, samples were obtained by removing the top 15 cm of soil at several random locations within the plot area and collating into six sets representing twenty grams of air dried soil each. Each twenty gm mixed sample was suspended in PT medium (a pectate based enrichment medium (7)), incubated anaerobically in light for 72 hours at 21^0C and plated in duplicate on CVP medium. The CVP plates were incubated in a manner similar to the PT medium and, after 72 hours, examined for the presence of Erwinia spp.. Water samples were assayed using CVP medium (10) and the techniques described by Jorge and Harrison (22).

Plants were monitored for the presence of blackleg symptoms throughout the season. At harvest, the actual number of tubers per plant plus total yield weight per plant were recorded. Two randomly selected tubers per treatment from each of three replications in the plot were collected at harvest time and assayed for Erwinia contamination using a toothpick puncture/wrap test. This consisted of puncturing each tuber up to 50 times with a sterile toothpick, wrapping the wounded tuber in a wet paper towel and covering this in saran wrap to make the sample air tight.

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[5]Ibid

Samples were incubated for 72 hours at 21^{0}C and presence or absence of softrot symptoms were recorded. Tissue from rotting areas was suspended in sterile distilled water and streaked onto CVP medium to verify the presence of Erwinia carotovora.

Results:

When Erwinia was introduced in irrigation water to plants during the three years of this experiment, there were no significant (P=0.05) effects on stand (Table 5.1 and Appendix-Figures 3.1 and 3.2), plant height, disease progress over the growing season, tuber number per plant (Table 5.2), yield (Tables 5.3 and 5.5) and daughter tuber infection (Table 5.4). The only significant, measurable effect was a decreased yield per plant in 1990 under the optimum irrigation regime when the highest level of Ecc inoculum was used. During this season a temporary, visible plant wilt was evident soon after the second and third applications of contaminated water.

When seedpieces were inoculated, stand was significantly reduced in two of three years by both inoculum levels of Ecc and Eca (Table 5.1). In years when stand reductions occurred there was a trend toward increased stand loss as the amount of irrigation water increased.

No significant differences in plant height were found during any of the three seasons. The rate of disease progress in plants grown from Erwinia inoculated seedpieces did not differ significantly from the normal maturation of controls. However, at the time of the final reading (100 - 114 DAP), these plants had vines which appeared more immature than those of the controls.

Tuber numbers per plant varied significantly among treatments and from year to year (Table 5.2). In 1988 tuber numbers were significantly reduced by Erwinia inoculum under both the deficient and excessive irrigation regimes, but were significantly increased under the optimum regime. In 1989 the only significant effect was a reduction in numbers of tubers per plant under the highest Eca inoculum level when excessively irrigated. In the third year of the study, 1990, Ecc inoculation produced significant increases in tuber numbers per plant under the deficient irrigation regime and decreased tuber numbers under the optimum irrigation regime.

Under optimum irrigation, a significant yield depression (P=0.05) was observed at: a) both the low and high Ecc and the high Eca seed treatments in 1990 and b) at the low Ecc seed treatment in 1989. Under excessive moisture a significant yield reduction by the high Eca seed was found in 1989. No other treatment significantly affected yields, but a trend toward yield reduction was evident. When all non-significant treatments were compared across the three years, yield was 17 percent lower than non-inoculated controls. Significant reductions in total yield, representing all microplots within a given replication and treatment, as affected by treatment were found in two of three years under all irrigation regimes (Table 5.5). Yield effects occurred only in 1989 and 1990 which were the warmer of the three years of the study. In 1988, a relatively cool season, no significant yield effects were found.

Daughter tuber infection was inconsistent from year to year and among treatments. Although there were no significant differences among treatments in any of the three years, there was a tendency for the highest amount of daughter tuber infection to occur at the highest Ecc and Eca inoculum levels. The amount of daughter tuber infection under the optimum irrigation regime was always equal to or lower than that found under the deficient and excessive irrigation regimes. Infection under plants irrigated with water containing Ecc was consistent over the three years of the project.

Table 5.1 **Effect of Three Levels of Erwinia carotovora Inoculum Present on Seed Pieces or in Irrigation Water on Percent Plant Stand Under Three Irrigation Regimes.**

	Irrigation Regime								
	1988			1989			1990		
Treatment	**DEF**[1]	**OPT**	**EXC**	**DEF**	**OPT**	**EXC**	**DEF**	**OPT**	**EXC**
	Percent Plant Stand[2]								
Control[3]	100	93	93	100	100	100	100	100	100
Low ECC W[4]	100	93	100	93	100	100	100	100	100
High ECC W	100	100	100	100	93	100	100	100	100
Low ECC S	100	93	100	53 *	27 *	40 *	33 *	40 *	53 *
High ECC S	87	93	93	40 *	33 *	27 *	33 *	67 *	27 *
Low ECA S	---	---	---	73 *	73 *	53 *	73 *	40 *	53 *
High ECA S	---	---	---	100	80 *	60 *	27 *	20 *	53 *

1/ DEF - deficient, OPT - optimum and EXC - excessive
2/ Stands represent mean percentage of plants emerged in 15 replications per treatment. Final stand counts taken 45 DAP.
3/ Control (0 cfu), Low (10^2 cfu) and High (10^6 cfu) Erwinia
4/ W = Irrigation inoculation and S = Seedpiece inoculation

Numbers followed by a * are significantly different from the control at P=0.05. Analysis conducted between control and each set of treatments only, not between Erwinia treatments.

Table 5.2 Effect of Three Levels of Erwinia carotovora Inoculum Present on Seed Pieces or in Irrigation Water on Tuber Number per Plant Under Three Irrigation Regimes.

	Irrigation Regime								
	1988			1989			1990		
Treatment	**DEF**[1]	**OPT**	**EXC**	**DEF**	**OPT**	**EXC**	**DEF**	**OPT**	**EXC**
	Mean Number of Tubers/Plant								
Control[2]	7.7	6.1	6.6	5.9	7.6	9.0	7.2	10.9	10.5
Low ECC W[3]	6.3	6.6	5.5	6.9	7.6	8.6	8.9	9.1	10.7
High ECC W	6.2	6.7	6.9	6.7	6.8	8.7	8.4	10.8	12.3
Low ECC S	5.6 *	6.3	5.8	5.4	5.5	11.2	11.8 *	7.6	9.0
High ECC S	6.2	7.9 *	5.1 *	4.5	5.2	5.0	9.8	7.7 *	8.8
Low ECA S	---	---	---	5.9	5.4	7.5	6.0	8.2	7.3
High ECA S	---	---	---	4.0	5.7	5.6 *	8.3	7.7	8.6

1/ DEF - deficient, OPT - optimum and EXC - excessive
2/ Control (0 cfu), Low (10^2 cfu) and High (10^6 cfu) Erwinia
3/ W = Irrigation inoculation and S = Seedpiece inoculation

Numbers followed by a * are significantly different from the control at P=0.05. Analysis conducted between control and each set of treatments only, not between Erwinia treatments.

Table 5.3 **Effect of Three Levels of *Erwinia carotovora* Inoculum Present on Seed Pieces or in Irrigation Water on Individual Plant Yield Under Three Irrigation Regimes.**

	Irrigation Regime								
		1988			1989			1990	
Treatment	**DEF**[1]	**OPT**	**EXC**	**DEF**	**OPT**	**EXC**	**DEF**	**OPT**	**EXC**
					Mean Yield (gm/plant)				
Control[2]	847	626	506	765	992	1588	426	739	1013
Low ECC W[3]	893	684	505	680	874	1775	420	632	1016
High ECC W	829	660	524	753	868	1654	345	523 *	1158
Low ECC S	766	649	451	873	386 *	1867	789 *	399 *	822
High ECC S	768	704	399	454	608	1270	227	481 *	692
Low ECA S	---	---	---	462	680	1191	408	620	850
High ECA S	---	---	---	505	627	983 *	242	363 *	754

1/ DEF - deficient, OPT - optimum and EXC - excessive
2/ Control (0 cfu), Low (10^2 cfu) and High (10^6 cfu) *Erwinia*
3/ W = Irrigation inoculation and S = Seedpiece inoculation

Numbers followed by a * are significantly different from the control at P=0.05. Analysis conducted between control and each set of treatments only, not between *Erwinia* treatments.

Table 5.4 **Effect of Three Levels of Erwinia carotovora Inoculum Present on Seed Pieces or in Irrigation Water on Daughter Tuber Infection Under Three Irrigation Regimes.**

	Irrigation Regime								
	1988			1989			1990		
Treatment	**DEF**[1]	**OPT**	**EXC**	**DEF**	**OPT**	**EXC**	**DEF**	**OPT**	**EXC**
	Soft Rot Potential Index[2]								
Control[3]	0.02	0	0	0	0	0	0.13	0	0
Low ECC W[4]	0.03	0.06	0.06	0.50	0	0	0.12	0.02	0.15
High ECC W	0.04	0.03	0.03	0.31	0	0.50	0.38	0.08	0.15
Low ECC S	0.05	0.08	0.04	0	0	0	0.04	0	0
High ECC S	0.07	0.03	0.14	0	0	0	0.58	0.08	0
Low ECA S	----	----	----	0	0	0	0.05	0.08	0
High ECA S	----	----	----	0	0	0.53	0.60	0	0.22

1/ DEF - deficient, OPT - optimum and EXC - excessive
2/ Data collected on three tubers per replication per treatment using an average of four replications. Soft rot potential index derived by multiplying the number of tubers rotted x area rotted divided by total number of tubers x 100
3/ Control (0 cfu), Low (10^2 cfu) and High (10^6 cfu) Erwinia
4/ W = Irrigation inoculation and S = Seedpiece inoculation

Numbers followed by a * are significantly different from the control at P=0.05. Analysis conducted between control and each set of treatments only, not between Erwinia treatments.

Table 5.5 **Effect of Three Levels of Erwinia carotovora Inoculum Present on Seed Pieces or in Irrigation Water on Total Yields per Treatment Under Three Irrigation Regimes.**

	Irrigation Regime								
	1988			1989			1990		
Treatment	DEF[1]	OPT	EXC	DEF	OPT	EXC	DEF	OPT	EXC
	Mean Total Yield per Plant (gm)								
Control[2]	12705	8232	7084	11475	14880	23820	6390	11085	15195
Low ECC W[3]	13395	9576	7575	9520	13110	26625	6300	9480	15240
High ECC W	12435	9900	7860	11295	12152	24810	5175	7845	17370
Low ECC S	11490	9086	6765	6984	1544*	11202*	3800*	2394*	6576*
High ECC S	9984	9856	5586	2724*	3040*	5080*	1135*	4810*	2768*
Low ECA S	----	----	----	5082*	7480*	9528*	4488	3720*	6856*
High ECA S	----	----	----	7575	7524*	8847*	968*	1089*	6032*

1/ DEF - deficient, OPT - optimum and EXC - excessive
2/ Control (0 cfu), Low (10^2 cfu) & High (10^6 cfu) Erwinia
3/ W = Irrigation inoculation and S = Seedpiece inoculation

Numbers followed by a * are significantly different from the control at P=0.05. Analysis conducted between control and each set of treatments only, not between Erwinia treatments.

Discussion:

Most North American certified seed potato programs have some of their seed stocks derived from limited generation, tissue-culture based sources of potatoes which are fully tested for pathogens prior to release and use for grower production. In many programs all seed stocks are derived from such sources. One of the major reasons for approaching seed production in this manner is to eliminate the threat from two subspecies of Erwinia carotovora, the causal agents of blackleg. In addition, most seed programs have instituted much more restrictive tolerances for blackleg in seed lots grown in the first few field generations to control the re-introduction of the pathogen.

There has been much discussion about the wisdom of this approach. It is known that visual detection of blackleg is at best imprecise and often misleading as a means to determine the actual amount of infection present. Most programs have, however, accepted this as a reasonable way to evaluate recontamination of clean stocks with Erwinia carotovora and to prevent further spread of the pathogen. Little is known, however, about the role of sub-symptomatic levels of Erwinia infection in seed stocks.

Results from this study show that stand loss may be the only visible field symptom of re-infection of seed tubers by Erwinia carotovora. Often, this is insufficient to arouse suspicions that re-infection may have occurred, especially when stand loss is slight. Furthermore, stand loss does not occur when the source of Erwinia inoculum for re-infection is the irrigation water.

Environment and amount of irrigation also play major roles in determining the potential for stand loss due to Erwinia. In the two years of this study when warm soil conditions prevailed (up to 30^0C) and excessive irrigation was practiced, stand losses were the greatest. This follows closely the reports by Aleck and Harrison (3) that as inoculum density is increased, under high soil moisture and high temperature conditions, pre-emergence tuber and shoot decay due to E. carotovora increased. Stand loss, by itself, can be a significant factor in yield reduction. Difficulty in determining whether blank areas in the seed bed are the result of eyeless seedpieces, seedpieces decayed by organisms other than Erwinia or actual skips from planter error compound the difficulty in verifying that Erwinia is actually recontaminating seed stocks. Because it is so hard to detect the exact point when Erwinia recontaminates the seed, especially if the only visual clues are stand loss, growers may not realize the potential for production loss until after it has occurred.

During three growing seasons in this study, seedpieces inoculated with Ecc and Eca never produced plants with blackleg symptoms yet, tuber numbers and yields were significantly affected under certain irrigation regimes. Tuber numbers of plants grown from inoculated seedpieces were generally decreased during this study with four cases having significantly reduced numbers. However, in two cases, high Ecc seed inoculation under optimum moisture (1988) and low Ecc seed inoculation under deficient moisture (1990), tuber numbers were significantly increased. Fluctuation in tuber numbers may have been a function of the disease process, that is, seedpiece decay or reduction in root mass. These in turn could have had an effect on root/stolon development in plants where Erwinia infection occurred. More likely, a mixture of environmental factors including over or under-watering of plants may have caused the fluctuations. In any case, the number of tubers produced per plant did not seem to indicate consistently an effect from inoculation with Erwinia.

Mean yields per plant in microplots planted with Erwinia inoculated seedpieces were significantly lower in five treatments during the two warmer years (1989 and 1990) of the study, but never during the coolest season (1988). Neither irrigation regime nor inoculum density had a noticeable effect on yields. Four of the five treatments where significant yield reductions occurred were under the optimum irrigation regime and the other under the excessive regime. As for inoculum density, two of the same five treatments were the low inoculum density while the other three were the high inoculum level. Also, yield reductions were comparable for both Ecc and Eca inoculations.

Plants contaminated by Ecc applied in irrigation water had significantly reduced yield per plant (P=0.05) in only one of three years and then only when the highest inoculum density was applied under the optimum irrigation regime. This was also the only treatment which produced visible disease symptoms which consisted of a temporary plant wilt evident within one week after inoculation. While recontamination of plants by irrigation water infested with Ecc may only cause significant yield reduction under optimum environmental and moisture conditions, it may act as an important source of Erwinia recontamination for daughter tubers since Ecc is widespread in surface water (8,18,22).

Total yield loss, resulting from both stand loss and effects of non-symptomatic infections of plants by Erwinia during the growing season was even more striking. During the coolest season (1988), no significant yield reductions were found. However, in the two warmer seasons (1989 and 1990), yield depressions of up to 90 percent were found when seedpieces were inoculated with Erwinia at either low or high inoculum levels. There was no clear relationship between inoculum density and yield. The lowest yields in four of twelve treatments were produced by microplots containing seedpieces with the low inoculum level and in eight of twelve treatments by microplots with the high inoculum level. There was also no clear difference among irrigation regimes. It is important to note that an inoculum level of 10^2 cfu of either Ecc or Eca per seedpiece (the lowest level used in this study) reduced significantly total yields by an average of 53 percent (Eca) to 60 percent (Ecc). In addition, total yield compared with uninoculated controls was decreased by the higher inoculum level by an average of 63 percent (Eca) to 76 percent (Ecc).

The extent of daughter tuber re-infection by Ecc and Eca was inconsistent, but re-infection generally occurred whenever bacteria were present. During the coolest growing season (1988), all Erwinia inoculations produced some re-infection of daughter tubers. This suggests that spread to daughter tubers from infected seedpieces and/or contaminated irrigation water can be a source of recontamination by E. carotovora. During the warmest growing season (1989), daughter tuber re-infection from either source of inoculum occurred only under the deficient or excessive moisture regimes and only in four of twelve treatments. This might be the result of the seedpiece rotting too quickly to provide the levels of Erwinia necessary for daughter tuber contamination or a function of solarization of the soil killing off or limiting the spread of bacteria down the stolons. In the year between the two temperature extremes (1990), daughter tuber re-infection was reasonably consistent under all irrigation regimes with some spread occurring in thirteen of eighteen treatments. Overall, as inoculum density increased, the amount of re-infection also increased. Again, these results track well with other reports in the literature which indicate that cool, moist conditions favor daughter tuber contamination from infected seedpieces or plants (12,17).

The primary conclusion from this work is that significant yield loss on a per plant basis due to sub-symptomatic levels of E. carotovora can occur under a variety of irrigation regimes and inoculum densities. This in itself should justify the use of tissue-culture based programs to eliminate the organism from seed stocks. But, in addition, it is evident that certification programs need to implement additional screening programs beyond routine field inspection to detect non-symptomatic, but potentially damaging Erwinia re-infections, especially when significant stand loss occurs. All Erwinia inoculations did not significantly reduce yields. However, there was a clear trend toward decreased yields per plant by both the low and high inoculum densities with the greatest loss occurring under the highest inoculum levels. Considering yield losses, both per plant and total (including stand loss), found in this study under the lowest inoculum level, it is interesting to speculate about the potential loss under San Luis Valley conditions with even lower inoculum levels. This could have significant ramifications for seed stocks recontaminated with low levels of Erwinia and grown in a season when optimal environmental conditions for disease expression occur. In addition, low levels of inoculum should have even more impact in growing areas with both warmer seasons and higher precipitation than usually occurs in the San Luis Valley. Under these conditions which would not be as favorable for reducing blackleg expression, especially seedpiece decay, a much greater loss could be associated with Erwinia infection.

These results should impress upon all growers and certification personnel that even a small amount of E. carotovora recontamination, which may not produce visible symptoms on infected plants, can produce the potential for large production/storage losses. Thus, sanitation and proper disinfestation at each point in production where potential for the spread of Erwinia may occur are critical!

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Figure 5.1 Mean Plant Stand

1988

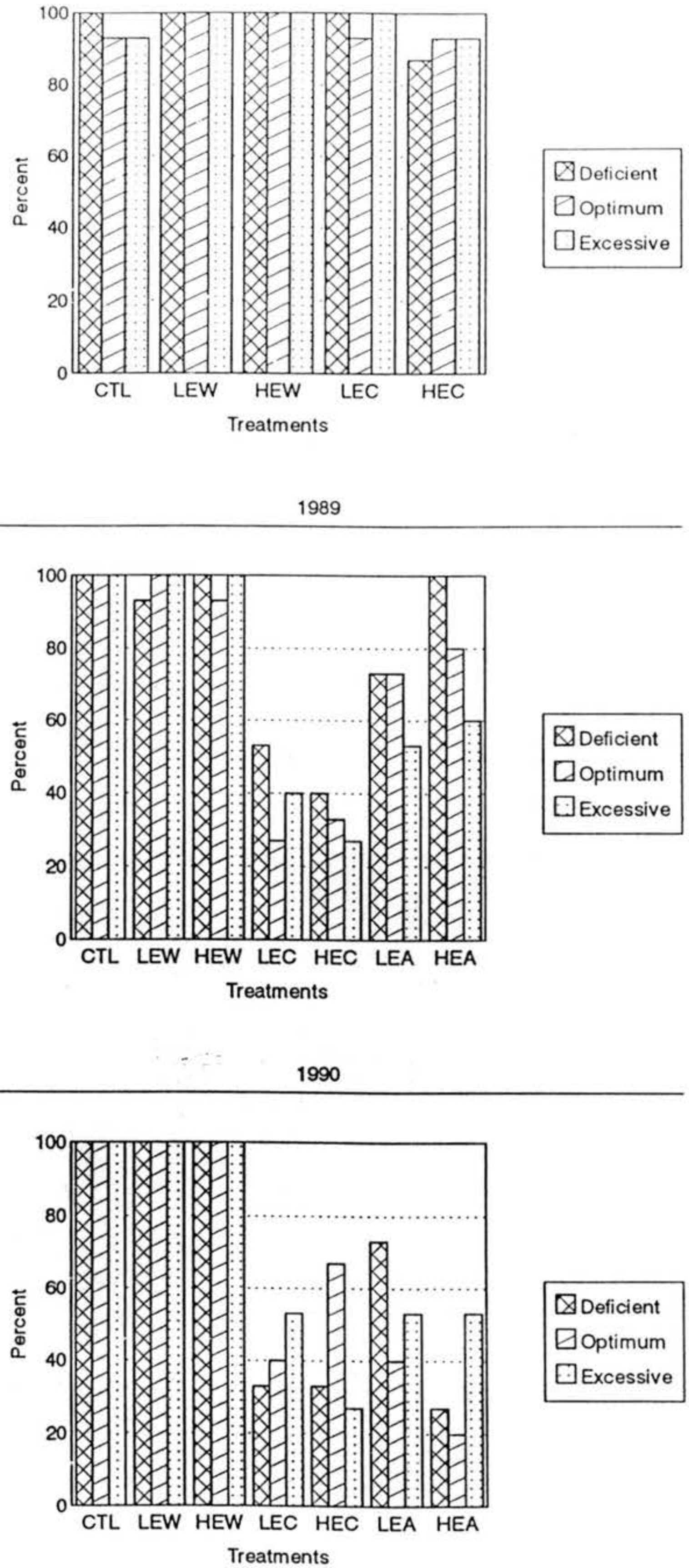
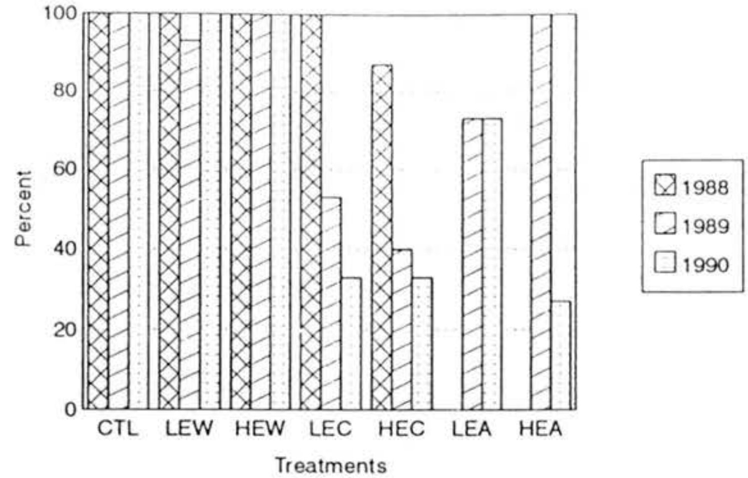
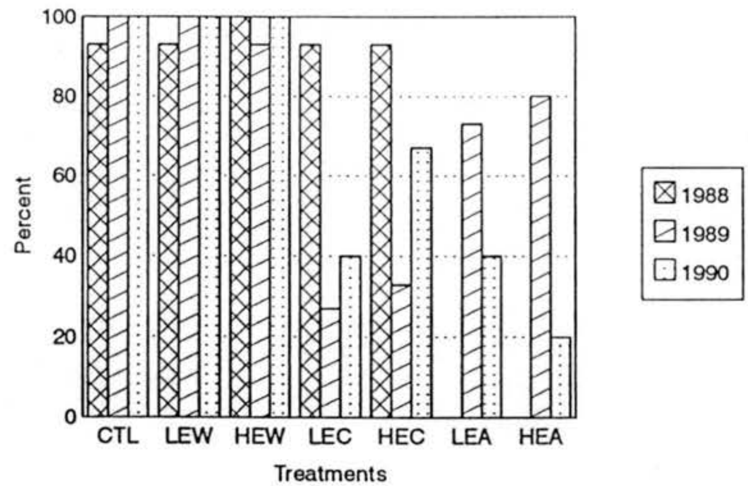


Figure 5.2 Mean Plant Stand

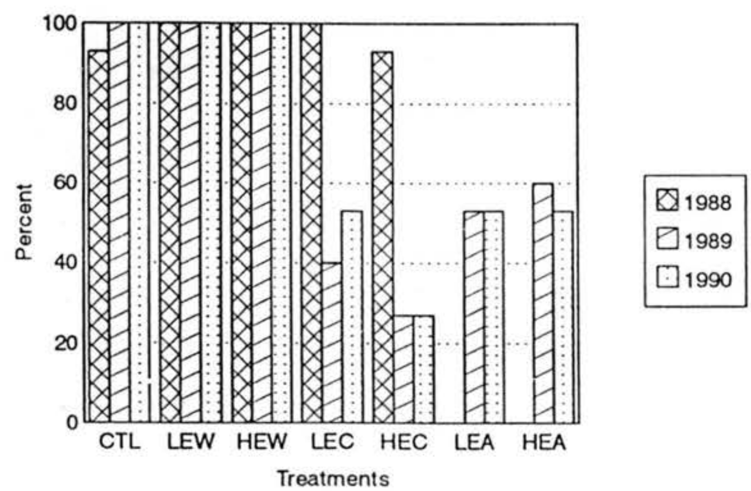
Deficient Moisture



Optimum Moisture



Excessive Moisture



CONCLUSION

Potato early dying (PED) caused by Verticillium dahliae (V) is responsible for significant yield reductions in potatoes in most production areas in North America. However, in the San Luis Valley, Colorado, the major potato production area in the state, this disease is rarely significant. Environmental factors such as air temperature, irrigation regime and density of Verticillium inoculum play a role in disease expression. A portion of this research was directed toward trying to clarify the relationship between these environmental factors and PED disease expression under growing conditions found in the San Luis Valley.

Another portion of this researh examined the role of other pathogens in the PED complex. Many researchers have documented the interactive effect on potatoes of other pathogens including nematodes, fungi and bacteria when in combination with Verticillium. However, little field research has been conducted using Erwinia carotovora, one of the main disease concerns in Colorado, in combination with Verticillium.

The last portion of this research was directed toward more fully understanding the role of differing inoculum levels of Erwinia carotovora subspp. and their effect on potatoes under cool season growing conditions. Blackleg of potato is considered to be one of the primary diseases of concern in the San Luis Valley. Overall losses from this disease are substantial and appear to be greatly influenced by the environmental conditions found in this production area.

A three year comparison of Russet Burbank potatoes grown in microplots containing combinations of three levels of Verticillium inoculum (zero, low and high), three levels of Erwinia carotovora subsp. carotovora (Ecc) and E. c. subsp. atroseptica (Eca) (zero, low and high) inoculated into the seed and, in the case of Ecc, also applied in the irrigation water was conducted. Each of the treatments was placed under one of three levels of irrigation (deficient, optimum and excessive). Each year of the study had slightly different environmental conditions. One of the three years was relatively cool while the other two years were relatively warm by San Luis Valley standards.

Verticillium appeared to have little impact on potatoes. Plant stand and height were unaffected by treatment. Verticillium wilt or PED disease progression was similar among treatments. In general, plants grown in microplots with Verticillium infested soil demonstrated higher levels of PED expression by the end of the season than normal senescence shown by the negative controls. There were no significant effects on tuber numbers and per plant yield was significantly depressed in only one of three years under deficient moisture and the highest Verticillium inoculum density. Irrigation regime affected symptom expression with deficient moisture demonstrating a significant increase in PED symptoms in the two warmest years for both low and high Verticillium inoculum densities. In the coolest year there were no effects due to irrigation. Per plant yield was also affected by irrigation regime. Again the two warmest years showed similar results with yields increasing as irrigation increased. In the coolest year, the reverse was true as yields increased with decreasing irrigation. This indicates that under cool environmental conditions the effect from Verticillium infections might outweigh the benefits of increasing irrigation, especially since excessive irrigation has been shown to increase damage by Verticillium (Cappaert *et al.*, 21).

Greenhouse studies completed in 1989 showed that air temperature can play a major role in PED symptom development. Specialized chambers were used which were held at three different air temperatures representing the mean San Luis Valley temperature (25^0C), mean "low" northern Colorado temperature (15^0C) and mean "high" northern Colorado temperature (30^0C). Verticillium treatments were similar to those used in the field studies. Stand was unaffected by temperature. Plant height increased as temperature increased, but there were no significant differences observed within each temperature regime. PED symptom progression was fastest under the highest temperature (30^0C), but did not reach the same level of severity as was found under the two lower temperatures. PED severity was greatest under the 25°C temperature. There were indications that plant physiological maturity was accelerated under the 30°C regime accounting for the increased rate of PED progression and peaking of symptoms prior to the two lower temperature regimes. Visible PED symptoms were almost non-existent in the lowest temperature regime. At the two higher temperature regimes, PED symptoms were significantly greater in plants grown in Verticillium-infested soil than in the controls. There were no significant differences evident in tuber number or yields among treatments within a given temperature regime.

The effect of soil fumigation to reduce Verticillium inoculum densities was examined on four fields in the San Luis Valley. Soil fumigation resulted in reductions in Verticillium microsclerotial counts which were maintained for at least two further growing seasons, even after growth of a cultivar susceptible to Verticillium wilt. However, unfumigated soil also showed reductions in microsclerotial counts during the same time period. This might have been the result of natural environmental factors found in the San Luis Valley or crop rotation. Do the factors resulting in microsclerotial reductions in the unfumigated soil have the potential for consistent control of Verticillium soil borne inoculum? Soil fumigation does appear to be effective, however, there are questions about its cost effectiveness, particularly when considering the role of other factors in inoculum reduction coupled with cool season growing conditions.

When examining the PED complex in the San Luis Valley, Ecc and Eca appeared to be the primary pathogens causing disease, while V. dahliae appeared to have a secondary role. Ecc and Eca seed inoculation treatments had reduced plant heights, stands and tuber numbers. Ecc irrigation treatments had no significant differences in these categories, in PED progress or plant yields. No V + Erwinia interactions were found in these categories. Irrigation appeared to have some effect on these factors with a trend toward greater stand losses and plant height reductions as irrigation increased.

PED progress during the growing season increased as irrigation increased. Progress of PED in V + Ecc and V + Eca seed treatments was similar to or sometimes significantly greater than PED progress in plants exposed to Verticillium alone. There was a trend toward greater PED expression during the season in plots with combinations of pathogens than plots with either Erwinia or Verticillium alone.

Per plant tuber yields were significantly lower than the control or either pathogen alone in three of the V + Ecc or V + Eca seed treatments. This interaction only occurred in the two warmest years of the study, indicating again a significant role for air temperature. While the majority of the V + Ecc or V + Eca seed combination treatments did not show significant reductions, there was an obvious trend toward this conclusion. There was no significant effect on the combination treatments by irrigation regime, even though a strong trend toward increased PED severity, PED disease progress and yield loss as irrigation increased was established. It is clear from this research that Verticillium + Erwinia interactions do occur. However, the effect of the interaction appears to be quite dependent upon having the correct environmental conditions present during the growing season and the proper mix of pathogen inoculum densities.

By breaking out the Erwinia carotovora treatments by themselves (no influence by Verticillium dahliae), some interesting information about these pathogens and how they act in the San Luis Valley was revealed. A significant reduction in stand occurred in two of three years by both inoculum levels of Ecc and Eca seed treatments, but in none of three years when Ecc was introduced into microplots in irrigation water. In general, as irrigation and Erwinia inoculum density increased, stand loss also increased. Plant heights were unaffected by the treatments. Foliar or stem symptoms of blackleg, except stand loss, were not seen in any of the

Ecc or Eca seed treatments. A temporary wilt was seen in 1990 at the highest inoculum level with the Ecc irrigation treatment. There was a general depression of tuber numbers under Ecc and Eca seed treatments with signficantly lower levels in four cases. There were also two cases of tuber number increases. Fluctuations in these results were probably the result of irrigation management rather than effect of disease. Ecc and Eca seed treatments generally decreased tuber yields, sometimes signficantly, in 1989 and 1990. There were no significant yield reductions in 1988. In 1990 the high Ecc irrigation treatment under optimum moisture also significantly decreased yields. This was the only case where this occurred during the three years of the study. By comparing total microplot yields, signficant yield reductions were seen with both inoculum levels of Ecc and Eca seed treatments in two of three years. The overwhelming trend was toward decreased yields with Ecc seed and Eca seed treatments. Ecc irrigation treatments did not have significant reductions in total yields.

Finally, daughter tuber contamination was examined. Infection of daughter tubers by <u>Erwinia</u> was greatest in the two coolest seasons. Overall, the higher the inoculum level used, the greater the amount of infection. Also, the greater the water stress, either deficient or excessive, the higher the infection rate. Infection in treatments irrigated with water containing Ecc was consistent in all years.

The role of <u>Erwinia</u> <u>carotovora</u> in the San Luis Valley potato production is complicated, but a lack of understanding of its role has serious implications. Results from this portion of the research indicate that very low inoculum levels of Ecc or Eca in the seed can significantly depress yield, both on an individual plant basis and more importantly on a total crop basis. In addition, high levels of Ecc present in the irrigation water can also reduce yields if conditions are right. There is a high potential for daughter tuber infection with either seed or irrigation inoculum, again, even with very low levels of bacteria.