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THE ECOLOGY AND CONTROL OF FUSARIUM YELLOWS OF CELERY IN MICHIGAN

Michigan State University PH.D. 1985

THE ECOLOGY AND CONTROL OF FUSARIUM YELLOWS OF CELERY IN MICHIGAN

by

Wade Howard Elmer

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ABSTRACT

THE ECOLOGY AND CONTROL OF FUSARIUM YELLOWS OF CELERY IN MICHIGAN

By

Wade Howard Elmer

Fusarium oxysporum f. sp. apii Race 2 (Nels. & Sherb.) Synd. and Hans (FOA2) was identified as causing the Fusarium yellows disease of celery on the resistant green cultivars in North Muskegon, MI in 1981. Race 1 of the pathogen had caused severe disease in Michigan during the 1930's and 1940's, but had virtually disappeared when the resistant green cultivar `Tall Utah 52-70 R Improved' was introduced in 1952. The relationship between inoculum density in soil and dry weights of celery fitted a linear equation (r^2 = .55). When the fraction of disease plants (X) was corrected for multiple infections {Ln (1/1-X)}, a better fit (r^2 = .99) predicted only 9 propagules/g soil to cause 10 % disease incidence in a 6-week period.

An orange mutant (OM) of FOA2 used in crop residue-amended soils demonstrated that FOA2 possessed a high degree of competitive saprophytic ability for celery residues, but OM populations decreased in onion- or mint-residue supplemented soils. FOA2 survived symtomlessly on the roots of nonsusceptible plants, and in artificially infested fallow soils, but populations decreased 90 % after 2 years. Naturally infested soils supplemented with residues of celery and sudax increased

the disease severity of celery, while onion and mint residues caused a decrease in disease when compared to nonamended soils.

FOA2 isolates from Michigan, California, and New York were compared for virulence, and for optimal soil temperature for disease development and growth in vitro. All isolates were virulent on self-blanching and green celery cultivars, but differed in virulence, and caused the greatest level of disease at a soil temperatures of 17-22 C. Isolates did not differ in growth rates.

Commercial and experimental celery germplasm were screened for resistance to Fusarium yellows in the greenhouse in artificially infested soil, and under field conditions in naturally infested soils. The moderately resistant commercial cultivars `Tall Utah 52-70 HK' and `Deacon' were the only cultivars that were horticulturally acceptible. The experimental celery lines 604, and 68-37 developed at Michigan State University were moderately to highly resistant.

TO

My Parents, Howard and

Marjorie

For

Their Inspiration

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Long before celery (Apium graveolens var dulce L.) was cultivated as a food crop, it was grown for its opiate-containing seed which was used in medicinal treatments. This property is inherent in its name which was derived from the Greek word "celinon" meaning fast-acting.

Vavilov (47) believed celery evolved in the low marshlands surrounding the Mediterranean Sea and must have appeared very similar to wild celery, known as smallage (A. leptophylum (Pers.) Muell.). Smallage is very leafy; the large succulent petioles of modern celery are obviously the result of selection over the past two hundred years (37). Celery was first mentioned as a food crop in 1623 in France, but Homer referred to celery as selinon in his Odyssey around 850 B.C. (37). The first written account of celery as a food crop in the United States was in 1806 (37). It was generally believed that celery was brought by the early European settlers when they immigrated to the New World.

The production of celery in the U.S. began in and around Kalamazoo, Michigan in the early 1900's where the well-drained organic soils and cool summers suited the crop well (28). Soon afterwards, celery production began to increase in California and Florida, and Michigan now ranks as the 3rd largest producer in the United States (37). Presently, Michigan produces celery on approximately 3,300 acres with annual production valued at over 16.4 million dollars (2). Cultivars such as `Florida 2-13', `Florida 683' and `Tall Green Light' are favored by

most Michigan growers.

Over 20 diseases of celery have been described (37); however, only a few are of economic importance in Michigan (24). One of the most serious foliar diseases of celery in Michigan is late blight incited by Septoria apiicola Briosi and Cav. (24). Early blight (Cercospora apii, Fres.) and bacterial blight (Pseudomonas apii, Fres.) of celery are of less concern, but can become severe in certain areas when environmental conditions are favorable (37). Pink rot (Sclerotinia sclerotiorum, (Lib.) d By.) and crater rot (Rhizoctonia solani, Kuehn) are soilborne fungal diseases that can affect the petioles of mature plants and may be devastating in seed beds and in the field if not controlled by fungicidal applications and proper sanitation (24). Fusarium yellows of celery (Fusarium oxysporum f. sp. apii Race 2 Nelson and Sherb. (Synder and Hansen), a soilborne vascular disease, is now considered to be the most important disease of celery in Michigan (11) and will be the subject of this dissertation.

FUSARIUM YELLOWS OF CELERY

History of Fusarium Yellows of Celery in Michigan

Fusarium yellows of celery was first reported in Kalamazoo, Michigan in 1914 by Coons (8) after a county agent asked for diagnosis of a diseased celery field. After surveys were completed in 1917, the disease appeared to be present in all celery-growing areas near Kalamazoo and in outlying areas (9). By 1931, Fusarium yellows was reported to be present in all celery-growing regions of the United States except Florida. The absence of the disease in Florida was unexplained, but suggested by Nelson et al. (28) to be due to

unfavorable soil temperatures. However, the differences in soil temperature, if any, between California and Florida would not completely explain the total lack of Fusarium yellows of celery in Florida since the disease developed over a wide range of soil temperatures (28).

Causal Agent

The causal agent of Fusarium yellows of celery is the fungus Fusarium oxysporum f. sp. apii (Nelson and Sherb.) Snyder and Hansen (FOA). There are two confirmed races of FOA: Race 1 (FOA1) causes disease on self-blanching celery cultivars, but is avirulent on the green cultivars (28); Race 2 (FOA2) causes disease on both self-blanching and green petioled celery cultivars (42). Race 3 (FOA3) has been reported, but not confirmed in California, and supposedly incites disease on the green cultivars, but not on the self-blanching cultivars (35).

Several attempts were made by Puhalla (35) and myself to isolate FOA1 from soils known to have harbored this race during the 1930's and 1940's, but these attempts were fruitless. Populations of FOA1 may have been reduced to levels not easily detected, since the total disapperance of FOA1 from Michigan and California soils seems unlikely.

The morphological characteristics of FOA1 were originally described by Nelson et al. (28) and designated as F. apii Nelson and Sherb. This species was later placed in the species Fusarium oxysporum (Synder and Hansen) (45). Nelson et al. (27) described F. oxysporum as having septate mycelium bearing branched or unbranched doliform conidiophores that produced oval to kidney shaped microconidia in false heads. Macroconidia appeared slightly sickle-shaped, and possessed an attenuated apical cell along with a foot-shaped basal cell. Although

macroconidia are considered abundant in F. oxysporum (25) it is interesting to note that most isolates of Race 1 (28) and Race 2 of FOA (6) do not produce abundant macroconidia. Chlamydospores are readily formed singly, or in pairs, on conidiophores or intercalarily in mycelium or macrocondia. Aerial mycelium was usually white on PDA which produced a creamy pigmentation visible from the undersides of the petri plate. The mycelium frequently produced blue sclerotia and/or erumpent orange-colored sporodochia. Michigan isolates of FOA2 consistently produced dark red colonies with a creamy white border on Komada's Selective Medium (KSM) (22). When propagules were incubated at 25 C on KSM, they became macroscopic in 2 days, but did not develop the red pigmentation until after 4 to 5 days.

The self-blanching celery cultivars predominated in the U.S in the 1930's; however, the green cultivars were more tolerant of the disease (29). In 1952, Ferry Morse Seed Co. introduced a green celery cultivar named `Tall Utah 52-70' that was highly resistant to Race 1 of the Fusarium yellows fungus (1). Celery cultivars selected from that orginal line replaced older cultivars in the U.S., and changed the status of Fusarium yellows of celery from an important disease to a minor disease during the period from 1952-1975.

In 1975, Hart and Endo (15) reported the reappearance of Fusarium yellows on the resistant green cultivar `Tall Utah 52-70 R Improved' in several celery-producing counties in California. Virulent isolates of FOA1 had virtually disappeared in the U.S.; therefore, it was not known if the green cultivars had lost their resistance to Fusarium yellows because seed producers had not routinely selected for resistance to race 1, or if a new race of FOA was responsible for the disease (14). An

isolate of Race 1 of FOA was finally obtained from France, where the disease was still present on the preferred self-blanching cultivars, and found to be avirulent on the green cultivars. This suggested that the new disease was incited by a new race of FOA, which was designated Race 2 (FOA2) (42).

The "new" disease became widespread in California's celery-growing region within a few years. Awuah et al. (4) reported Fusarium yellows on formerly resistant green cultivars in Orange County, New York in 1982. Concurrent with this observation, the author reported the reappearance of the disease on the formerly resistant green cultivars in a celery field in North Muskegon, MI in the summer of 1981 (11). Judging from the severity of the symptoms and the grower's observations, it appeared that the disease had been present in the field for at least ten years. Likewise, Awuah and Lorbeer (4) suspected that the disease may have been present for many years in New York. These observations suggested that the new race (FOA2) of the Fusarium yellows fungus appeared in California, Michigan and New York within a relatively short time. As of this writing, the author has demonstrated that Fusarium yellows of celery is present in 11 Michigan celery fields and in one celery field in Wisconsin (Chapter II). The plants in the majority of these infested fields have only begun to exhibit symptoms of Fusarium yellows in the last few years; therefore, the fungus may have been recently introduced into these fields.

Symptoms

In the early stages of Fusarium yellows disease, a stunting of the celery plant is the most noticeable symptom (14, 28). The retarded growth rate is commonly observable before any foliar symptoms are

evident; however, when inoculum levels are very high, celery seedlings rapidly wilt and die. Stunting is reported to be more severe when younger plants are infected compared to older plants (16, 28).

The next symptom of Fusarium yellows commonly seen is a chlorosis of the older (outer) petioles. Generally, the leaves begin losing their bright green coloration from the margin and the chlorosis progresses inward until the entire petiole has a dull green appearance. Occasionally, the entire petiole becomes uniformly chlorotic without the initial localization of the chlorosis. Development of chlorosis in the younger (inner) petioles may depend on the cultivar. Highly susceptible cultivars such as `Tall Green Light', `Transgreen' and `Tall Utah 52-70 R Improved' often developed chlorosis in the youngest petioles in my field trials, while cultivars more tolerant of the disease such as `Florida 683 K', `Tendercrisp' and `Bishop' often developed chlorosis in the older petioles only (12).

Red to brown discoloration of the internal vascular tissues is a diagnostic symptom of the Fusarium yellows disease. Nelson et al., (28) reported that the discoloration was coextensive with fungal colonization, and that most infected plants developed vascular discoloration in the primary roots before the crown region. Hart and Endo (16) agreed with this and stated that primary infection occurred through the young root tips followed by colonization of the vascular tissues of the primary roots, crowns and petioles. With highly susceptible cultivars, vascular discoloration can extend past the crown region and can be detected in the petioles, and in some instances, was observed up to the first node on the petiole. Frequently, discoloration was unilateral in the crown region and could be traced to a large

lateral primary root (16). When this occurred, no vascular discoloration could be seen in the crown extending below the point of entry. When the crown was extensively discolored, a decay was often seen, giving rise to the name "crown rot" which has been used in the past to describe the disease (28, 34). The decayed tissue was composed of a soft spongy material heavily infested with soft rotting bacteria. Occasionally, the decay area was outlined with a dark red coloration. Nelson et al. (28) believed this was an attempt by the plant to prevent lateral colonization of other tissues.

Wilt of the plant often accompanied the internal breakdown. The outer petioles most frequently exhibited the first signs of wilting, then as the disease continued, and/or if soil moisture became limiting, the remaining petioles would display signs of wilting. Wilt was not as common as stunting or chlorosis, but was often observed in severely infected plants or when periods of drought occurred. Wilting, like internal breakdown of the crown, was generally the final stage of the disease and usually (but not always) preceded death.

Another symptom noticed with plants artificially inoculated in greenhouse experiments was the fragility of young leaves. Nelson et al. (28) emphasized this symptom as a means of differentiating two forms of FOA1 which they thought existed in the soil in equal proportions in Michigan. Form 1 of the fungus caused a curling of the older outer leaflets, leaving them very brittle, but the leaves retained the green pigmentation and appeared turgid and erect. Younger leaflets were relatively unaffected. Conversely, Form 2 caused a curling downward of younger inner leaflets, gradually moving on the outer leaflets. Nelson et al. (28) also noted that the degree of leaf brittleness was more

severe on the seedlings infected with Form 2. California isolates of the fungus tested in 1934 did not exhibit these symptoms on celery leaves, but only stunted the plants until they wilted and died. Since these isolates were virulent on all cultivars tested, no race classification based on cultivar resistance was proposed. The author has occasionally observed brittleness of celery leaves as a symptom of Fusarium yellows, but has never associated this symptom with any specific isolate and questioned the biological validity of the two forms of FOA1.

In Michigan, celery transplanted into naturally infested soil (about 75-100 colony forming units/g soil) exhibited stunting as early as one month after transplanting. A high degree of symptom variability was common. With most cultivars all stages of the disease could be observed in a single field. Whether this was due to genetic variability of the host or a non-uniform inoculum level in the soil was not clear. Schneider (40) suggested disease suppression could be occurring from nonpathogenic strains of F. oxysporum which colonized celery roots prior to infection by the pathogen, and prevented disease expression on celery in parts of the field referred to as "green islands". The actual factors governing symptom variability are still unclear.

Etiology of Fusarium yellows of celery

Fusarium yellows of celery has not received the attention that other F. oxysporum diseases have (27), partly due to the use of cultivars resistant to FOA1 which have rendered the disease unimportant. However, the emergence of Race 2 of FOA in California, Michigan, Wisconsin and New York, has encouraged new work on the biology of the disease (5, 11, 14).

Chlamydospores are believed to the principle overwintering structure of F. oxysporum (39). These resting structures are usually formed in diseased tissue and are released into the soil upon plant residue decomposition. Chlamydospores are known to survive for many years in the soil, even in the absence of a host plant (26, 39). FOA2 has good competitive saprophytic ability and can effectively compete for colonization of plant residues in soil (13, 31). Likewise, several researchers have shown that F. oxysporum can invade the cortex of roots of nonsusceptible plants without causing symptoms and thus provide another means of long term survival (3, 17, 21). Chapter III of this dissertation will investigate this aspect in more detail.

Infection of celery by FOA2 was investigated by Hart (14). He believed the main infection court was the area behind the young celery root apices in the zone of elongation. Exudates leak from rapidly dividing cells behind the root apex and stimulate chlamydospore germination and growth of the infection hyphae to the root. The fungus probably penetrates directly since wounding has little effect on disease development (16); however, natural wounds produced around emerging rootlets may also provide suitable infection courts.

The only detailed histological study of the vascular colonization of celery by FOA1 was conducted by Nelson et al. (28). The hyphae of the fungus invaded the tracheal vessels of the young root and colonized each successive cell, entering new vessels through pits in the tracheal wall. The amount of observable hyphae per vessel varied from a few strands to complete occlusion of the vessel. Rarely were the number of trachea so completely occluded that vascular plugging could be inferred as the cause of wilt; therefore, diffusible toxins were suspected to contribute

to the development of wilt and chlorosis (28, 36). Colonization continued into the crown region and basal portions of the petioles. The fungus has been isolated from the upper foliar portions, but colonization of this tissue was thought to be minimal (36). Neither FOA1 or FOA2 have been reported sporulating on the plant tissue, as seen with other Fusarium wilt diseases (44); however, the author has noted that in periods of high humidity, mycelium bearing microcondia may be seen around the base of severely rotted crowns and petioles. The infected celery plants either die from the disease or are eventually plowed under at the end of the season, providing the principal overwintering inoculum for the next year.

Soil temperatures optimal for disease development were reported by Hart (14) to be 20-24 C. These findings differed from those of Nelson (28) and Ryker (30) who reported soil temperatures of 26-28 C to be optimum for disease development. Whether these differences were related to differences between FOA1 and FOA2 is still unclear and will be discussed further in Chapter IV.

Soil-water potential can affect the severity of Fusarium yellows. Nelson (28) and Ryker (30) reported high soil moisture was favorable for disease incidence and severity. However, Hart (14) found little difference in disease levels between a continuously wet soil and an alternating wet and dry cycle. Welch (48) studied the effects of oxygen tension on the severity of Fusarium yellows of celery and consistently observed greater disease when celery was grown under low oxygen tensions induced by high soil moisture conditions. Since celery was believed to have evolved in low wet areas where oxygen levels are minimal (47), these observations suggested that the greatest disease occurred under

soil moisture conditions most optimal for the host plant.

The severity of Fusarium yellows of celery can also be affected by pH of the soil. Edgerton (10) first demonstrated that high soil acidity increased the severity of Fusarium wilt of tomato. Several other researchers have also shown the high correlation of low soil pH and increased disease severity with the Fusarium vascular wilt diseases (43, 46). Opgenorth and Endo (32) investigated the mechanism of the increased Fusarium yellows of celery in acidic soils. They suggested that in neutral and alkaline soils bacteria competed with FOA2 at the root surface and suppressed infection, but were unable to survive and compete with the pathogen in acidic soils. In contrast, Jones and Woltz (20) have proposed that the Fusarium wilt pathogens are more virulent under acidic conditions because of increased availability of micronutrients to the fungus. Manganese, zinc and copper increased growth and sporulation of F. oxysporum f. sp. lycopersici and infection of the host. Opgenorth and Endo (32) found that dilute micronutrient applications to infested soil adjusted at 7.5 pH did not increase Fusarium yellows of celery; therefore, the micronutrient availability hypothesis was not believed to be a major factor in the Fusarium yellows disease complex.

The emergence of race 2 of FOA in North America has demanded new strategies to control Fusarium yellows of celery. Presently, highly resistant cultivars that are horticulturally acceptable do not exist (12, 30). Orton et al. (33) have introduced UC1, a line of celery that segregates into resistant and susceptible progeny, but commercially acceptable cultivars are not yet available. Fungicides and soil fumigants have also been investigated and found to be ineffective in

providing any disease suppression (5, 23).

Several researchers have noted effects of fertilizers on Fusarium vascular diseases (18, 19, 46). Schneider (41) found that adjusting the soil fertility levels suppressed severity of Fusarium yellows of celery. When nitrogen (N) in the form of NO_3^- was applied in conjuction with high levels of potassium salts (Cl^-, NO_3^-, SO_4^{-2}), the rate of root infection by FOA2 was greatly reduced. However, when 20 % or more of the N was in the form of NH_4^- as $(NH_4)_2SO_4$, NH_4Cl or NH_4NO_3, the effects were completely nullified. The mechanism(s) underlying this phenomenon are poorly understood since the concentrations of the nutrients did not directly affect the pathogen. Awuah and Lorbeer (4) found that the nitrate forms of N and high rates of potassium fertilizers were ineffective in suppressing Fusarium yellows of celery on muck soil in New York. A similar study was conducted in Michigan where $CaNo_3$ was compared to the NH_4- forms of N but no noticeable differences were observed (23). Perhaps the high cation-ion exchange capacity of the organic soils of Michigan and New York (7) prevented any real changes in the nutrients available to the pathogen and/or the host.

OBJECTIVES

In this dissertation, the author has investigated other mechanisms to suppress Fusarium yellows of celery such as incorporating residues from rotation crops into the soil (Chapter III), and evaluating genetic sources of celery germplasm for resistance (Chapter V). Other studies were conducted to explore the ecology of FOA2 in celery, nonhosts and fallow soils (Chapter III) and to understanding the relationship of disease incidence and severity to inoculum levels (Chapter II). Lastly, isolates of FOA2 and FOA1 from different geographic areas were compared

for virulence, for optimal soil temperature for disease development and for growth rates *in vitro* to detemined if there were measurable differences (Chapter IV).

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CHAPTER II

DISCOVERY, DISTRIBUTION AND SOIL POPULATIONS OF FUSARIUM YELLOWS OF CELERY IN MICHIGAN

INTRODUCTION

Fusarium yellows of celery (Apium graveolens var dulce L.) is incited by two races of Fusarium oxysporum f. sp. apii (FOA) (Nels. & Sherb.) Synder and Hansen. Race 1 of FOA (FOA1) was first observed in Michigan in 1913 by Coons (4) and caused severe losses on self-blanching celery cultivars near Kalamazoo, MI (13). The introduction of the resistant green cultivar `Tall Utah 52-70' in 1952 (2) and cultivars derived from it in later years resulted in the virtual disappearance of Fusarium yellows from the United States until its reappearance in California in 1978 (8) on FOA1-resistant green cultivars.

In Michigan, Fusarium yellows was discovered on the FOA1-resistant cultivars `Florida 2-13' and `Florida 683' on the William Willbrandt farm in North Muskegon, MI during the summer of 1981 (5). Symptoms included stunting, chlorosis, occasional wilting, vascular discoloration in roots, crown and petioles, and necrosis of plant tissue.

To determine whether the recent celery disease outbreak in Michigan was due to a loss of resistance in the FOA1-resistant cultivars or to a new race of the pathogen, virulence tests were conducted. Virulent isolates of FOA1 were unobtainable in the United States. A French isolate of FOA1 was obtained and used in these tests, since race 1 was still causing disease on FOA1-susceptible self-blanching celery

cultivars grown in France.

Fusarium yellows of celery was discovered on two additional farms in Michigan in 1982, one in Muskegon and one in Ottawa county. The following year the disease was found on four different farms: one in Muskegon, two in Ottawa and one in Van Buren county (6). In 1984 Fusarium yellows was identified on three additional farms in Kent, Muskegon and Ingham counties (Figure 2.1). Many of these fields had no prior history of Fusarium yellows, but exhibited severe symptoms where 50 - 100 % of the plants were unmarketable.

Researchers have associated rather high inoculum levels of 1000 propagules/ g soil (P/G) or more with high disease severity for some diseases caused by F. oxysporum (1, 18). However, other investigations revealed that very low inoculum densities of F. oxysporum can cause significant disease loss (14). Welch (17) examined the relationship of inoculum levels of FOA2 to disease incidence of celery in artificially infested field soil. By extrapolating disease incidence-inoculum density curves, severe disease loss was predicted to result from inoculum levels of one P/G. Welch (17) demonstrated that inoculum density of FOA2 was linearly proportional to the fraction of diseased celery plants (X), when corrected for multiple infections [Ln 1/(1-X)] (16). This relationship predicted that inoculum levels above 2.8 P/G would result in 100 % disease incidence after 14 weeks. An estimated average of 0.47 infections per major celery root 6 weeks after transplanting resulted in significant vascular discoloration in the crown at harvest (17). Such an infection would result in an unmarketable plant. Welch (17) concluded that sudden outbreaks of Fusarium yellows in California could result from very low inoculum

levels.

The objectives of this research were to identify the race of the F. oxysporum f. sp. apii causing the disease, and to determine its distribution in Michigan's celery-growing areas, to determine the inoculum densities and disease potentials of naturally and artificially infested muck (organic) soils.

MATERIALS AND METHODS

Discovery and Distribution of Fusarium Yellows in Michigan

Surveys were conducted over the 1981-1984 growing seasons to identify celery fields with Fusarium yellows (Figure 2.1). Celery plants exhibiting symptoms of stunting and chlorosis of the foliage, and vascular discoloration in the roots, crown area and/or basal portions of the petioles were removed from celery fields for isolation of the causal organism. Isolations were made from discolored tissue by rinsing pieces (2 X 2 x 2 mm) of infected tissue for several hours in tap water followed by a 1 min. surface sterilization in 10 % household bleach (5.25 % sodium hypochlorite). Potato dextrose agar (PDA), water agar, and Komada's selective medium (KSM) (11) were used for isolations. Plates were incubated at 25 C under a 12 hour photoperiod. Fusarium oxysporum was identified by colony characteristics and spore morphology on PDA and carnation leaf agar (12), then single-spored and stored in sterile soil tubes according to the procedures of Nelson et al. (12).

Since pathogenic and nonpathogenic isolates of F. oxysporum cannot be differentiated morphologically, isolates of FOA2 were identified by subjecting them to a pathogenicity test. Inoculum was increased by transferring a colonized PDA plug (produced by placing a small quantity

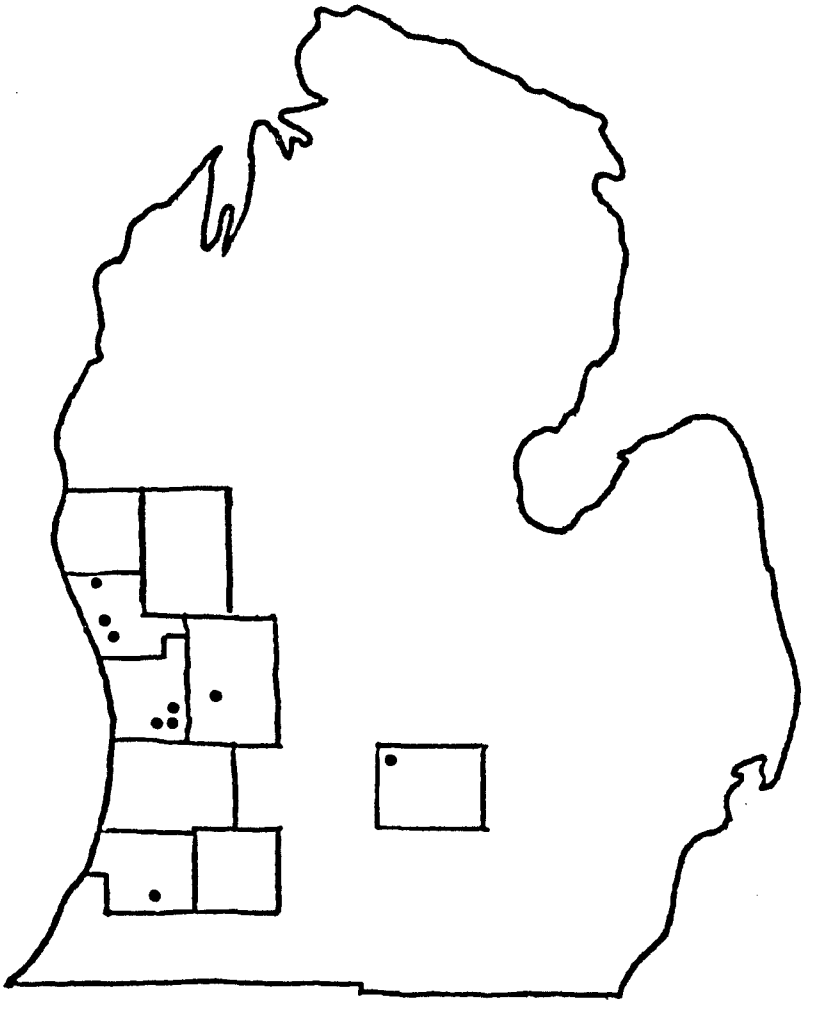

Figure 2.1. Distribution of Fusarium yellows of celery in Michigan.

of infested soil from soil tubes onto a PDA plate) to flasks containing sterile ground wheat straw moistened with 0.025 M asparagine (2 ml/g wheat straw) (15). After 3 weeks incubation at 25 C under a 12 hour photoperiod, the colonized wheat straw was slowly air-dried at 21-23 C, passed through a # 12-mesh sieve (1.41 mm) and thoroughly mixed into field muck soil at 0.5 or 2.0 g wheat straw/kg soil (oven dry weight equivalent). Inoculum contained free microconidia and macroconidia, and chlamydospores.

One month-old celery seedlings of the self-blanching cultivar `Golden Detroit' (susceptible to FOA1) and the green cultivar `Tall Utah 52-70 R Improved' (resistant to FOA1) were transplanted into 10 cm pots containing infested muck soil. Ten replicate plants of each cultivar were maintained in the greenhouse and plants transplanted into noninfested soil served as controls. After 6 weeks, plants were removed, split longitudinally through the crown with a knife and rated for vascular discoloration in the roots and crown. Disease ratings were based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown area, 4 = VD in 11-25 % of the crown area, 5 = VD in 26-75 % of the crown area, 6 = VD in 76-100 % of the crown area or death of the plant. Dry weights of the foliar portions were also recorded.

The experiment was repeated when an isolate of FOA1 was obtained from France via Dr. Puhalla of University of California, Berkeley (isolate A8). Only one inoculum rate [0.2 g wheat straw/liter sieved (2 mm mesh) potting mix] was used. Inoculum was incorporated into soil on a volume:volume basis to be consistent between experiments that used muck soils and commercial potting soils having bulk densities of 0.3

g/cc and 0.2 g/cc, respectively.

Enumeration and Disease Potential of FOA2 in Naturally Infested Soil

Soil naturally infested with F. oxysporum f. sp. apii Race 2 (FOA2) was randomly removed from a depth of 15 cm using a shovel in a celery field where disease had been severe for several years. The soil was sieved through a 2-mm-mesh screen, mixed in a cement mixer for 20-30 min. and stored in plastic bags. The soil was determined to contain approximately 32 % moisture.

Populations of FOA2 were enumerated by conventional soil dilutions into KSM (11). Mixing the soil suspension into molten KSM was found in preliminary trials to give more consistent results than surface applications onto solidified agar. Five-5 g soil samples (oven dry weight equivalent) were diluted with 500 ml sterile H_2O (1×10^{-2} g soil/ml) and agitated at high speed with a magnetic stir bar for 30 min. A ten-fold dilution (1×10^{-3} g soil/ml) was prepared from each sample from which a 5 ml aliquot was added to 50 ml of cooled molten (48-50 C) KSM and immediately poured into 5 petri plates (10 ml per plate). Two or three aliquots were assayed per sample. Plates were incubated as before and colonies of F. oxysporum were differentiated from other Fusarium species by colony characteristics and spore morphology (12).

FOA2 populations were estimated by assaying a random sample (15-25 %) of the F. oxysporum colonies growing in KSM for pathogenicity on `Golden Detroit' and `Tall Utah 52-70 R Improved'. Inocula originating from single-spored condia or hyphal tips of the colonies were increased on PDA plates and incubated in polyethylene bags on laboratory benches for 2-3 weeks. Then each colonized agar plate was comminuted with 100 ml of sterile H_2O and incorporated into approximately 0.35 kg of potting

mix with a garden stake. Two 1-month-old seedlings of `Golden Detroit' and `Tall Utah 52-70 R Improved' were transplanted into two 10 cm pots filled with infested soil and maintained in the greenhouse. Seedlings of `Golden Detroit' and `Tall Utah 52-70 R Improved' were also transplanted into soil infested with comminuted PDA from plates colonized by known Michigan isolates of FOA2. Soil incorporated with sterile PDA plates served as controls. When symptoms became severe in soils infested with the known isolates of FOA2 (4-6 weeks), the isolates were scored as pathogenic if they caused death or vascular discoloration in the crown or primary roots of the plants. Stunting alone was not considered good evidence that an isolate was FOA2 since sterile PDA-supplemented soils occasionally caused some stunting in the celery plants.

The percentage of isolates from soils that were pathogenic on `Tall Utah 52-70 R Improved' and `Golden Detroit' was used to estimate the populations of FOA2 in naturally infested soils. Inoculum density was expressed as propagules/g soil (P/G) (Experiment I). The experiment was repeated (Experiment II) using a new batch of infested soil removed from the same area in the field.

The relationship between soil inoculum levels and infection incidence, disease severity and dry weights of celery was examined in the greenhouse using the same naturally infested soil used in Experiment I. Steam-disinfested field soil was used to dilute the naturally infested field soil into the following proportions of naturally infested:disinfested soil: 1.0, 0.75, 0.50, 0.25, 0.10 and 0. Each soil proportion was placed in a concrete mixer for 20 min. and dispensed into twelve 15 cm pots whereupon three healthy 1-month-old seedlings of

`Tall Utah 52-70 R Improved' were transplanted into each pot and arranged in the greenhouse in a randomized block design. Plants received three 100 ml applications of a 20-20-20 soluble fertilizer at 2-week intervals begining one week after transplanting. Six weeks later each plant was rated for vascular discoloration in the crown and foliar portion were removed, oven-dried at 80 C for 24 hours and the weights recorded. For comparison the experiment was repeated using the soil enumerated for FOA2 populations in Experiment II.

Disease Reaction to FOA2 in Artificially Infested Soil

Incremental amounts of wheat straw inoculum were mixed into steamed muck soil in a concrete mixer and allowed to rotate for 20 min. before dispensing into 10 cm pots. The amount of inoculum to be added was determined by assays of densities of pathogen propagules in artificially infested muck soils (1 g FOA2-colonized wheat straw/kg muck soil) using standard soil dilution techniques described earlier. Inoculum rates were then adjusted to yield inoculum densities of 0, 1 X 10^2, 1 X 10^3, 1 X 10^4, 1 X 10^5 and 1 X 10^6 CFU of FOA2/g soil in the final mixture. The actual amounts of wheat straw incorporated per kg muck soil were: 0, 0.003, 0.03, 0.28, 2.80 and 28.0 g, respectively. One-month-old seedlings of `Tall Utah 52-70 R Improved' were transplanted into each of 10 pots containing the various soil inoculum levels and were maintained in the greenhouse for 6 weeks. Dry weights and disease ratings were taken as described above.

Vertical Distribution of FOA2 in a Naturally Infested Celery Field

On May 17th, 1984 soil core samples were individually removed with a soil sampling probe from a celery field in North Muskegon, MI where

100 % of the plants were diseased the previous year. Six cylindrical (7.6 diam. X 15 cm) samples were removed consecutively per site down to a total depth of 90 cm. Five sites in the field were sampled and replicate soil cores were combined into composite samples. Each soil sample was air-dried, ground in a mortar with a pestle, passed through a 40 mesh sieve, and stored in plastic bags at 21-23 C until used.

Populations of FOA2 were estimated by diluting one 5 g sample from each of the six composite soil samples and assaying as before. Three-5 ml aliquots were assayed per soil sample. Due to the enormous numbers of F. oxysporum isolates in this soil and to the decrease of numbers with increasing depth, the following percentages of total isolates from each 15 cm soil section were tested for pathogenicity: 0-15 cm, 10 %, 15-30 cm, 10 %, 30-45 cm, 10 %, 45-60 cm, 20 %, 60-75 cm, 33 %, 75-90 cm, 100 %. Populations of FOA2 were estimated for each 15 cm sample.

On Sept 18th, 1984 the experiment was repeated in the same area of the field, but the soil had been plowed to a depth of 38 cm (15 inches) prior to the celery transplanting operation in late May. FOA2 population assays were conducted as before, but 100 % of the F. oxysporum isolates were tested for pathogenicity since the populations were lower.

Relationship of Various Concentrations of Conidial Suspensions and Time of Exposure to Celery on Disease Expression

Macrocondia of FOA2 were produced on carnation leaf agar (12) by placing a small quantity of soil from stored soil tubes on the surface of 20 plates and incubating for 12-17 days at 25 C under 12 hour photoperiod. After incubation the plates were washed with 10 ml of sterile water, and spore suspensions were filtered through 4 layers of

cheesecloth to remove mycelial fragments. Conidia were washed three times with sterile distilled water by centrifugation at 3000 g for 5 min. The resuspended pellet consisted of microcondia and macrocondia at a ratio of approximately 10:1. Macrocondia were enumerated with a hemacytometer, and the suspension was diluted with sterile distilled water to yield the concentrations of 0, 1×10^2, 1×10^3, 5×10^3, 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6 macroconidia/ml. Twenty ml aliquots of each concentration were placed in 40 ml beakers.

A minimum of twenty five 3-week-old celery seedlings (`Tall Utah 52-70 R Improved') were carefully removed from the germinating medium to avoid injury to the roots, and were gently rinsed to removed all adhering soil particles. The seedlings were wrapped in cotton and placed in the beakers containing the spore suspensions so all the roots were submerged. The beakers were set on a platform shaker at low speed for 24 hours to agitate the suspensions and promote spore contact with the roots.

Fifteen seedlings of similar size were selected from each beaker and transplanted into 10 cm pots filled with potting mix and were maintained in the greenhouse for 6 weeks under the same fertilizer regime mentioned before. There were 5 replicate pots per concentration with 3 seedlings/pot.

To examine the effects of duration of root exposure to the conidial suspensions, a minimum of 25 seedlings of `Tall Utah 52-70 R Improved' were placed in eight 40 ml beakers containing 20 ml of spore suspensions adjusted to 1×10^4 macroconidia/ml. Seedlings placed into 8 beakers filled with sterile distilled water served as controls. The beakers were set on the platform shaker as before, but one treated and one

control beaker were removed after 0 and 1 min, and after 1, 6, 12, 24, 48, and 72 hours. Fifteen uniform seedlings were selected and transplanted into 10 cm pots (3 plants per pot) and rated for disease symptoms, and dry weights were determined after 6 weeks. The experiments was repeated once.

RESULTS

Discovery and Distribition of Fusarium Yellows in Michigan

The disease symptoms of stunting, chlorosis and vascular discoloration in green celery cultivars found at several locations in Michigan's celery-growing areas (Figure 2.1) were shown to be incited by the fungus *Fusarium oxysporum* f. sp. *apii* Race 2 (FOA2). All symptoms were reproducible in the greenhouse on `Golden Detroit' and `Tall Utah 52-70 R Improved' grown in soil artificially infested with FOA2-colonized wheat straw (Table 2.1). Since FOA1 only causes Fusarium yellows on the older susceptible self-blanching cultivars such as `Golden Detroit', but not on `Tall Utah 52-70' or its descendants, the Michigan isolate was confirmed to be Race 2. The French FOA1 isolate was avirulent on `Tall Utah 52-70 R Improved', but caused severe stunting and vascular discoloration on `Golden Detroit' (Table 2.2). The pathogen was consistently recovered from the discolored vascular tissue and was morphologically identical to the original isolate used to increase inoculum.

Symptoms were variable among individual plants in greenhouse tests. Wilting and death were occasionally observed in severe cases, but stunting was the most common symptom. No significant differences were noted between the dry weights or disease ratings of the two celery

Table 2.1. Effects of two inoculum densities of FOA2 on dry weights and disease ratings of Fusarium yellows of celery on `Golden Detroit' (susceptible to FOA1) and `Tall Utah 52-70 R Improved' (resistant to FOA1) cultivars.

Cultivar	Inoculum Rate (g wheat straw /kg soil)	Disease[x] Rating	Dry weight(g)[y]
`Golden Detroit'	0	1.0 a[z]	4.4 a
	0.5	3.5 b	1.7 b
	2.0	4.3 b	1.1 b
`Tall Utah 52-70 R'	0	1.0 a	4.7 a
	0.5	5.1 b	1.0 b
	2.0	4.8 b	1.9 b

[x]Based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown, 4 = VD in 11-25 % of the crown, 5 = VD in 26-75 % of the crown, 6 = VD in 76-100 % of the crown or dead.

[y]Represents the dry weights of the foliar plant portion.

[z]Values represent the means of 10 plants; values follwed by differing letters are significantly different by Duncan's multiple range test at $\underline{P}$ = 0.05.

Table 2.2. Disease ratings and dry weights of `Golden Detroit' (susceptible to FOA1) and `Tall Utah 52-70 R Improved' (resistant to FOA1) grown in soil infested with a Michigan isolate of FOA2 or a French isolate of FOA1.

Cultivar	Race[w]	Disease[x] Rating	Dry weight (g)[y]
`Golden Detroit'	Control	1.0 a[z]	3.1 a
	Race 1	5.3 b	0.4 b
	Race 2	5.4 b	1.2 b
`Tall Utah 52-70 R Improved'	Control	1.0 a	3.0 a
	Race 1	1.0 a	3.1 a
	Race 2	5.3 b	0.8 b

[w]Soil was infested with colonized wheat straw at the rate of 0.2 g/liter soil with either race 1 or race 2.

[x]Disease ratings based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown, 4 = VD in 11-25 % of the crown, 5 = VD in 26-75 % of the crown, 6 = VD in 76-100 % of the crown or dead.

[y]Represent the dry weights of the foliar plant portion (g).

[z]Values represent the mean of seven pots consiting of 3 plants/pot; values followed by differing letters are significantly different by the Student-Newman-Keul's test at $P = 0.05$.

Table 2.3. Enumeration of FOA2 in naturally infested soil.

	F. oxysporum /g soil	No. tested	No. pathogenic[x]	EID[y]
Experiment I	3.63×10^2	60	12	73
Experiment II	2.45×10^2	100	42	103

[x]Pathogenic on `Tall Utah 52-70 R Improved' and `Golden Detroit'

[y]Estimated Inoculum Density/g soil.

cultivars grown in infested soil; however, `Tall Utah 52-70 R Improved' was more susceptible than `Golden Detroit' judging from the higher degree of stunting and chlorosis on `Tall Utah 52-70 R Improved'.

Enumeration and Disease Potential of FOA2 in Naturally Infested Soil

Several morphological types of F. oxysporum developed on KSM after 7 days incubation of soil dilution plates. Aerial mycelium associated with F. oxysporum colonies varied from sparse and low-growing to raised fluffy colonies, but all produced a red pigmentation surrounded with a creamy white border visible from the underside of the agar plate. Colonies of known isolates of FOA2 were indistinguishable from F. oxysporum colonies.

In the first of two identical experiments (Experiment I), 60 isolates of F. oxysporum from naturally infested soil were assayed for pathogenicity on `Golden Detroit' and `Tall Utah 52-70 R Improved'. Of these, 20 % were pathogenic on both `Tall Utah 52-70 R Improved' and `Golden Detroit' (Table 2.3). Soil population of FOA2 was estimated to be 73 propagules/g soil (P/G). In Experiment II, 100 isolates were assayed for pathogenicity and 42 % were identified as FOA2. Inoculum density of FOA2 was estimated to be 103 P/G (Table 2.3). When dry weights of celery plants grown in various proportions of naturally infested:steamed disinfested soil were regressed against the estimated inoculum densities (EID) in Experiment I (Figure 2.2) and Experiment II (Not illustrated), regression equations for the best fit were:

Experiment I, $Y = 3.9 - 0.02X$ ($R^2 = .55$, $P = 0.05$) Eq. 1

Experiment II, $Y = 8.0 - 0.03X$ ($R^2 = .51$, $P = 0.05$) Eq. 2

A statistical comparison of the slope of Eq. 1 (where X = EID and Y = Dry weights (g)) with the slope of Eq. 2 yielded no significant

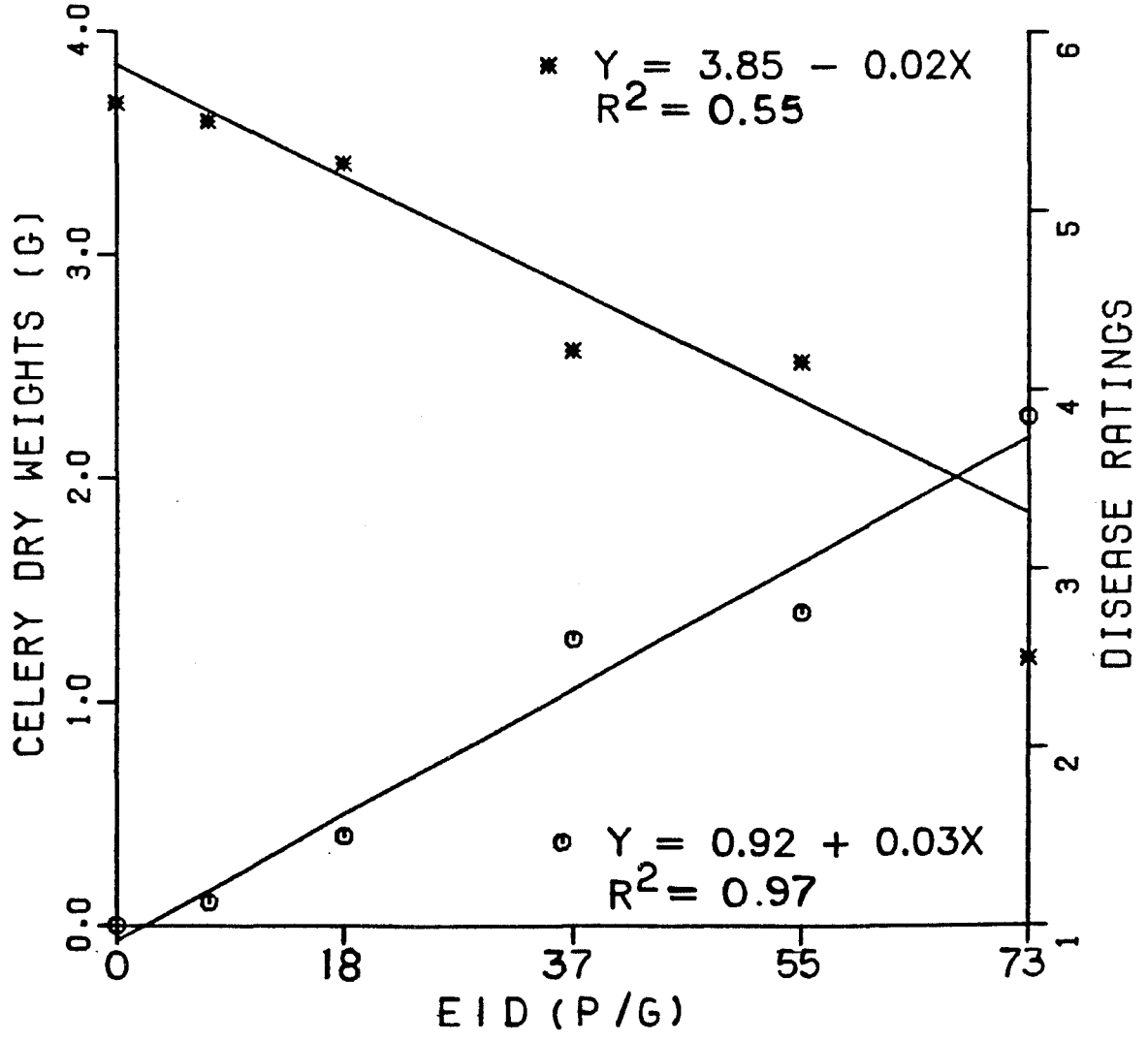

Figure 2.2. Relation of celery dry weights (✱) and disease ratings (⊖) after 6 weeks to increasing amounts of estimated inoculum densities (EID) of FOA2 in naturally infested soil.

differences at $\underline{P}$ = 0.05. The EID required to cause a significant decrease in the dry weight of celery in 6 weeks in Experiment I was estimated to be 36.5 P/G by Student-Newman-Keul's test ($\underline{P}$ = 0.05).

Disease severity ratings also increased linearly as the EID increased in Experiment I (Figure 2.2) and Experiment II (Not illustrated), and were best described by the equations:

Experiment I, $Y = 0.91 + 0.03X$ ($R^2 = .97$, $\underline{P} = 0.05$) Eq. 3

Experiment II, $Y = 1.31 + 0.03X$ ($R^2 = .73$, $\underline{P} = 0.05$) Eq. 4

No statistical difference was detected between the slope of the line for Eq. 3 (where X = EID and Y = disease severity) with the slope in Eq. 4 at $\underline{P}$ = 0.05. Since vascular discoloration was detected in 3 plants out of 36 plants at the 10 % naturally infested:disinfested soil in Experiment I (data not shown), the disease severity rating of 1.1 for that treatment can be substituted for Y in Eq. 3, and it can be calculated that an EID of 6.5 P/G would be necessary to incite that level of disease severity after 6 weeks. This value is quite close to the EID of 7.3 P/G calculated to be present in the 10 % naturally infested:disinfested soil mixture as determined by the soil dilution assays and pathogenicity tests (Table 2.3).

When the fraction of plants exhibiting vascular discoloration in the crown (X) was corrected for multiple infections {ln 1/(1-X)} (16), redesignated as Y, and regressed against the EID (X) (Figure 2.3), the relationship was described by the equation:

$$Y = -0.17 + 0.03X \quad (R^2 = .98,\ \underline{P} = 0.05) \qquad \text{Eq. 5}$$

This transformation predicts disease incidence from the estimated number of crown infections that would be detected as vascular discoloration after 6 weeks. Only 9 P/G were predicted by this linear equation to

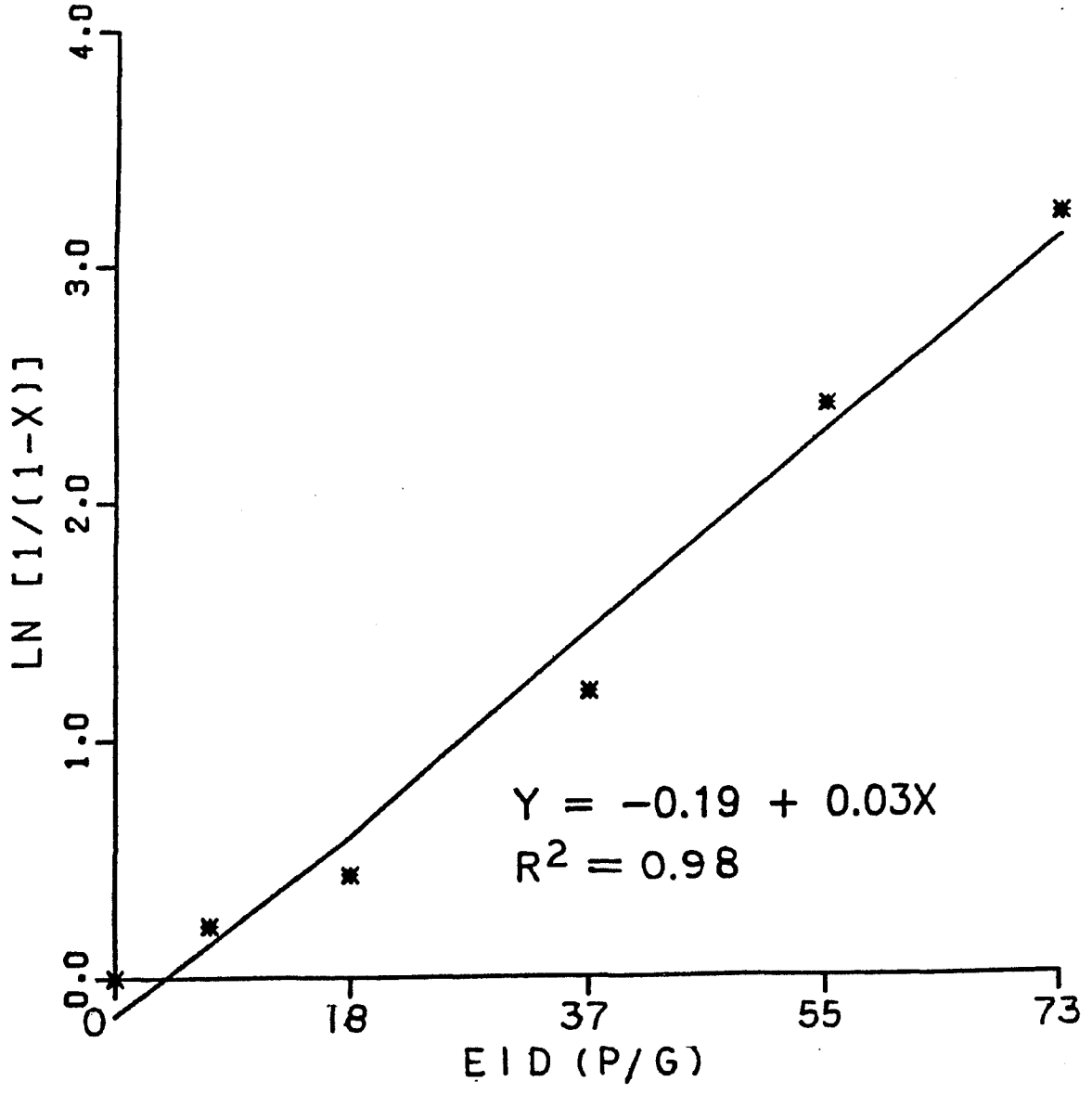

Figure 2.3. Relation of infection incidence (Ln 1/(1-X)) in celery plants after 6 weeks to increasing amounts of estimated inoculum densities (EID) of FOA2 in naturally infested soil.

incite a 10 % disease incidence after 6 weeks resulting from an estimated averaged 0.10 infections per plant. Using Eq. 5 an EID of 39.0 P/G was predicted necessary to incite one major crown infection (Y = 1) detectable as vascular discoloration in every plant after 6 weeks. However, this estimated value was statistically based on detecting vascular discoloration in 63 % of the plants which represents a high incidence of disease for such a relatively short time. Infections occurring after 6 weeks would likely increase the disease severity and lower actual number of propagules required to incite severe disease by harvest time. These results suggest that very low levels of FOA2 can cause significant crop failure.

Disease Potential in Artificially Infested Soils

Vascular discoloration was not detected in celery plants grown in soil artificially infested with FOA2-colonized wheat straw adjusted to 1 X 10^2 P/G, but was observed at and above 1 X 10^3 P/G (Table 2.4). Significant decreases in dry weights were observed only at the highest inoculum density of 1 X 10^6 P/G when compared to control plants (Student-Newman-Keul's test at $P = 0.05$). Since disease severity was not significantly different (Student-Newman-Keul's test, $P = 0.05$) from the control except at the highest inoculum density, the fraction of plants with vascular discoloration (X) was corrected for multiple infections {ln 1/(1-X)}, redeisgnated as Y, and regressed against the log cfu of FOA2/g soil (X) (Figure 2.4). The best fit was the linear equation:

$$Y = -0.60 + 0.34X \quad (R^2 = .79,\ P = 0.05) \qquad \text{Eq. 6}$$

It can be calculated from Eq. 6 that 4.7 X 10^4 P/G of the FOA2-colonized wheat straw (1.3 g wheat straw inoculum/kg soil) was required to incite

Table 2.4. Effects of different artificial inoculum densities of FOA2 on dry weights and disease ratings of `Tall Utah 52-70 R Improved'.

Inoculum rate[w] cfu/g soil	Disease[x] Ratings	Dry Weights(g)[y]
Control	1.0 a[z]	2.5 a
1 X 10^2	1.0 a	2.5 a
1 X 10^3	1.9 ab	2.4 a
1 X 10^4	2.2 ab	2.4 a
1 X 10^5	3.3 ab	1.8 ab
1 X 10^6	5.7 b	0.4 b

[w]Cfu determined by prelimary experiment; 1 g FOA2-colonized wheat straw /kg soil = 3.57 X 10^4 cfu/g soil.

[x]Disease ratings based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown, 4 = VD in 11-25 % of the crown, 5 = VD in 26-75 % of the crown, 6 = 76-100 % of the crown or death of the plant.

[y]Represents the dry weights of foliar plant portion.

[z]Values represent the mean of 10 plants; values followed by differing letters are significantly different by Student-Newman-Keul's test at $P = 0.05$.

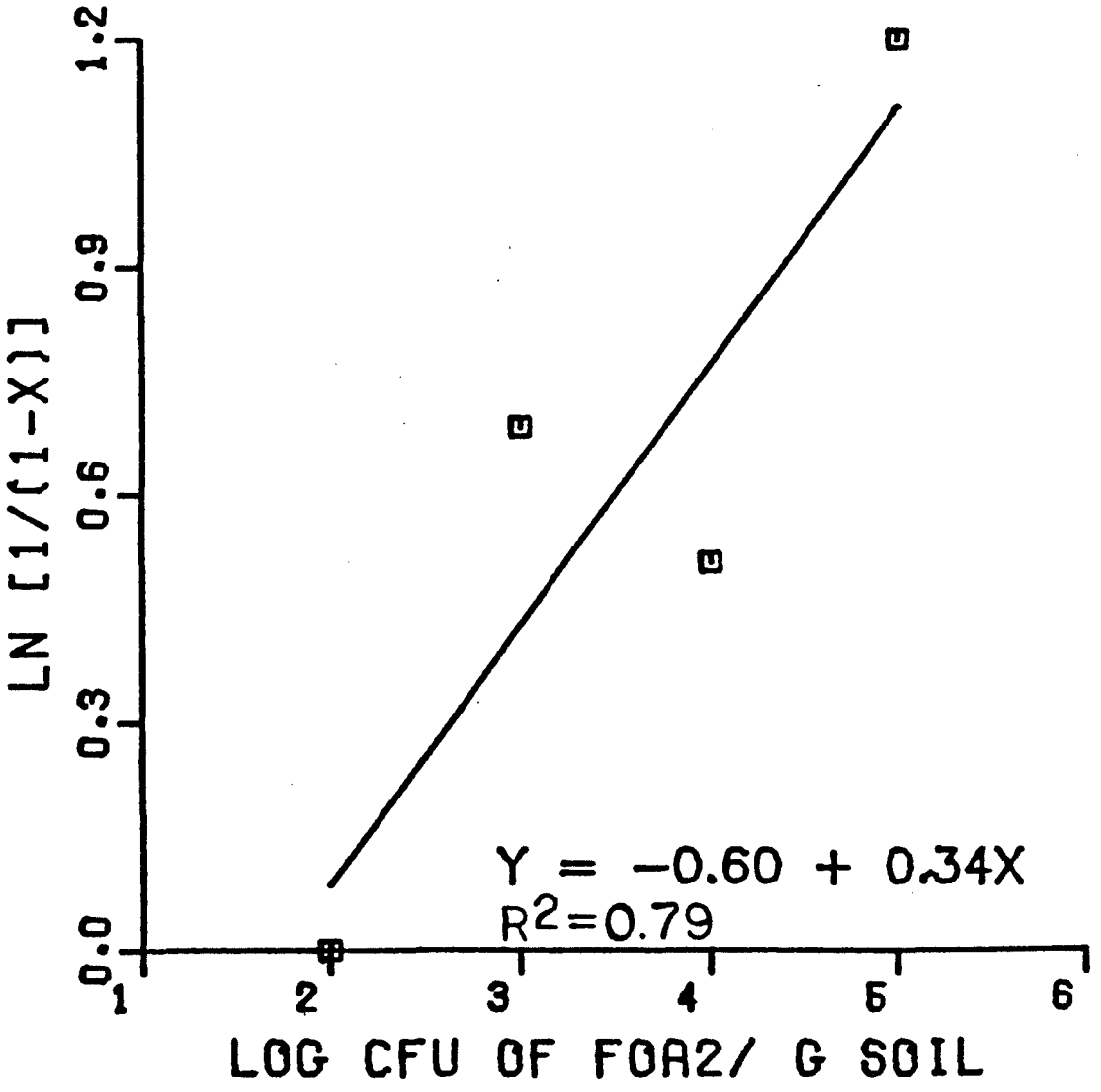

Figure 2.4. Relation of infection incidence (Ln 1/(1-X)) in celery plants after 6 weeks to increasing amounts of FOA2 inoculum in artificially infested soil.

vascular discoloration in the celery crown after 6 weeks.

Vertical Distribution of FOA2 in Naturally Infested Field Soil

When populations of F. oxysporum were assayed in cylindrical soil sections sampled from an infested field, total populations decreased by approximately 50 % in each 7.6 diam. X 15 cm soil section as soil depth increased (Figure 2.5). When FOA2 populations were estimated by subjecting a random sample to pathogenicity tests on `Golden Detroit' and `Tall Utah 52-70 R Improved', the greatest populations of FOA2 were found at the 15-30 and 30-45 cm soil depths. The pathogen was detected in all soil samples down to 90 cm in the May 17th sampling.

After the field was deep plowed in late May and soil samples were retaken in September, a significant decrease in the populations of F. oxysporum was observed. The F. oxysporum populations were approximately 25 % of those detected in May. Consequently, estimated populations of FOA2 were less in all soil layers except at the 0-15 cm soil depth where no significant difference was detected from the May sampling. No isolates obtained from the soil layers of 30-45 cm, 45-60 cm and 75-90 cm depth were pathogenic on either celery cultivar. The small number of FOA2 isolates detected in the 60-75 cm layer could again be due to contamination from upper soil layers when the soil cores were removed, since no FOA2 isolates were detected in the 30-45 cm or 45-60 cm layers.

Relationship of Various Concentrations of Conidia of FOA2 and Time of Exposure to Celery Seedling Roots to Disease After 6 Weeks

When celery seedling roots were exposed to increasing concentrations of conidia of FOA2 in aqueous suspensions, disease severity ratings (Y) did not increase significantly with inoculum concentrations (X) after 6 weeks growth in the greenhouse (Table 2.5).

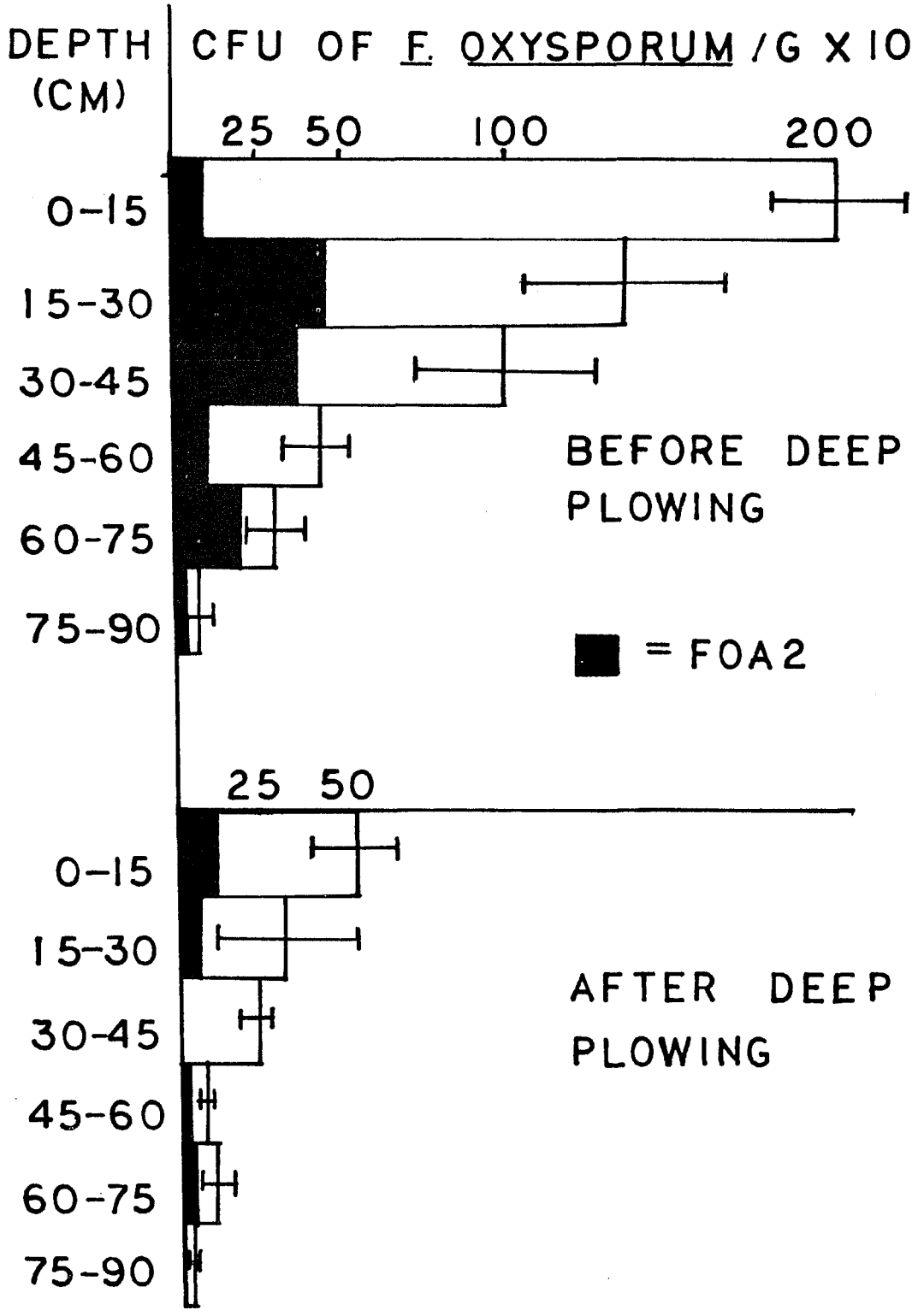

Figure 2.5. Vertical distribution of Fusarium oxysporum and FOA2 before (May 17, 1984) and after (September 18, 1985) deep plowing (38 cm) of soil naturally infested with FOA2.

Table 2.5. Effects of exposing celery seedlings to increasing concentrations of conidia of FOA2 for 24 hours on dry weights and disease ratings of 'Tall Utah 52-70 R Improved'.

Inoculum Concentrations (macroconidia/ml)	Disease[x] Ratings	Dry Weights(g)[y]
Distilled water (Control)	1.0 a[z]	3.1 a
1 X 10^2	2.6 ab	2.9 a
1 X 10^3	4.2 b	2.6 a
5X 10^3	3.5 ab	2.6 a
1 X 10^4	3.8 ab	2.8 a
5 X 10^4	5.0 b	2.0 a
1 X 10^5	4.6 ab	2.2 a
5 X 10^5	5.4 b	1.0 b
1 X 10^6	4.7 ab	1.7 ab

[x]Disease ratings based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown, 4 = VD in 11-25 % of the crown, 5 = VD in 26-75 % of the crown, 6 = VD in 76-100 % of the crown or death of the plant.

[y]Represents the dry weights of the foliar plant portion.

[z]Values represent the mean of 5 pots consisting of 3 plants/pot; values followed by differing letters are significantly different by Student-Newman-Keul's test at $P = 0.05$.

The best mathematical fit followed the 2nd order equation;

$$Y = 0.94 + 0.99X - 0.05X^2 \quad (R^2 = .41, \underline{P} = 0.05) \qquad \text{Eq. 7}$$

Likewise, a similar mathematical relationship was seen when celery dry weights were regressed against log concentration of macroconidia (Figure 2.6), and was best described by the equation:

$$Y = 3.00 + 0.12X - 0.06X^2 \quad (R^2 = .41, \underline{P} = 0.05) \qquad \text{Eq. 8}$$

Decreases in dry weights of celery were not significantly different from the control plants until the inoculum suspensions reached 5 X 10^5 macrocondia/ml.

When the percentage of plants with vascular discoloration (X) was corrected for multiple infections {ln 1/(1-X)}, redesignated as Y, and regressed against log macroconidia/ml (X), a more sensitive linear relationship was derived (Figure 2.7) and best described by the equation:

$$Y = -0.09 + 0.56X \quad (R^2 = .78, \underline{P} = 0.05) \qquad \text{Eq. 9}$$

The estimated number of macroconidia required to cause one infection after 6 weeks detectable as vascular discoloration in the crown was predicted to be 88 macroconidia/ml. However, this represents a very low amount of inoculum and may not represent the actual disease-causing potential of the macroconidal suspensions since many healthy escapes were noted among the plants.

The time of exposure of celery seedling roots to a fixed concentration of macroconidia (1 X 10^4 spores/ml) influenced the dry weights of celery; however, the celery seedlings exposed to distilled water were also affected (Figure 2.8). The greatest decrease in dry weights compared to the respective control treatment occurred between a 12 and 48 hour exposure period. Longer exposure periods to distilled water adversely affected the growth of celery after 6 weeks; therefore,

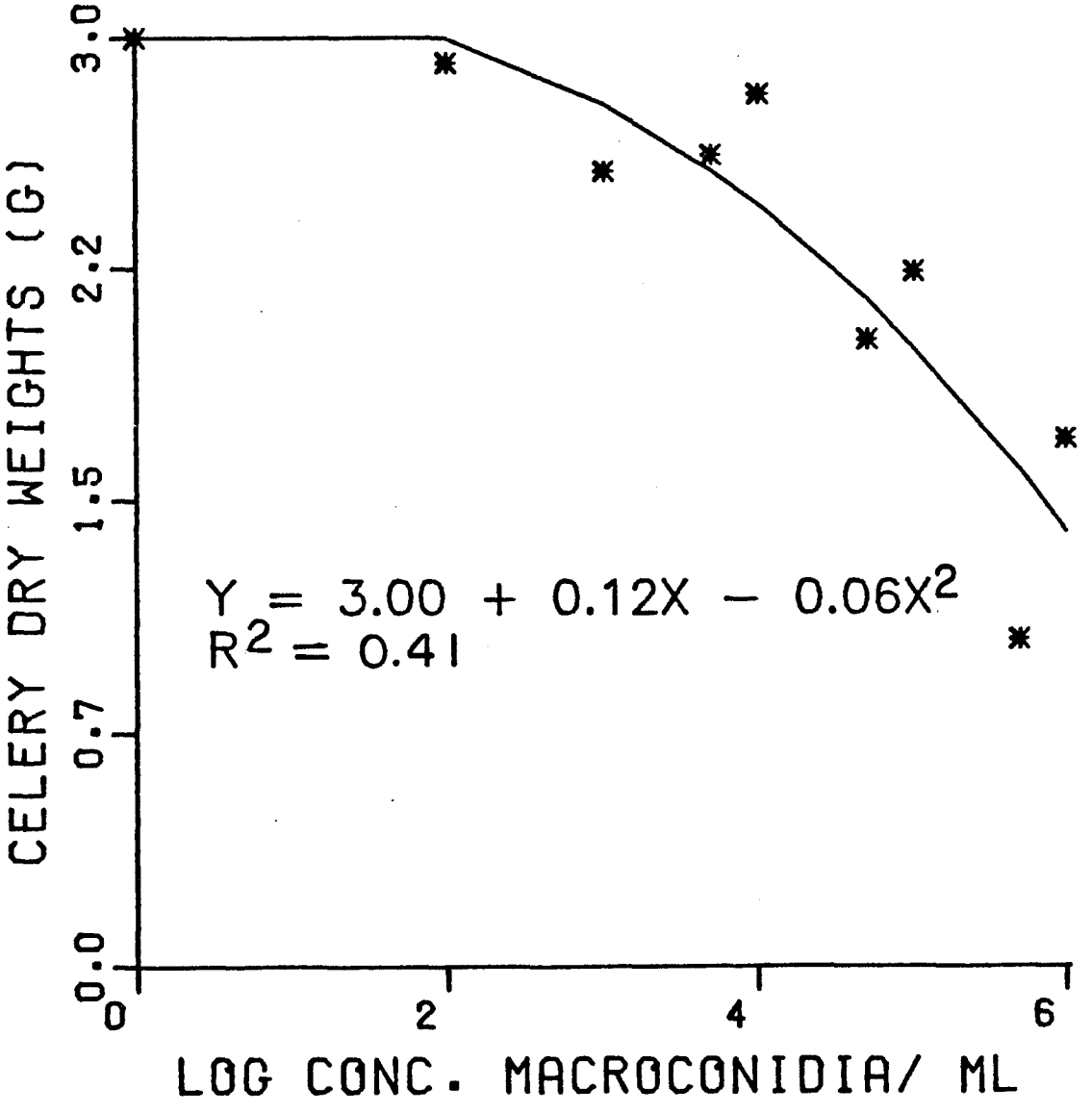

Figure 2.6. Effects of soaking celery seedlings in increasing concentrations of macroconidia of FOA2 for 24 hours on the dry weights of celery after 6 weeks growth.

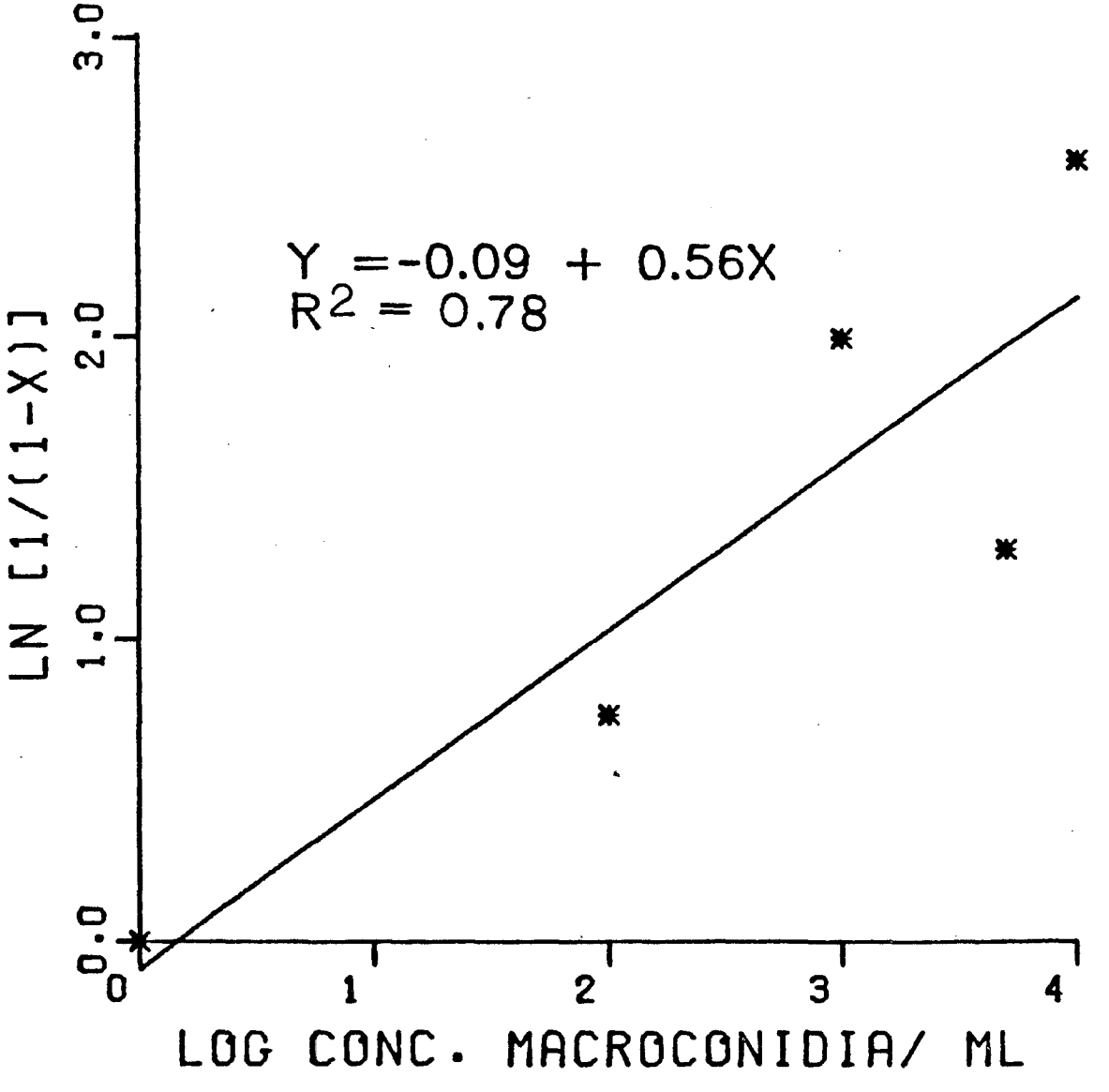

Figure 2.7. Relation of infection incidence (Ln 1/(1-X)) in celery plants after 6 weeks to increasing concentrations of macroconidial suspensions inoculated by soaking celery seedlings for 24 hours.

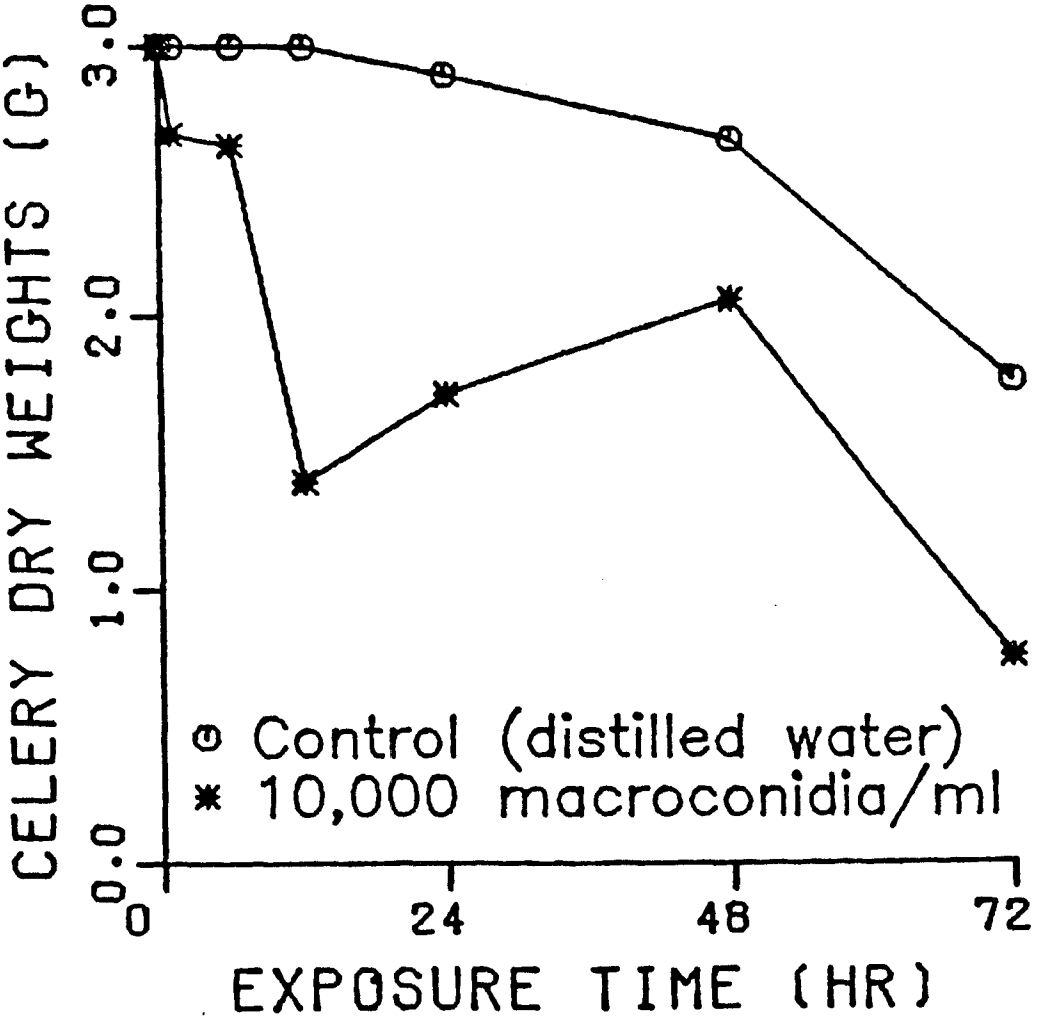

Figure 2.8. Effects of exposure time of celery seedling roots to macroconidial suspensions on dry weights of celery after 6 weeks growth.

the actual cause of the stunting after 48 hours could not be exclusively due to Fusarium yellows alone. After 72 hours of exposure to the spore suspensions, the seedlings were badly stressed and over 50 % of the plants died within 4 weeks. No control plants died when exposed to distilled water for 72 hours exposure, but their dry weights after 6 weeks was reduced.

DISCUSSION

Fusarium oxysporum f. sp. apii Race 2 (FOA2) was identified as the causal agent of the stunting, yellowing and vascular discoloration on the FOA1 resistant-green celery cultivars in five Michigan counties. The reappearance of the disease on the Race 1-resistant cultivars occurred after approximately 25 years of virtual immunity to Fusarium yellows (5).

The original source of the Fusarium yellows inoculum is unknown. However, the sudden appearance of the disease in several of Michigan's celery-growing areas within a short time suggested that the pathogen was recently introduced into the these fields. Since most commercial celery operations in Michigan share transplants and harvesting equipment (Personal communication with Mr. William Willbrandt and Dr. M. L. Lacy), infested soil and transplants could have aided in disseminating the pathogen into these fields. However, the detection of Fusarium yellows in isolated disease foci in a field, usually at the field edge, suggested that infested soil might have been introduced on farm equipment and contaminated the soil at the field edge. One would expect a more uniform distribution of field disease symtoms if infected transplants were the primary inoculum source.

Our results on the effects of FOA2-inoculum densities on the

Fusarium yellows disease supported Welch's (17) findings that low inoculum levels of FOA2 can incite severe disease loss in celery. Welch's (17) prediction that crop failure due to Fusarium yellows could result from 1 P/G may, in fact, be very similar to our findings of 9 P/G inciting a low incidence of disease in 6 weeks. Since celery roots occupy a large volume of soil and their mass increases exponentially over time (10), the probability of additional infections occurring after 6 weeks are high. Thus the actual P/G needed to incite a certain level of disease would decrease with longer growing periods. Also, root infections not yet detectable as vascular discoloration would have more time to develop. Even if these infections did not cause stunting, it is likely they would discolor the crown at harvest and produce an unmarketable crop. Therefore, it is reasonable to assume that the actual P/G required to cause serious crop loss at harvest could be less than 9 P/G. More importantly, the relative bulk densities of the California mineral soil where celery is grown and the Michigan muck soil used in these assays are very different. The highly expandable muck soils of Michigan have bulk densities of 0.2 to 0.3 g/cc, whereas the mineral soils of California are approximately 1.25 to 1.45 g/cc (3). Since the volume of 1 g of muck soil surrounding a suitable infection court on a celery root tip would occupy 5 to 7 times as much space as a mineral soil, it would appear that a higher inoculum density in muck soil would be required to incite the same level of disease as in the mineral soil. Propagules in muck soil would be at a greater average distance from the infection court than in the mineral soil, and therefore, would be less likely to germinate in response to root exudates from the celery root.

The tremendous difference between the disease potential of the natural soil inoculum and the FOA2-colonized wheat straw demonstrated the low infectivity of artificially produced propagules. Since the highest percentage of propagules in the wheat straw inoculum were microconidia, and their role in the Fusarium yellows of celery disease was thought to be minimal (7), the elevated EID predicted to incite disease was expected. This information can allow for more accurate prediction of disease levels for greenhouse cultivar screening and other soil assays with Fusarium yellows of celery.

The highest populations of FOA2 estimated before the deep plowing were observed between the 15-30 cm soil depths. This area corresponded approximately to the root zone where infected roots and crowns along with plowed under colonized celery trimmings from last years crop were left for decay. Although contamination from upper soil sections could have contributed to the detection of the pathogen at lower soil depths, it is possible that infected celery roots could reach such depths in the soil. Since muck soil horizons are not well defined as in mineral soils (3), physical soil barriers of lower horizons in muck soils are less prominent and infected roots may possibly extend into such horizons.

The significant decrease in the populations of F. oxysporum and FOA2 between the two sampling periods suggested that populations might be reduced if buried deep enough. Since significant disease loss still occurred on the susceptible celery cultivars grown in the field after the deep plowing, inoculum levels were apparently not reduced enough to decrease disease. However, deep plowing could aid in managing FOA2 populations in fields with low infestations to slow the rapid inoculum increase that occurs.

Endo (7) reported that macroconidia were less infective than chlamydospores in the Fusarium yellows of celery disease. Since the macroconidial suspensions did not cause as much disease as did similar levels of chlamydospores assumed to present in the naturally infested soils, these data would concur with Endo (7). However, an accurate comparsion of disease severity caused by these two propagules was not investigated.

Although, macroconidia were able to cause low incidence of disease by a brief dip at 1 X 10^{4} macroconidia/ml, a period of 12 hours was optimal for infection. Hart and Endo (9) observed that disease ratings of Fusarium yellows of celery also increased as the exposure time to soil inoculum increased. In other studies, Hart and Endo (8) concluded that root dips were ineffective in inciting consistent disease symptoms due to the lack of undamaged root apices to served as infection courts. We did not directly compare the macroconidial root dip with the artificially infested soil technique for inciting disease, but the number of disease escapes observed in the spore dip procedure confirmed Hart and Endo's (8) conclusion that this procedure has little use in studies with Fusarium yellows of celery.

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CHAPTER III

SURVIVAL AND ROOT COLONIZATION OF FUSARIUM OXYSPORUM F. SP. APII RACE 2, AND EFFECTS OF CROP RESIDUES ON SOIL POPULATIONS AND RESULTING DISEASE IN CELERY IN MICHIGAN

INTRODUCTION

Pathogenic formae of Fusarium oxysporum are reported to persist in soil for long periods of time after the host crop has been removed (30, 32). Armstrong and Armstrong (2) demonstrated that cotton wilt incidence was still very high in an infested field after a 12 year absence from cotton. Katan (15) showed that F. oxysporum f. sp. lycopersici was still present in a field in which tomatoes had not been grown for 10 years. Several researchers have suggested that the high capacity for survival was due in part to the ability of the wilt Fusaria to colonize the roots and stems of nonsusceptible hosts (1, 2, 12, 15, 33, 37, 41). Studies on the survival of populations of pathogenic strains of F. oxysporum in fallow soils without any plant roots or residues have been mostly limited to greenhouse (9, 38) and laboratory (3) studies where environmental parameters could be monitored. Likewise, available field survival data of wilt Fusaria in natural soil are often clouded by the inability to accurately distinguish the pathogen from the morphologically identical saprophytes (13). These obstacles have made long term field population studies with the pathogenic Fusaria cumbersome and they are often avoided.

Fusarium yellows of celery incited by F. oxysporum f. sp. apii (Nels. and Sherb.) Synd. and Hans. Race 2 (FOA2) has recently become the most destructive disease of celery in Michigan (7). Soil fungicides and fumigants were ineffective in controlling the disease (17), and genetically stable highly resistant cultivars are not available (6, 22). Therefore, relocation to noninfested fields provides the only alternative for control to the grower other than accepting a partial disease loss by growing moderately resistant celery cultivars, such as `Deacon' and `Tall Utah 52-70 HK'. However, if populations of FOA2 continue to increase on infected celery roots, and on celery trimmings returned to the soil, soil populations may increase until disease levels make these cultivars unmarketable as was demonstrated with other wilt pathogens on crops with some resistance (5, 20). Celery cultivation in Michigan exists exclusively on valuable organic muck soils and infested fields are not likely to be left fallow, but rotated into alternate or cover crops.

Several investigations have revealed that plant residues in soil can either increase (35, 40) or decrease (24, 31) populations of soilborne fungal pathogens. Since FOA2, like most wilt Fusaria, has competitive saprophytic ability (23), information on the influence of alternate crop residues on soil populations of FOA2 would aid in planning crop rotation strategies to decrease disease severity when infested fields were rotated back into celery.

The objectives of this work were to determine field survival in fallow and in cropped soil, and root colonization ability of FOA2 in natural muck soil, and the influence of residues of celery and

nonsusceptible crops on the populations of FOA2 and resulting effects on severity of Fusarium yellows of celery.

MATERIALS AND METHODS

Soil and Root Assays For F. oxysporum

Soil was assayed for F. oxysporum by conventional soil dilutions. Five g soil samples (oven dry weight equivalent) were suspended in 500 ml of distilled water (0.01 g soil/ml suspension) and agitated with a magnetic stir bar for 30 min. Ten ml of the suspension was diluted with 90 ml of distilled water from which three to five replicate 5 ml aliquots were mixed into 50 ml of molten cooled (48-50 C) Komada's Selective Medium (KSM) (16). Five plates were poured (10 ml each) and incubated at 25 C under a 12 hour photoperiod. The total number of colonies of F. oxysporum on the five plates were recorded after 5-7 days. Colonies of F. oxysporum were identified by pigmentation and spore morphology (21) and expressed as colony-forming units (cfu)/g soil (oven dry weight equivalent).

Colonization of plant roots by F. oxysporum was assessed by washing roots in running tap water for 2 hours, surface sterilizing roots in 10 % household bleach for 1 min., and rinsing with distilled water. The roots were blotted dry on absorbent paper, weighed, and homogenized with 250 ml of distilled water in a Waring blender for 1 min. Another 250 ml of distilled water were added to the root suspension and agitated with a magnetic stir bar from which 10 ml were removed and added to 90 ml of distilled water. Three to five replicate aliquots (5 ml each) of the root suspension were mixed into 50 ml of

cooled molten (48-50 C) KSM, poured into 5 petri plates and incubated as above. Colonies of F. oxysporum were enumerated as before and expressed as cfu/g fresh root tissue.

Plant Materials and Growing Conditions

In addition to the highly susceptible and moderately resistant celery (Apium graveolens var dulce L.) cultivars `Tall Utah 52-70 R Improved' and `Tall Utah 52-70 HK', respectively, the following alternate crops, cover crops and weed species were investigated for their role in the ecology of FOA2: Onion (Allium cepae L.) `Spartan Banner', carrot (Daucus carota L.) `Gold Pak', parsley (Petroselinum crispum (Mill) Nym.) `Moss Curl', peppermint (Mentha piperita L.) `Black Mitchem', lettuce (Lactuca sativa L.) `Minetto', cabbage (Brassica oleracea L.) `Golden Acre', sweet corn (Zea mays L.) `Harmony', sudax (Sorghum sp.) `Haygrazer', rye (Secale cereale L.), purslane (Portulaca oleracea L.), barnyardgrass (Echinochloa crusgalli (L.) Beauv.), lambsquarters (Chenopodium album L.), smartweed (Polygonum pennsylvanicum L.), crabgrass (Digitaria sanguinalis (L.) Scop.), redroot pigweed (Amarathus retroflexus L.) and prostrate pigweed (A. blitoides S. Wort.) All plants were tested by planting seeds in naturally infested soil, except for celery and parsley where 1-month-old transplants were used, and mint, where cuttings rooted in sterile sand under intermittent mist were used for transplanting into infested soil. Transplants were grown in a commercial potting soil. All plants were incubated in a greenhouse maintained at 18-30 C. Three applications of 20-20-20 soluble fertilizer were made at 2-week intervals begining 1 week after germination or transplanting. Soil and roots were then

assayed for pathogen populations and root colonization, and celery cultivars were rated for disease.

Disease Ratings

Disease ratings were made visually on all plants after 6 weeks growth in the greenhouse, and were based on a scale of 1-6 where 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown area, 4 = VD in 11-25 % of the crown area, 5 = VD in 26-75 % of the crown area, 6 = VD in 76-100 % of the crown area or death of the plant. A longitudinal slice was made with a knife through the center of the plant crown prior to rating plants for percentage of vascular discoloration.

Pathogenicity Tests

Isolates of F. oxysporum were assayed for pathogenicity by transferring a single spore or hyphal tip to the center of a PDA plate and incubating for 2-3 weeks under a 12 hour photoperiod. Colonized plates were homogenized with 100 ml of sterile distilled water in a blender and mixed into 0.35 kg of potting soil. The infested soil was dispensed into two-10 cm pots and healthy 1-month-old seedlings of `Golden Detroit' or `Tall Utah 52-70 R Improved' were transplanted into the soil. Accurate race identification of FOA2 required that vascular discoloration be detected in the roots or crown of both the self-blanching cultivar `Golden Detroit' and the green cultivar `Tall Utah 52-70 R Improved'. Stunting alone was not considered to be sufficient evidence of disease. Seedlings placed into either soil supplemented with PDA colonized by known isolates of FOA2 or with sterile PDA served

as controls.

Survival of FOA2 in Fallow Soil

Two different samples of muck soils were used to study pathogen survival: one gave no detectable level of Fusarium yellows disease with susceptibile celery and the other was naturally infested with FOA2 (73 cfu/g soil) and had had severe disease for several years. Both soils were artificially infested by incorporating FOA2-colonized celery trimmings. A FOA2-colonized agar plug removed with a sterile # 3 cork borer was transferred to autoclaved leaves and petioles of `Florida 683' celery and incubated for three weeks, air-dried, ground in a Wiley Mill, and passed through a 3 mm sieve. The colonized residues produced free microconidia and macroconidia along with abundant chlamydospores. To distinguish FOA2 from the other F. oxysporum colonies without resorting to extensive pathogenicity tests, inoculum was incorporated into each soil at a high level (5 g colonized celery trimmings/100 g soil) so natural populations would be diluted out below detection in any significant amount. Noninfested soils of each type were also included as controls. Two g aliquots of each soil were placed in small nylon mesh (15 μm) bags and buried in galvanized cans filled with their respective muck soil and placed 5 to 10 cm below the soil surface at three locations: North Muskegon, MI (Site 1) and two sites on the Michigan State University campus in East Lansing, MI (Sites 2 and 3).

When the experiment was begun and every three months for 2 years thereafter, three replicate bags of each soil were recovered from each site and assayed for populations of F. oxysporum. On month 15, 50 isolates were tested for pathogenicity to confirm that colonies

recovered were FOA2 and not saprophytic F. oxysporum.

Detection of Symptomless Carriers of FOA2

Weeds and several crop plants were collected on three occasions from a FOA2-infested field in North Muskegon, MI that had been rotated out of celery for two years. The field had been planted entirely to onions the first year and onions, sweet corn and cabbage the second year. Depending on availability, ten to twenty plants of several weed species and crop plants were collected per sampling and examined for signs of vascular discoloration.

Roots were assayed as mentioned above. Stems were sampled by flaming 5-cm stem segments with 95 % ethanol, peeling back the outer tissue, removing wedge-shaped pieces of vascular tissue, and placing these pieces on KSM. Over 60 pieces of stem tissue were plated out per plant species. Depending on the number of isolates of F. oxysporum obtained from each plant species, a proportion (3-60 %) was assayed for pathogenicity on `Golden Detroit' and `Tall Utah 52-70 R Improved' celery.

Disease Reaction of Celery and Nonsusceptible Plants in Soils Infested with FOA2

Eight crop plants were tested for susceptibility to FOA2 using soil infested with colonized wheat straw inoculum described in Chapter II. Inoculum was incorporated into potting soil at the rate of 0.2 g inoculum/liter soil. Six replicate pots of celery, parsley, peppermint, cabbage, lettuce, sweet corn, sudax and rye were used containing five plants per pot. Controls consisted of seeds or transplants grown in noninfested soil. Corn, sudax and rye were examined for disease symptoms

after 2 weeks because of their rapid growth rate, while all other plants (celery, parsley, peppermint, cabbage, and lettuce) were evaluated after 6 weeks. Signs of vascular discoloration and dry weights were recorded for all plants and comparisons were made with Student's t-test.

Effect of Root Exudates of Celery and Other Crop Plants on Germination of Chlamydospores of FOA2 in a Chlamydospore-Agar-Soil Overlay (CSAO) System

A modification of the soil overlay system utilized by Hart (10) was employed to study chlamydospore behavior in soil near roots of various plants. A highly virulent isolate of FOA2 (FA-3, ATCC 52626) was transferred from test tubes of autoclaved muck soil to carnation leaf agar (21) or potato-carrot agar (36) and incubated at 25 C for 15-17 days under a 12 hour photoperiod. Conidia were washed from the surface of plates with 10 ml of sterile distilled water, filtered through 4 layers of cheesecloth to removed mycelial fragments, and washed three times by centrifugation at 3000 g for 5 min. The resuspended pellet consisted of microconidia and macroconidia at an approximate ratio of 10:1. Macroconidia were enumerated with a hemacytometer, the conidial suspension was mixed with cooled molten (48-50 C) water agar in an amount sufficient to yield 2.5 X 10^5 macroconidia/ml, and the agar was immediately poured into 6.0 cm petri plates (3 ml/plate). Chlamydospores were induced by placing a layer of field mineral soil (Sphinks loamy sand) over the surface of the agar. The soil had been moistened with water 2 hours prior to use to yield a final moisture content of 20 %. Plates were incubated for 3 days in the dark, air-dried for 2-3 days to facilitate maturation of chlamydospores, and soil was then removed from the agar surface with a paint brush under a gentle

stream of water. Chlamydospores were easily observed microscopically in the agar matrix under a magnification of 200 X and had developed terminally at the end of short macroconidial germ tubes, or less frequently were intercalary within macroconidia. Chlamydospores were distinguished from hyphal swellings by the thick cell walls and dense cytoplasm.

Seedlings of highly susceptible and moderately resistant celery cultivars along with nine other alternate crops were tested for effects of their root exduates on chlamydospore germination. Seeds of celery, carrot, parsley, cabbage, and lettuce were germinated in a commercial potting mix and removed after 3 weeks. Corn, rye and sudax were germinated on sterile filter paper and removed after 5 days. Onion was similarly germinated and removed after 7 days. Mint cuttings were rooted in sterile sand under intermittent mist. Roots were gently washed to remove soil particles and seedlings were placed on top of the CASO system so that the foliar portions of the seedling extended out of the petri plate through a hole in the side of the plastic petri dish. The seedlings were anchored to the surface of the agar by placing a small quantity of soil on the stem, but the roots were left exposed. After 2 days incubation at 25 C under a 12 hour photoperiod, the region behind the root apices was placed at the bottom of the field of microscopic view under 200 X magnification, and the total number of germinated chlamydospores out of 25 counted in this field was enumerated. Both sides behind the root tip were examined for chlamydospore germination. This region was reported to be the infection court for FOA2 (11) and other Fusarium wilt pathogens. (29, 34).

Chlamydospores were scored as germinated if the length of the germ tube was at least the diameter of the spore. At least 3 root tips of 4 seedlings were examined. Untreated CASO system plates served as controls.

Root Colonization by an Orange Mutant of FOA2 of Celery and Other Crops and Weed Plants

To eliminate the need for extensive pathogenicity tests to distinguish FOA2 from the nonpathogenic strains of F. oxysporum in soil, a pathogenic orange mutant (OM) of FOA2 was produced from Michigan isolate FA-3 (ATCC 52626) (8) by exposure to ultra-violet light according to the procedures of Puhalla (25) and increased on wheat straw as mentioned in Chapter II. OM-colonized wheat straw was incorporated into natural muck field soil (3 g wheat straw/kg soil) by rotating the soil in a cement mixer for 20 min. Soil was stored in plastic bags at 30 % moisture content until use. Following a 6 week incubation at 18-30 C to allow time for the OM populations to stabilize, a highly susceptible (`Tall Utah 52-70 R Improved') and moderately resistant (`Tall Utah 52-70 HK') cultivar of celery, along with seven agronomic crops and four weed species, were transplanted into the soil. All plants were seeded in the OM-infested soil except for celery, parsley and mint, which were placed into the soil as transplants. Three pots for each species were prepared, each containing five seedlings per pot.

The roots of all plants of each species in the 3 replicate pots were excised after 6 weeks, combined into a composite sample, and assayed for cfu of OM/g root tissue. Care was taken to remove all adhering soil particles by washing. Preliminary experiments were

performed to determine the proper dilution factor, which depended on quantity of roots available and degree of colonization by the OM. Five-5 ml aliquots of the final dilution were mixed into KSM and plates were incubated and colonies enumerated as described above.

Effect of Crop Residues on Populations of an Orange Mutant of FOA2

Crop residues were collected at the Michigan State University Muck Farm in Bath, MI. Celery trimmings (`Florida 683'), small onion culls (`Spartan Banner'), peppermint foliage (`Black Mitchem'), potato vines (`Russet Burbank'), and rye and sudax residues were air-dried, passed through a Wiley Mill and stored in plastic bags at 4 C.

The OM-infested soil used above was used to study the effect of crop residues on populations of FOA2 so that colonies could be identified without having to resort to pathogenicity tests. Five hundred-g aliquots of the soil (30 % moisture content) were supplemented with celery, onion, and mint residues at the rate of 0.5 g residue/100 g soil (oven dry weight equivalent). The residue-supplemented soils were moistened with distilled water to bring the moisture content up to 50 % and soils were incubated in plastic bags loosely tied to allow for gas exchange at 25 C in the dark. Non-supplemented soils served as controls. Five-g samples (oven-dry-weight equivalent) were assayed for OM populations at day 0 and day 1, and at week 1, 2, and 3. The experiment was repeated once.

Effect of Water Extracts of Crop Residues on Germination of Chlamydospores of FOA2 in a CASO System

The CASO described above was used to examine the effects of water extracts from crop residues on chlamydospores. Water extracts from

celery, onion, mint, sudax and rye were prepared by suspending 1 or 2 g of the dried plant residues in 100 ml of distilled water and agitating the suspension with a magnetic stir bar for 2-3 hours. The suspension was filtered through 4 layers of cheesecloth and centrifuged for 10 min at 10,000 g. The filtrate from 1 g/100 ml distilled water residue supension was designated as X concentration. Each treatment was placed in four agar wells (200 µl/well) produced in the CASO with a # 1 cork borer. Following a 48 hour incubation in the dark at 25 C, plates were stained with phenolic rose bengal, and the area surrounding the agar well was examined microscopically under a 200 X magnification. The number of germinated chlamydospores out of 100 counted along the perimeter of the well's edge was recorded. The mean of four wells represented one replicate, and four replicates were examined per treatment. Wells filled with distilled water served as controls. The experiment was repeated once.

Effect of Water Extracts of Crop Residues on Growth of FOA2 in a Minimal Broth Medium

Water extracts of celery, onion and mint were prepared as described earlier and treatments were expressed as X or 2X rates. Extracts were amended with salts and sucrose to yield a 25 % strength modified (15 g sucrose/ liter) Czapek-Dox broth (36). Twenty five ml were dispensed into 125 ml flasks and autoclaved. Each flask received a FOA2 agar plug (#1 cork borer) colonized with FA-3 (ATCC 52626) from the outer margins of a 3-day-old FOA2 colony produced on PDA at 25 C.

Flasks were incubated in the dark at 25 C for 3 or 6 days whereupon the fungal mats of four replicate flasks were harvested on preweighed

glass fiber filter papers and rinsed twice with 150 ml of distilled water. Controls were flasks that contained each extract and received a sterile PDA agar plug, and flasks that contained only a 25 % Czapeks Dox solution, no residue extracts, and a sterile or a colonized agar plug. Fungal mats were dried at 80 C for 24 hr and weighed.

Influence of Crop Residues on Fusarium Yellows of Celery in Naturally Infested Soils

Soil naturally infested with FOA2 (73-103 cfu/g soil) was dried, passed through a 2 mm sieve, and supplemented with residues of celery, onion, mint, potato, rye and sudax (1 g residue/liter soil). These soils were allowed to incubate for 1 month in the greenhouse at 18-30 C. Three one month-old celery seedlings (`Tall Utah 52-70 R Improved') were then transplanted into 10-cm pots filled with the residue-supplemented soils. Steamed field soil and a 1:1 mixture of steamed:naturally infested field soil were also supplemented with the residues and celery seedlings were transplanted into them. There were ten replicates. Celery seedlings grown in soil not supplemented with residues served as controls. Disease ratings and dry weights were taken and analyzed for differences after 6 weeks.

Another study was conducted to determine the effect of plant residues in soils with low inoculum densities on disease expression. Naturally infested soil containing approximately 73 cfu of FOA2/g soil was diluted with steamed soil to yield the proportions of 0, 10, 25, 50 and 100 % naturally infested soil. Each soil mixture was supplememted with residues of celery, onion and mint (1 g residue/liter soil). Soil mixtures without residues served as controls. Healthy celery seedlings

were transplanted into the soil and held in the greenhouse for 6 weeks. Disease expression was assessed as dry weights of plants and regressed against the estimated inoculum densities (EID) in the different soil mixtures.

The effects of different concentrations of plant residues in naturally infested soil on disease severity and celery dry weights were also investigated. Soils were supplemented with residues at the rate of 0.5, 1.0 and 2.0 g residue per liter of soil, celery seedlings were transplanted into the soil, and seedlings were assessed for Fusarium yellows by disease ratings and dry weights.

RESULTS

Survival of FOA2 in Fallow Soil

Populations of FOA2 in artificially infested soil from the 3 fallow field sites fluctuated widely, but generally remained greater than the respective noninfested soil controls over the 2 year period (Figures 3.1, 3.2 and 3.3). Overall, the pathogen appeared to survive slightly better in the muck soil having no natural population of FOA2 than in soil having a natural population at sites 1 and 2. Site 3 (Figure 3.3) experienced periodic flooding during the first 6 months which resulted in approximately 95 % reductions in the FOA2 populations, but at other sampling times populations in both infested and noninfested soils were essentially equal. *F. oxysporum* populations increased rapidly after flooding periods; the reasons for these increases are unclear. Pathogenicity tests conducted on recovered *F. oxysporum* colonies confirmed that FOA2 was still present after the flooding periods, and

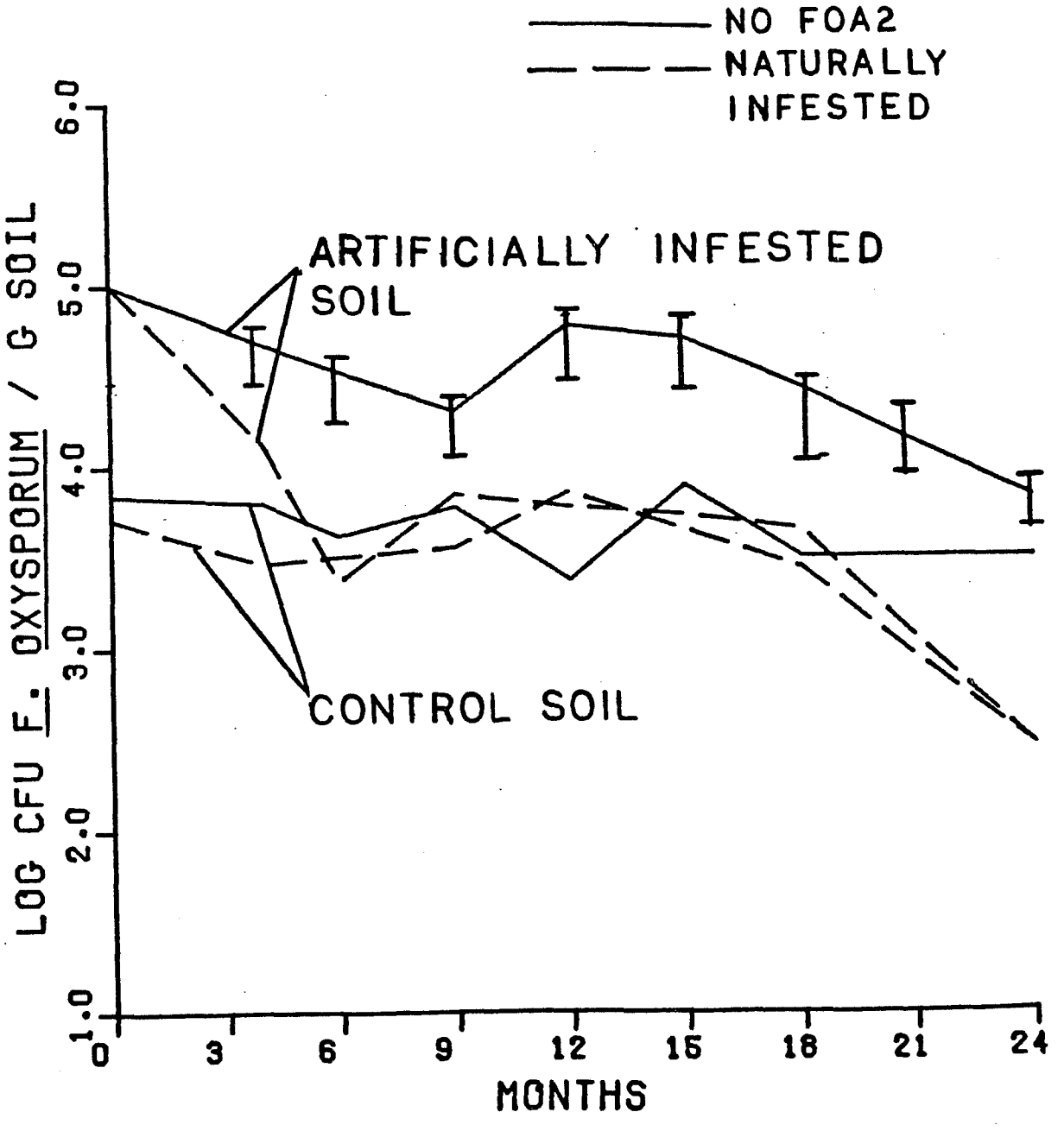

Figure 3.1, Survival Site 1: North Muskegon, MI, Persistence of FOA2 in artificially infested and noninfested muck soil having no FOA2, and in muck soil having FOA2 as determined by conventional soil dilutions. Standard errors of means are provided for one soil only, but represent the variation of the populations in all soils assayed.

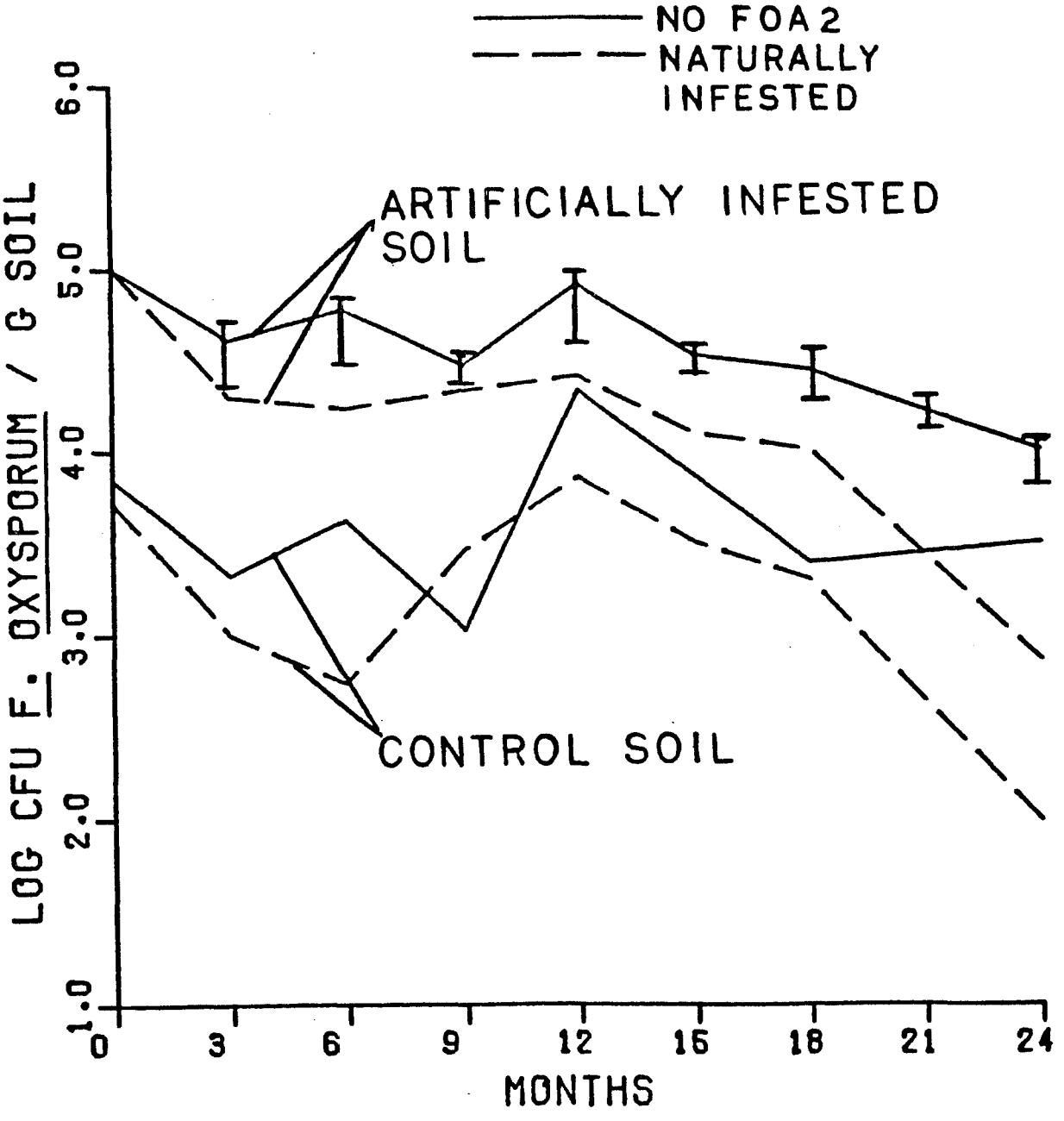

Figure 3.2. Survival Site 2: East Lansing, MI, Persistence of FOA2 in artificially infested and noninfested muck soil having no FOA2, and in muck soil having FOA2 as determined by conventional soil dilutions. Standard errors of means are provided for one soil only, but represent the variation of the population in all soils assayed.

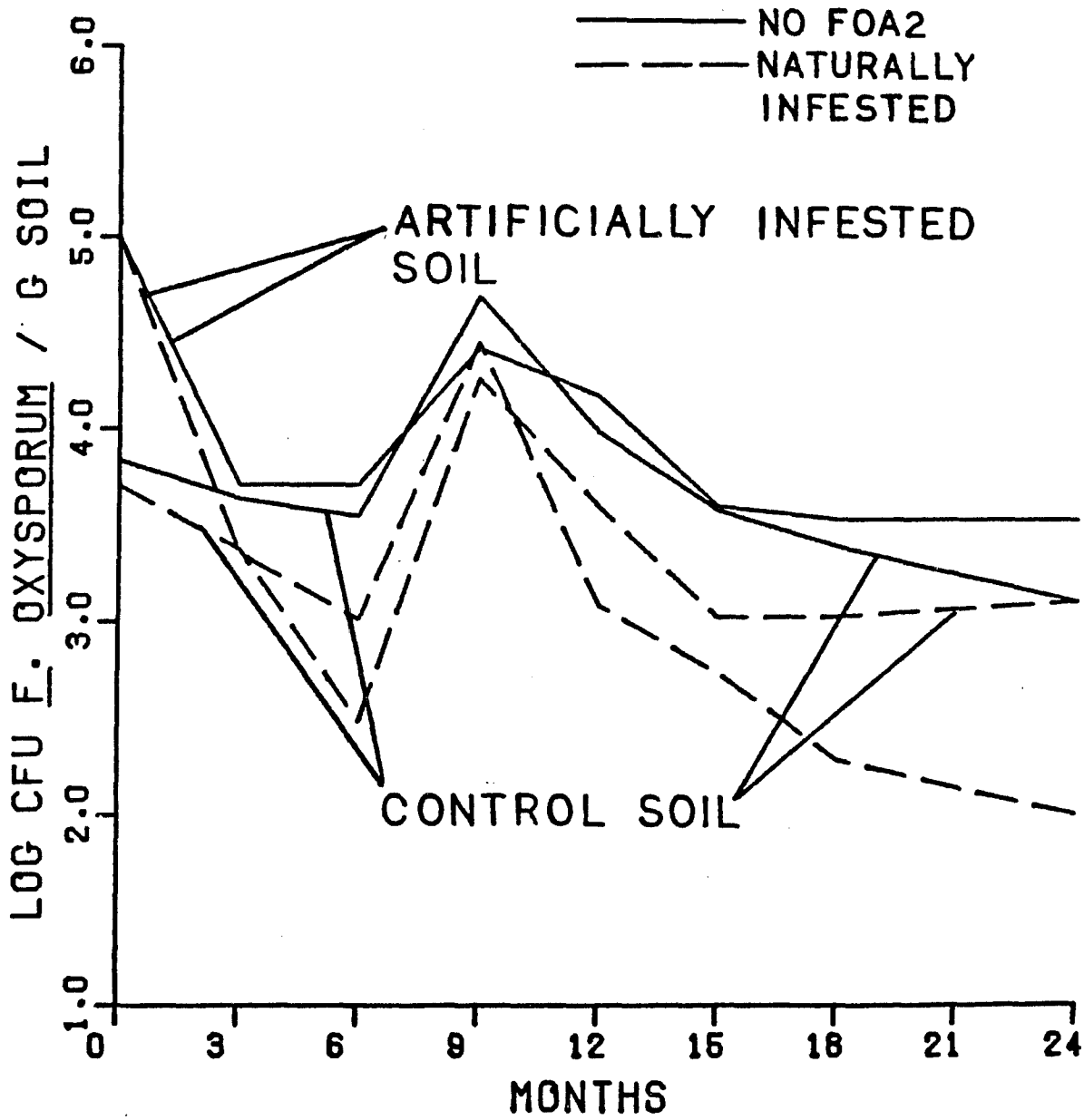

Figure 3.3. Survival Site 3: East Lansing, MI Persistence of FOA2 in artificially infested and noninfested muck soil having no FOA2 and muck soil having FOA2 as determined by conventional soil dilutions.

that colonies were not saprophytic F. oxysporum.

Symptomless Colonization of Crop Plants and Weeds in the Field

No vascular discoloration could be observed in plants sampled from the infested field that had been rotated out of celery 2 years previously. However, isolates of FOA2 were found to symptomlessly colonize roots and stems of plants and weeds (Table 3.1). Roots yielded predominately F. oxysporum and F. solani. FOA2 was detected in the stems of onion, cabbage and smartweed, while no Fusaria were recovered from stems of redroot pigweed (data not shown), prostrate pigweed (data not shown), lambsquarters, or purslane. Barnyard grass roots were consistently more heavily colonized by F. oxysporum than those of other plants (Table 3.1). Findings were consistent between sampling periods.

Symptoms and Growth of Celery and Nonsusceptible Crop Plants Grown in Artificially Infested Soil

No signs of stunting, wilting or vascular discoloration were observed on crop plants tested in artificially infested soil except for celery (Table 3.2). Likewise, no significant differences were detected between the dry weights of plants grown in infested soil and those grown in noninfested soil. Celery plants grown in infested soil exhibited a high degree of vascular discoloration, but differences in dry weights were not significant.

Effect of Root Exudates of Celery and Nonsusceptible Plants on Chlamydospore Germination

All plant roots tested in the CASO system produced exudates that stimulated germination of chlamydospores (Table 3.3). No significant differences were detectable in stimulation of chlamydospores between

Table 3.1. Symptomless Colonization of Weeds and Crop Plants by F. oxysporum and FOA2 Under Field Conditions.

Plant	Tissue	Total F. oxysporum/g root tissue[a]	# isolates tested[b]	% FOA2
Lambsquarters	Root	93.5	14	71
	Taproot	-	2	100
Purslane	Root	56.6	9	33
Smartweed	Root	27.0	18	77
	Stem	-	5	80
Barnyardgrass	Root	485.5	17	5
	Stem	-	1	0
Sweet Corn	Root	29.2	14	50
Cabbage	Root	4.5	3	33
	Stem	-	3	67
Onion	Root	15.1	3	67
	Stem	-	1	100

[a]Root colonization was determined by diluting homogenized root suspensions into KSM, incubating the plates, and enumerating colonies of F. oxysporum. Stems and taproot were sampled by placing pieces of stem and taproot segments on solidified KSM, incubating the plates, and enumerating colonies of F. oxysporum.

[b]Isolates were tested for pathogenicity on `Tall Utah 52-70 R Improved' and `Golden Detroit'.

Table 3.2. Symptoms and Growth of Nonsusceptible Crop Plants in Soil Artificially Infested with FOA2.

Plant	Disease[a] Rating	Dry Weights (g) Control	Infested[b]
Celery	4.5	3.5	3.0 ns
Parsley	1.0	2.5	3.3 ns
Cabbage	1.0	3.9	4.1 ns
Lettuce	1.0	3.3	3.6 ns
Mint	1.0	7.7	9.1 ns
Sweet corn	1.0	8.8	8.7 ns
Sudax	1.0	5.6	5.9 ns
Rye	1.0	1.9	1.6 ns

[a]Values represent the mean of 6 pots consisting of 5 plants/pot; ns = not significantly different from its respective control by student's *t*-test at *P* = 0.05. Disease ratings based on the scale: 1= no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown area. 4 = VD in 11-25 % of the crown area, 5 = VD in 26-75 % of the crown area, 6 = VD in 76-100 % of the crown area

[b]Soilwasinfested with 0.2 g of FOA2-colonized wheat straw inoculum /liter soil.

Table 3.3. Effects of Root Exudates of Celery Cultivars and Other Plants on Germination of Chlamydospores of FOA2 in a CASO System.

Plant	Germination Percent[a]	Standard Error
1. Control	1.6[c]	1.7
2. Celery (HS)[b]	48.2	20.0
3. Celery (MR)[b]	61.0	5.9
4. Carrot	25.8	18.8
5. Parsley	34.5	15.9
6. Onion	43.5	14.9
7. Cabbage	19.1	18.4
8. Lettuce	28.0	9.2
9. Mint	27.0	14.8
10. Rye	64.8	22.8
11. Corn	97.0	1.7
12. Sudax	80.4	10.9

[a]Indicates the percentage germinated out of 25 chlamydospores counted per reading.

[b]HS = Highly susceptible to FOA2 (`Tall Utah 52-70 R Improved'), MR = Moderately resistant to FOA2 (`Tall Utah 52-70 HK').

[c]Values represent the mean of 4 samples consisting of 3 root apices counted/sample.

root exudates of the highly susceptible celery cultivar, 'Tall Utah 52-70 R Improved', and the moderately resistant celery cultivar, 'Tall Utah 52-70 HK'.

Chlamydospore germination occurred to some degree all around the root area of all plants, but behind the root apices the percentage of germinating chlamydospores and the lengths of the germ tubes were greater. In almost every case, the germ tube was oriented toward the root indicating a chemotactic response. Wide variability in germination occurred between roots of the same plant and between roots of different plants of the same species despite care to standardize every treatment.

Colonization of Celery and Nonsusceptible Plant Roots by an Orange Mutant of FOA2

Celery, carrot, onion, cabbage, mint, corn, sudax, purslane, barnyardgrass, and lambsquarters harbored the orange mutant (OM) in their roots. Parsley and crabgrass had no detectable colonization of the OM while lettuce had very little (Table 3.4). Three times more cfu of the OM was detected in the roots of susceptible celery cultivars than in the roots of the moderately resistant cultivar. However, ten times greater root colonization occurred in carrots than in susceptible celery roots. Roots of corn and barnyardgrass sustained an extremely high level of OM colonization. Cabbage, mint and sudax supported levels of colonization by the OM approximately equal to those in susceptible celery, whereas a higher level of root invasion occurred in the weeds lambsquarters and purslane than in susceptible celery.

Effect of Crop Residues on Soil Populations of OM

Residues of celery, onion and mint promoted an increase in the cfu

Table 3.4. Colonization of Roots of Celery Cultivars and Other Plants by OM in Natural Soil.

Plant	Total Orange Mutant CFU/g fresh root
1. Celery (HS)[x]	19.2[y]
2. Celery (MR)[x]	6.6
3. Carrot	198.0
4. Parsley	0.0
5. Onion	4.0[z]
6. Cabbage	13.3
7. Lettuce	1.5
8. Mint	16.4
9. Corn	458.7
10. Sudax	15.2
11. Purslane	67.7
12. Barnyardgrass	4093.6
13. Lambsquarters	78.7
14. Crabgrass	0.0

[x]HS = Highly susceptible (`Tall Utah 52-70 R Improved'), MR = Moderately Resistant ('Tall Utah 52-70 HK').

[y]Values represent the mean of 3 sample aliquots from a composite root sample of 15 plants grown in OM-infested muck soil.

[z]Onion roots were not comminuted, but placed on KMS and represented 4 cfu of OM from 21 roots.

of OM in soil 1 day after incorporation (Figure 3.4). Onion residues stimulated the greatest increase in OM plate counts. Populations of OM declined rapidly 1 week after incorporation of the mint and onion residues into soil, but declined less than in the celery-supplemented soil. Populations of the OM were also suppressed in the onion and mint residue-supplemented soils after 2 and 3 weeks, but the celery-supplemented soils had populations equal to or greater than the nonsupplemented control.

Effect of Water Extracts of Crop Residues on Germination of Chlamydospores of FOA2 in a CASO System

Water extracts prepared from all crop residues examined stimulated germination of chlamydospores on the CASO system (Table 3.5). Celery extracts promoted the least amount of germination while onion extracts led to approximately 75 % germination, and were significantly greater at both rates than other extracts.

The concentration of extracts also influenced germination. The higher rate (2X) stimulated more germination and resulted in longer germ tubes in celery, onion and rye extracts, whereas in mint and sudax extracts, a decrease was observed at the 2X rate.

Effect of Water Extracts of Crop Residues on Growth of FOA2 in Broth Culture

All broth media made with residue-extracts caused an increase in the dry weights of FOA2 over the nonresidue-supplemented broth, but the type of residue broth and the rate greatly affected the fungal dry weights (Figures 3.5, 3.6, and 3.7). Celery and mint extract broths at both concentrations stimulated a significant increase ($P = 0.05$) in

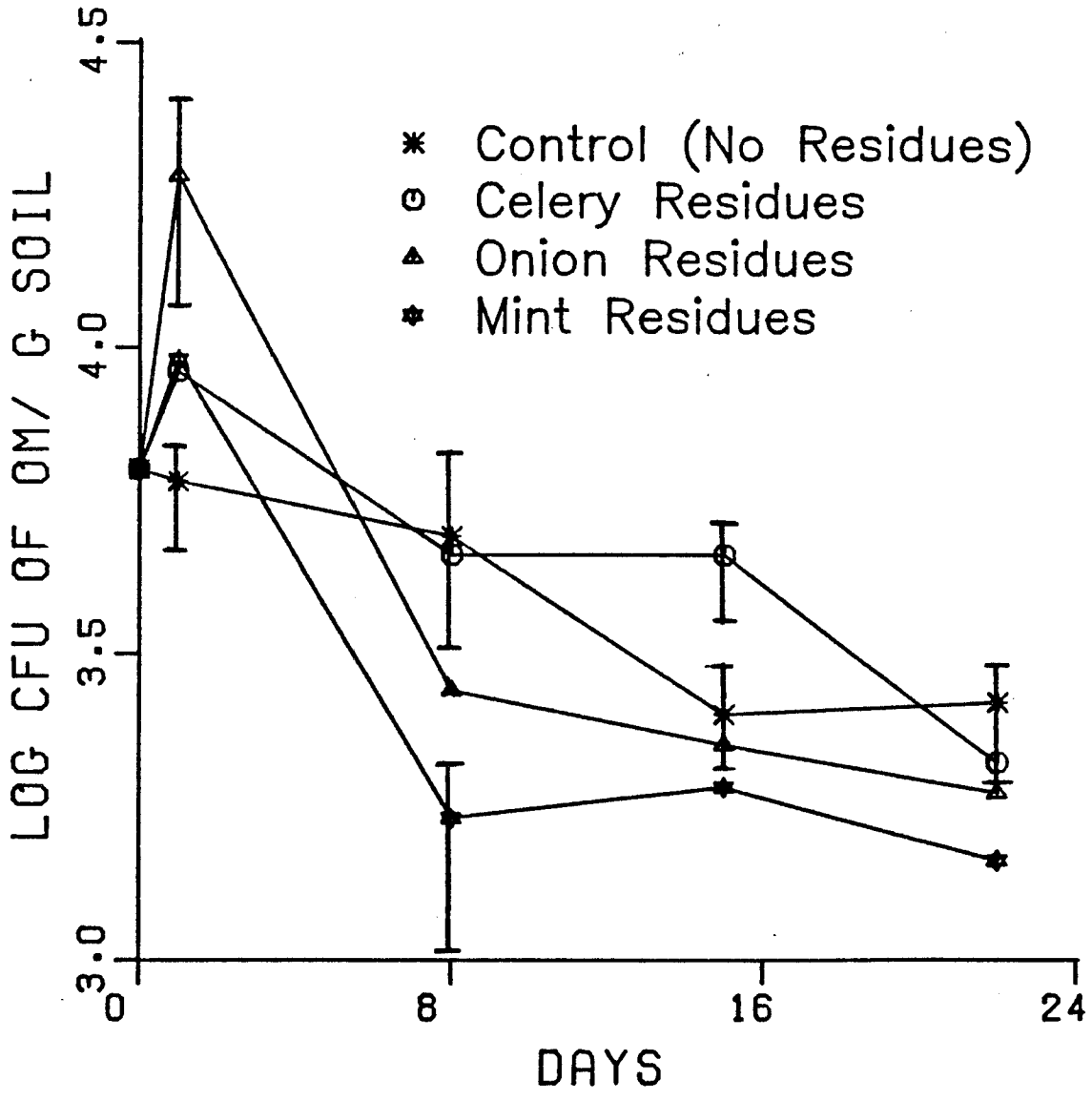

Figure 3.4. Effects of crop residues incorporated into artificially infested soil on populations of an orange mutant of FOA2 (OM) over time as determined by soil dilutions into KSM. Standard errors are provided for specific means only.

Table 3.5. Effects of Water Extracts of Plant Residues on Germination of Chlamydospores of FOA2 in a CASO System.

Plant	Rate[x]	% Germination of Chlamydospores	Standard Errors
Water Control	-	3.7[y]	1.0
Celery	X	4.5	3.9
	2X	6.8	0.3
Onion	X	61.8	4.3
	2X	72.8	11.6
Mint	X	25.4	3.6
	2X	13.2	6.3
Rye	X	9.0	0.9
	2X	22.8	14.0
Sudax	X	29.6	5.7
	2X	19.6	4.3

[x]X = filtrate of 1 g of dried residue/100 ml distilled water, 2X = filtrate of 2 g of dried residue/100 ml of distilled water.

[y]Values represent the mean of 3 replicate, each consisting of 4 samples 50 chlamydospores.

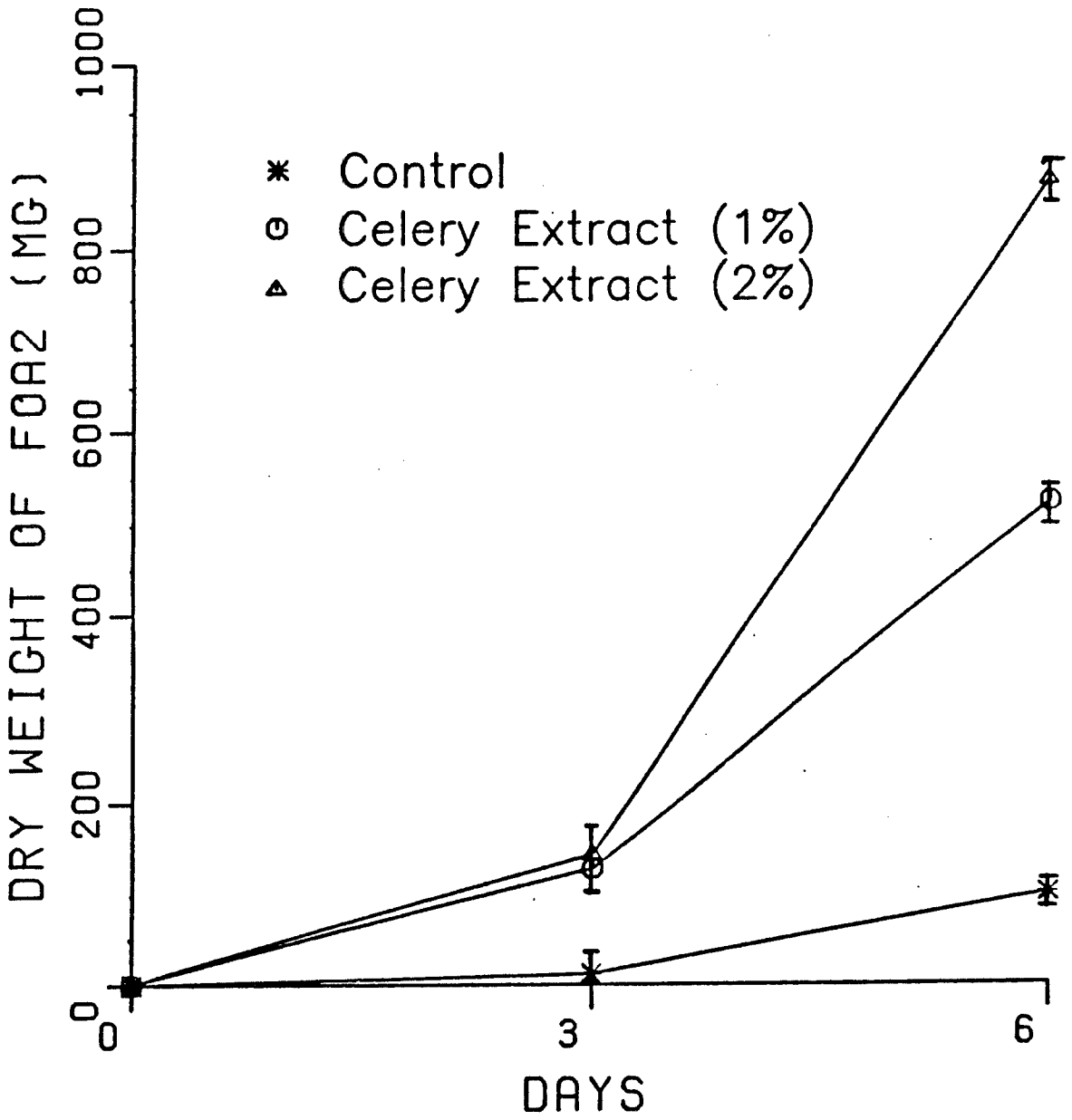

Figure 3.5. Growth of FOA2 in two concentrations of celery-extract broth over time. Extract concentration X represents the filtrate of 1 g of celery residue/100 ml distilled water; extract concentration 2X represents the filtrate of 2 g celery residue/100 ml distilled water. Standard errors are provided for the means.

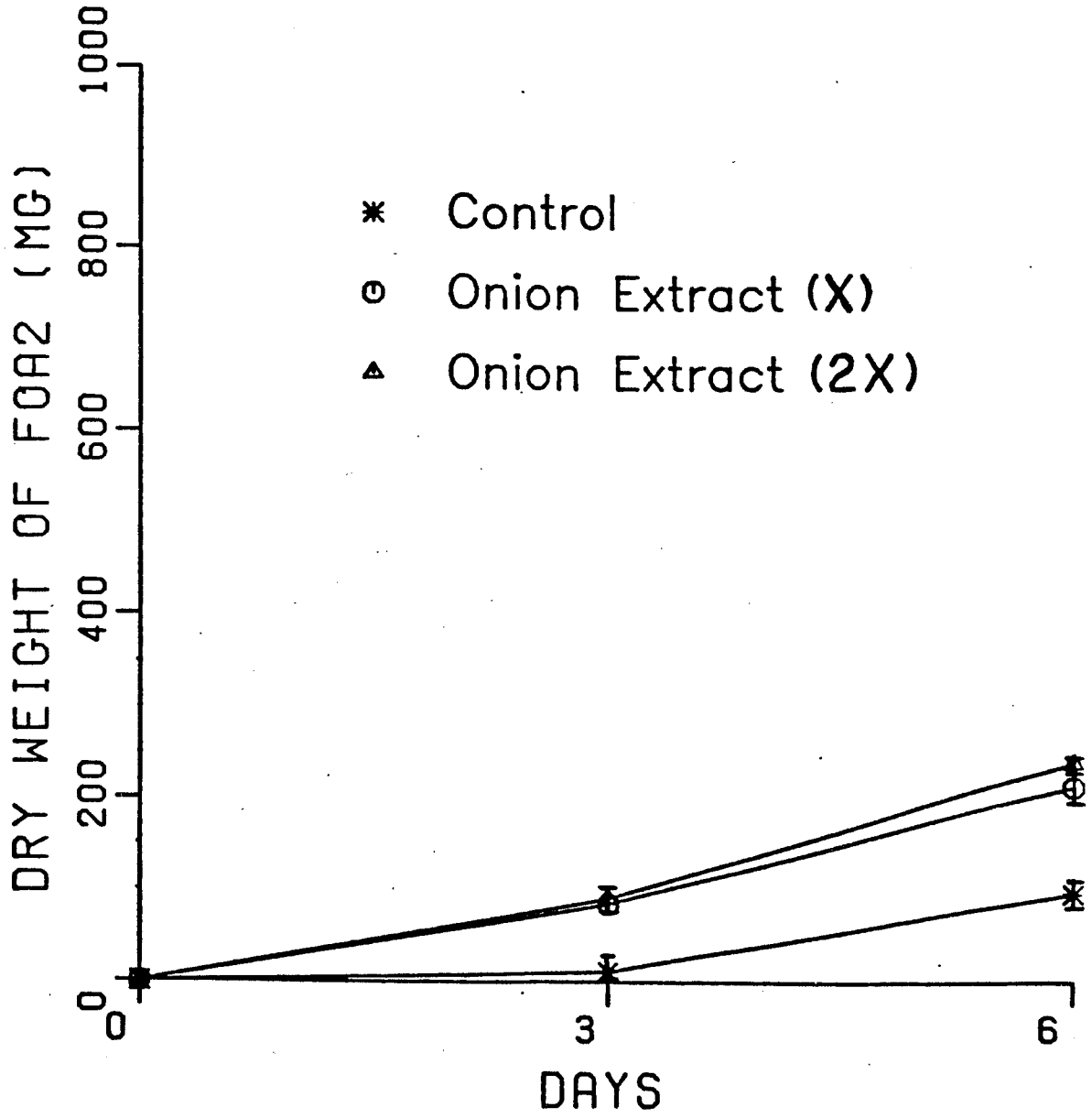

Figure 3.6. Growth of FOA2 in two concentrations of onion-extract broth over time. Extract concentration X represents the filtrate of 1 g of onion residue/100 ml distilled water; extract concentration 2X represents the filtrate of 2 g onion residue/100 ml distilled water. Standard errors are provided for the means.

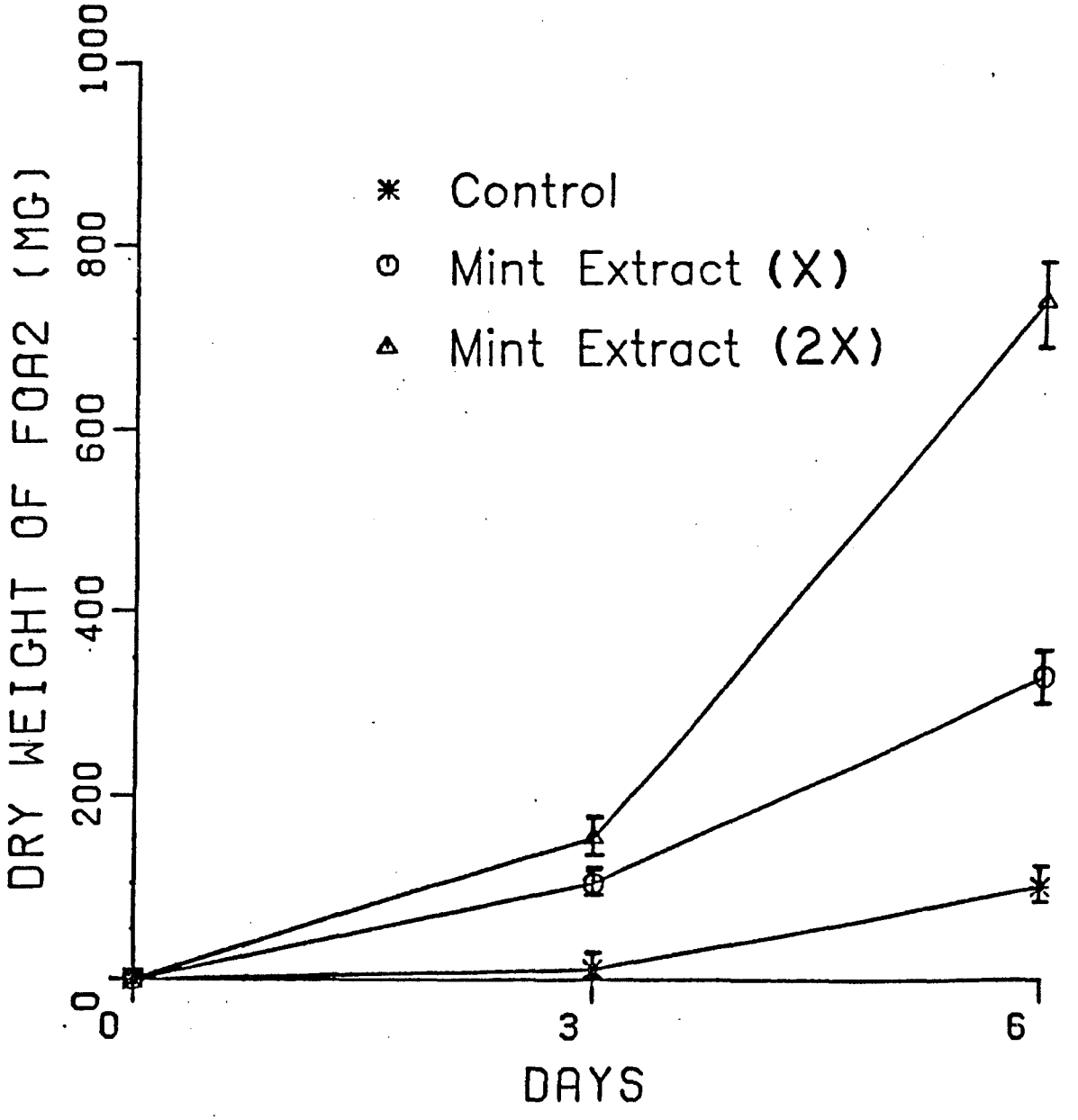

Figure 3.7. Growth of FOA2 in two concentrations of mint-extract broth over time. Extract concentration X represents the filtrate of 1 g of mint residue/100 ml distilled water; extract concentration 2X represents the filtrate of 2 g of mint residue/100 ml distilled water. Standard errors are provided for the means.

growth of FOA2 over the control after 6 days. Onion extract broth promoted a slight increase in fungal dry weight when compared to the control broth, but no significant increase occurred at the higher concentration.

Aerial mycelium in the broth culture was greatest in the celery extract broth treatments and least in the onion extract broth. Differences in dry weights were always greater after 6 days than after 3 days incubation.

Effects of Crop Residues on Fusarium Yellows of Celery

Dry weights of celery plants grown in 1:1 steamed:naturally infested soil supplemented with celery residues were less than those in the nonsupplemented soil (Table 3.6). Celery residues also decreased the dry weights of celery in 100 % naturally infested soil when compared to the control, but not significantly. Sudax residues also decreased the dry weights of celery, but not significantly. Significant increases in dry weights of celery grown in onion-supplemented soil at the 1:1 infested-steamed soil mixture and in mint-supplemented soil in the 100 % naturally infested soil were detected, indicating that these residues suppressed the disease. Celery plants grown in celery and sudax residue-supplemented soil had higher disease ratings, whereas onion and mint residues seemed to suppress the vascular discoloration of test plants when grown in soil supplemented with these residues, but values were not statistically significant (Table 3.6).

Likewise, when naturally infested soil was diluted with steamed soil to 10 % (approximately 7 propagules/g), more stunting of celery occurred when celery residues were incorporated into the soil than in

Table 3.6. Effects of Crop Residues on Fusarium Yellows of Celery in Different Percentages of Naturally Infested Soil.

	Percent Naturally Infested Soil[w]					
	0		50		100	
Residue	DR[x]	DW[y]	DR	DW	DR	DW
No Residues	1.0[z]	4.4	3.4	2.0	4.0	1.4
Celery	1.0	3.9	5.3	0.7 *	5.0	0.9
Onion	1.0	3.4	3.3	2.4 *	3.5	1.6
Mint	1.0	3.7	4.5	1.6	3.2	2.4
Potato	1.0	3.0	3.6	2.3	3.5	1.6
Rye	1.0	3.3	4.7	1.4	4.4	1.7
Sudax	1.0	4.0	4.8	1.1	4.8	1.1

[v]Naturally infested soil had 73-103 cfu of FOA2/g soil determined by conventional soil dilutions and pathogenicity tests and was mixed with steamed soil.

[x]Disease rating based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown area, 4 = VD in 11-25 % of the crown area, 5 = VD in 26-75 % of the crown area, 6 = VD in 76-100 % of the crown area or death of the plant.

[y]Dry weight (g) of the foliar plant portion.

[z]Values represent the mean of 12 pots consisting a 3 plants/pot; values followed by * are significantly different from their respective control by Student-Newman-Keul's test at $P = 0.05$.

nonsupplemented soil which suggests more disease resulted from a rapid increase of FOA2 (Figure 3.8). Onion residues suppressed the disease in 100 % natural soil. In contrast, mint residues did not cause significant differences, but tended to decrease dry weights of celery at low inoculum levels, and increased the dry weights of celery at higher natural inoculum levels.

When crop residues were added in increasing concentrations to naturally infested soil, no significant decreases were noted in disease ratings of plants grown in celery- or mint-supplemented soils (Table 3.7). Celery plants grown in soil supplemented with residues of onion at the rates of 1 or 2 g onion residue/liter of soil did cause a suppression of vascular discoloration and stunting.

DISCUSSION

These investigations provide an understanding of the potential impact of continuous celery cultivation versus rotation with alternate crops on the Fusarium yellow disease of celery. Pathogen populations in fallow soils persisted in a dormant state for at least 2 years. The initial population decrease of 50-90 % of the propagules in the first 6 months could partially be explained by the loss of the nonpersistent microconidia. These spores are less sensitive to fungistasis (19) and conversion of microconidia to persistent chlamydospores is rare. Chlamydospores already present in the celery residues and those presumably produced from macroconidia declined at Sites 1 and 2 to 5-10 % of their original counts after 2 years. The possibility that chlamydospores germinated and grew saprophytically on celery or other

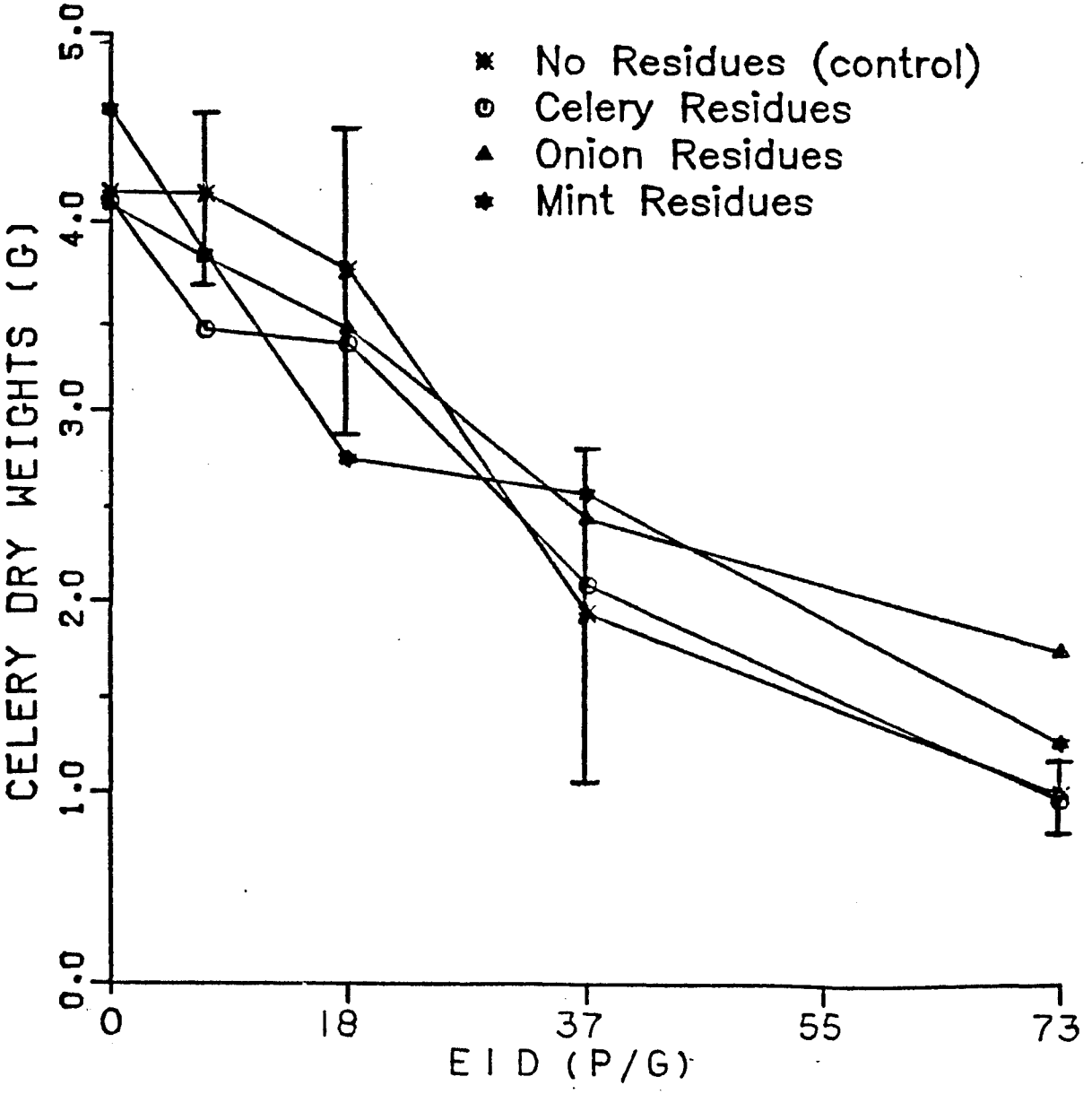

Figure 3.8. Effects of crop residues on Fusarium yellows of celery in increasing amounts of estimated inoculum densities (EID) of FOA2 in naturally infested soil as assessed by celery dry weights.

Table 3.7. Effects of Increasing Concentrations of Crop Residues on Expression of Fusarium Yellows Disease in Celery in Naturally Infested Soil.

		Steamed Soil		Naturally Infested Soil	
Residue	Level[w]	DR[x]	DW[y]	DR	DW
No Residues	-	1.0[z]	4.3	4.1	2.2
Celery	0.5	1.0	4.1	4.3	3.1
	1.0	1.0	4.5	2.7	3.0
	2.0	1.0	4.5	3.3	2.8
Onion	0.5	1.0	4.5	2.6	3.2
	1.0	1.0	3.6	1.8 *	3.3
	2.0	1.0	4.1	2.0 *	3.2
Mint	0.5	1.0	4.1	3.2	2.7
	1.0	1.0	4.4	3.6	2.6
	2.0	1.0	3.7	2.4	2.9

[v]Naturally infested soil had approximately 73-103 cfu of FOA2/g soil determined by conventional soil dilutions and pathogenicity tests.

[w]Represents the amount of residue in g added to 1 liter of soil.

[x]Disease ratings based on the scale 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown area, 4 = VD in 11-25 % of the crown area, 5 = VD in 26-75 % of the crown area, 6 = VD in 76-100 % of the crown area or death of the plant.

[y]Dry weights (g) of the foliar portion.

[z]Values represent the mean of 5 pots consisting of 3 plants/pot; values followed by * are significantly different from their respective control by Student-Newman-Keul's test at $P = 0.05$.

organic substrates, and then formed new chlamydospores as reported (28) has not been excluded. It is doubtful that short term fallowing of infested fields would provide any effective disease control. Long term survival may be more dependent on colonizing roots of nonsusceptible plants, and on competing saprophytically with other microbes for residues substrates than persisting as free chlamydospores as previously argued (2, 12, 15).

Other investigators have concluded that cultivar resistance to Fusarium pathogens was not a result of exudate specificity (39), which agreed with our findings since both the moderately resistant and highly susceptible celery cultivars equally stimulated chlamydospore germination in the CASO system. The high degree of chlamydospore germination in response to corn and sudax exudates seemed to be a result of excessive carbohydrate leakage from the attached seed. In these treatments germination occurred at distances far from the plant roots and germ tube direction was random. The nonspecificity of the various plant root exudates on chlamydospore germination is in agreement with the high occurrence of FOA2 on symptomless carriers in the field.

Symptomless colonization of the crop plants by the orange mutant (OM) of FOA2 (Table 3.4) demonstrated that the degree of colonization was dependent on plant species. The absence of any detectable colonization of parsley and crabgrass roots by the OM could have been due to the surface disinfestation procedure which have may killed the OM, or perhaps to too small a root sample to reveal colonization. The greater root colonization of the highly susceptible celery cultivar than in the moderately resistant one suggested that the resistance of `Tall

Utah 52-70 HK' is in its ability to slow the colonization of roots by the pathogen. These findings support the hypothesis of Rappaport et al. (27) that the tolerance of the moderately resistant celery cultivar `Tall Utah 52-70 HK' resids inside rather than at the surface of the root.

Although celery roots were expected to contain relatively high levels of colonization by the OM, it appeared that root colonization was actually more extensive in barnyardgrass, corn and carrot. The pathogen may have a preference for colonizing roots of certain plants. Katan (15) demonstrated that F. oxysporum f. sp. lycospersici colonized the roots of monocots more heavily than those of dicots which (with the exception of crabgrass), agreed with our findings. Since carrot and celery are both members of the family Apiaceae (Umbelliferae), there could be some specificity for similar members of the host's family; however, parsley is also a member of the Apiaceae and no colonization of its roots was detected. Populations of FOA2 may build up saprophytically more rapidly on roots of some nonsusceptible plants than on celery, even though they are not invaded systemically by FOA2 as celery is. Smith and Snyder (30) suggested that populations of F. oxysporum f. sp. vasinfectum could increase faster in soils planted to nonsuscepts (monocots) than under continuous cotton. They implied that this phenomenon could relate to the ease in which F. oxysporum can invade the cortical cells of nonsusceptible plant roots. However, it seems that many other plants will also allow FOA2 to persist and possibly increase inside the root tissue.

Utilizing the OM in residue-supplemented soil allowed a convenient

assay to monitor FOA2 populations while eliminating the need for pathogenicity tests required for identification of FOA2. These studies revealed that the competitive saprophytic ability of FOA2 was affected differently by different residues. The strong stimulation of onion residues on the OM populations (Figure 3.4) was also observed on germination of chlamydospores in the CASO system. The initial stimulation was probably a direct response to the high soluble sugar level in onion tissue (14). Nevertheless, the sharp decline in the plate counts of the OM after 1 week of incubation suggested that other factors may be involved. Many plant residues have been demonstrated to be toxic to microorganisms (4) and these could have been responsible for the population decline. Alternately, the OM may be unable to colonize the onion residue after the stimulation, and germ tubes may lyse before inducing new chlamydospores to form. Lastly, the possible role of antagonistic microbes has not been excluded since onion residues could have also selected for specific populations of competitive or mycoparasitic organisms. However, since the onion extract broth did not favor growth of the FOA2, we suspect that inhibitory compounds in onion influenced the population decline.

The celery and mint residues did not generate as much increase in OM populations (Figure 3.4) or chlamydospore germination (Table 3.7) as did the onion residues, but did stimulate the growth of FOA2 in broth culture more than onion extracts (Figure 3.5 and 3.7). The sharp decrease in the OM populations caused by mint residues 1 week after incorporation could be due to some indirect effect of the residue on the populations. Since a substantial amount of growth of FOA2 occurred in

broth media containing soluble extracts of mint residues, it appeared that mint substrates would support colonization by FOA2. Perhaps degradation products from high oil content of mint residues could become inhibitory to the OM in the soil. Several other researchers have demonstrated that volatile and nonvolatile degradation products of plant residue supplements can inhibit species of Fusarium (42) and other fungi (18). The effects of these residues on the populations of FOA2 could help explain the overall trends of disease severity of celery plants grown in the residue-supplemented soils.

Our investigations revealed some important findings that could be beneficial to celery growers with FOA2-infested fields. Since continuous celery cultivation will accelerate the increase in FOA2 populations from decaying infected celery roots and colonized celery trimmings returned to the soil after harvest, this practice should be avoided. Alternate crop plants such as corn or carrots, along with the cover crops rye and sudax should also be excluded since root colonization of these plants by FOA2 was high, and alleolpathic responses of these crops may eventually decrease crop yield (26). In addition, effective weed control would also aid in denying the pathogen nonsusceptible hosts for survival since barnyard grass supported a high degree of colonization in naturally and artificially infested soils.

However, rotation with onions for periods of 2 or more years would slow, and possibly even decrease the population of FOA2 so that disease severity would be less when the field were rotated back into celery with the moderately resistant cultivars. The possibility that inoculum densities would reach levels that were damaging to all celery cultivars

would be lessened. In addition, the damage from other celery pests and pathogens could be minimized by crop rotation.

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CHAPTER IV

COMPARISON OF ISOLATES OF FUSARIUM OXYSPORUM F. SP. APII FROM SEVERAL GEOGRAPHIC AREAS FOR VIRULENCE, AND FOR OPTIMAL TEMPERATURES FOR DISEASE DEVELOPMENT IN SOIL AND GROWTH IN VITRO

INTRODUCTION

Fusarium yellows of celery (Apium graveolens var dulce L.) is reported to be incited by at least two races of Fusarium oxysporum f. sp. apii (18, 23). A third race was reported by Puhalla (20), but has not been confirmed. Race 1 of F. oxysporum f. sp. apii (FOA1) caused serious disease symptoms on the golden self-blanching celery cultivars grown in the U. S. during the 1930's and 1940's, and in France today, but is avirulent on the green cultivar `Tall Utah 52-70' and cultivars derived from it since its introduction in 1952 (1). Race 2 of F. oxysporum f. sp. apii (FOA2) was discovered in 1975 by Hart and Endo (9) in California on the FOA1-resistant green celery cultivars. FOA2 attacks both the self-blanching and green cultivars. In 1981-83 Fusarium yellows of celery incited by FOA2 was discovered on several farms in Michigan, and in Wisconsin (7) and New York (3) on FOA1-resistant celery cultivars. Race 3 reportedly causes disease on the green cultivars of celery, but not on the self-blanching celery cultivars. Little information exists on the biological significance of Race 3. The report of its existence is based on greenhouse studies by Puhalla (20) using inoculation techniques that the author (Chapter II)

and others (9) have found to give inconsistent results.

There has been considerable confusion in the celery industry regarding resistance to the Fusarium yellows disease in celery cultivars grown in different parts of the country. The celery cultivar `Summit' was reported by a commercial seed company to be tolerant to the Fusarium yellows disease (2); however, field evaluations made in Michigan indicated that `Summit' is highly susceptible (Chapter V). Reports from New York concluded that `Tall Utah 52-70 HK' lacked resistance to the disease (Personal communication with Dr. J. W. Lorbeer), whereas field tests in Michigan demonstrated that this cultivar was one of two cultivars tested that had the highest level of resistance currently available (7). These conflicting data could be due to several possibilites which are discussed below.

Isolates of FOA2 from California or New York could be different from Michigan isolates in virulence, or could be different races of the pathogen. Puhalla (20) concluded on the basis of laboratory tests that 2 out of 4 Michigan isolates of FOA2 (FA-2, FA-3) that were isolated from diseased celery crowns growing in the same field were atypical when compared by means of laboratory tests with other Michigan and California isolates of FOA2. Although these 2 Michigan isolates caused disease on both self-blanching and green celery cultivars, they were defined as exceptional FOA2 isolates (designated 2E) because they failed to form heterokaryons with color mutants of FOA2 on a pairing medium, and produced colonies larger than a standard California isolate of FOA2 when conidia of each were placed on a specialized medium. Puhalla (20) suggested these 2E Michigan isolates could pose a threat in the future,

but did not explain how.

Another possible explanation of different disease ratings on celery plants grown different geographical areas is differing concentrations of inoculum in different types of soil, which would differ in their disease-causing capacity. Inoculum density can greatly affect the amount of disease obtained on celery with FOA2 (25) and other wilt Fusaria (16). Martyn and McLaughlin (13) demonstrated that cultivars of watermelon resistant to F. oxysporum f. sp. niveum would becomed diseased if inoculum concentrations were increased sufficiently. Douglas (5) showed a similar phenomenon with resistant muskmelon cultivars and F. oxysporum f. sp. melonis. My studies (Chapter II) and others (25) indicated that there is an inoculum density threshold, which when exceeded, can lead to a sudden dramatic increase in disease.

Furthermore, differences in disease ratings on cultivars can result when soil temperatures vary (15, 26). Otto et al. (19) demonstrated that moderately resistant celery cultivars were more susceptible to Fusarium yellows of celery when grown during the warmer summer months than during the winter months in California.

Nelson et al. (18) and Ryker (21) reported that soil temperatures of 26-28 C were optimal for Fusarium yellows of celery on the self-blanching cultivars infected with FOA1, but agreed that optimal growth of celery plants occurred at lower temperatures. However, Hart (8) reported that cooler (20-24 C) soil temperatures resulted in more disease with FOA2 on `Tall Utah 52-70 R Improved'. These findings suggested that different races of F. oxysporum f. sp. apii could have different optimal soil temperatures, and that green celery cultivars

would respond differently to FOA2 under different soil temperatures.

The objectives of these studies were: to compare isolates of FOA2 from California, New York and Michigan, along with an isolate of FOA1 from France, for virulence on several self-blanching and green celery cultivars under standardized greenhouse conditions, to determine if differences in optimal soil temperature for disease development exist, and to compare the isolates in broth culture for differences in growth rates at different temperatures.

MATERIALS AND METHODS

Plant Materials, Growing Conditions and Disease Ratings

Celery cultivars were seeded in commercial potting mix under a 14 hour photoperiod, and were removed for transplanting one month after seeding. Cultivars had been previously ranked in greenhouse tests and under field conditions in naturally infested soils as being moderately resistant (MR = slight vascular discoloration (VD) in the crown and slight stunting), moderately susceptible (MS = 10 % or more VD in the crown and slight to moderate level of stunting) or highly susceptible (HS = extensive VD and severe stunting or death of the plant) to the Michigan isolates of FOA2 (6). The following cultivars of celery were used for study: `Tall Utah 52-70 R Improved' (HS), `Florida 683' (HS), `Tall Green Light' (HS), `Golden Detroit' (HS), `Summit' (HS), `Bishop' (MS), `Golden Spartan' (MR), `Deacon' (MR) and `Tall Utah 52-70 HK' (MR). `Golden Detroit' and `Golden Spartan' were self-blanching types while all others were green cultivars. `Golden Detroit' is also highly susceptible to FOA1 while `Golden Spartan' susceptibility to FOA1 is

unknown.

Seedlings were transplanted into a commercial potting mix in 10 cm plastic pots for greenhouse tests, or in 2 liter plastic pots without drainage holes for soil temperature studies. Plants received three-100 ml applications of a 20-20-20 soluble fertilizer, with the first application occurring 1 week after transplanting and continuing at 2-week intervals. Greenhouse experiments were terminated 6 weeks after transplanting, uprooted, and plants were rated for disease.

Disease was rated on a scale of 1-6 where 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown area, 4 = VD in 11-25 % of the crown area, 5 = VD in 26-75 % of the crown area, and 6 = VD in 76-100 % of the crown area or death of the plant. Dry weights of the foliar portions of the plant were also taken and recorded.

Collection, Storage and Production of Inoculum

Michigan isolates of FOA2 (FA-3, FA-8) were isolated from diseased celery crowns from the North Muskegon and Whitehall areas of Michigan by washing pieces (2 X 2 X 2 mm) of infected tissue in tap water for several hours, surface disinfesting in 10 % household bleach for 1 min., rinsing in distilled water, and placing on Komada's Selective Medium (KMS) (12). Plates were incubated at 25 C under a 12 hour photoperiod. Five to 7 days later colonies of F. oxysporum were identified, singled spored, and stored in sterile soil tubes according to the procedures of Nelson et al. (17).

California isolates of FOA2 (A1, A2) and a French isolate of FOA1 (A8) were provided by Dr. J. Puhalla, University of California,

Berkeley. The French isolate was used because no virulent isolates of FOA1 could be found in the U.S. New York isolates of FOA2 (NR1, JC1, P-13) were provided by Dr. J. Lorbeer of Cornell University. All isolates were single-spored and stored in sterile soil tubes as above.

Inoculum for greenhouse assays and soil temperature studies consisted of FOA1- or FOA2-colonized wheat straw or macroconidia. Procedures for producing the two types of artificial inoculum were identical to those described in Chapter II.

Comparison of Isolates for Virulence

Two greenhouse experiments were conducted to compare virulence of isolates of FOA1 and FOA2 on several self-blanching and green celery cultivars. The first experiment compared three FOA2 isolates from Michigan, California and New York and the French isolate of FOA1 for virulence on different celery cultivars. The second experiment compared different isolates of FOA2 from Michigan, California and New York; no isolate of FOA1 was included.

In the first experiment, three seedlings of each celery cultivar were transplanted into pots containing potting mix artificially infested with wheat straw colonized by the isolate of FOA1 (A8), or the FOA2 isolate from Michigan (FA-8), California (A1) or New York (NR1, P-13). Soil was artificially infested with inoculum by incorporating 0.8 g of colonized wheat straw into four liters of sieved (2 mm mesh) soil (0.2 g wheat straw/liter soil) by rotating the soil mixture in a cement mixer for 30 min. This yielded approximately 1 X 10^4 to 5 X 10^4 cfu of FOA1 or FOA2/g of soil as determined by conventional soil dilutions into Komada's Selective medium (12). Each cultivar-isolate treatment

combination was replicated 7 times and cultivars transplanted into soil with sterile wheat straw incorporated served as the control.

The second experiment was conducted exactly as above, except that different isolates of FOA2 from Michigan (FA-3), California (A2) and New York (JC1) were compared for virulence on the celery cultivars. Disease ratings and dry weights were recorded after 6 weeks. Data were subjected to analysis of variance (ANOVA) and analyzed as a randomized complete block with means compared by Duncan's multiple range test ($P = 0.05$).

Optimal Soil Temperature for Disease Development

Two liter plastic pots were filled to approximately 25 % of volume with sterile sand to provide a drainage basin for excess soil water, and to provide ballast necessary to prevent floating of the pots in the temperature tanks. Soil was placed on the sand up to the surface of the pot and firmed by smartly tapping the pot on the surface of the bench.

The effect of soil temperature on root infection and subsequent disease development was studied by transplanting healthy celery seedlings of `Tall Utah 52-70 R Improved' into soil artificially infested with wheat straw colonized by the FOA2 isolates from Michigan (FA-8), California (A1) and New York (NR1). Inoculum was incorporated into the potting medium at the rate of 0.25 g wheat straw/liter soil by rotating the soil mixture in a cement mixture for 30 min. Disease ratings and dry weights were recorded after 6 weeks as mentioned above. To study the effect of temperature on disease development, plants were inoculated before being placed in sterile soil by allowing 1-month-old celery seedlings of `Tall Utah 52-70 R Improved' to soak for 24 hours in

a conidial suspension of FOA2 (1 X 10^4 macroconidia/ml) produced from the Michigan isolate FA-3, California isolate A2 and the New York isolate JC1. Procedures for producing and preparing the spore suspensions were identical to methods outlined in Chapter II.

Since the optimal temperature for disease development of FOA1 on the self-blanching celery cultivars was reported to be 28 C (17, 21), and cooler temperatures of 20-24 C were reported by Hart (8) to be favorable for disease development on green celery cultivars infected with FOA2, a comparison of temperature effects on disease incited by the two races was made in the greenhouse. The celery cultivar `Golden Detroit' was used since it was highly susceptible to both races. One-month-old seedlings were grown in potting soil infested with the wheat straw colonized by FOA1 (A8) and FOA2 (FA-3) (0.25 g wheat straw/liter soil) and placed in temperature tanks so that a soil temperature of 22 or 28 C was obtained. Each treatment was replicated 8 times with seedlings grown in potting mix infested with sterile wheat straw serving as controls.

Growth In Vitro of Isolates of FOA2 and FOA1 From Different Geographic Areas

PDA agar plugs colonized by the French isolate of FOA1 (A8) or the FOA2 isolates from Michigan (FA-3, FA-8), California (A1, A2) or New York (JC1, NR1) were removed with a # 1 cork borer from the outer margins of 3 day-old colonies grown on PDA plates at 25 C under a 12 hour photoperiod. One plug was aseptically transferred into each flask (125 ml) containing 25 ml of autoclaved modified Czapek-Dox broth (15 g sucrose/liter) (24). Flasks were incubated at 17, 22, 27, or 32 C in

still culture for 72 or 144 hours. Dry weights were determined by harvesting fungal mats on preweighed glass fiber filter papers, rinsing with 150 ml of distilled water twice, and drying in a forced air drier at 80 C for 24 hours before weighing. Four replicate flasks were assayed per isolate-temperature combination at each sampling time. Flasks treated with sterile PDA agar plugs served as controls, and their weights were subtracted from each treatment to provide the fungal dry weight only. The experiment was repeated, all data were pooled, and a split-plot analysis of variance was performed.

RESULTS

Comparison of Isolates for Virulence

When self-blanching and green celery cultivars were grown in soil artificially infested with different isolates of FOA2 and FOA1, significant differences in pathogenicity and virulence were observed. Isolate, cultivar and their interaction all generated significant F values for disease ratings and dry weights (Table 4.1).

When A8 (FOA1) and FOA2 isolates from Michigan (FA-8), California (A1) and New York (P-13, NR1) were compared for pathogenicity and virulence on the self-blanching and green cultivars, disease ratings ranged from 1 (no disease) to 6 (severely diseased) among the cultivars. (Table 4.2). All FOA2 isolates were virulent to some extent on all celery cultivars, although some were less virulent than expected on certain cultivars. Disease ratings made on the highly susceptible (HS) celery plants grown in soil artificially infested with the FOA2 isolates from Michigan (FA-8) and California (A1) were greater than, but not

Table 4.1. Anaylsis of variance for disease ratings and dry weights of self-blanching and green celery cultivars grown in soils artificially infested with isolates of FOA2 and one of FOA1.

	Disease Ratings			Dry Weights		
Source	df	Mean square	F value	df	Mean square	F value
Blocks	5	4.0	3.6 *	5	1.9	3.6 *
Isolate X Cultivar	40	7.0	6.4 *	40	2.5	4.4 *
Isolate	5	70.8	64.4 *	5	22.1	39.6 *
Cultivar	8	22.8	20.7 *	8	21.1	37.7 *
Error	323	1.1		323	0.6	

* Represents a significant F value at $P = 0.01$.

Table 4.2. Disease reaction of self-blanching and green celery cultivars of varying susceptibility to Fusarium yellows to several isolates of FOA2 from different geographic areas and one French isolate of FOA1.

	Isolate[w]					
Cultivar[x]	Control	FA-8	A1	NR1	P-13	A8
TU (HS)	1.0 a[y] j	5.3 a k	5.2 a k	3.3 ab k	5.1 b k	1.0 a j
TGL (HS)	1.0 a j	6.0 a k	5.8 a k	5.1 b k	5.7 b k	1.0 a j
Sum (HS)	1.0 a j	6.0 a k	6.0 a k	5.3 b k	6.0 b k	1.0 a j
683 (HS)	1.0 a j	4.7 a k	4.9 a k	3.5 ab k	5.2 b k	1.0 a j
Bis (MS)	1.0 a j	4.9 a k	4.4 a k	1.7 a jk	3.9 ab k	5.1 b k
Dea (MR)	1.0 a j	3.9 a k	3.2 a k	3.9 ab k	2.3 b jk	1.0 a j
HK (MR)	1.0 a j	4.7 a k	3.2 a k	2.9 ab k	4.1 ab k	1.0 a j
GD (HS)[z]	1.0 a j	5.4 a k	5.7 a k	5.1 b k	6.0 b k	5.3 b k
GS (MR)[z]	1.0 a j	3.8 a k	4.9 a k	2.8 ab jk	4.7 b k	1.0 a j

[w]FA-8 = FOA2 (Michigan), A1 = FOA2 (California), NR1 = FOA2 (New York), P-13 = FOA2 (New York), A8 = FOA1 (France).

[x]TU = `Tall Utah 52-70 R Improved', TGL = `Tall Green Light', Sum = `Summit', 683 = `Florida 683', Bis = `Bishop', Dea = `Deacon', HK = `Tall Utah 52-70 HK', GD = `Golden Detroit', GS = `Golden Spartan', HS = Highly susceptible, MS = Moderately susceptible, MR = Moderately resistant; Ratings were based on field reaction to Fusarium yellows in Michigan.

[y]Values represent the mean of 6 pots consisting of 3 plants/pot. Values in each vertical column (or horizontal row) followed by differing letters to the right of (a,b) (or underneath (j,k)) are significantly different by Duncan's multiple range test at $\underline{P} = 0.05$. Disease ratings based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown area, 4 = VD in 11-25 % the crown area, 5 = VD in 26-75 % of the crown area, 6 = VD in 76 % of the crown area or death of the plant.

[z]`Golden Detroit' and `Golden Spartan' are self-blanching cultivars. All others are green cultivars.

significantly different (P = 0.05) from those ratings made on the moderately resistant (MR) cultivars. `Bishop' appeared to be more resistant to the NR1 isolate from New York than to any other isolate tested. `Deacon' and `Tall Utah 52-70 HK' had higher disease ratings when grown in soil infested with the Michigan (FA-8), California (A1), and New York isolates (NR1, P-13) than would have been expected based on field studies (Chapter V). Most disease ratings of celery cultivars grown in soil infested with the NR1 isolate from New York were less than, but not significantly different from, the other FOA2 isolate from New York (P-13), and those from Michigan and California. The French isolate of FOA1 (A8) was avirulent on all green cultivars except `Bishop' where VD was present on every plant (FOA1 isolate (A8) was found to be virulent on `Bishop' again when retested). Isolate A8 was highly virulent on the self-blanching cultivar `Golden Detroit', as expected. The other self-blanching cultivar (`Golden Spartan') showed no disease symptoms when exposed to the FOA1 isolate (A8). This cultivar was assumed to have resistance to FOA1 since one of its parents was a FOA1-resistant green celery cultivar (10).

Significant differences were observed among the dry weights of celery cultivars when the plants were grown in soil infested with isolates mentioned above (Table 4.3). `Deacon' (MR) had significantly higher dry weights than the HS cultivars `Summit' and `Tall Green Light' when exposed to the different FOA2 isolates, while surprisingly, this was not true for the MR cultivar `Tall Utah 52-70 HK'. With the NR1 isolate, cultivars such as `Golden Spartan' and `Bishop' had higher dry weights than `Summit', but this was not true for the other HS cultivars

Table 4.3. Dry weights of self-blanching and green celery cultivars of varying susceptibility to Fusarium yellows grown in soil artificially infested with several isolates of FOA2 from different geographic areas and one French isolate of FOA1.

Cultivar[x]	Control (g)	Percent Dry Weight Reduction from the Control — Isolate[w] FA-8	A1	NR1	P-13	A8
TU (HS)	3.0[y] a	74 a k	60 ab k	17 b jk	58 ab k	0 a j
TGL (HS)	3.8 a	82 a k	82 a k	61 ab k	100 a k	3 a j
Sum (HS)	3.0 a	70 a k	100 a k	70 a k	100 a k	0 a j
683 (HS)	3.7 a	60 ab k	65 ab k	44 ab jk	68 ab k	11 a j
Bis (MS)	3.3 a	55 ab k	40 ab jk	10 b j	43 ab jk	39 ab jk
Dea (MR)	3.9 a	24 b j	24 b j	21 b j	34 b j	6 a j
HK (MR)	3.5 a	54 ab k	75 a k	54 ab k	62 ab k	31 a jk
GD (HS)[z]	3.1 a	62 ab k	71 a k	52 ab k	97 a k	82 b k
GS (MR)[z]	3.0 a	20 b j	74 ab j	20 b j	60 ab k	0 ab j

[w]TU = `Tall Utah 52-70 R Improved', TGL = `Tall Green Light', Sum = `Summit', 683 = `Florida 683', Bis = `Bishop', Dea = `Deacon', HK = `Tall Utah 52-70 HK', GD = 'Golden Detroit', GS = `Golden Spartan', HS = Highly susceptible, MS = Moderately susceptible, MR = Moderately resistant; Ratings were based on field reaction to Fusarium yellows in Michigan.

[x] FA-8 = FOA2 (Michigan), A1 = FOA2 (California), NR1 = FOA2 (New York), P-13 = FOA2 (New York), A8 = FOA1 (France).

[y]Values represent the mean of 6 pots consisting of 3 plants/pots. Values in each vertical column (or horizontal row) followed by the differing letters to the right of (a,b) (or underneath (j,k)) are significantly different by Duncan's multiple range test at $P = 0.05$.

[z] `Golden Detroit' and `Golden Spartan' are self-blanching celery cultivars. All others are green cultivars.

`Tall Utah 52-70 R Improved', `Tall Green Light' and `Florida 683'. `Golden Spartan' and `Deacon' were the only cultivars exposed to the Michigan isolate FA-8 that were not significantly reduced below the dry weights of control plants. Any given cultivar grown in soil infested with any of the FOA2 isolates exhibited no real difference in growth (dry weights) from each other. The only exceptions were `Bishop', which was significantly more stunted in the Michigan isolate (FA-8)-infested soil than with other FOA2 isolates, and `Golden Spartan' which was stunted by the New York isolate (P-13). The self-blanching cultivar `Golden Detroit' was the only one significantly reduced in dry weights by the FOA1 isolate A8 when compared to the other cultivars.

Similar results developed among the FOA2 isolates when the experiment was repeated using new FOA2 isolates from Michigan (FA-3), California (A2), and New York (JC1) (Table 4.4). All FOA2 isolates caused disease on all cultivars when examined for VD. No differences were detected in disease ratings among cultivars that were exposed to the Michigan FOA2 isolate FA-3 or the California isolate A2. However, `Deacon' and `Tall Utah 52-70 HK' exhibited less VD in the FA-3-infested soil than other cultivars did, but values were not statistically different. When `Deacon' was grown in soil infested with the FOA2 isolate from New York (JC1), it appeared to be more resistant than the HS cultivars `Tall Utah 52-70 R Improved', `Tall Green Light', `Summit' and `Golden Detroit'. More VD was also observed in the MR cultivar `Tall Utah 52-70 HK' than in the other MR cultivar `Deacon' when grown in soil infested with the New York isolate, which may support claims from New York that `Tall Utah 52-70 HK' lacked resistance to the

Table 4.4. Disease reaction of self-blanching and green celery cultivars of varying susceptibility to Fusarium yellows to several isolates of FOA2 from different geographic areas.

	Isolate[w]			
Cultivar[x]	Control	FA-3	A2	JC1
TU (HS)	1.0[y] a j	5.8 a k	5.8 a k	4.7 b k
TGL (HS)	1.0 a j	5.9 a k	6.0 a k	5.1 b k
Sum (HS)	1.0 a j	6.0 a k	6.0 a k	5.2 b k
683 (HS)	1.0 a j	5.3 a k	6.0 a k	3.0 ab k
Bis (MS)	1.0 a j	5.8 a k	5.8 a k	3.8 ab k
Dea (MR)	1.0 a j	4.3 a k	6.0 a k	1.5 a j
HK (MR)	1.0 a j	4.3 a k	5.5 a k	4.4 b k
GD (HS)[z]	1.0 a j	5.7 a k	6.0 a k	5.3 b k
GS (MR)[z]	1.0 a j	5.3 a k	6.0 a k	3.0 ab k

[w]FA-3 = FOA2 (Michigan), A2 = FOA2 (California), JC1 = FOA2 (New York)

[x]TU = `Tall Utah 52-70 R Improved', TGL = `Tall Green Light', Sum = `Summit', 683 = `Florida 683', Bis = `Bishop', Dea = `Deacon', HK = `Tall Utah 52-70 HK', GD = `Golden Detroit', GS = `Golden Spartan', HS = Highly susceptible, MS = Moderately susceptible, MR = Moderately resistant; Ratings were based on disease reaction to Michigan isolates of FOA2.

[y]Values represent the mean of 7 pots consisting of 3 plants/pot. Values in each vertical column (or horizontal row) followed by differing letters to the right (a,b) (or underneath (j,k)) are significantly different by Duncan's multiple range test at P = 0.05. Disease ratings based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown area, 4 = VD in 11-25 % of the crown area, 5 = VD in 26-75 % of the crown area, 6 = VD in 76-100 % of the crown area or death of the plant.

[z]`Golden Detroit' and `Golden Spartan' are self-blanching celery cultivars. All others are green cultivars.

Fusarium yellows disease in infested soils in New York (Personal communication with Dr. J. L. Lorbeer).

The Michigan isolate FA-3 significantly reduced the dry weight of the HS cultivars `Tall Green Light' and `Summit' when compared to `Deacon' and `Tall Utah 52-70 HK', but did not cause any real stunting in the HS cultivar `Florida 683' or `Golden Detroit' when compared to the control (Table 4.5). All cultivars except `Tall Utah 52-70 HK' were stunted when grown in soil infested with the California isolate (A2) including the MR cultivar `Deacon', which appeared much more resistant to the other California isolate (A1) (Table 4.3). All dry weights of celery cultivars grown in soil with the New York isolate (JC1) were not significantly different ($P = 0.05$) from each other, and only `Summit' and `Tall Utah 52-70 HK' were significantly stunted compared to controls.

Effects of Soil Temperature on Fusarium Yellows Development Incited by Isolates of FOA2 From Different Geographic Areas

Vascular discoloration (VD) was detected in all plants grown in soil infested with each isolate of FOA2 at all soil temperatures, but was significantly less at 32 C than at other temperatures (Table 4.6). Significant reductions in dry weights of celery plants occurred at 17 and 22 C, whereas only slight nonsignificant decreases in dry weights were observed at 27 C. No reduction in celery dry weights occurred at 32 C for any of the isolates. Isolates were equally virulent at all soil temperatures (17, 22, 27, 32 C).

When plants were inoculated by soaking roots of seedlings in conidial suspensions and were than transplanted into soil adjusted to

Table 4.5. Dry weights of self-blanching and green celery cultivars of varying susceptibility to Fusarium yellows grown in soil artificially infested with several isolates of FOA2 from different geographic areas.

Cultivar[x]	Control (g)	Percent Dry Weight Reduction From the Control — Isolate[w] FA-3	A2	JC1
TU (HS)	3.4[y] a	36 b jk	98 a k	33 a jk
TGL (HS)	3.1 a	87 a k	94 a k	58 a jk
Sum (HS)	3.6 a	95 a k	95 a k	64 a k
683 (HS)	3.2 a	0 b j	94 a k	13 a j
Bis (MS)	2.3 a	18 ab jk	60 a k	0 a j
Dea (MR)	3.8 a	19 b j	85 a k	19 a j
HK (MR)	3.6 a	14 b j	28 b jk	5 a k
GD (HS)[z]	3.2 a	35 ab j	85 a k	54 a jk
GS (MR)[z]	3.4 a	36 ab jk	71 a k	39 a jk

[w] FA-3 = FOA2 (Michigan), A2 = FOA2 (California), JC1 = FOA2 (New York).

[x]TU = `Tall Utah 52-70 R Improved', TGL = `Tall Green Light', Sum = `Summit', 683 = `Florida 683', Bis = `Bishop', Dea = `Deacon', HK = `Tall Utah 52-70 HK', GD = `Golden Detroit', GS = `Golden Spartan', HS = Highly susceptible, MS = Moderately susceptible, MR = Moderately resistant; Ratings were based on field reaction to Fusarium yellows in Michigan.

[y]Values represent the mean of 7 pots consisting of 3 plants/pot. Values in each vertical column (or horizontal row) followed by differing letters to the right of (a,b) (or underneath (j,k)) are significantly different by Duncan's multiple range test at $P = 0.05$.

[z]`Golden Detroit' and `Golden Spartan' are self-blanching celery cultivars. All others are green cultivars.

Table 4.6. The effect of soil temperature on disease ratings and dry weights of `Tall Utah 52-70 R Improved' grown in soil artificially infested with isolates of FOA2 from different geographic areas.

	Temperature (C)							
	17		22		27		32	
FOA2 Isolate[w]	DR[x]	DW[y]	DR	DW	DR	DW	DR	DW
Control	1.0[z]a	5.2 a	1.0 a	5.7 a	1.6 a	2.2 a	1.0 a	1.2 a
FA-8	5.8 b	0.5 b	5.9 b	0.3 b	5.1 b	1.6 a	3.9 b	1.3 a
A1	6.0 b	0.0 b	6.0 b	0.0 b	5.1 b	1.6 a	4.6 b	1.4 a
NR1	5.7 b	0.4 b	6.0 b	0.2 b	4.8 b	1.9 a	2.9 b	1.7 a

[w]Isolate FA-8 from Michigan, Isolate A1 from California, Isolate NR1 from New York.

[x]Disease ratings based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown area, 4 = VD in 11-25 % of the crown area, 5 = VD in 26-75 % of the crown area, 6 = VD in 76-100 % of the crown areas of death of the plant.

[y]Represents the dry weight of the foliar portions (g)

[z]Values represent the mean of 6 pots consisting of 3 plants/pot. Values in columns followed by differing letters are significantly different by Duncan's multiple range test at $P = 0.05$. Differences between temperature (rows) were not significantly different ($P = 0.05$) from each other.

17, 22 or 27 C, similar trends were observed with respect to temperature (Table 4.7). All plants inoculated with isolates of FOA2 had slighly higher, but not significantly different disease ratings when grown in soils held at 22 C than other soil temperatures; however, significant VD was still observed in plants held at soil temperatures of 17 or 27 C. Dry weights were also significantly reduced by all isolates at all soil temperatures except the New York isolate at 27 C. Although no differences in dry weights could be detected after 6 weeks at the various temperatures, plants held at 22 C exhibited more initial stunting and developed chlorosis usually 7 to 10 days before the time these symptoms became evident in plants maintained at 17 or 27 C.

The optimal temperature for growth of healthy celery was difficult to determine since different experiments gave different optimal values for dry weights (Tables 4.6 and 4.7). However, it appeared that cooler soil temperatures favored the growth of celery since plants initially produced more vegetative growth at 17 or 22 C than at 27 or 32 C.

When FOA1 and FOA2 were compared for disease development on `Golden Detroit' at 22 and 28 C, no significant difference in disease ratings was observed (Table 4.8). Likewise, no real differences in dry weights could be detected between the two races of the pathogen at either soil temperature. Symptoms appeared to develop earlier in plants growing at the 22 C soil temperature than at 28 C. Dry weights of `Golden Detroit' grown in noninfested soils at 22 C were significantly greater ($\underline{P}$ = 0.05) than those of plants incubated at 28 C.

Table 4.7. The effects of soil temperature on disease ratings and dry weights of `Tall Utah 52-70 R Improved' artificially inoculated by soaking seedlings in conidia of isolates of FOA2 from different geographic areas.

	Temperature (C)					
	17		22		27	
FOA2 Isolate[w]	DR[x]	DW[y]	DR	DW	DR	DW
Control	1.0[z]a	4.7 a	1.0 a	5.5 a	1.0 a	5.7 a
FA-3	3.5 b	3.8 b	3.8 b	3.9 b	2.3 a	4.4 b
A2	3.6 b	2.7 b	5.0 b	2.4 b	4.3 b	3.4 b
JC1	2.4 b	3.4 b	3.8 b	4.1 b	2.7 b	5.3 a

[w]Isolate FA-3 from Michigan, Isolate A2 from California, Isolate JC1 from New York.

[x]Disease rating based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown area, 4 = VD in 11-25 % of the crown area, 5 = VD in 26-75 % of the crown area, 6 = VD in 76-100 % of the crown area or death of the plant.

[y]Represents the dry weights of the foliar portions (g).

[z]Values represent the mean of 6 pots consisting of 3 plants/pot. Values in each column followed be differing letters are significantly different from the control by Duncan's multiple range test ($P = 0.05$). Differences between temperature (rows) were not significantly different ($P = 0.05$).

Table 4.8. Comparison of FOA1 (A8) and FOA2 (FA-3) isolates for effects on disease ratings and dry weights of `Golden Detroit' seedlings grown at soil temperatures 22 or 28 C.

	Soil Temperature (C)			
	22		28	
Isolate of FOA2	DR[x]	DW[y]	DR	DW
Control	1.3[z]a	3.8 a	1.1 a	2.1 a
FOA1 (A8)	6.0 b	0.1 b	4.9 b	0.4 b
FOA2 (FA-3)	5.6 b	1.3 b	5.1 b	0.7 b

[x]Disease ratings based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown area, 4 = VD in 11-25 % of the crown area, 5 = VD in 26-75 % of the crown area, 6 = VD in 76-100 % of the crown area or death of the plant.

[y]Represents the dry weights of the foliar portions.

[z]Values represent the means of 8 pots consisting 2 plants/pot. Values in each column followed by differing letters are significantly different from the control by Duncan's multiple range test at $P = 0.05$.

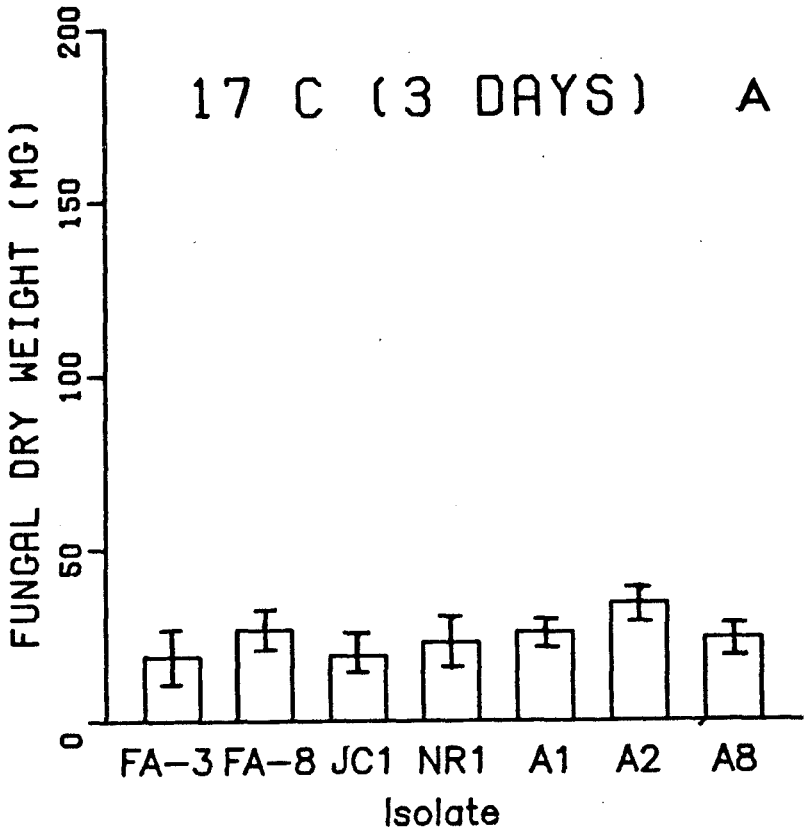
Growth of Several Isolates of FOA2 From Different Locations and a French Isolate of FOA1 in Broth Culture

Dry weights of all of FOA2 isolates and the French isolate of FOA1 (A8) became greater as temperature increased from 17 to 32 C after 3 days incubation in broth culture (Figures 4.1a, 4.2a, 4.3a, and 4.4a). After 6 days incubation, only a slight nonsignificant increase in dry weights of the F. oxysporum f. sp. apii isolates occurred as the incubation temperature increased from 17 to 32 C (Figures 4.1b, 4.2b, 4.3b and 4.4b).

Significant increases (P = 0.05) in dry weights of every isolate of FOA2 or FOA1 at all temperatures were detected after 6 days incubation as compared to their weights after 3 days. Following 6 days incubation at 17 or 22 C, fungal weights of each isolate increased as much as 4 times above their weights after 3 day sampling period (Figure 4.1), while weights increased approximately three times as much at 27 C or twice as much at 32 C. (Figure 4.4). This suggests that the lag phase of fungal growth in still broth culture was longer at lower temperatures of 17 and 22 C (Figures 4.1 and 4.2) than at 27 or 32 C (Figures 4.3 and 4.4).

Standard errors assigned to the mean dry weight data for each isolate demonstrated that considerable variation occurred between replicates. As a result of this variation, statistically significant differences between isolates at any incubation temperature were not detectable.

Figure 4.1. Dry weights of several isolates of FOA2 from different geographic areas and one isolate of FOA1 (A8) incubated in Czapek's Dox broth at 17 C after 3 days (A) and 6 days (B). FA-3 and FA-8 are FOA2 isolates from New York; A1 and A2 are FOA2 isolates from California; A8 is a FOA1 isolate from France. Standard errors are provided for the means.


17 C (3 DAYS) A
FUNGAL DRY WEIGHT (MG)
0
50
100
150
200
FA-3 FA-8 JC1 NR1 A1 A2 A8
Isolate

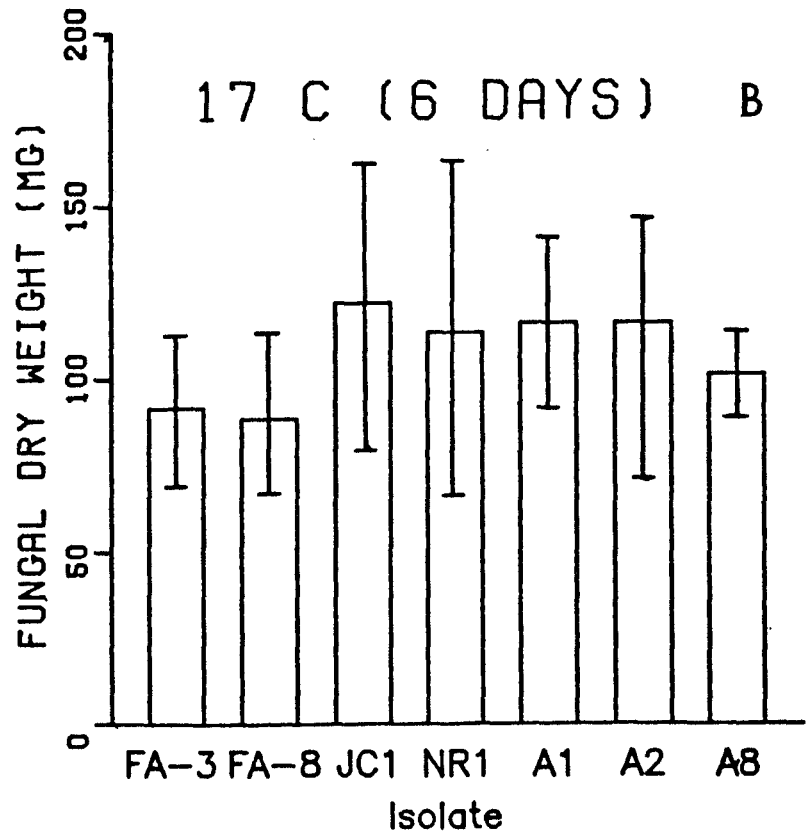
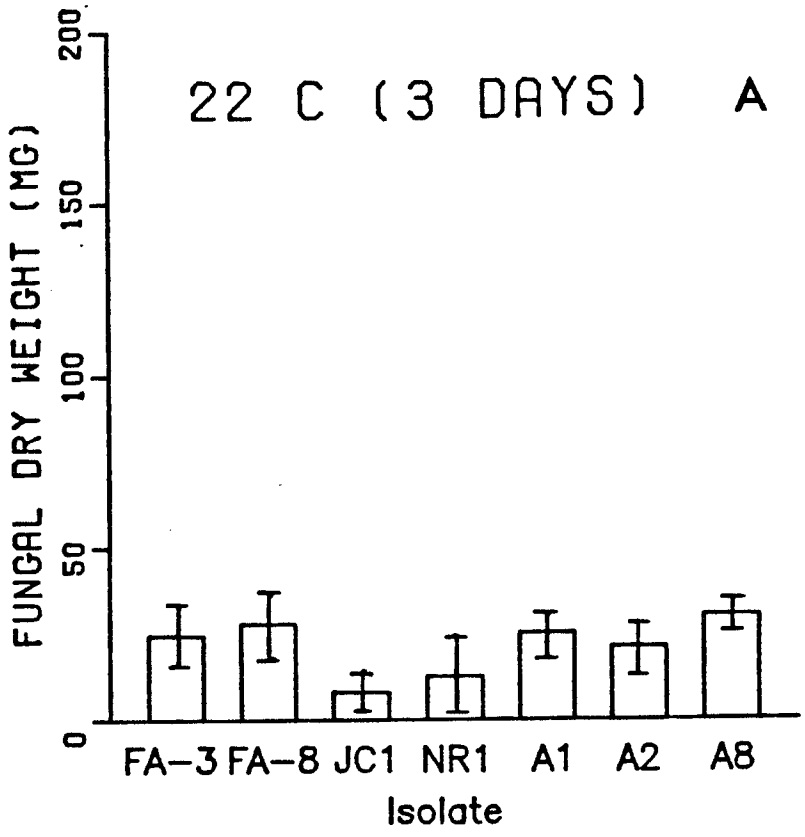

17 C (6 DAYS) B
FUNGAL DRY WEIGHT (MG)
0
50
100
150
200
FA-3 FA-8 JC1 NR1 A1 A2 A8
Isolate

Figure 4.2. Dry weights of several isolates of FOA2 from different geographic areas and one isolate of FOA1 (A8) incubated in Czapek's Dox broth at 22 C after 3 days (A) and 6 days (B). FA-3 and FA-8 are FOA2 isolates from New York; A1 and A2 are FOA2 isolates from California; A8 is a FOA1 isolate from France. Standard errors are provided for the means.


22 C (3 DAYS) A
FUNGAL DRY WEIGHT (MG)
0
50
100
150
200
FA-3 FA-8 JC1 NR1 A1 A2 A8
Isolate

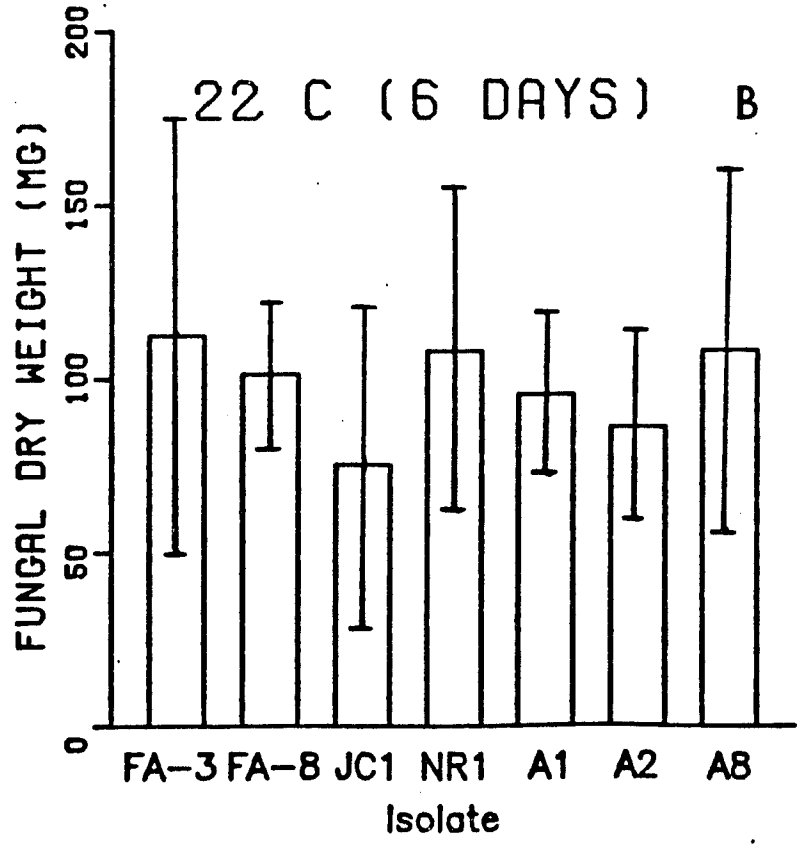
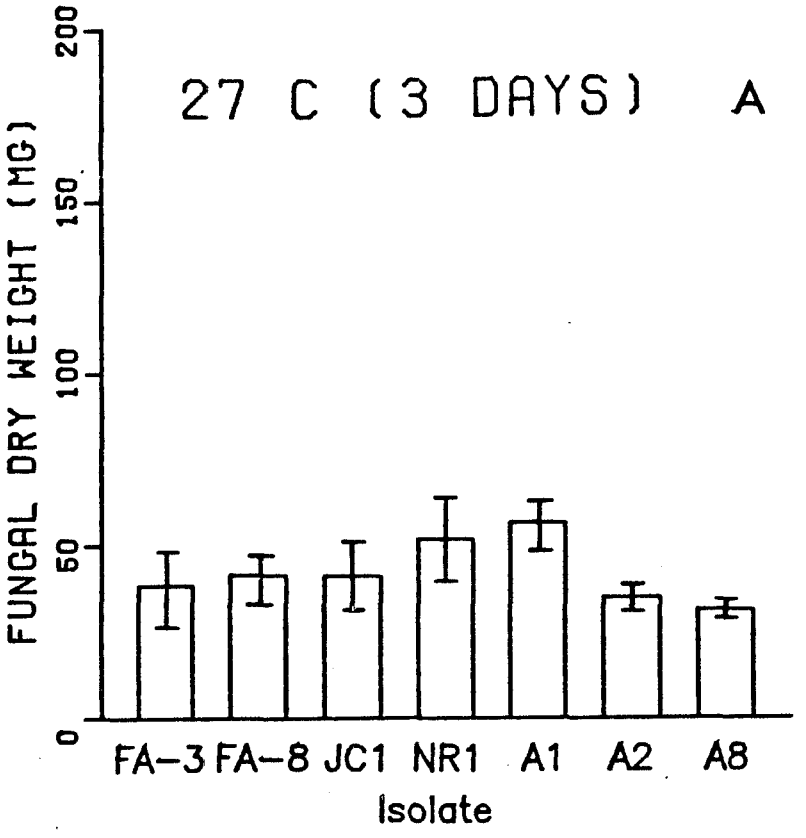

22 C (6 DAYS) B
FUNGAL DRY WEIGHT (MG)
0
50
100
150
200
FA-3 FA-8 JC1 NR1 A1 A2 A8
Isolate

Figure 4.3. Dry weights of several isolates of FOA2 from different geographic areas and one isolate of FOA1 (A8) incubated in Czapek's Dox broth at 27 C after 3 days (A) and 6 days (B). FA-3 and FA-8 are FOA2 isolates from New York; A1 and A2 are FOA2 isolates from California; A8 is a FOA1 isolate from France. Standard errors are provided for the means.


27 C (3 DAYS) A
FUNGAL DRY WEIGHT (MG)
0
50
100
150
200
FA-3 FA-8 JC1 NR1 A1 A2 A8
Isolate

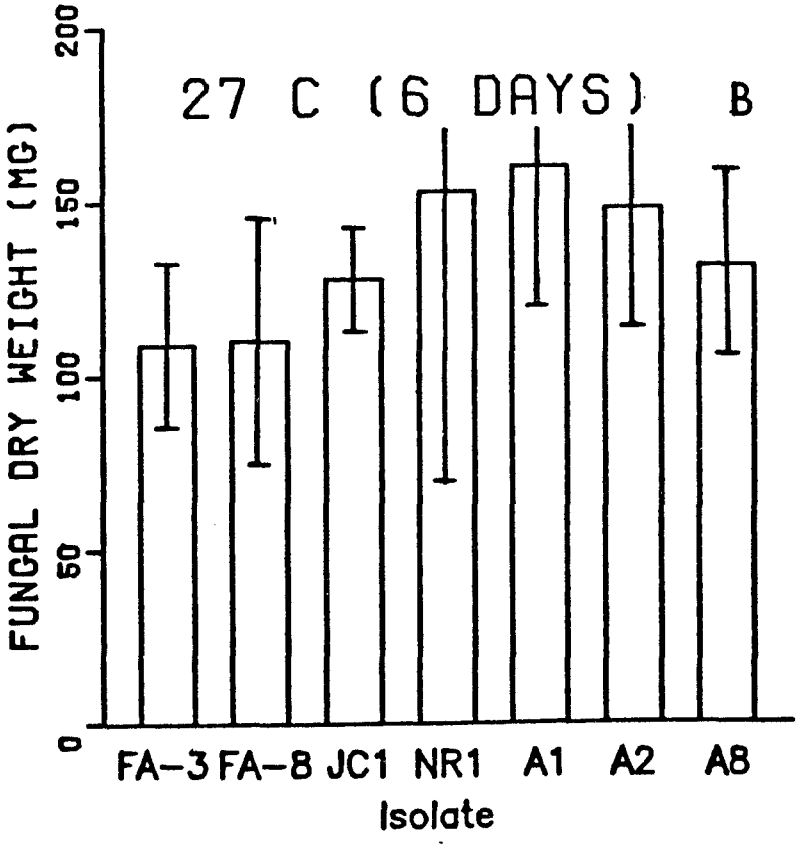
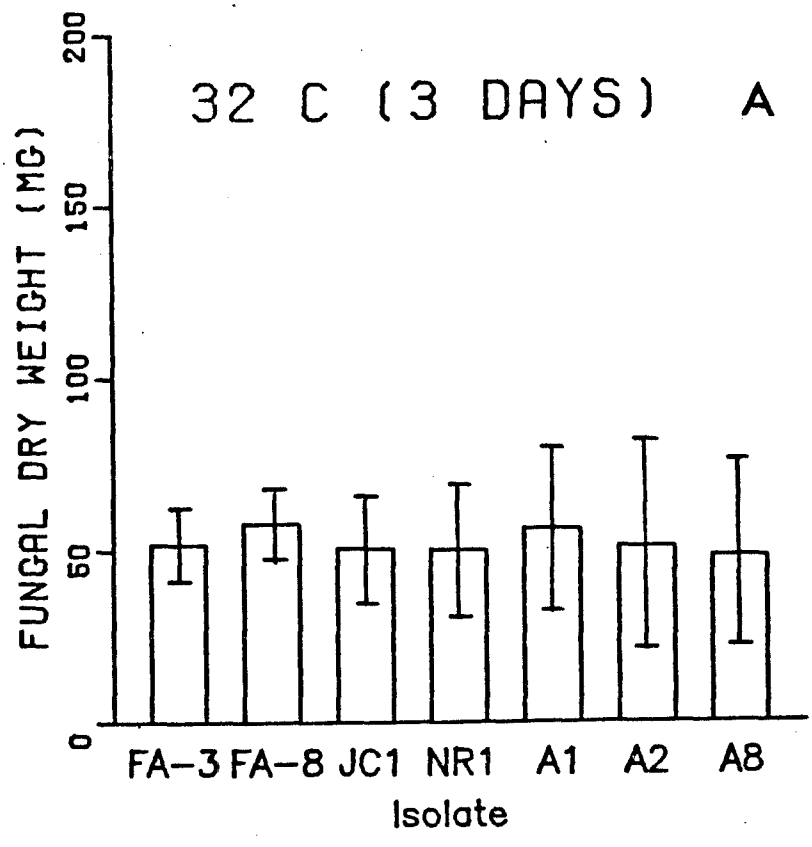
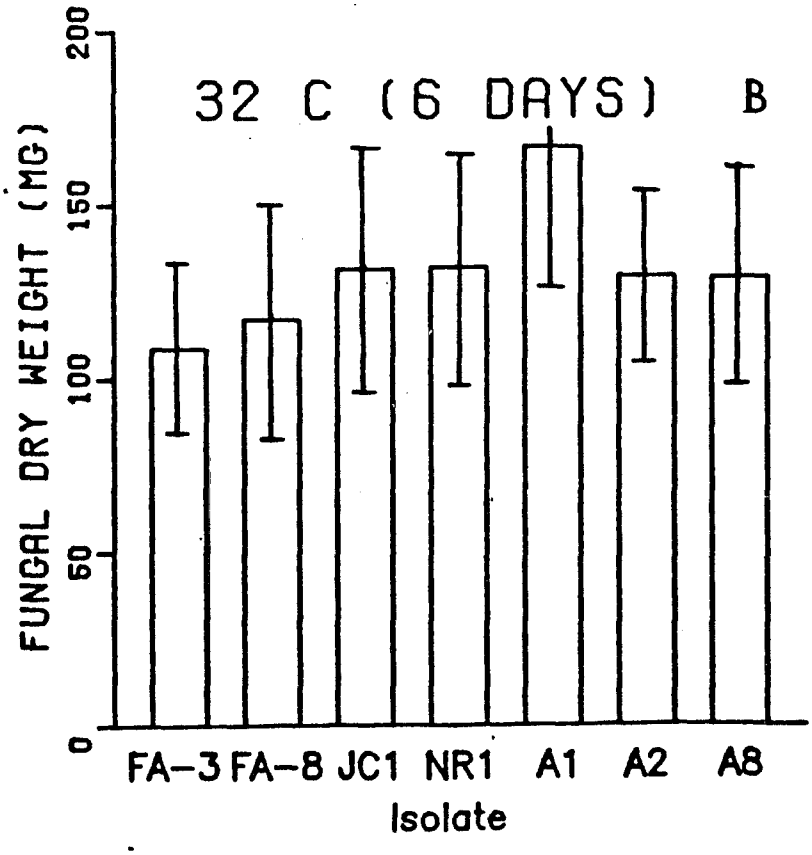

27 C (6 DAYS) B
FUNGAL DRY WEIGHT (MG)
0
50
100
150
200
FA-3 FA-8 JC1 NR1 A1 A2 A8
Isolate

Figure 4.4. Dry weights of several isolates of FOA2 from different geographic areas and one isolate of FOA1 (A8) incubated in Czapek's Dox broth at 32 C after 3 days (A) and 6 days (B). FA-3 and FA-8 are FOA2 isolates from New York; A1 and A2 are FOA2 isolates from California; A8 is a FOA1 isolate from France. Standard errors are provided for the means.


32 C (3 DAYS) A
FUNGAL DRY WEIGHT (MG)
0
50
100
150
200
FA-3 FA-8 JC1 NR1 A1 A2 A8
Isolate


32 C (6 DAYS) B
FUNGAL DRY WEIGHT (MG)
0
50
100
150
200
FA-3 FA-8 JC1 NR1 A1 A2 A8
Isolate

DISCUSSION

All isolates of FOA2 caused disease on the HS self-blanching cultivar `Golden Detroit' and the HS green cultivar `Tall Utah 52-70 R Improved', which demonstrated that all FOA2 isolates were Race 2 of *F. oxysporum* f. sp. *apii* (23). The significant F value ($P = 0.01$) for the interaction term of cultivar X isolate for disease ratings and dry weights demonstrated that the different levels of resistance were exhibited in the self-blanching and green celery cultivars to different races and different isolates of the pathogen.

Green celery cultivars that were highly susceptible to FOA2 were virtually immune to FOA1. Surprisingly, the green cultivar `Bishop' which exhibited some resistance to the FOA2 in field trials in Michigan's naturally infested soils (Chapter V) showed susceptibility to FOA1 (Table 4.2). `Bishop' (Moran Seed Company) was a selection from `Tall Utah 52-70' introduced in 1952 as highly resistant to FOA1 (1). Since several green celery cultivars existed in the 1950's that were not derived from `Tall Utah 52-70', but were slighty susceptible to FOA1 (14), celery breeders may have lost resistance to FOA1 in certain lines of green celery germplasm because they did not routinely select for resistance. These findings could have important effects on the Michigan celery industry if FOA1 reappeared since `Bishop' has received a higher resistance rating to Fusarium yellows of celery in Michigan than most other cultivars (Chapter V).

Significant differences in disease ratings among the FOA2 isolates for any one cultivar were rarely detected. However the New York isolates JC1 and NR1 seemed to cause more stunting and VD on the MR

cultivar `Tall Utah 52-70 HK', similar to the HS cultivars (Tables 4.4 and 4.5), while exhibiting lower virulence on other cultivars. The author feels that the JC1 and NR1 isolates from New York were atypical when compared to other FOA2 isolates for virulence on `Tall Utah 52-70 HK', and on some other cultivars, but it still was classified as Race 2 since it was pathogenic on `Tall Utah 52-70 R Improved' and `Golden Detroit' (23). Therefore, it is possible that greenhouse or laboratory screening of celery cultivars for resistance to Fusarium yellows in soil infested with these isolates of FOA2 could generate different disease ratings than if another isolate was used. Since reports from New York stated that `Tall Utah 52-70 HK' lacked resistance to Fusarium yellows of celery (Personal communication with Dr. J. W. Lorbeer), it may be possible that certain fields are prodominantely inhabited by clonal progenitors of these isolates. However, since all the New York isolates were from the same geographic area (Personal communication with Dr. J. W. Lorbeer), and the New York isolate P-13 appeared as virulent as those from Michigan or California, infested fields may be inhabited by strains of FOA2 that vary in virulence to different celery cultivars. Puhalla (20) found that differences in virulence existed among isolates of FOA2 and subsequently described a new race of F. oxysporum f. sp. apii based on these differences in virulence and laboratory tests. The author does not feel that new races should be erected based on differences in virulence, but should be based on distinct differences in pathogenicity on specific cultivars of celery.

These findings agreed with Hart's (8) that cooler soil temperatures (20-24 C) were optimal for disease development of FOA2 on the green

cultivar `Tall Utah 52-70 R Improved' since the most disease in our studies seemed to occur around 22 C. This temperature also appeared to be most favorable for growth of celery. Several other researchers have also reported that Fusarium wilt diseases were most severe at soil temperature(s) optimal for growth of the host (4, 11, 27). Adjusting soil temperatures to 22 C could improve the evaluations of celery cultivars for resistance to Fusarium yellows in the greenhouse, and decrease the number of disease escapes which occur.

Findings of Nelson et al. (18) and Ryker (21) that the Michigan isolates of FOA1 caused greater disease at soil temperatures of 28 C differed from our results comparing FOA1 and FOA2 on `Golden Detroit', but agreed that cooler temperatures are more favorable for growth of the self-blanching celery cultivars. Since disease ratings are believed to be more dependable for assessing the amount of disease in celery plants than stunting (8, 22), these findings suggested that both isolates may be slightly more virulent on `Golden Detroit' when grown at 22 C than at 28 C. We are not excluding the possibility that the French isolate of FOA1 used in our studies differed from Michigan isolates of FOA1 used by Nelson et al. (18) and Ryker (21) in optimal temperature for disease development on celery. In addition, differences in environmental conditions such as soil moisture or lighting may have influenced the results since these factors can affect the development of Fusarium yellows of celery under greenhouse conditions (22, 25).

Growth of each isolate in still broth culture revealed no distinct differences among the FOA2 isolates from the different geographic regions of the U.S., or between the French isolate of FOA1 and the FOA2

isolates. If real differences in growth rates of these isolates do exist, they were not detectable on account of replicate variation.

No evidence was found in these studies that suggested that isolates of FOA2 are of different races. All isolates of FOA2 caused disease on `Tall Utah 52-70 R Improved' and `Golden Detroit', and seemed to vary only in virulence on the cultivars tested. These conclusions suggested that screening celery cultivars in other areas of the country may not generate the same results as in Michigan. Difference in disease ratings could be due to FOA2 strains that differ in virulence. The author feels that evaluations should be made on cultivars under field conditions and not under greenhouse conditions since a cultivar's resistance rating would be more accurate if it can be rated at harvest. A celery cultivar's adaptibility to a specific area may also influence its level of resistance and could contribute to the confusion on resistance of cultivars to Fusarium yellows of celery.

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CHAPTER V

EVALUATIONS OF CELERY GERMPLASM AS A SOURCE OF RESISTANCE TO FUSARIUM OXYSPORUM F. SP. APII RACE 2 IN MICHIGAN

INTRODUCTION

Fusarium yellows of celery (Apium graveolens var. dulce L.), incited by Fusarium oxysporum f. sp. apii Race 2 (Nels. & Sherb.) Synd. & Hans (FOA2), was reported in California in 1978 (6), in Michigan in 1982 (3), and in New York in 1984 (2). Fusarium yellows was not seen in Michigan celery for 20-25 years because of the introduction of immunity (or a high level of resistance) to Race 1 of the fungus (FOA1). FOA1 caused widespread losses in the self-blanching cultivars grown in Michigan in the 1930's (11), but virtually disappeared when the highly resistant green cultivar `Tall Utah 52-70' and its descendants were introduced beginning in 1952 (1). Race 2 (FOA2) causes disease on both the self-blanching cultivars and on the currently grown green cultivars derived from the Race 1-resistant cultivar `Tall Utah 52-70' (18).

The number of celery fields in Michigan with Fusarium yellows has increased from 1 in 1981 to 11 in 1984 (4), and the spread of the disease to noninfested areas will likely continue. Several attempts to control the disease with soil fungicides have been ineffective (2, 8, 15).

Since all celery cultivars grown in Michigan were susceptible to race 2 at the time Fusarium yellows was identified (12), a greenhouse

and field screening program was initiated at Michigan State University in 1981 to evaluate celery germplasm for resistance. Several experimental lines of celery and some celeriac (A. graveolens var rapaceum (Mill.) Gaudich cultivars were also evaluated as potential sources of resistance for future breeding.

MATERIALS AND METHODS

Thirty commercial cultivars of celery, six lines of celeriac, and one cultivar of parsley (Petroselium crispum (Mill.) Nym. ('Festival 68') were screened for resistance to Fusarium yellows under greenhouse conditions in artificially infested soil. Field screening was carried out at the William Willbrandt farm in North Muskegon, MI where the disease has been severe for several years. In addition, several experimental lines of celery that were developed at Michigan State University for resistance to early blight (Cercospora apii Fres.) (600 series) (10), to late blight (Septoria appicola Fres.) (C X P series) (9) and to bolting were evaluated. The 600 series consisted of crosses of (Danish celeriac X Cornell 619 celery) X Utah celery and the C X P series were crosses between celery ('Golden Spartan') and parsley ('Festival 68'). The screening program extended over a 3 year period and involved many greenhouse and field experiments.

Colonized wheat straw inoculum was prepared as described in Chapter II. Inoculum contained mycelial fragments, abundant microconidia, and some macroconidia and chlamydospores embedded in the wheat straw tissue. Soil inoculum densities resulting from 1 g wheat straw/kg soil ranged from 1×10^4 to 5×10^4 cfu/g soil as determined by placing soil dilutions into Komada's Selective Medium (7).

Seeds were germinated in the greenhouse in steamed potting mix under a 12 hour photoperiod. Three- to 4-week-old seedlings were transplanted into 10 or 15 cm pots containing steamed muck soil or potting mix infested with FOA2-colonized wheat straw (1 g straw/ kg soil) and placed on greenhouse benches. Ten replicates of each cultivar were used (1-3 plants per replicate) and `Tall Utah 52-70 R Improved' served as the susceptible control. After 5-6 weeks when symptoms were severe in `Tall Utah 52-70 R Improved', the plants were rated for % vascular discoloration in the roots and crowns, and the tops of the plants were removed and dried. Plants were rated for disease by slicing the crown longitudinally and rating the vascular discoloration (VD) on a scale of 1-6 where 1 = no disease, 2 = VD in the primary roots only, 3 = VD in less than 10 % of the crown area, 4 = VD in 11 - 25 % of the crown area, 5 = VD in 26 - 75 % of the crown area, and 6 = VD in 76 - 100 % of the crown or death of the plant. All greenhouse evaluations were repeated at least once.

Field evaluations for resistance to Fusarium yellows were conducted with 9- to 11-week-old transplants grown in flats in the greenhouse and transplanted into a field at the Willbrandt farm in North Muskegon, MI where the disease had been severe for at least 5 years. The soil contained approximately 73-103 cfu of FOA2/g soil according to assays using conventional soil dilution techniques and testing representative samples of F. oxysporum colonies for pathogenicity. A randomized block design was employed with four replicates per cultivar. Each replicate consisted of a 6-meter section of row. Plots received the same cultural and chemical treatments as the surrounding commercial plantings.

Disease symptoms were monitored periodically during the season. Nine to 10 weeks after transplanting, disease ratings were made using the scale of 1-5 where 1 = no disease symptoms (Highly Resistant (HR)), 2 = slight stunting and/or slight vascular discoloration (VD) in the primary roots and crown (Moderately Resistant (MR)), 3 = moderate level of stunting, chlorosis in the older petioles and VD in the crown area (Moderately Susceptible (MS)), 4 = severe stunting, chlorosis extending into younger petioles and extensive VD with rotted crowns (Highly Susceptible (HS)), and 5 = plants near death or dead (Dead (D)). Trimmed weight yields were taken on all marketable cultivars for the 1983 and 1984 field studies. All data were subjected to analysis of variance (ANOVA) and means were compared by Duncan's multiple range or Student's t-test.

RESULTS

Greenhouse evaluations revealed that five commercial cultivars, namely `Tall Utah 52-70 HK', Ferry Morse 1217, `Golden Spartan', `Summer Pascal', and `Tendercrisp', exhibited little or no vascular discoloration in the crowns or roots when grown in artificially infested soil (Table 5.1). Several of the C X P lines also exhibited resistance to the pathogen. Cultivars `Tall Utah 52-70 R Improved' and `Summit' were badly stunted and contained severely rotted crowns and petioles.

Parsley and two of the celeriac lines possessed high resistance (Table 5.2) to FOA2 since no vascular discoloration or stunting could be detected. Other celeriac cultivars had varying degrees of disease resistance.

Field trials in 1982, 1983 and 1984 (Tables 5.3, 5.4 and 5.5) provided data on cultivar resistance similar to those derived in

Table 5.1. Dry weights and disease ratings of thirty celery lines evaluated in the greenhouse in soil artificially infested with FOA2.

	Cultivar	Dry weights (g)	Disease Rating[x]
1.	C X P 1[y]	2.25[z]a	1.75 abcdef
2.	C X P 2	1.93 ab	2.85 efg
3.	C X P 5	1.91 abc	1.20 abc
4.	Summer Pascal	1.89 abc	1.10 ab
5.	C X P 3	1.76 abc	2.90 efghi
6.	C X P 6	1.64 bcd	2.55 cdefgh
7.	Calmario	1.62 bcde	1.90 abcdef
8.	Florida 683 K	1.53 bcde	2.95 efghi
9.	Tall Utah 52-70	1.52 bcdef	4.55 k
10.	Tall Utah 52-70 H K	1.51 bcdef	1.00 a
11.	Golden Detroit	1.48 bcdef	2.85 efghi
12.	C X P 4	1.48 bcdef	1.65 abcd
13.	Tall Green Light	1.47 bcdef	3.60 ghijk
14.	June Belle	1.46 bcdef	2.60 defgh
15.	Ferry Morse 1217	1.46 bcdef	1.00 a
16.	Tall Golden Self Blanching	1.44 bcdef	3.10 fghij
17.	Golden Spartan	1.35 bcdefg	1.40 abcd
18.	Florida 683	1.35 bcdefg	3.30 ghijk
19.	C X P 7	1.31 cdefg	4.60 k
20.	Tall Utah 52-75	1.23 defg	4.20 ijk
21.	Tendercrisp	1.15 defg	2.25 abcdefgh
22.	Earibelle	1.15 defg	3.65 hijk
23.	Ventura	1.09 defgh	4.00 ijk
24.	Clean Cut	1.08 defgh	3.80 hijk
25.	Grande	1.03 fgh	2.45 bcdefgh
26.	Transgreen	0.99 fgh	3.30 ghijk
27.	Florida 2-13	0.96 fgh	4.50 k
28.	Florimart 19	0.85 gh	2.55 cdefgh
29.	Tall Utah 52-70	0.57 hi	4.55 jk
30.	Summit	0.53 i	4.60 k

[x]Disease ratings based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown, 4 = VD in 11-25 % of the crown area, 5 = VD in 25-75 % of the crown area, 6 = VD in 75-100 % of the crown or death of the plant.

[y]C X P lines 1, 2, 3 and 4 are F_2 generation of `Festival 68' (parsley) and `Golden Spartan' (celery); C X P lines 5, 6 and 7 are F_2 generation of `Festival 68' and `Golden Detroit' (celery).

[z]Each value represents the mean of 10 pots consisting of 3 plants/pot. Values followed by differing letters are significantly different by Duncan's multiple range test (P = 0.05).

Table 5.2. The effects of FOA2 on disease ratings and dry weights of parsley and celeriac cultivars

Cultivar	Disease[x] Rating	Dry Weights (g) Noninfested soil	Dry Weights (g) Infested soil
1. `Festival 68' (Parsley)	1.00[y]a	0.91	1.07 ns[z]
2. Schittsellerie `Amersterdamer'	1.00 a	1.08	1.11 ns
3. Knolselderij `Prager'	1.00 a	0.71	1.07 ns
4. Knolselderij `Arvi'	1.20 a	1.35	1.57 ns
5. Knolselderij `Albatros'	1.30 a	1.03	1.30 ns
6. Bleichsellerie `Improved'	2.90 ab	0.99	0.98 ns
7. Bleichsellerie `Alma'	2.90 ab	1.01	0.95 ns
8. `Tall Utah 52-70 R Improved' (Celery)	4.55 c	2.25	0.57 s

[x]Disease ratings based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown area, 4 = VD in 11-25 % of the crown area, 5 = VD in 25-75 % of the crown area, 6 = VD in 76-100 % of the crown area or death of the plant.

[y]Values represent the mean of 10 pots consisting of 1 plant/pot. Values followed by differing letters are significantly different by Duncan's multiple range test (P = 0.05).

[z]ns (s) = not significantly different (= is significantly different) from its respective control using Tukey's test (P = 0.05).

Table 5.3. Yields and disease ratings of celery cultivars in 1982 field trials.

	Cultivar	Yield (lbs/3 meter row)	Disease[w] Ratings	Disease[w] Reaction Ratings
1.	Summer Pascal	45.6[x]a	2.0 a	MR
2.	Tall Utah 52-70 H K	40.5 ab	2.0 a	MR
3.	Tendercrisp	36.6 bc	2.5 ab	MR
4.	Golden Spartan	32.4 c	3.5 cde	MS
5.	C X P[y]	32.1 c	2.5 ab	-
6.	Florida 683 K	25.0 d	3.5 cde	HS
7.	Grande	24.3 d	2.8 bc	MS
8.	Tall Utah 52-75	19.9 de	3.3 cd	MS
9.	Calmario	18.1 def	4.0 efg	HS
10.	Florimart 19	16.9 efg	4.0 efg	HS
11.	Florida 2-13	15.1 efgh	4.0 efg	HS
12.	Tall Utah 52-70 R Improved	14.1 efghi	4.0 efg	HS
13.	Earlibelle	13.7 efghij	3.8 def	HS
14.	Clean Cut	12.3 fghijk	3.8 def	HS
15.	June Belle	11.1 fghijkl	4.3 fgh	HS
16.	Transgreen	8.7 hijkl	4.5 gh	HS
17.	Tall Green Light	7.3 ijkl	4.8 h	HS
18.	C X P[z]	3.9 l	4.8 h	-

[w]Disease ratings based on the scale: 1 = Highly resistant (HR), no disease symptoms, 2 = Moderately resistant (MR), very slight chlorosis stunting, slight vascular discoloration (VD), 3 = Moderately susceptible (MS), uniformly chlorotic and somewhat stunted, VD moderate, 4 = Highly susceptible (HS), badly stunted and chlorotic, VD severe, 5 = Highly susceptible (HS), plants severely stunted to dead, crowns rotted.

[x]Values represent the mean of four replicates; values followed by differing letter are significantly different by Duncan's multiple range test ($P = 0.05$).

[y]F_2 generation of Golden Spartan X Festival 68.

[z]F_2 generation of Golden Detroit X Festival 68.

Table 5.4. Yields and disease ratings of celery cultivar in 1983 field trials.

Cultivar	Trimmed weight[x] (lbs/3 meter row)	Disease[y] Rating
1. Tall Utah 52-70 H K	17.7 a	2.0 a
2. Deacon	16.6 ab	2.0 a
3. Ferry Morse 1217	14.3 abc	2.3 ab
4. Summer Pascal	13.7 abc	2.3 ab
5. Tendercrisp	13.3 abc	3.5 de
6. Bishop	12.6 bcd	2.9 bc
7. Tall Utah 52-75	10.2 cd	4.0 ef
8. Summit	7.3 de	4.0 ef
9. Florida 683	5.2 ef	4.5 f
10. Ventura	1.6 f	4.5 f

[x]Values represent the mean of 4 replicates; values followed by differing letters are significantly different by Duncan's multiple range test ($P = 0.05$).

[y]Disease ratings based on the scale: 1 = no disease, 2 = very slight chlorosis and stunting, slight vascular discoloration (VD), 3 = uniformily chlorotic and stunted, VD moderate, 4 = badly stunted and chlorotic, VD severe, 5 = plants severely stunted to dead, crown rotted.

Table 5.5. Yield and disease ratings of 1984 celery cultivar field trials.

Cultivar	Trimmed weight[x] (lbs/1.5 meter row)	Disease[y] Ratings
1. Ferry Morse 1217	19.0 a	2.0 a
2. Deacon	16.9 ab	2.0 a
3. Tall Utah 52-70 H K	15.6 ab	2.0 a
4. Florida 683 K	15.5 ab	2.5 a
5. Bishop	14.2 b	2.5 a

[x]Values represent the mean of four replicates; values followed by differing letters are significantly different by Duncan's multiple range test ($P = 0.05$).

[y]Disease ratings based on the scale: 1 = no disease, 2 = very slight stunting, chlorosis and vascular discoloration (VD) in the crown, 3 = uniformly chlorotic and stunted with moderate VD, 4 = stunted and chlorotic with extensive VD, 5 = plants severely stunted to dead with rotted crowns.

greenhouse trials, but revealed a much wider array of symptom severity among cultivars and allowed for greater differentiation of susceptiblity to Fusarium yellows. Cultivars `Deacon' and `Tall Utah 52-70 HK' were consistently classified as MR and the most horticulturally desirable of the cultivars tested. `Bishop', `Tendercrisp', and `Florida 683 K' were ranked as being MS cultivars. `Golden Spartan', Ferry Morse 1217 and `Summer Pascal' were rated as MR, but were not horticulturally desirable due to excessive suckering, brittleness and spreading growth habits. Consistently, `Tall Utah 52-70', `Tall Utah 52-70 R Improved', `Florida 2-13', `Tall Green Light', `June Belle', `Transgreen', `Calmario', `Earlibelle', `Golden Detroit', `Summit', `Ventura' and `Florida 683' were highly susceptible (HS), but differences in symptom severity were still evident.

Several experimental lines of celery germplasm exhibited some resistance to Fusarium yellows. Of the 40 early blight-resistant lines (line 601 through 640) tested in single plots in naturally infested soil in 1983, two lines (604 and 638) were rated as MR (data not shown). In 1984, another seed lot of the 600 series (line 601 throught 667) was screened in the field and all lines were rated from HS to MS; however, several lines were segregating into individuals that ranged from moderately stunted to severely wilted and dead. The 604 and 638 lines screened in 1983 were retested in 1984 and only the 604 line was rated as MR. Several slow bolting lines and the 604 and 638 lines were compared in another field where Fusarium yellows was present and lines 68-37 and 604 exhibited a level of tolerance greater than Deacon (Table 5.6). Slow-bolting line 68-37 was compared to other commercial

Table 5.6. Field Reaction of Experimental and Commercial Lines of Celery to Fusarium Yellows of Celery.

Cultivar	Trimmed weight[x] (lbs/1.5 meter row)	Disease[y] Rating
1. 68-37[z]	23.8	MR-HR
2. 604[z]	16.4	MR
3. Tendercrisp	14.2	MR
4. Deacon	13.1	MR
5. 638[z]	8.6	MS
6. Florida 683	6.3	HS
7. Ventura	2.2	HS

[x]Represents the total trimmed weights of a single plot.

[y]HR = Highly Resistant, No stunting or chlorosis or VD in the crown or roots; MR = Moderately Resistant, slight stunting and VD in the crown or roots; MS = Moderately Susceptible, stunting and chlorosis with extensive VD in the crown and roots; HS = Highly Susceptible, severely stunted and wilted with rotted crowns.

[z]Line 68-37 is a slow-bolting line and lines 604 and 638 are early blight-resistant lines developed at Michigan State University.

cultivars in the greenhouse in artificially infested soils (Table 5.7) and was more resistant than `Deacon' or `Tall Utah 52-70 HK', which are presently the most resistant commercial cultivars of celery.

DISCUSSION

Of the several inoculation techniques tested by other researchers (5, 13, 14, 17) and in my studies (Chapter II), the author feels that the FOA2-colonized wheat straw soil infestation procedure was the most dependable for evaluating resistance to Fusarium yellows in the greenhouse. However, field evaluations were more reliable than greenhouse tests for assessing disease reaction, even though soil inoculum densities were higher in artificially infested soils in the greenhouse. This was thought to be in part due to a longer growing period in the field, allowing the disease to develop further and aid in distinguishing differences in stunting and symptom severity. Horticultural characteristics could also be better evaluated at harvest in the field. In addition, environmental parameters surrounding the greenhouse tests may not have been optimal for disease since lighting, temperature, and moisture levels can affect the development of Fusarium yellows of celery in the greenhouse (5, 17, 19).

Generally, top weights did not correlate well with disease ratings in the greenhouse tests and were not considered as reliable as vascular discoloration in evaluating resistance. In addition, chlorosis of the outer petioles was not consistently observed in susceptible cultivars and did not provide a good criterion for distinguishing moderately resistant (MR) from moderately susceptible (MS) ratings.

No commercial cultivar tested so far has exhibited the level of

Table 5.7. Evaluation of Experimental and Commercial Celery Lines for Resistance to Fusarium yellows of Celery in the Greenhouse.

Cultivar	Disease[x] Ratings	Dry Weights (g) Control	Dry Weights (g) Infested Soil
1. 68-37	1.6[y]a	3.5	2.9 ns[z]
2. Deacon	2.0 a	3.2	2.8 ns
3. Tall Utah 52-70 H K	2.1 a	3.3	2.3 ns
4. Tall Utah 52-70 R Improved	5.2 b	3.0	1.1 s
5. Tall Green Light	5.4 b	3.2	0.2 s

[x]Based on the scale: 1 = no disease, 2 = vascular discoloration (VD) in the primary roots only, 3 = VD in less than 10 % of the crown area, 4 = VD in 11 - 25 % of the crown area, 5 = VD in 26 - 75 % of the crown area, 6 = VD in 76 - 100 % of the crown area or dead.

[y]Values represent the mean of 7 pots consisting of 3 plants/pot. Values followed by differing letters are significantly different by Student-Newman-Keul's test ($P = 0.05$).

[z]ns = not significantly different from its respective control by Student's t test ($P = 0.05$).

resistance to FOA2 that was discovered in `Tall Utah 52-70' to Race 1 of FOA (1). `Deacon' and `Tall Utah 52-70 HK' were rated as having the highest level of resistance of the horticulturally acceptable commercially available cultivars that were tested. Their growth was vigorous and uniform, and vascular discoloration in the crown was slight. These cultivars would be suitable for fresh market when grown in lightly to moderately infested soils, and would still be acceptable in heavily infested soils for processing where healthy white celery crowns are not essential.

Most experimental lines of celery were highly susceptible to FOA2; however, since their sources of resistance to early blight (10) and late blight (9) were obtained from FOA2-resistant celeriac and parsley, respectively, it was not surprising to find some progeny possessing resistance to Fusarium yellows. The high level of resistance to Fusarium yellows discovered in the slow-bolting line 68-37 is not understood, since it was derived solely from celery parents (Personal communication with Dr. S. Honma).

Other studies have shown that isolates of FOA2 from California, New York and Michigan can differ in virulence on green cultivars (16, Chapter IV). Therefore, resistant germplasm should be evaluated in all areas of celery production where the disease occurs. Orton et al. (14) reported that inheritance of resistance to Fusarium yellows in celery was conferred by a single dominant gene accompanied by an undetermined number of quantifying genes; therefore, it is probable that additional breeding could produce a stable genotypic line of celery that would

possess resistance to FOA2 equal to that found in `Tall Utah 52-70' to Race 1 of Fusarium yellows of celery pathogen.

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