This is to certify that the

dissertation entitled

Biology and Control of Fusarium oxysporum f. sp. apii Race 2

presented by

Karen Faye Ireland Toth

has been accepted towards fulfillment
of the requirements for

PhD degree in Botany and Plant Pathology

Melvyn L. Lacey
Major professor

Date 9/28/89

BIOLOGY AND CONTROL OF *FUSARIUM OXYSPORUM* F. SP. *APII* RACE 2

By

Karen Faye Ireland Toth

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1989

ABSTRACT

BIOLOGY AND CONTROL OF *FUSARIUM OXYSPORUM* F. SP. *APII* RACE 2

By

Karen Faye Ireland Toth

Fusarium oxysporum f. sp. *apii* race 2, the causal agent of Fusarium yellows of celery, is the limiting factor in celery production in Michigan. First identified in Michigan in 1981, Fusarium yellows has spread to all the celery-growing regions of the state. Control measures are presently inadequate for celery fields highly infested with race 2.

Determination of soil populations of *F. oxysporum* f. sp. *apii* race 2 is difficult with present methods. Vegetative compatibility and protein banding patterns were investigated as alternatives. *F. oxysporum* f. sp. *apii* race 2 isolates from Michigan, California, and New York were placed within a single vegetative compatibility group based on heterokaryon formation between chlorate-induced nitrate auxotrophics. *F. oxysporum* f. sp. *apii* race 1 and 11 other form species of *F. oxysporum* were not vegetatively compatible with race 2. Protein banding patterns on polyacrylamide gels were similar for all form species of *F. oxysporum* tested. Vegetative compatibility more accurately identified race 2 cultures isolated from soil than gel electrophoresis or pathogenicity tests.

Somaclones regenerated from cell suspensions derived from a moderately resistant celery cultivar expressed high levels of resistance

to race 2. The high resistance was passed onto some progeny via self-fertilization. These lines could provide a source of resistance for celery growers.

Seven celery cultivars and 21 experimental lines were screened for resistance to *F. oxysporum* f. sp. *apii* race 2 in field trials in Michigan. Lines highly resistant to race 2 were identified, but most were horticulturally inferior to susceptible cultivars. Soil temperatures above 30° C reduced celery growth and resistance to race 2.

Rotation to onions, leeks, and other vegetables reduced *F. oxysporum* f. sp. *apii* race 2 populations in Michigan muck fields. Incorporation of dried or green residues of various crops into infested muck fields did not reduce Fusarium yellows severity on celery. A race 2-suppressive field was discovered in Michigan.

A disease management strategy involving resistant cultivars, rotation with other crops, and assessment of soil populations of *F. oxysporum* f. sp. *apii* race 2 was evolved using data generated in this study.

ACKNOWLEDGEMENTS

I would like to sincerely thank Dr. Melvyn Lacy, my major professor, for all his advice, direction, understanding, and friendship during my graduate career at Michigan State. An appreciation is also extended to my guidance committee, Drs. John Lockwood, Christine Stephens, and Amy Iezzoni, for their interest and advice. A special thanks goes to Dr. Raymond Hammerschmidt for offering many helpful suggestions and for substituting at my dissertation defense.

A very special thanks goes to Mr. Richard Crum, Mr. Brian Cortright, and numerous undergraduate students who provided helping hands for all aspects of this project.

Much appreciation is also extended to Celery Research, Incorporated for providing the funds for this study, and to Mr. Brian Willbrandt, Mr. Tom Willbrandt, and Mr. Gordon Martinie for generously providing space on their commercial farms and added technical assistance for my field research.

My parents, Jane and Croswell Ireland, deserve a special round of applause for supporting me in every way possible to allow me to pursue my goals.

Most of all, I give my thanks, appreciation, and love to my number-one editor and husband, Dr. Peter P. Toth, for believing in me and always encouraging me to do my best.

For Peter

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 4

CHAPTER 5

LIST OF FIGURES

CHAPTER 5

LIST OF ABBREVIATIONS

cm	centimeters
C	degrees celsius
g	grams
ha	hectares
LSD	least significant difference
l	liters
m	meters
μM	micromoles
ml	milliliters
mm	millimeters
mM	millimoles
M	moles
R_F	electrophoretic mobility of protein on SDS-PAGE relative to bromophenol dye front
rpm	rotations per minute
t	metric tonnes
Tris	tris(hydroxymethyl)aminomethane
v/v	volume for volume
w/v	weight per volume

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Celery (*Apium graveolens* L. var *dulce* (Mill.) Pers.) probably originated in marshlands around the Mediterranean Sea (Ryder 1979). Celery was first grown for medicinal purposes, most likely because its bitter flavor and odor suggested curative properties (Ryder 1979) and because celery seed contains an opiate (Hart 1977). During the late 1500's to early 1600's celery began to be cultivated as a food product to add flavor to cooked foods (Ryder 1979, Ware and McCollum 1975).

As celery began to be cultivated as a fresh vegetable in the 1700's, its horticultural characteristics began to change (Ryder 1979). Wild celery was a leafy plant, probably resembling the leafy vegetable smallage which is still planted in gardens as a cooking herb. Selection and breeding lead to the development of the large, succulent, and highly edible petioles for which commercial celery is grown today.

Celery belongs to the family Umbelliferae, which also contains celeriac, parsley, carrot, parsnip, and several weeds (Ryder 1979). Celery grows by producing a close rosette of petioles on a compressed stem forming a tight or loose head. Commercial celery cultivars are biennials, but will bolt and flower in the first year with a vernalization treatment (below 10° C for 6 to 8 weeks). Bolting occurs when the main stem elongates to form a branched flower stalk containing

numerous umbels of small white perfect flowers which have 5 petals and 5 stamens. Celery seeds are actually fruits made up of two compressed carpels which contain the true seed.

Like most *Apium* species, celery plants are diploid with 2n = 2x = 22. Celery cultivars are generally open-pollinated. Celery plants can be self-pollinated, although individual flowers do not self-pollinate because the styles remain immature until after the pollen from that flower is shed (Ryder 1979).

Commercial celery cultivars fall into three categories: yellow types which contain some chlorophyll when blanched, easy-blanching or self-blanching which are white when blanched, and green types which are not blanched (Hart 1977). The yellow and self-blanching cultivars were grown in the United States until the mid 1940's. Growers turned to green cultivars during World War II because of the lack of field hands for blanching. After World War II, celery growers continued growing the green cultivars because consumers had accepted them and because the green cultivars were highly resistant to the fungal disease Fusarium yellows and some diseases caused by mineral deficiencies (Hart 1977). Yellow celery cultivars are still most common in Europe (Ryder 1979).

There are three recognized types of green celery: Pascals, Utahs, and Crystal Jumbos (Hart 1977). Utahs are yellow-green in color and have a more open growth habit than the other two types. Pascals are also yellow-green with petioles which curve in towards the top. Crystal Jumbos are more gray-green in color with a closed, upright growth habit.

Green celery is one of the most used salad vegetables in the United States (Ryder 1979). Celery is 94% water and contains small amounts of

carbohydrates and fat, which is why it is a popular diet food (Schneider 1982). One cup of chopped celery contains approximately 20 calories, 1.1 g protein, 0.1 g fat, 4.7 g carbohydrate, 47 mg calcium, 151 mg sodium, 320 mg vitamin A, 409 mg potassium, 11 mg vitamin C, and trace amounts of other vitamins and minerals.

Celery production in the United States began near Kalamazoo, Michigan in the 1800's (Hart 1977). Michigan remains the third largest celery-producing state, producing 6.4% of the total United States production in 1987 (Shapley and Dudek 1989). Other celery producing states include California, Florida, Texas, Ohio, Wisconsin, and New York.

In Michigan, celery is grown on highly organic muck soils. Almost all of the celery produced in Michigan is grown in the western half of central Michigan (Shapley and Dudek 1989). Most of the celery farms are family owned and operated, and an average-sized farm would contain approximately 75 acres of tillable muck (Shapley and Dudek 1989). Celery seedlings are started in greenhouses or outdoor beds, transplanted into the fields from May until July, and harvested from late June until October.

Celery is an intensively managed crop, requiring a high input expenditure compared to net return in order to produce a profitable crop (Shapley and Dudek 1989). Celery is generally monocropped, and few vegetables are as profitable as celery to grow (Lorenz and Maynard 1988, Ryder 1979, Ware and McCollum 1975).

The climate in Michigan is favorable for the production of high quality processing and fresh market celery (Shapley and Dudek 1989), yet

insect pests and diseases take their toll. The disease problems on celery in Michigan include: late blight caused by *Septoria apiicola*, early blight incited by *Cercospora apii*, bacterial blight caused by *Pseudomonas apii* and *P. cichorii*, *Sclerotinia* pink rot, cucumber mosaic and common celery mosaic viruses, and aster yellows (Lacy and Grafius 1980). Black heart caused by calcium deficiency and cracked stem due to boron deficiency are two nutritional problems common in Michigan celery (Lacy and Grafius 1980). Fusarium yellows caused by *F. oxysporum* f. sp. *apii* (Lacy and Elmer 1985) is the most important disease of celery in Michigan and is the topic of this dissertation.

I. FUSARIUM YELLOWS OF CELERY

History and biology. *Fusarium oxysporum* f. sp. *apii* (R. Nels. & Sherb.) Snyd. and Hans., the causal agent of Fusarium yellows of celery was the limiting factor in production of yellow and self-blanching celery cultivars grown during the first half of this century (Nelson et al. 1937, Ryker 1935), but the disease vanished after the introduction of highly resistant green celery cultivars in the 1940-1950's (Opgenorth and Endo 1985). In 1978, Fusarium yellows of celery reappeared in California due to a new race of the fungus (race 2) (Hart and Endo 1978). Race 2 attacked previously resistant green cultivars as well as yellow and self-blanching celery cultivars, whereas the original Fusarium yellows pathogen attacked only the yellow and self-blanching cultivars (Schneider and Norelli 1981).

F. oxysporum f. sp. *apii* race 2 was identified in Michigan by 1981 (Elmer and Lacy 1984) and had spread to all the celery growing regions

in Michigan by 1987 (Figure 1) (Toth unpublished). Fusarium yellows is also an important disease of celery in New York (Awuah et al. 1986), Ohio (R. Rowe, personal communication), Wisconsin (Elmer and Lacy 1984), and Texas (Martyn 1987). Fusarium yellows was observed on celery grown in soil from Florida (Toth unpublished), but has not caused important losses in that state.

The most common diagnostic symptom of Fusarium yellows is a red to brown discoloration in the vascular tissue of the roots and crowns of infected plants (Awuah et al. 1986, Hart and Endo 1978, Lacy and Elmer 1985). The discoloration may extend into the petioles in severely infected plants, and be accompanied by rotting of the central crown area. As the disease progresses, plants become stunted and chlorotic. Severely infected plants can wilt and die, some of them because of bacterial soft rot which develops in the diseased crowns. Even slightly infected plants are of low quality because they are often stunted and bitter tasting (Awuah et al. 1986, Toth unpublished).

F. oxysporum f. sp. *apii* race 2 infects celery roots through the root tips (Hart and Endo 1981). Although numerous infections were required for severe disease to develop (Hart and Endo 1981), a *F. oxysporum* f. sp. *apii* race 2 population of only 42 propagules per gram of muck soil was predicted to be sufficient to incite severe disease in susceptible celery (Elmer and Lacy 1987b).

F. oxysporum f. sp. *apii* race 2 is a soil-borne pathogen. *F. oxysporum* isolates are imperfect fungi which produce single-celled, oval-shaped microconidia in false heads on relatively short monophialides (Nelson et al. 1983). Infectious spores of *F. oxysporum*

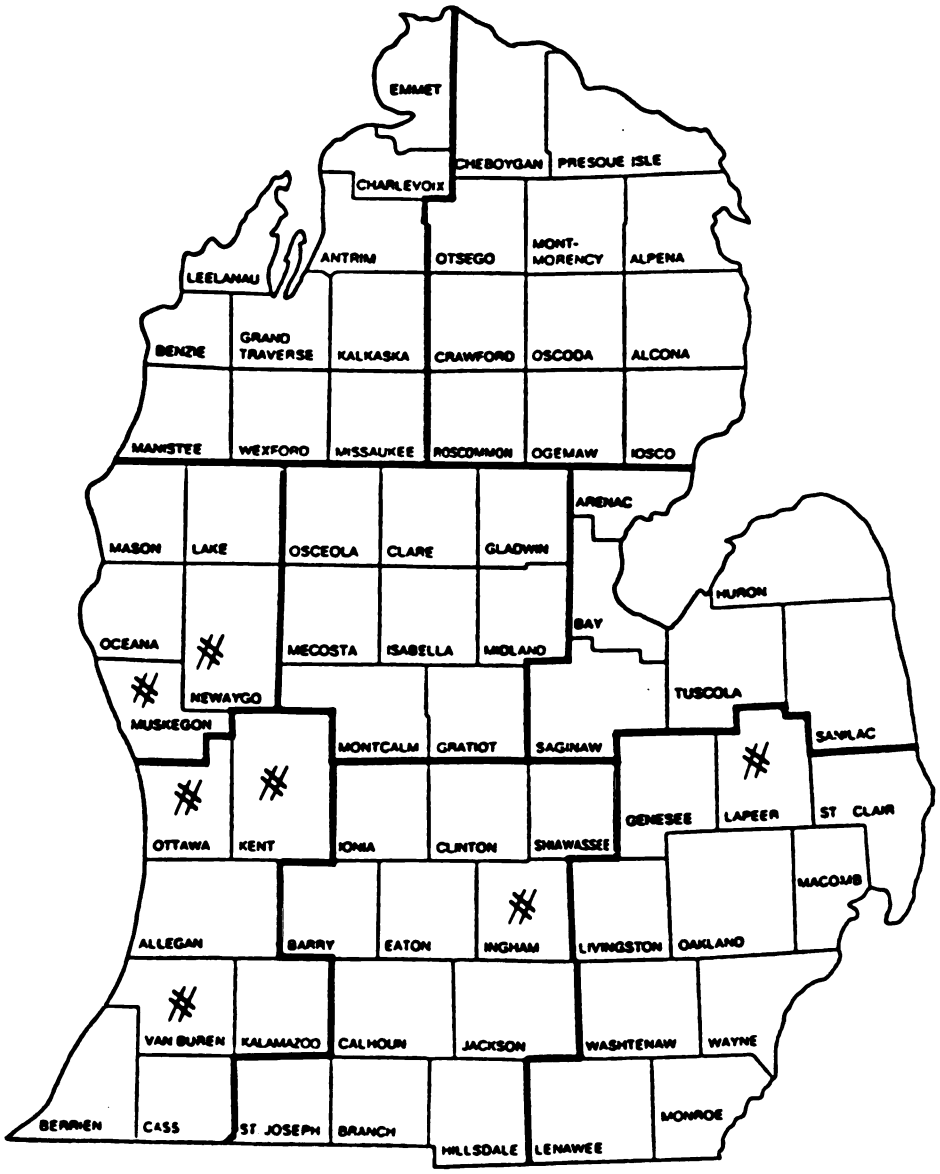

Figure 1. Counties in Michigan (✻) with confirmed *F. oxysporum* f. sp. *apii* race 2-infested celery fields as of 1987.

include chlamydospores and macrospores; the latter are slightly sickle shaped and have an attenuated apical cell and a foot-shaped basal cell. *F. oxysporum* f. sp. *apii* race 2 produces abundant microconidia and less abundant chlamydospores, but rarely produces macroconidia on commonly used culturing media.

Control. *F. oxysporum* f. sp. *apii* race 2 is difficult to control on celery. Soil fumigation is expensive, and has not yet proven to be a viable means of controlling the pathogen (Awuah et al. 1986, Greathead 1988, Otto et al. 1976). Likewise, no commercially available fungicides have controlled this pathogen (Awuah et al. 1986).

Host resistance is the most practical method of controlling *F. oxysporum* f. sp. *apii* race 2 (Lorenz and Maynard 1988, Opgenorth and Endo 1985). Field trials in California (Hart and Endo 1978, Opgenorth and Endo, 1979 & 1985), Michigan (Elmer et al. 1986), and New York (Awuah et al. 1986) demonstrated that most yellow and green celery cultivars were susceptible to *F. oxysporum* f. sp. *apii* race 2. The green celery cultivars grown today were all derived from a single plant designated number 52-70 selected from the cultivar Crystal Jumbo for resistance to *F. oxysporum* f. sp. *apii* race 1 (Hart 1977, Opgenorth and Endo 1985). Although 52-70 was very heterozygous (Hart 1977), further selections from 52-70 which lead to a number of green cultivars narrowed the genetic pool in celery which possibly was the basis for the nearly uniform susceptibility of celery cultivars to *F. oxysporum* f. sp. *apii* race 2.

Cultivars Tall Utah 52-70 HK and Florida 683 K were selected from line 52-70 H for possible resistance to *F. oxysporum* f. sp. *apii* race 2

(Hart 1977). Tall Utah 52-70 HK was rated as moderately resistant and Florida 683 K as susceptible to *F. oxysporum* f. sp. *apii* race 2 in field trials conducted in Michigan (Elmer et al. 1986). Deacon is another cultivar which has a moderate level of resistance to *F. oxysporum* f. sp. *apii* race 2 (Elmer et al. 1986).

UC-1 was a breeding line released from the University of California at Davis in 1984 because of its high resistance to *F. oxysporum* f. sp. *apii* race 2 (Orton et al. 1984). UC-1 was derived from crosses between several race 2-resistant celeriac (*A. graveolens* var. *rapaceum*) accessions, race 2-resistant celery, and susceptible celery. Although tall and vigorous, UC-1 contains many undesirable characteristics like hollow petioles and numerous side shoots carried over from the celeriac parents. UC-1 is currently being used in celery breeding programs as a source of resistance to *F. oxysporum* f. sp. *apii* race 2.

Researchers were able to increase resistance in celery to *F. oxysporum* f. sp. *apii* race 2 (Heath-Pagliuso et al. 1988, Wright and Lacy 1988), *Septoria apiicola*, *Cercospora apii*, and *Pseudomonas cichorii* (Wright and Lacy 1988) by regenerating celery plants from cell suspensions. In some somaclones, the increased resistance was stable and passed onto the progeny via self-fertilization (Heath-Pagliuso et al. 1988). Heath-Pagliuso et al. (1989) recently released a highly resistant celery line, UC-T3, which was derived from a somaclone regenerated from cells from susceptible cultivar Tall Utah 52-70 R.

The mechanisms of resistance in the moderately resistant celery cultivars that are commercially available is not known. *F. oxysporum* f. sp. *apii* race 2 colonized the roots and crowns of susceptible and

moderately resistant celery equally, but colonized the petioles of susceptible plants to a greater extent than moderately resistant plants (Elmer and Lacy 1987a). Likewise, vascular discoloration and rotting readily occurs in petioles of susceptible cultivars, but are rarely observed in resistant plants (Elmer and Lacy 1987a).

Researchers have explored other strategies for controlling *F. oxysporum* f. sp. *apii* race 2 in celery. Endo (1982) reported that increasing the pH of soil to 7.5-7.8 decreased severity of Fusarium yellows in the greenhouse. In celery fields, *F. oxysporum* f. sp. *apii* race 2 propagules were found to exist and remain viable at soil depths of at least 30 cm. The amount of lime needed to raise the pH from the normal average of 6.6 for the California celery fields examined to a slightly alkaline pH to a depth of 30 cm proved to be economically prohibitive.

Schneider (1982,1985) examined the effects of different forms of nitrogen and their associated ions on the development of Fusarium yellows in celery in sandy loam soils in California. Ammonium salts were more conducive to Fusarium yellows than nitrate salts, regardless of the accompanying ions. A regime of KCl with $Ca(NO_3)_2$ almost completely controlled Fusarium yellows in the greenhouse and in some field trials. Disease suppression was related to a K:Cl ratio of 1:1 in treated petioles, rather than total amount of each ion. Awuah and Lorbeer (1982) reported that nitrogen and potassium fertilizers were ineffective in controlling Fusarium yellows in muck fields in New York. Likewise, nitrate fertilizers did not reduce the severity of Fusarium yellows in celery grown in a muck field in Michigan (Lacy 1982).

Crop rotation is another control tactic used for many soil-borne pathogens. Fusarium yellows incidence and severity increased when celery residues were added to soils infested with *F. oxysporum* f. sp. *apii* race 2 (Elmer and Lacy 1987a, Opgenorth and Endo 1981) suggesting that monocropping celery, especially when celery trimmings are incorporated back into the fields, would rapidly increase populations of *F. oxysporum* f. sp. *apii* race 2 in celery fields. Unfortunately, *F. oxysporum* f. sp. *apii* race 2 readily colonizes the roots of a number of plant genera (including weeds) without causing obvious symptoms (Elmer and Lacy 1987a). This leaves a potential source of inoculum even after rotation out of celery.

Crop rotation could reduce *F. oxysporum* f. sp. *apii* race 2 populations if the pathogen was unable to colonize the roots of the crop of choice, or if residues left after the crop was harvested were deleterious to the pathogen. Roots of onion and lettuce were colonized by a UV-light induced color mutant of *F. oxysporum* f. sp. *apii* race 2 to a significantly lesser extent than were roots of susceptible celery (Elmer and Lacy 1987a). No mutant colonies were recovered from parsley or crabgrass roots. Less severe Fusarium yellows developed on susceptible celery grown in muck soil infested with *F. oxysporum* f. sp. *apii* race 2 and to which onion residues were added than when grown in soil with celery residues (Elmer and Lacy 1987a). Likewise, *F. oxysporum* f. sp. *apii* race 2 populations decreased more rapidly in soils with onion residues than soils with celery residues (Elmer and Lacy 1987a).

II. OBJECTIVES

The main objectives of this doctoral project were to a develop laboratory technique for identifying *F. oxysporum* f. sp. *apii* race 2 cultures and to examine various methods for controlling the pathogen. Chapter 2 examines the potential of laboratory procedures involving vegetative compatibility or gel electrophoresis in identifying *F. oxysporum* f. sp. *apii* race 2 cultures isolated from soil dilutions. Chapters 3 to 5 encompass various strategies examined for controlling *F. oxysporum* f. sp. *apii* race 2. The control methods studied include the use of tissue culture techniques in an attempt to increase resistance in celery to *F. oxysporum* f. sp. *apii* race 2 (Chapter 3) and the testing of experimental celery lines for resistance in field trials under Michigan growing conditions (Chapter 4). The effects of crop rotation, cover crop choice, and organic amendments on *F. oxysporum* f. sp. *apii* race 2 populations in celery fields are presented in Chapter 5. Chapter 5 also covers some preliminary studies on a possible race 2-suppressive field in Michigan.

LITERATURE CITED

Awuah, R. T. and Lorbeer, J. W. 1982. Occurrence of *Fusarium oxysporum* f. sp. *apii* race 2 in New York and attempts to control Fusarium yellows of celery caused by this pathogen. National Celery Workshop Proceedings. 1981-82 Annual Report, California Celery Research Program. California Celery Research Advisory Board, Dinuba, CA. 207 pp.

Awuah, R. T., Lorbeer, J. W., and Ellerbrock, L. A. 1986. Occurrence of Fusarium yellows of celery caused by *Fusarium oxysporum* f. sp. *apii* race 2 in New York and its control. Plant Dis. 70:1154-1158.

Endo, R. M. 1982. The biology and control of the Fusarium yellows disease of celery. 1980-81 Annual Report, California Celery Research Program. California Celery Research Advisory Board, Dinuba, CA. 83 pp.

Elmer, W. H. and Lacy M. L. 1984. Fusarium yellows (*Fusarium oxysporum* f. sp. *apii* race 2) of celery in Michigan. Plant Dis. 68:537.

Elmer, W. H. and Lacy, M. L. 1987a. Effects of crop residues and colonization of plant tissues on propagule survival and soil populations of *Fusarium oxysporum* f. sp. *apii* race 2. Phytopathology 77:381-387.

Elmer, W. H. and Lacy, M. L. 1987b. Effects of inoculum densities of *Fusarium oxysporum* f. sp. *apii* in organic soil on disease expression in celery. Plant Dis. 71:1086-1089.

Elmer, W. H., Lacy M. L., and Honma, S. 1986. Evaluations of celery germ plasm for resistance to *Fusarium oxysporum* f. sp. *apii* race 2 in Michigan. Plant Dis. 70:416-419.

Greathead, A. 1988. Control of Fusarium yellows. California Celery. California Celery Research Advisory Board, Dinuba, California. 2:3-5.

Hart, L. P. 1977. History of celery according to Gene Hill. Unpublished.

Hart, L. P. and Endo R. M. 1978. The reappearance of Fusarium yellows of celery in California. Plant Dis. Reptr. 62:138-142.

Hart, L. P. and Endo, R. M. 1981. The effect of time of exposure to inoculum, plant age, root development, and root wounding on Fusarium yellows of celery. Phytopathology 71:77-79.

Heath-Pagliuso, S., Pullman, J., and Rappaport, L. 1988. Somaclonal variation in celery: screening for resistance to *Fusarium oxysporum* f. sp. *apii*. Theor. Appl. Genet. 75:446-451.

Heath-Pagliuso, S., Pullman, J., and Rappaport, L. 1989. UC-T3 somaclone: celery somaclone resistant to *Fusarium oxysporum* f. sp. *apii* race 2. HortScience 24:(In press).

Lacy, M. L. 1982. Reappearence of Fusarium yellows disease in Michigan Celery. National Celery Workshop Proceedings. 1981-82 Annual Report, California Celery Research Program. California Celery Research Advisory Board, Dinuba, CA. 207 pp.

Lacy, M. L. and Grafius, E. J. 1980. Disease and Insect Pests of Celery. Extension Bulletin E-1427. Cooperative Extension Service, Michigan State University. 8 pp.

Lacy, M. L. and Elmer, W. H. 1985. Fusarium yellows of celery in Michigan. Michigan Extension Bulletin E-1823. 2 pg.

Lorenz, O. A. and Maynard, D. N. 1988. Knott's Handbook for Vegetable Growers. Third edition. John Wiley and Sons, New York. 456 pp.

Martyn, R. D. 1987. Fusarium yellows of celery in Texas. Plant Dis. 71:651.

Nelson, R., Coons, G. H., and Cochran, L. C. 1937. The Fusarium yellows disease of celery. Mich. Agric. Exp. Sta. Tech. Bull. #155. 74 pp.

Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* species: An Illustrated Manual for Identification. The Pennsylvania State University Press. University Park, Pennsylvania. 193 pp.

Opgenorth, D. C. and Endo, R. M. 1979. Sources of resistance to Fusarium yellows of celery in California. Plant Dis. Reptr. 63:165-169.

Opgenorth, D. C. and Endo, R. M. 1981. Competitive saprophytic ability (CSA) of *Fusarium oxysporum* f. sp. *apii*. (Abstr.) Phytopathology 71:246-247.

Opgenorth, D. C., and Endo, R. M. 1985. Additional sources of resistance to race 2 of *Fusarium oxysporum* f. sp. *apii*. Plant Dis. 69:882-884.

Orton, T. J., Hulbert, S. H., Durgan, M. E., and Quiros, C. F. 1984. UC1, Fusarium yellows-resistant celery breeding line. HortScience 19:594.

Otto, H. W., Paulus, A. O., Snyder, M. J., Endo, R. M., Hart, L. P., and Nelson, J. 1976. A crown rot of celery. Calif. Agric. 30:10-11.

Quiros, C. F., D'Antonio, V., and Ochoa, O. 1988. Breeding celery for disease resistance and improved quality. Annual Report of California Celery Research Advisory Board, Dinuba. California. 2:1-3.

Ryder, E. J. 1979. Leafy Salad Vegatables. Avi Publishing Company, Inc., Westport, Connecticut. 266 pp.

Ryker, T. C. 1935. Fusarium yellows of celery. Phytopathology 25:578-600.

Shapley, A. E. and Dudek, T. A. 1989. The cost of producing celery, West Central Michigan: 1990. Staff Paper, Department of Agricultural Economics, Michigan State University. 8 pp.

Schneider, R. W. 1982. Use of potassium and competitive root colonizers in the control of Fusarium yellows of celery. 1980-81 Annual Report, California Celery Research Program. California Celery Research Advisory Board, Dinuba, CA. 83 pp.

Schneider, R. W. 1985. Suppression of Fusarium yellows of celery with potassium, chloride, and nitrate. Phytopathology 75:40-48.

Schneider, R. W. and Norelli, J. L. 1981. A new race of *Fusarium oxysporum* f. sp. *apii* in California. (Abstr.) Phytopathology 71:108.

Ware, G. W. and McCollum, J. P. 1975. Producing Vegetable Crops. The Interstate Printers and Publishers, Inc. Danville, Illnois. 599 pp.

Wright, J. C. and Lacy M. L. 1988. Increase of disease resistance in celery cultivars by regeneration of whole plants from cell suspension cultures. Plant Dis. 72:256-259.

CHAPTER 2

POTENTIAL OF NITRATE NONUTILIZING MUTANTS AND POLYACRYLAMIDE GEL ELECTROPHORESIS IN IDENTIFYING *FORMAE SPECIALES* OF *FUSARIUM OXYSPORUM*.

INTRODUCTION

Fusarium oxysporum (Schlect.) emend. Snyd. & Hans. is a soil-borne pathogen of a wide variety of agricultural crops (Snyder and Hansen 1940). *F. oxysporum* f. sp. *apii* (R. Nels. & Sherb.) Snyd. and Hans., the cause of Fusarium yellows of celery (*Apium graveolens* L. var *dulce* (Pers.) Miller), was the limiting factor in production of yellow and self-blanching celery cultivars grown during the first half of this century (Nelson et al. 1937, Ryker 1935), but the disease vanished after the introduction of highly resistant green celery cultivars in the 1950's (Opgenorth and Endo 1985).

In 1975, Fusarium yellows of celery reappeared in California (Hart and Endo 1978). The reappearance of Fusarium yellows appeared to be caused by a new race of the fungus which was designated race 2 because it caused disease in both race 1-resistant green cultivars and race 1-susceptible self-blanching celery cultivars (Schneider and Norelli 1981). *F. oxysporum* f. sp. *apii* race 2 has also been isolated from the celery growing states of Michigan (Elmer and Lacy 1984), New York (Awuah et al. 1986), Ohio (R. Rowe, personal communication), Wisconsin (Elmer 1985), and Texas (Martyn 1987). Fusarium yellows is currently the most important disease of celery in Michigan, making its study a high priority in celery research.

Most of the 122 strains and races of *F. oxysporum* are morphologically indistinguishable from each other (Nelson et al. 1983,

Snyder and Hansen 1940), and from saprophytic *F. oxysporum* (Nelson et al. 1983). The most common technique used to identify a particular pathogenic strain or race of *F. oxysporum* is to carry out a pathogenicity test with the appropriate host(s) (Armstrong and Armstrong 1968). These procedures are laborious and time-consuming since it usually takes several weeks for symptoms to appear, and, in the case of celery, require rather exacting greenhouse conditions for symptom expression (Endo et al. 1978). Other characteristics used to identify fungi include vegetative compatibility (Puhalla 1985) and electrophoretic separation of proteins (Micales et al. 1986), which were investigated in this work as alternatives to pathogenicity tests.

I. VEGETATIVE COMPATIBILITY GROUPING

Heterokaryosis and the resulting parasexual cycle are mechanisms for gene exchange in fungi (Alexopoulus and Mims 1979). Heterokaryosis is the only means known for genetic recombination in imperfect fungi such as *F. oxysporum* f. sp. *apii* race 2. Heterokaryosis with resulting genetic recombination was reported for auxotrophic mutants of the imperfect fungi *F. oxysporum* f. sp. *pisi* (Buxton 1956), *F. oxysporum* f. sp. *cubense* (Buxton 1962), *Verticillium albo-atrum* (Hastie 1962, Hastie 1964), and *V. dahliae* (Puhalla and Mayfield 1974). Parasexual recombination was also reported for *Aspergillus niger* (Pontecorvo et al. 1953) and *Penicillium chrysogenum* (Pontecorvo and Sermonti 1954).

Vegetative compatibility is the ability of genetically similar fungal strains to anastomose and form a stable heterokaryon. Mutually compatible strains are assigned to one vegetative compatibility group

(VCG). Strains which cannot form a heterokaryon are said to be vegetatively incompatible. Vegetative compatibility is often controlled by several genes, and two strains must have the same alleles at each locus in order for a stable heterokaryon to form (Anagnostakis 1982, Croft and Jinks 1977, Perkins et al. 1982, Puhalla and Spieth 1983, Puhalla and Spieth 1985). Vegetatively incompatible strains of imperfect fungi would be genetically isolated from one another. Thus, vegetatively compatible strains should be more alike in other morphological and biochemical traits than vegetatively incompatible strains. Assignment of strains to VC groups was positively correlated with pathogenicity tests for *F. oxysporum* (Katan and Katan 1988, Ploetz and Correll 1988, Puhalla 1979), *Gibberella fujikuori* (= *F. moniliforme*) (Puhalla and Spieth 1983), and for *V. dahliae* (Corsini et al. 1985, Puhalla 1979). Vegetatively compatible strains of *F. oxysporum* were also similar for colony size (Correll et al. 1986a) and isozyme banding patterns (Bosland and Williams 1987). Strains of *Aspergillus nidulans* placed in the same VCG had similar growth patterns and antibiotic production (Croft and Jinks 1977).

Forced heterokaryosis between different auxotrophic or color mutants is the primary technique used to study vegetative compatibility. Vigorous growth or complementary pigmentation from such pairings is taken as evidence of a forced, balanced heterokaryon. Prototrophic growth confirming heterokaryosis has been reported between UV-light or mutagenically-induced auxotrophic mutants of *V. albo-atrum* (Hastie 1962, Hastie 1964, Typas and Heale 1976), *V. dahliae* (Puhalla 1979, Puhalla and Mayfield 1974, Typas and Heale 1976), *G. zeae* (= *F.*

graminearum) (Leslie 1987), *F. oxysporum* f. sp. *gladioli* (Buxton 1954), *F. oxysporum* f. sp. *lycopersici* (Sanchez et al. 1976), *F. oxysporum* f. sp. *pisi* (Buxton 1956), and *G. fujikuori* (= *F. moniliforme*) (Puhalla and Speith 1983, Puhalla and Speith 1985). Heterokaryosis between UV-induced color mutants has been demonstrated for *Neurospora* (Huang 1964), *V. dahliae* (Puhalla 1979), *A. nidulans* (Croft and Jinks 1977), and *F. oxysporum* f. sp. *apii* (Puhalla 1984).

The generation of mutants by UV light and chemical mutagen treatment is too laborious to be useful in screening populations of field isolates for vegetative compatibility. Likewise, a researcher has no control over the type of mutations induced, and often several genes may be affected by the mutagenic treatment. Mutants induced by these techniques must be carefully characterized to determine what mutations are present. The number of mutated genes is virtually impossible to determine for fungi which have no sexual stage.

Recently, researchers have employed a technique which produces nitrate nonutilizing (*nit*) mutants without the use of a mutagen for studying heterokaryosis (Bosland and Williams 1986, Correll et al. 1986, Elmer and Stephens 1989, Jacobson and Gordon 1988, Joaquim et al. 1987, Katan and Katan 1988, Ploetz and Correll 1988, Puhalla 1985, Puhalla and Spieth 1985). Fungi like *F. oxysporum* f. sp. *apii* race 2 are normally able to utilize nitrate as a nitrogen source by reducing it to ammonium via the enzymes nitrate reductase and nitrite reductase (Figure 1). Two proteins which regulate the nitrate-assimilation pathway (Cove 1976, Klittich et al. 1986) and a molybdenum-containing cofactor required by

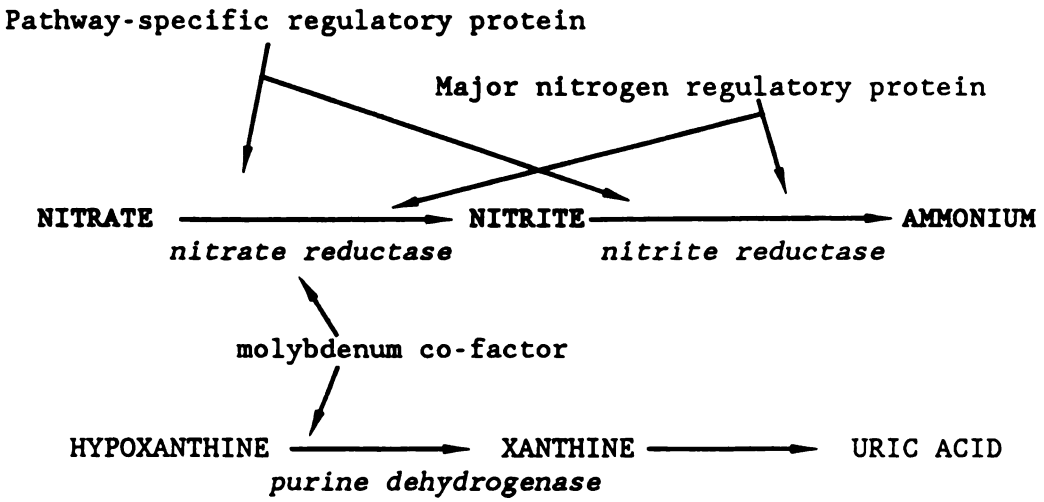

Figure 1. Nitrate assimilation pathway in fungi (Cove 1979, Marzluf 1981)

nitrate reductase and xanthine dehydrogenase (Cove 1976, Klittich et al. 1986, Pateman et al. 1964) are also required for nitrate reduction (Figure 1).

To produce *nit* mutants, wild type fungal strains are grown on a medium containing 1.5% potassium chlorate ($KClO_3$) (Puhalla 1985). Chlorate, a nitrate analogue, is presumably reduced within fungal cells by nitrate reductase to toxic chlorite which severely restricts colony growth (Cove 1976, Klittich et al. 1986). Mutants resistant to chlorate display a faster, more expansive growth on chlorate medium, and most chlorate-resistant mutants are unable to utilize nitrate as a nitrogen source (Correll et al. 1987, Cove 1976, Klittich et al. 1986, Puhalla 1985). Exposure to chlorate selects for mutations in the nitrate reductase locus, any of the 4 to 5 loci which code for the molybdenum-containing cofactor, or in the locus coding for the pathway regulatory protein (Cove 1976, Klittich et al. 1986). Individual *nits* usually possess only one mutation.

Nits which are produced from an individual fungal isolate and which have mutations in different areas of the nitrate-assimilation pathway are able to complement one another and form a nitrate-utilizing heterokaryon when their hyphae anastomose (Correll et al. 1987), unless the isolate is self-incompatible (Bosland and Williams 1987, Correll et al. 1988, Jacobson and Gordon 1988). Complementary *nit* mutants from vegetatively compatible fungal strains form nitrate-utilizing heterokaryons (Correll et al. 1987, Puhalla 1985). Strains whose complementary *nit* mutants do not form a nitrate-utilizing heterokaryon are vegetatively incompatible (Puhalla 1985).

Nits have been recovered from a number of different fungi including various form species of *F. oxysporum* (Bosland and Williams 1987, Correll et al. 1986a, Elmer and Stephens 1989, Jacobson and Gordon 1988, Katan and Katan 1988, Larkin et al. 1988, Ploetz and Correll 1988, Puhalla 1985), *G. fujikuori* (Puhalla and Spieth 1985), *V. albo-atrum* (Correll et al. 1988), and *V. dahliae* (Joaquim et al. 1987, Joaquim and Rowe 1988). Division of isolates into VCG corresponded to division by host range in most cases (Bosland and Williams 1987, Correll et al. 1988, Katan and Katan 1988, Larkin et al. 1987, Ploetz and Correll 1988, Puhalla 1985), although isolates from some form species and races of *F. oxysporum* (Elmer and Stephens 1989, Jacobson and Gordon 1988, Ploetz and Correll 1988) and isolates of *V. dahliae* from potato (Joaquim et al. 1987) were divided into more than one VCG. Saprophytic isolates were also placed within different VCG's than pathogenic isolates of the same species (Correll et al. 1986a, Katan and Katan 1988, Larkin et al. 1988).

II. GEL ELECTROPHORESIS

Gel electrophoresis techniques were shown to be reliable for classifying fungi (Micales et al. 1986). Enzymes coded by different alleles or separate genetic loci possess different electrophoretic mobilities due to variations in their respective amino acid content and molecular weight (Chrambach and Rodbard 1971, Micales et al. 1986). Banding patterns from different fungal strains can be compared by counting the number of bands, looking for bands present in some strains

which are absent in others, or by staining for different enzymes and comparing relative number and position of the bands for each isozyme (Micales et al. 1986).

Comparing protein banding patterns on polyacrylamide and starch gels has proven useful in distinguishing between some species of fungi, and even between different strains within a species. Banding patterns on starch gels of 103 pathogenic *F. oxysporum* isolates from crucifers divided them into 4 groups comparable to separation by host specificity and vegetative compatibility (Bosland and Williams 1987). Considerable variation was observed in protein banding patterns from 11 species of *Puccinia*, but less variation was noted between *formae speciales* within a species (Burdon and Marshall 1981). Differences were noted in protein banding patterns for different species of *Phytophthora* (Bielenin et al. 1988, Erselius and De Vallavieille 1984, Gill and Powell 1968a), but within a species banding patterns were similar or identical (Bielenin et al. 1988, Gill and Powell 1968b). Based on protein patterns it was possible to separate 3 species of *Neurospora* (Chang et al. 1962), and *F. oxysporum* from 13 other *Fusarium* species, *V. albo-atrum*, and *Graphium* (Glynn and Reid 1969).

This study was undertaken to determine if vegetative compatibility and electrophoretic separation of proteins have potential as techniques to identify *F. oxysporum* f. sp. *apii* race 2. Vegetative compatibility was studied by complementation, or lack thereof, between *nit* mutants of several *F. oxysporum* f. sp. *apii* race 2 isolates and other form species of *F. oxysporum*. Soluble proteins from these same isolates were separated in sodium dodecyl sulfate-polyacrylamide gels and their

banding patterns compared. *F. oxysporum* f. sp. *apii* race 2 isolated from muck soils were identified using these two techniques, and the results compared with those obtained from greenhouse pathogenicity tests.

MATERIALS AND METHODS

I. FUNGAL ISOLATES

The same fungal isolates were used in all experiments. Isolates *Foa* 2, 3-9, 11, 12, 18 and W31 were *Fusarium oxysporum* f. sp. *apii* race 2 isolates obtained from symptomatic celery collected throughout Michigan and tested for pathogenicity in the greenhouse. *Foa* A1 and A2 were race 2 isolates obtained from celery in New York and provided by J. Lorbeer (Cornell University). *Foa* P13 and NR-1 were race 2 which originated in California and were compliments of J. Puhalla (University of California at Berkeley). *Foa* A8 was a *F. oxysporum* f. sp. *apii* race 1 isolate (pathogenic only on yellow celery cultivars) originally obtained from France and was also provided by J. Puhalla.

F. oxysporum f. sp. *melonis* race 2, *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *cepae*, and *F. oxysporum* f. sp. *conglutinans* were provided by W. Elmer (Michigan State University). *F. oxysporum* f. sp. *chrysanthemi*, *F. oxysporum* f. sp. *redolans*, *F. oxysporum* f. sp. *glycine*, *F. oxysporum* f. sp. *medicaginis*, *F. oxysporum* f. sp. *gladioli*, *F. oxysporum* f. sp. *tuberosi*, *F. oxysporum* f. sp. *dianthi*, and *F. solani* f. sp. *phaesoli* were from the collection of T. Isakeit (Michigan State University). *F. solani* f. sp. *pisi* was previously collected from an unknown location in Michigan.

II. MEDIA

Potato carrot agar (PCA) was prepared by autoclaving 20 g carrots and 20 g potatoes in 500 ml distilled water for 30 min. The autoclaved broth was filtered through cheesecloth and brought to a final volume of 1 l with distilled water. 20 grams of agar were added, then the medium autoclaved for 20 min, and then dispensed into Petri plates.

Minimal medium (MM) (Puhalla 1985) contained 0.9 M sucrose, 0.2 M $NaNO_3$, 7.3 mM KH_2PO_4, 2.0 mM $MgSO_4 \cdot 7H_2O$, 6.7 mM KCl, 0.0002% (v/v) sterile trace elements solution, and 20 g agar/liter medium. Sterile trace elements solution contained 0.3 M citric acid, 0.2 M $ZnSO_4 \cdot 7H_2O$, 0.2 M $FeSO_4 \cdot 7H_2O$, 0.03 M $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 1.0 mM $CuSO_4 \cdot 5H_2O$, 0.21 mM $MnSO_4 \cdot H_2O$, 0.85 mM H_3BO_3, and 0.25 mM $Na_2MoO_4 \cdot H_2O$. The trace elements solution was stored at 4° C. Minimal medium broth contained the same ingredients except agar.

III. NITRATE-NONUTILIZING MUTANTS

Selection of nitrate-nonutilizing mutants. Nitrate-nonutilizing (*nit*) mutants were selected for on a potato-sucrose medium containing 15 g $KClO_3$/ liter medium (KPS) (Puhalla 1985). A sterile flattened needle was scraped across colonies on PCA and then used to stab-inoculate KPS medium. The isolates first exhibited slow, restricted growth on KPS, but within 5 to 15 days faster growing, chlorate-tolerant sectors were observed. Aerial mycelium from some sectors was transferred to MM which contained nitrate as the sole nitrogen source. Nitrate-nonutilizing mutants were distinguished by their thin, but normally expansive growth and lack of aerial mycelium on MM (Figure 2).

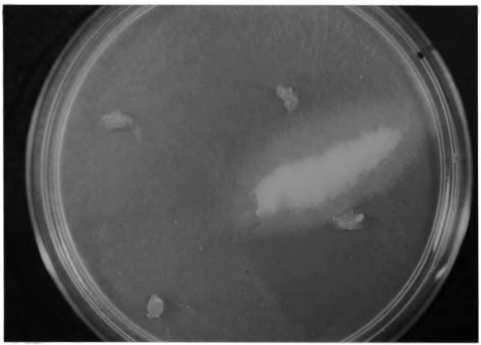

Figure 2. Heterokaryon formation between complementary nitrate-nonutilizing mutants from two vegetatively compatible *Fusarium oxysporum* isolates. Note thin, expansive growth of mutants, and dense, wild-type growth of heterokaryon (middle of right side of culture).

Complementation tests. Heterokaryons were forced on MM with nitrate as the sole nitrogen source. Blocks approximately 0.5 cm^2 cut from *nit* cultures on MM were placed approximately 1.0 cm apart on fresh MM plates and kept in an incubator at 25 to 28° C and a 12 hr photoperiod. Prototrophic heterokaryons were identified as wild-type growth where the hyphae from two *nit* mutants intergrew (Figure 2). Heterokaryons were first observed after 1 week and no new heterokaryons were observed after 3 weeks following transfer to MM.

Nit mutants obtained from an individual fungal strain were grown together on MM in all possible combinations. Two *nit* auxotrophs with complementary mutations were selected for pairing (complementation testing) with all other *F. oxysporum* f. sp. *apii* isolates. The complementary *nits* for each *F. oxysporum* f. sp. *apii* race 2 isolate and the race 1 isolate were paired by growing them together in all combinations on MM for 3 weeks. The complementary mutants for 4 *F. oxysporum* f. sp. *apii* race 2 isolates and the *F. oxysporum* f. sp. *apii* race 1 isolate were paired with 2 to 4 *nits* from each of the 11 other *formae speciales* of *F. oxysporum*.

Classification of mutants. The terminology used for the *nit* mutations described herein are based on those of Correll et al. (1987) (Table 1). Mutants with mutations in the nitrate reductase structural locus were designated as *nit1* mutants. Those with a mutation in any of the genes coding for the molybdenum cofactor were called NitM mutants, and any with a mutation in a pathway-specific regulatory locus were termed *nit3* mutants.

Table 1. Classification of nitrate-nonutiliting mutants based on growth on different nitrogen sources.

Mutant class[2]	Mutation	Growth on nitrogen sources[1] Nitrate	Nitrite	Hypoxanthine	Uric acid
Wild-type	---	+[3]	+	+	+
Nit1	Nitrate reductase structural locus	-	+	+	+
Nit3	Pathway-specific regulatory locus	-	-	+	+
NitM	Molybdenum cofactor loci	-	+	-	+

[1] Based on growth on a minimal medium supplemented with 0.2 M sodium nitrate, 0.03 M sodium nitrite, 0.7 M hypoxanthine (Cove 1976), or 0.0012 M uric acid (Correll et al. 1987).

[2] Based on designations of Correll (Correll et al. 1987).

[3] Nonutilizing of nitrogen source (-) was distinguished by lack of growth or thin expansive growth. Utilizing of nitrogen source (+) was determined by dense, wild-type growth.

In order to determine which enzymes were nonfunctional test isolates were grown on MM with 0.7 M hypoxanthine (Cove 1976), MM with 0.03 M $NaNO_2$ (Cove 1976), and MM with 1.2 mM uric acid (Correll et al. 1987) in place of $NaNO_3$. The colonies were examined for nonutilization of the nitrogen source (distinguished by the lack of growth or thin expansive growth) or dense wild type growth after 1 to 3 days. *Nits* unable to utilize hypoxanthine would be NitM mutants since the molybdenum cofactor required for nitrate reductase is also required for xanthine dehydrogenase which reduces hypoxanthine to xanthine (Figure 1) (Pateman et al. 1964). *Nits* unable to grow on $NaNO_2$ would contain a mutation in the locus which regulates both nitrate and nitrite reductase (*nit3* mutants) (Figure 1) (Cove 1976, Klittich et al. 1986). Remaining *nits* would fall into the *nit1* category. Since all *nit* mutants are able to grow on uric acid, it was included to determine if any other mutation had occurred which might prevent growth on MM.

IV. POLYACRYLAMIDE GEL ELECTROPHORESIS

Cultures. Because culture medium ingredients affect the number and placement of bands on polyacrylamide gels (Glynn and Reid 1969), isolates for polyacrylamide gel electrophoresis were grown in a defined medium, MM broth, to reduce this source of variability. Flasks (250 ml) containing 100 ml MM broth were inoculated with one 13-mm-diameter plug from a PCA or MM agar culture. In preliminary experiments, the broth cultures were kept on a rotary shaker at 100 rpm for 5, 8 or 10 days in the dark. The 5-day-old cultures contained the highest concentration of

soluble proteins and gave the most consistent results with electrophoresis. They were used in all subsequent experiments.

Protein isolation. The mycelium from 5-day-old MM broth cultures was collected by vacuum filtration and washed with 150-200 ml distilled water. Mycelium from 3 cultures for each isolate were combined and homogenized in 5 ml of 0.02 M Tris-HCl (pH 7.4) in a glass vessel with a motor driven Potter-Elvejhem pestle set at 500 rpm. The homogenate was centrifuged in a Sorvall refrigerated centrifuge at 2000 rpm for 5 min, and the supernatant extracted and stored frozen (-14° C).

Electrophoresis procedure. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a discontinuous buffer system (Laemmli 1970) with a Sturdier Slab Gel Electrophoresis Unit SE400 (Hoefer Scientific Instruments, San Francisco, California). Resolving gels were 14 cm wide X 12 cm high X 1.5 mm thick. Stacking gels were 4 cm high X 12 cm wide X 1.5 mm thick.

In preliminary experiments, resolving gels contained either (1) 8% (w/v) acrylamide and 0.21% (w/v) bisacrylamide, (2) 12% acrylamide and 0.32% bisacrylamide, or (3) 15% acrylamide and 0.40% bisacrylamide. The protein bands spread throughout the 12% acrylamide, but clustered together at the bottom and top of the 8% and 15% gels, respectively. Consequently, 12% acrylamide/0.32% bisacrylamide gels were used in all experiments reported in this paper.

The resolving gel also contained 0.375 M Tris-HCl (pH 8.8), 0.1% (w/v) SDS, 0.5 M Urea, and was polymerized with 0.03% (w/v) ammonium persulfate and 0.1% (v/v) N,N,N'N'-tetramethylethylenediamine (TEMED).

The stacking gel consisted of 3% acrylamide, 0.08% bisacrylamide, 0.125 M Tris-HCl (pH 6.8), 0.1% SDS, and was polymerized with 0.05% ammonium persulfate and 0.2% TEMED.

The optimal protein concentration in preliminary gels was 400 ug protein/electrophoresis well, giving distinct, darkly stained bands. This protein concentration was used in all gels described in this report. The protein concentration of each sample was determined by the manual assay of Markwell et al. (1981), using bovine serum albumin as the standard. Samples were mixed with sample buffer and placed in a boiling water bath for 5 min. The sample buffer contained 0.125 M Tris-HCl (pH 6.8), 4% (w/v) SDS, 20% (v/v) glycerol, 0.1 M DL-dithiothreitol and 0.002% bromophenol blue (Toth 1988).

Molecular weight standards included lysozyme (approximate molecular weight of 14,300), ß-lactoglobulin (18,400), trypsinogen (24,000), pepsin (34,700), egg albumin (45,000), and bovine albumin (66,000) (Dalton Mark VI®, Sigma Chemical, St. Louis). Pepsin migrates anomalously in the Laemmli system used (Sigma Technical Bulletin # MWS-887) and so was excluded from the regression analysis. Plots of log molecular weight vs R_F were linear ($r = .99$) ($P < 0.01$) for all gels presented in this report.

Gels were run for 1 hr at 25 mA and then for 4 hr at 30 mA. Gels were fixed overnight in a solution of 50% (v/v) methanol, 40% acetic acid, and 10% water; stained with 0.25% (w/v) Coomassie brilliant blue R250; destained in a solution of 5% (v/v) methanol, 7.5% acetic acid, and 87.5% water; and stored in 7% acetic acid. All procedures were carried out at room temperature (approximately 21-24° C).

V. GREENHOUSE PATHOGENICITY TESTS

Test isolates were grown for 3 to 4 weeks on PCA. One half of a 10-cm Petri dish culture was mixed into a 10-cm-diameter pot containing Baccto® professional planting mix (Michigan Peat Company, Houston, Texas) and two 4-week-old celery plants were transplanted into each pot. Uninoculated PCA and cultures of a *F. oxysporum* f. sp. *apii* race 2 isolate were the controls. The plants were kept in the greenhouse under sodium vapor lights with a 16 hr photoperiod for 9 weeks. Then the plants were uprooted and their crowns and tap roots cut open to reveal any vascular discoloration. Isolates which caused vascular discoloration in any inoculated plants were identified as *F. oxysporum* f. sp. *apii* race 2.

VI. ISOLATION AND IDENTIFICATION OF UNKNOWN FUNGAL ISOLATES

Soil samples from muck fields containing infected celery plants were passed through a sieve with a 2 mm diameter pore size. Thirty grams of each sample were air-dried for 2 days. Five grams of air-dried soil were stirred into 500 ml of a 1% (w/v) solution of carboxymethyl cellulose for 30 min. One ml of the soil suspension was diluted with 9 ml sterile distilled water. A 5 ml aliquot from the dilution was added to 50 ml molten (50° C) Komada's medium (Komada 1975) and immediately poured into 5 Petri dishes (10 X 1.5 cm). Three samples were taken from each soil suspension. All cultures were kept in an incubator at 25-28° C under a 12 hr photoperiod.

After 7 days, a few aerial hyphal fragments from colonies which had white aerial mycelium with pink, purple, or orange pigment on Komada's

medium were transferred to PCA. Colonies on PCA identified as *F. oxysporum* based on microscopic examination (Nelson et al. 1983) were transferred to KPS for auxotrophic mutant selection. One to two *nit* mutants from an unknown isolate were paired on minimal medium with complementary *nits* from two different known *F. oxysporum* f. sp. *apii* race 2 isolates. *F. oxysporum* colonies were also transferred to minimal medium broth for SDS-PAGE, and to PCA for greenhouse pathogenicity tests.

RESULTS

I. VEGETATIVE COMPATIBILITY TEST

Complementation tests between *F. oxysporum* f. sp. *apii* race 2 and other *formae speciales* of F. oxysporum. All 14 *F. oxysporum* f. sp. *apii* race 2 isolates, the *F. oxysporum* f. sp. *apii* race 1 isolate, and the 11 other *formae speciales* of *F. oxysporum* tested produced several *nit* mutants, and all produced at least one set of complementary *nit* mutants. The *nit* mutants were stable, and did not revert back to wild-type prototrophic growth during storage on MM for several weeks.

At least one *nit* mutant from *F. oxysporum* f. sp. *apii* race 2 isolates *Foa* 2, *Foa* 6, *Foa* 11, and *Foa* P13 formed a nitrate-utilizing heterokaryon with at least one *nit* mutant from every other *F. oxysporum* f. sp. *apii* race 2 isolate (Table 2). The *nits* from the remaining 10 *F. oxysporum* f. sp. *apii* race 2 isolates formed nitrate utilizing heterokaryons only with some of the other isolates (Table 2). The *nit* mutants from *Foa* 2, *Foa* 6, *Foa* 11, and *Foa* P13 which formed nitrate-utilizing heterokaryons with all other isolates were the NitM phenotype (Table 1) (Correll et al. 1987). The remaining 10 *F. oxysporum* f. sp. *apii* race 2 isolates produced only *nit1* and *nit3* mutants (Correll et al. 1987) (Table 1). *F. oxysporum* f. sp. *apii* race 2 isolates *Foa* 2, *Foa* 6, *Foa* 11, and *Foa* P13 were designated as tester isolates and their *nit* mutants used in further studies reported below.

Table 2. Heterokaryon formation between nitrate-nonutilizing mutants from 14 *Fusarium oxysporum* f. sp. *apii* (*Foa*) race 2 isolates and one *F. oxysporum* f. sp. *apii* race 1 isolate.

	Foa isolate[1]													
Foa isolate	2	3	5	6	7	8	9	11	12	18	A1	A2	P13	NR1
2	+[2]	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+		-	+	+	-	-	+	-	+	+	+	+	-
5	+	-		+	-	-	-	+	-	-	-	+	+	-
6	+	+	+		+	+	+	+	+	+	+	+	+	+
7	+	+	-	+		+	-	+	+	+	-	-	+	-
8	+	-	-	+	+		-	+	+	-	-	+	+	-
9	+	-	-	+	-	-		+	+	+	+	+	+	-
11	+	+	+	+	+	+	+		+	+	+	+	+	+
12	+	-	-	+	+	+	+	+		+	-	-	+	-
18	+	+	-	+	+	-	+	+	+		-	+	+	-
A1	+	+	-	+	-	-	+	+	-	-		+	+	+
A2	+	+	+	+	-	+	+	+	-	+	+		+	+
P13	+	+	+	+	+	+	+	+	+	+	+	+		+
A8	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] *Foa* A8 was a *F. oxysporum* f. sp. *apii* race 1 isolate from France. The rest were *F. oxysporum* f. sp. *apii* race 2 isolates from the United States.

[2] Presence (+) or absence (-) of a nitrate utilizing heterokaryon between *nit* mutants of the test isolates when grown together on medium with nitrate as sole nitrogen source.

The NitM mutants for *Foa* 2, *Foa* 6, *Foa* 11, and *Foa* P13, and a *nit1* from *Foa* 9 incited the same severity of disease on susceptible celery in the greenhouse as their respective wild-type parents (Table 3). Isolates *Foa* 9, *Foa* 11, and *Foa* P13 had lost virulence during long term culturing, and this was also observed in their corresponding *nit* mutants. Retention of pathogenicity by *nit* mutants has also been reported for other isolates of *F. oxysporum* (Katan and Katan 1988).

None of the *nit* mutants from the 14 *F. oxysporum* f. sp. *apii* race 2 isolates formed a nitrate-utilizing heterokaryon with the *nit* mutants from the *F. oxysporum* f. sp. *apii* race 1 strain (*Foa* A8) from France (Table 2). *F. oxysporum* f. sp. *apii* race 1 was unable to cause disease in the greenhouse on green celery cultivars Tall Utah 52-70 R and Tall Utah 52-70 HK which were highly susceptible and moderately resistant to *F. oxysporum* f. sp. *apii* race 2, respectively (Table 4). Likewise, none of the *nits* from the 4 *F. oxysporum* f. sp. *apii* race 2 tester isolates or the *F. oxysporum* f. sp. *apii* race 1 strain formed nitrate-utilizing heterokaryons with *nit* mutants from 11 other *formae speciales* of *F. oxysporum* (Table 5). No Fusarium yellows disease symptoms were observed on Tall Utah 52-70 R and Tall Utah 52-70 HK plants inoculated with any of the 11 other *formae speciales* of *F. oxysporum* in greenhouse tests (Table 4).

Identification of unknown isolates with vegetative complementation. Not all unknown *F. oxysporum* cultures isolated by soil dilution produced *nit* mutants on KPS. Some isolates were naturally tolerant of chlorate and readily grew on KPS. The growth of other isolates remained restricted, not producing any chlorate-tolerant sectors within 5 weeks.

Table 3. Comparison of disease severity caused by wild-type and nitrate-nonutilizing (*nit*) mutants for 5 isolates of *Fusarium oxysporum* f. sp. *apii* race 2.

Isolate[1]	Mean disease rating[2]	Corresponding *Nit*	Mean disease rating
Foa 2	1.38[3]	2-16	1.57
Foa 6	2.63	6-3	2.38
Foa 9	1.25	9-5	1.00
Foa 11	1.00	11-3	1.00
Foa P13	1.25	P13-3	1.00

[1] *Foa* 2, *Foa* 6, *Foa* 9, and *Foa* 11 were *F. oxysporum* f. sp. *apii* race 2 isolates from Michigan. *Foa* P13 was an isolate from California.

[2] Plants were rated on a 1 to 5 class scale: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant.

[3] Disease ratings for celery cultivar Tall Utah 52-70 R which was highly susceptible to *F. oxysporum* f. sp. *apii* race 2. Mean of 8 plants/ isolate.

Table 4. Comparison of disease severity on celery by different *formae speciales* of *Fusarium oxysporum*.

Formae speciales	Mean disease rating[1]	
	Tall Utah 52-70 HK[2]	Tall Utah 52-70 R
apii race 1	1.0[3]	1.0
apii race 2	1.5	4.5
cepae	1.0	1.0
chrysanthemi	1.0	1.0
conglutinans	1.0	1.0
dianthi	1.0	1.0
gladioli	1.0	1.0
glycine	1.0	1.0
lycopersici	1.0	1.0
medicaginis	1.0	1.0
melonis race 2	1.0	1.0
redolans	1.0	1.0
tuberosi	1.0	1.0
Uninoculated	1.0	1.0
PCA	1.0	1.0

[1] Plants were rated on a 1 to 5 class scale: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant.

[2] Tall Utah 52-70 HK and Tall Utah 52-70 R are celery cultivars moderately resistant and highly susceptible to *F. oxysporum* f. sp. *apii* race 2, respectively.

[3] Mean for 8 plants/cultivar/*formae speciales*.

Table 5. Complementation between nitrate-nonutilizing mutants (*nit*) of *Fusarium oxysporum* f. sp. *apii* race 2 or *F. oxysporum* f. sp. *apii* race 1 isolates with 11 other *formae speciales* of *F. oxysporum*.

	F. oxysporum f. sp. *apii* strain				
formae speciales	*Foa* 2[1]	*Foa* 6	*Foa* 11	*Foa* P13	*Foa* A8
cepae	-[2]	-	-	-	-
chrysanthemi	-	-	-	-	-
conglutinans	-	-	-	-	-
dianthi	-	-	-	-	-
gladioli	-	-	-	-	-
glycine	-	-	-	-	-
lycopersici	-	-	-	-	-
medicaginis	-	-	-	-	-
melonis race 2	-	-	-	-	-
redolans	-	-	-	-	-
tuberosi	-	-	-	-	-

[1] *Foa* 2, *Foa* 6, and *Foa* 11 were *F. oxysporum* f. sp. *apii* race 2 isolates from Michigan, *Foa* P13 was a *F. oxysporum* f. sp. *apii* race 2 isolate from California, and *Foa* A8 was a *F. oxysporum* f. sp. *apii* race 1 isolate from France.

[2] Presence (+) or absence (-) of a nitrate utilizing heterokaryon between *nit* mutants of the test isolates when grown together on medium with nitrate as sole nitrogen source.

Still others produced chlorate-tolerant sectors which were able to utilize nitrate, which has been noted for chlorate-tolerant sectors from other fungi (Correll et al. 1987, Cove 1976, Klittich and Leslie 1987). The two tester *nits* used to identify unknown isolates were complementary NitM mutants from different *F. oxysporum* f. sp. *apii* race 2 tester isolates. Two different NitM mutants were used to decrease the chances of false negative identifications which would occur if the same gene was inoperative in both tester *nit* and the unknown *nit*.

A total of 128 unknown *F. oxysporum* isolates were identified by the VCG test and greenhouse pathogenicity test (Table 6). Of these, 27 were identified as *F. oxysporum* f. sp. *apii* race 2, and 96 as not *F. oxysporum* f. sp. *apii* race 2 by both procedures (Table 6). Only 4 isolates were identified as *F. oxysporum* f. sp. *apii* race 2 by vegetative compatibility test which were apparently not *F. oxysporum* f. sp. *apii* race 2 by greenhouse pathogenicity tests. These four may be isolates with very low virulence which the greenhouse pathogenicity test fails to identify. One isolate was identified as *F. oxysporum* f. sp. *apii* race 2 by pathogenicity tests but not by the vegetative compatibility test. This isolate might have been a self-incompatible isolate which would be unable to anastomose with itself or any other isolate (Bosland and Williams 1987, Correll et al. 1988, Jacobson and Gordon 1988).

Table 6. Identification by vegetative compatibility and greenhouse pathogenicity tests of *F. oxysporum* f. sp. *apii* race 2 cultures isolated from muck soil.

Number of cultures[1]	Procedure	
	Vegetative compatibility	Greenhouse pathogenicity
27	+[2]	+
96	-	-
4	+	-
1	-	+

[1] *F. oxysporum* cultures isolated from muck soil collected from celery fields.

[2] Identified as *F. oxysporum* f. sp. *apii* race 2 (+), or determined not to be *F. oxysporum* f. sp. *apii* race 2 (-).

II. ELECTROPHORESIS

Banding patterns for *F. oxysporum* f. sp. *apii* race 2 and other *formae speciales F. oxysporum.* A 100% homology in number and placement of bands occurred between *F. oxysporum* f. sp. *apii* race 2 isolates *Foa* 20, *Foa* W31, *Foa* A1, and *Foa* P13 (Figure 3). Isolate *Foa* 2 appeared to be missing 3 bands present in all other *F. oxysporum* f. sp. *apii* race 2 isolates (Figure 3). Since *Foa* 2 did not have as high a concentration of proteins within the mol. wts. examined as did the other isolates (evidenced by lightly stained bands), it is possible that the bands were present but the concentration of protein was too low to detect with Coomassie blue.

Four prominent bands in the *F. oxysporum* f. sp. *apii* race 2 isolates were either missing or faintly stained in *F. oxysporum* f. sp. *apii* race 1 (*Foa* A8) (Figure 3). These bands corresponded to proteins at the approximate weights of 67,300, 45,000, 38,600 and 19,600 based on the linear regression equation developed from the molecular weight standards.

A 90 to 100% homology in number and placement of bands was obtained between *F. oxysporum* f. sp. *apii* race 2 isolates and 10 other *formae speciales* of *F. oxysporum* (Figures 4,5,6). *Foa* A8 had a band at an approximate molecular weight of 61,000 which was absent in the banding patterns of *Foa* P13, *F. oxysporum* f. sp. *redolans*, *F. oxysporum* f. sp. *glycine*, *F. oxysporum* f. sp. *gladioli*, and *F. oxysporum* f. sp. *conglutinans* (Figure 4). *F. oxysporum* f. sp. *melonis* race 2 and *F. oxysporum* f. sp. *medicaginis* contained a band corresponding to a molecular weight of 31,800 which was absent in all other isolates on

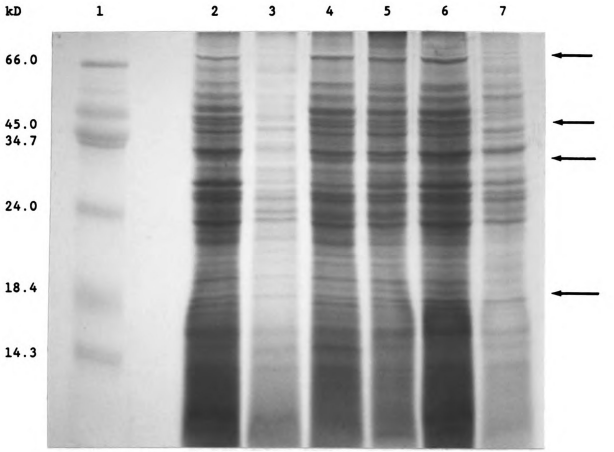

Figure 3. Sodium dodecylsulfate polyacrylamide gel electrophoresis of soluble proteins from *Fusarium oxysporum* f. sp. *apii* isolates and molecular weight standards in 12% polyacrylamide gels. Samples were: (lane 1) molecular weight standards (from top to bottom) bovine albumin, egg albumin, pepsin, trypsinogen, β lactoglobulin, and lysozyme; (lanes 2-6) *F. oxysporum* f. sp. *apii* race 2 isolates (left to right) *Foa* 20, *Foa* 2, *Foa* W31, *Foa* P13, *Foa* A1; and (lane 7) *F. oxysporum* f. sp. *apii* race 1 (*Foa* A8). Arrows on right mark differences in protein bands between race 1 and race 2.

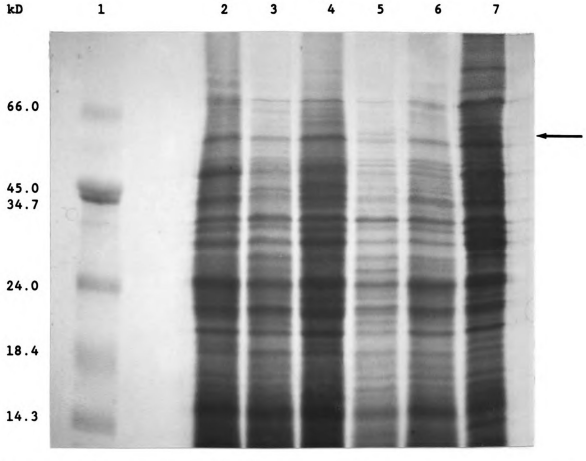

Figure 4. Sodium dodecylsulfate polyacrylamide gel electrophoresis of soluble proteins from *F. oxysporum* isolates and molecular weight standards in 12% polyacrylamide gels. Samples were: (lane 1) molecular weight standards (from top to bottom) bovine albumin, egg albumin, pepsin, trypsinogen, ß lactoglobulin, and lysozyme; (lane 2) *F. oxysporum* f. sp. *redolans*; (lane 3) *F. oxysporum* f. sp. *glycine*; (lane 4) *F. oxysporum* f. sp. *gladioli*; (lane 5) *F. oxysporum* f. sp. *apii* race 1 (*Foa* A8); (lane 6) *F. oxysporum* f. sp. *conglutinans*; and (lane 7) *F. oxysporum* f. sp. *apii* race 2 (*Foa* P13). Arrows on right mark differences in protein banding patterns between lanes.

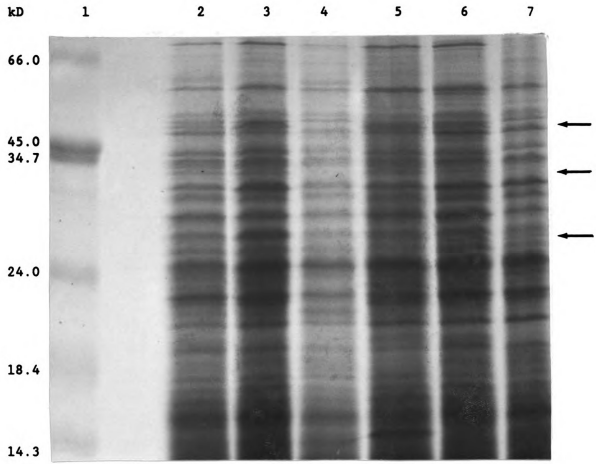

Figure 5. Sodium dodecylsulfate polyacrylamide gel electrophoresis of soluble proteins from *F. oxysporum* isolates and molecular weight standards in 12% polyacrylamide gels. Samples were: (lane 1) molecular weight standards (from top to bottom) bovine albumin, egg albumin, pepsin, trypsinogen, ß lactoglobulin, and lysozyme; (lane 2) *F. oxysporum* f. sp. *apii* race 2 (*Foa* 20); (lane 3) *F. oxysporum* f. sp. *dianthi*; (lane 4) *F. oxysporum* f. sp. *apii* race 2 (*Foa* 2); (lane 5) *F. oxysporum* f. sp. *apii* race 2 (*Foa* W31); (lane 6) *F. oxysporum* f. sp. *melonis* race 2; and (lane 7) *F. oxysporum* f. sp. *medicaginis*. Arrows on right mark differences in protein banding patterns between lanes.

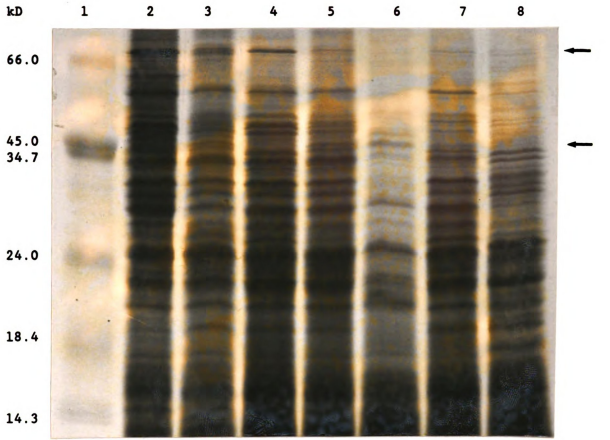

Figure 6. Sodium dodecylsulfate polyacrylamide gel electrophoresis of soluble proteins from *F. oxysporum* isolates and molecular weight standards in 12% polyacrylamide gels. Samples were: (lane 1) molecular weight standards (from top to bottom) bovine albumin, egg albumin, pepsin, trypsinogen, ß lactoglobulin, and lysozyme; (lane 2) *F. oxysporum* f. sp. *apii* race 2 (*Foa* P13); (lane 3) *F. oxysporum* f. sp. *chrysanthemi*; (lane 4) *F. oxysporum* f. sp. *apii* race 2 (*Foa* A1); (lane 5) *F. oxysporum* f. sp. *tuberosi*; (lane 6) *F. oxysporum* f. sp. *cepae*; (lane 7) *F. solani* f. sp. *phaesoli*; and (lane 8) *F. solani* f. sp. *pisi*. Arrows on right mark differences in protein banding patterns between lanes.

that gel, including *Foa* 2, 20 and W31 (Figure 5). *F. oxysporum* f. sp. *melonis* race 2 was missing a band at molecular weight 48,000 which was present in all other isolates on the gel (Figure 5) and the band corresponding to a molecular weight of 41,000 was less prominent in *F. oxysporum* f. sp. *melonis* and *F. oxysporum* f. sp. *medicaginis* than the other lanes (Figure 5). No differences in number or placement of bands was observed between banding patterns for *Foa* 20, *Foa* W31, and *F. oxysporum* f. sp. *dianthi* (Figure 5).

Foa P13, *Foa* A1 and *F. oxysporum* f. sp. *cepae* were missing a band corresponding to a molecular weight of roughly 44,500 which was present in the other form species on the same gel (Figure 6). *F. oxysporum* f. sp. *chrysanthemi* and *F. solani* f. sp. *pisi* had a double band at molecular weight of 65,600 which was only a single band, corresponding to the smaller of the double bands, in the other lanes (Figure 6). *F. solani* f. sp. *phaesoli* had a banding pattern more similar to *F. oxysporum* isolates than did *F. solani* f. sp. *pisi* (Figure 6).

Identification of unknowns with SDS-PAGE. The protein banding patterns for 4 of the 12 unknown isolates were indistinguishable from the banding patterns for *F. oxysporum* f. sp. *apii* race 2 isolates (Table 7). These 4 isolates were also identified as being *F. oxysporum* f. sp. *apii* race 2 isolates by the *nit*-mutant procedure and pathogenicity tests (Table 7). Seven of the unknown isolates had banding patterns different from *F. oxysporum* f. sp. *apii* race 2, and were also identified as not *F. oxysporum* f. sp. *apii* race 2 with the other two procedures (Table 7). One isolate had a banding pattern similar to that of *F. oxysporum* f. sp. *apii* race 2 isolates, but it was missing one of the

prominent bands, and so was labeled as not *F. oxysporum* f. sp. *apii* race 2 using SDS-PAGE. This isolate was identified as being *F. oxysporum* f. sp. *apii* race 2 by the *nit*-mutant and pathogenicity methods (Table 7).

Table 7. Identification by SDS-PAGE, vegetative compatibility, and greenhouse pathogenicity tests of *F. oxysporum* f. sp. *apii* race 2 cultures isolated from muck soil .

Number of cultures[1]	Procedure		
	SDS-PAGE	Vegetative compatibility	Greenhouse pathogenicity
4	+[2]	+	+
7	-	-	-
1	-	+	+

[1] *F. oxysporum* cultures isolated from muck soil collected from celery fields.

[2] Identified as *F. oxysporum* f. sp. *apii* race 2 (+), or determined not to be *F. oxysporum* f. sp. *apii* race 2 (-). For SDS-PAGE, + = protein banding pattern not different from that of *F. oxysporum* f. sp. *apii* race 2, and - = banding pattern very different from that of *F. oxysporum* f. sp. *apii* race 2.

DISCUSSION

A successful identification technique relies on a characteristic which is unique to the strain being identified. Pathogenicity tests achieve this since individual isolates of *F. oxysporum* show a high degree of host specificity (Snyder and Hansen 1940). Vegetative compatibility tests and gel electrophoresis also capitalize on unique gene products.

Complementation between *nit* mutants of vegetatively compatible isolates is dependent on the type of mutation present in the *nits* being paired. NitM mutants readily complement *nit1*, *nit3*, and some other NitM mutants from vegetatively compatible isolates (Correll et al. 1987). *Nit1* mutants infrequently complemented each other, and *nit3* mutants never complemented other *nit3* mutants. Complementation between *nit1* and *nit3* mutants is slow and weak, and sometimes no complementation is observed between *nit1* and *nit3* mutants from the same fungal isolate (Correll et al. 1987). It is highly probable that the 10 *F. oxysporum* f. sp. *apii* race 2 isolates which were rated as negative for heterokaryon production (Table 2) would have formed nitrate-utilizing heterokaryons with *nits* from every other *F. oxysporum* f. sp. *apii* race 2 isolate if they has produced NitM mutants.

The *F. oxysporum* f. sp. *apii* race 2 isolates from Michigan, California, and New York examined in this report appeared to all belong to a single vegetative compatibility group, and no other form species or

saprophytic strain of *F. oxysporum* tested was placed within that VCG. Puhalla (1985) and Correll et al. (1986a) also reported that the *F. oxysporum* f. sp. *apii* race 2 isolates they studied belonged to one unique vegetative compatibility group. Thus, *F. oxysporum* f. sp. *apii* race 2 contains at least one unique allele for vegetative compatibility.

Although there were a few differences between banding patterns for *F. oxysporum* f. sp. *apii* race 2 isolates and some other form species of *F. oxysporum*, there was no particular banding pattern unique to *F. oxysporum* f. sp. *apii* race 2 by which to distinguish this pathogen from all other *F. oxysporum* isolates. Comparing isozyme patterns might have given more distinctive results since different proteins can have the same electrophoretic mobility (Micales et al. 1986). Isozyme patterns have been used to distinguish different *formae speciales* within *F. oxysporum* (Biles and Martyn 1988, Reddy and Stachmann 1972, Scala et al. 1981). Likewise, esterase isozyme patterns distinguished isolates of *F. oxysporum* f. sp. *spinaciae* which caused spinach decline from those responsible for spinach wilt (Madhosingh 1980).

The results of vegetative compatibility test and SDS-PAGE banding patterns provides some information on the biology of *F. oxysporum* f. sp. *apii* race 2. The data suggest that *F. oxysporum* f. sp. *apii* race 2 arose from a single mutant, which probably originated in California and quickly spread to the other states. How it spread to other states is not known, but it probably spread within Michigan in infested muck soil (Elmer 1985).

Whether *F. oxysporum* f. sp. *apii* race 2 originated from *F. oxysporum* f. sp. *apii* race 1 cannot be determined. Since no isolates of *F.*

oxysporum f. sp. *apii* race 1 from the United States can be found (P. Hart, personal communication), and researchers in the United States were unable to isolate *F. oxysporum* f. sp. *apii* race 1, even from fields in continual celery production for more than 50 years (Elmer 1985), direct comparisons cannot be made. Isolates taxonomically placed within a particular form species of *F. oxysporum* yet separated into races based on host specificity are usually vegetatively incompatible from one another (Bosland and Williams 1987, Katan and Katan 1988, Jacobson and Gordon 1988, Larkin et al. 1988).

Like host specificity, vegetative incompatibility may be a mechanism for adaptation (Bosland and Williams 1987). Host specificity allows a new genotype to proliferate in an environment with reduced competition from possibly more fit sibling species. Likewise, a genetic change leading to vegetative incompatibility would also reduce the chance of genetic recombination between a wild-type isolate and a mutant, decreasing the chance of genetic recombination with the resulting loss of fitness that many new genotypes would have. Different VCG's would also be observed within a species, form species, or race if pathogenic genotypes arose independently yet sympatrically, and so would remained genetically isolated from each other due to vegetative incompatibility.

The vegetative compatibility test accurately identified 127/128 of the unknown *F. oxysporum* cultures (either + or - for being *F. oxysporum* f. sp. *apii* race 2). The greenhouse pathogenicity procedure only identified 124/128 accurately, since isolates of low virulence rarely cause noticeable disease in the greenhouse. With gel electrophoresis, 11/12 of the unknowns tested were identified the same as with vegetative

compatibility and pathogenicity methods, but a larger number of unidentified *F. oxysporum* isolates would need to by examined to adequately test the accuracy of SDS-PAGE in identifying *F. oxysporum* f. sp. *apii* race 2 isolates.

Gel electrophoresis, if reliable, would identify *F. oxysporum* f. sp. *apii* race 2 cultures isolated from soil samples or plant tissue faster than the vegetative compatibility test or the greenhouse pathogenicity procedure. SDS-PAGE could identify cultures 10 to 12 days after transfer of colonies from Komada's medium. With the *nit* mutant procedure, isolates could be identified within a minimum of 20 days after transfer of cultures from Komada's medium, whereas the greenhouse pathogenicity method would take at least 12 weeks.

Although slower than gel electrophoresis, the vegetative compatibility test proved to be a better procedure for identifying *F. oxysporum* f. sp. *apii* race 2 isolates in the laboratory. The *nit*-mutant results were clear-cut (either a heterokaryon formed or it did not). Analyzing the gels left more room for subjective interpretations. The *nit* test also is less labor intensive than gel electrophoresis. Several more person-hours were needed per isolate to homogenize the MM broth cultures and prepare the samples for electrophoresis than were needed to transfer cultures to KPS, to identify *nits*, and to pair mutants with the tester *nits*.

LITERATURE CITED

Alexopoulus C. J. and Mims, C. W. 1979. Introductory Mycology. John Wiley and Sons, Inc. New York. 632 pp.

Anagnostakis, S. L. 1982. Genetic analyses of *Endothia parasitica*: Linkage data for four single genes and three vegetative compatibility types. Genetics 102:25-28.

Armstrong, G. M. and Armstrong, J. K. 1968. *Formae speciales* and races of *Fusarium oxysporum* causing a trachemoycosis in the syndrome of disease. Phytopathology 58:1242-1246.

Awuah, R. T., Lorbeer, J. W., and Ellerbrock, L. A. 1986. Occurrence of Fusarium yellows of celery caused by *Fusarium oxysporum* f. sp. *apii* race 2 in New York and its control. Plant Dis. 70:1154-1158.

Bielenin, A., Jeffers, S. N., Wilcox, W. F., and Jones, A. L. 1988. Separation by protein electrophoresis of six species of *Phytophthora* associated with deciduous fruit crops. Phytopathology 78:1402-1408.

Biles, C. L. and Martyn, R. D. 1988. Isozyme analysis of *Fusarium oxysporum* f. sp. *niveum* races and selected *Fusarium* sp. (Abstr.) Phytopathology 78:625.

Bosland, P. W. and Williams, P. H. 1987. An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibility, and geographic origin. Can. J. Bot. 65:2067-2073.

Burdon, J. J. and Marshall, D. R. 1981. Isozyme variation between species and *formae speciales* of the genus *Puccinia*. Can. J. Bot. 59:2628-2634.

Buxton, E. W. 1954. Heterocaryosis and variability in *Fusarium oxysporum* f. sp. *gladioli* (Snyder and Hansen). J. Gen. Microbiol. 10:71-84.

Buxton, E. W. 1956. Heterokaryosis and parasexual recombination in pathogenic strains of *Fusarium oxysporum*. J. Gen. Microbiol. 15:133-139.

Buxton, E. W. 1962. Parasexual recombination in the banana-wilt Fusarium. Trans. Brit. Mycol. Soc. 45:274-279.

Chrambach, A. and Rodard, D. 1971 Polyacrylamide gel electrophoresis. Science 172:440-451.

Chang, L. O., Srb, A. M., and Steward, F. C. 1962. Electrophoretic separations of the soluble proteins of *Neurospora*. Nature 193:756-759.

Correll, J. C., Gordon, T. R., and McCain, A. H. 1988. Vegetative compatibility and pathogenicity of *Verticillium albo-atrum*. Phytopathology 78:1017-1021.

Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77:1640-1646.

Correll, J. C., Puhalla, J. E., and Schneider, R. W. 1986a. Identification of *Fusarium oxysporum* f. sp. *apii* on the basis of colony size, virulence, and vegetative compatibility. Phytopathology 79:396-400.

Correll, J. C., Puhalla, J. E., and Schneider, R. W. 1986b. Vegetative compatibility groups among nonpathogenic root-colonizing strains of *Fusarium oxysporum*. Can. J. Bot. 64:2358-2361.

Corsini, D. F., Davis, J. R., and Pavek, J. J. 1985. Stability of resistance of potato to strains of *Verticillium dahliae* from different vegetative compatibility groups. Plant Dis. 69:980-982.

Cove, D. J. 1976. Chlorate toxicity in *Aspergillus nidulans*: the selection and characterization of chlorate resistant mutants. Heredity 36:191-203.

Cove, D. J. 1979. Genetic studies of nitrate assimilation in *Aspergillus nidulans*. Biol. Rev. 54:291-327.

Croft, J. H. and Jinks, J. L. 1977. Aspects of the population genetics in *Aspergillus nidulans*. pgs 339-360. In: Genetics and Physiology of *Aspergillus*. (J. E. Smith and J. A. Pateman eds.) Academic Press , New York. 552 pp.

Elmer, W. H. 1985. The ecology and control of Fusarium yellows of celery in Michigan. Doctoral Dissertation. Michigan State University. 146 pp.

Elmer, W. H. and Lacy M. L. 1984. Fusarium yellows (*Fusarium oxysporum* f. sp. *apii*) of celery in Michigan. Plant Dis. 68:537.

Elmer, W. H. and Stephens, C. T. 1989. Classification of *Fusarium oxysporum* f. sp. *asparagi* into vegetatively compatible groups. Phytopathology 79:88-93.

Endo, R. M., Hart, L. P., and Opgenorth, D. C. 1978. The etiology, biology, and control of the Fusarium yellows disease of celery. 1977-78 Annual Report, California Celery Research Program. California Celery Research Advisory Board, Dinuba, CA. 75 pp.

Erselius, L. J. and De Vallavieille, C. 1984. Variation in protein profiles of *Phytophthora*: comparison of six species. Trans. Br. Mycol. Soc. 83:463-472.

Gill, H. S. and Powell, D. 1968a. The use of polyacrylamide gel disc electrophoresis in delimiting three species of *Phytophthora*. Phytopath. Z. 63:23-29.

Gill, H. S. and Powell, D. 1968b. Polyacrylamide gel (disc) electrophoresis of physiologic races A-1 to A-8 of *Phytophthora fragariae*. Phytopathology 58:722-723.

Glynn, A. N. and Reid, J. 1969. Electrophoretic patterns of soluble fungal proteins and their possible use as taxonomic criteria in the genus *Fusarium*. Can. J. Bot. 47:1823-1831.

Hart, L. P. and Endo, R. M. 1978. The reappearance of Fusarium yellows of celery in California. Plant Dis. Reptr. 62:138-142.

Hastie, A. C. 1962. Genetic recombination in the hop-wilt fungus *Verticillium albo-atrum*. J. Gen. Microbiol. 27:373-382.

Hastie, A. C. 1964. The parasexual cycle in *Verticillium albo-atrum*. Genet. Res. Camb. 5:305-315.

Huang, P. C. 1964. Recombination and complementation of albino mutants in *Neurospora*. Genetics 49:453-469.

Jacobson, D. J. and Gordon, T. R. 1988. Vegetative compatibility and self-incompatibility within *Fusarium oxysporum* f. sp. *melonis*. Phytopathology 78:668-672.

Joaquim, T. R. and Rowe, R. C. 1988. Use of nitrate non-utilizing mutants of *Verticillium dahliae* in vegetative compatibility analysis to detect pathotypes from potato. (Abstr.) Phytopathology 78:1519.

Joaquim, T. R., Rowe, R. C., and Puhalla, J. E. 1987. Subspecific classification of isolates of *Verticillium dahliae* based on vegetative compatibility. (Abstr.) Phytopathology 77:1750.

Katan, T. and Katan, J. 1988. Vegetative-compatibility grouping of *Fusarium oxysporum* f. sp. *vasinfectum* from tissue and the rhizosphere of cotton plants. Phytopathology 78:852-855.

Klittich, C. J. R. and Leslie, J. F. 1987. Chlorate-resistant, nitrate utilizing mutants in *Fusarium moniliforme*. (Abstr.) Phytopathology 77:1772.

Klittich, C. J. R., Leslie, J. F., and Wilson, J. D. 1986. Spontaneous chlorate-resistant mutants of *Gibberella fujikuroi* (*Fusarium moniliforme*). (Abstr.) Phytopathology 76:1142.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. of Plant Protection Res. 8:114-124.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Larkin, R. P., Hopkins, D. L., and Martin, F. M. 1988. Differentiation of strains and pathogenic races of *Fusarium oxysporum* f. sp. *niveum* based on vegetative compatibility. (Abstr.) Phytopathology 78:1542.

Leslie, J. F. 1987. A nitrate non-utilizing mutant of *Gibberella zeae*. J. Gen. Micro. 113:1279-1287.

Madhosingh, C. 1980. Isoenzymes in isolates of *Fusarium oxysporum* causing spinach diseases. Phytopath. Z. 97:56-67.

Markwell, M. A. K., Haas, S. M., Tolbert, N. E., and Bieber, L. L. 1981. Protein determination in membrane and lipoprotein samples: manual and automated procedures. Methods in Enzymology 72:296-303.

Martyn, R. D. 1987. Fusarium yellows of celery in Texas. Plant Dis. 71:651.

Marzluf, G. A. 1981. Regulation of nitrogen metabolism and gene expression in fungi. Microbiol. Rev. 45:437-461.

Micales, J. A., Bonde, M. R., and Peterson, G. L. 1986. The use of isozyme analysis in fungal taxonomy and genetics. Mycotaxon 27:405-449.

Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* species: An Illustrated Manual for Identification. The Pennsylvania State University Press. University Park, PA. 193 pp.

Pateman, J. A., Cove, D. J., Rever, B. M., and Roberts, D. B. 1964. A common co-factor for nitrate reductase and xanthine dehydrogenase which also regulates the synthesis of nitrate reductase. Nature 201:58-60.

Perkins, D. D., Radford, A., Newmeyer, D. and Bjorkman, M. 1982. Chromosomal loci of *Neurospora crassa*. Microbiol. Rev. 46:426-570.

Ploetz, R. C. and Correll, J. C. 1988. Vegetative compatibility among races of *Fusarium oxysporum* f. sp. *cubense*. Plant Dis. 72:325-328.

Pontecorvo, G., Roper, J. A., and Forbes, E. 1953. Genetic recombination without sexual reproduction in *Aspergillus niger*. J. Gen. Microbiol. 8:198-210.

Pontecorvo, G. and Sermonti, G. 1954. Parasexual recombination in *Penicillium chrysogenum*. J. Gen. Microbiol. 11:94-104.

Puhalla, J. E. 1979. Classification of isolates of *Verticillium dahliae* based on heterokaryon incompatibility. Phytopathology 69:1186-1189.

Puhalla, J. E. 1984. A visual indicator of heterokaryosis in *Fusarium oxysporum* from celery. Can. J. Bot. 62:540-545.

Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.

Puhalla, J. E. and Mayfield, J. E. 1974. The mechanism of heterokaryotic growth in *Verticillium dahliae*. Genetics 76:411-422.

Puhalla, J. E. and Spieth, P. T. 1983. Heterokaryosis in *Fusarium moniliforme*. Exp. Mycol. 7:328-335.

Puhalla, J. E. and Spieth, P. T. 1985. A comparison of heterokaryosis and vegetative incompatibility among varieties of *Gibberella fujikuroi*. Exp. Mycol. 9:39-47.

Reddy, M. N. and Stahmann, M. A. 1972. Isozyme patterns of *Fusarium* species and their significance in taxonomy. Phytopath. Z. 74:115-125.

Sanchez, L. E., Leary, J. V., and Endo, R. M. 1976. Heterokaryosis in *Fusarium oxysporum* f. sp. *lycopersici*. J. Gen. Microbiol. 93:219-226.

Scala, F., Cristinzio, G., Marziano, F., and Noviello, C. 1981. Endopolygalacturonase zymograms of *Fusarium* species. Trans. Br. Mycol. Soc. 77:587-591.

Snyder, W. C. and Hansen, H. N. 1940. The species concept in Fusarium. Am. J. Bot. 27:64-67.

Toth, P. P. 1988. Characterization of the factors regulating the coupling and respiratory control of isolated chick heart mitochondria. Doctoral Dissertation, Michigan State University. 360 pp.

Typas, M. A. and Heale, J. B. 1976. Heterokaryosis and the role of cytoplasmic inheritance in dark resting structure formation in *Verticillium* spp. Molec. Gen. Genet. 146:17-26.

CHAPTER 3

SOMACLONAL VARIATION AS A MEANS FOR INCREASING RESISTANCE IN CELERY TO *FUSARIUM OXYSPORUM* F. SP. *APII* RACE 2

INTRODUCTION

Fusarium oxysporum f. sp. *apii* race 2 (R. Nels. & Sherb.) Snyd. and Hans., the causal agent of Fusarium yellows of celery (*Apium graveolens* L. var *dulce* (Mill.) Pers.), has become the limiting factor in celery production throughout much of the United States (Awuah et al. 1986, Elmer and Lacy 1984, Hart and Endo 1978, Martyn 1987). Infection of celery by *F. oxysporum* f. sp. *apii* race 2 leads to vascular discoloration, chlorosis, stunting, and wilt in susceptible plants, eventually progressing to necrosis and rot in roots and crowns (Hart and Endo 1978). Host resistance is the only feasible means for controlling *F. oxysporum* f. sp. *apii* race 2, but to date there are few cultivars available which have the high level of resistance necessary for growing celery in Fusarium infested fields.

Potential sources of resistance to *F. oxysporum* f. sp. *apii* race 2 include celeriac (*A. graveolens* var. *rapaceum* (Mill.) DC) (Awuah et al. 1986, Elmer et al. 1986, Opgenorth and Endo 1979, 1985, Quiros et al. 1988), parsley (*Petroselinum cripsum* (Hoffm.) Nym.) (Elmer et al. 1986), smallage (*A. graveolens* var. *secalinum*) (Opgenorth and Endo 1985), and *Apium* species from other countries (Opgenorth and Endo 1979, 1985). When these plants are crossed with domestic celery cultivars, many undesirable phenotypic characteristics may be inherited along with resistance (Honma and Lacy 1980, Orton et al. 1984, Opgenorth and Endo

1985). Several rounds of backcrossing and selection are necessary to produce a highly resistant cultivar which is horticulturally acceptable to celery growers.

Although celery is a biennial, it can be forced to flower the first year by a vernalization treatment (Honma 1959). Even so, it still takes 12 to 14 months to go from seed to seed, and developing a new celery cultivar takes a number of years. Unfortunately, celery growers that have *Fusarium*-infested fields cannot wait the number of years required for highly resistant cultivars to be produced by conventional breeding methods.

Tissue culture techniques offer an alternative way to induce heritable changes in existing cultivars. Tissue culture techniques were first used as a means of asexual propagation to produce clones (genetically identical organisms) for research purposes and for planting stock (Chaleff 1983, Larkin and Scowcroft 1981). It was quickly observed that phenotypic variants were often found in clonal populations (Chaleff 1983, Larkin and Scowcroft 1981). Researchers have utilized this variability to produce new genotypes of plants, including resistant genotypes from susceptible hosts (Brettell and Ingram 1979, Daub 1986, Evans et al. 1984, Larkin and Scowcroft 1981).

The term "plant tissue culture" refers to the *in vitro* cultivation of plant parts under aseptic conditions (Biondi and Thorpe 1981). Most tissue culture techniques involve the induction of undifferentiated cell cultures from differentiated plant tissues. The initial explant may come from any plant organ or cell type, including, but not limited to, roots, leaves, buds, embryos and protoplasts. Undifferentiated cell

cultures grow in the form of callus, which is a mass of undifferentiated cells on a solid medium, or cell suspensions in a liquid medium. Auxins and cytokinins are usually necessary for initiation and maintenence of undifferentiated cell cultures (Ammirato 1983, Flick et al. 1983, Gamborg and Schluk 1981).

Usually the most difficult step in a tissue culture cycle is the regeneration of plants from cultured cells, and cell cultures which lack the ability to regenerate plants have limited application (Flick et al. 1983). Regeneration often involves the manipulation of culturing conditions to induce or select for differentiating cells (Ammirato 1983, Flick et al. 1983, Evans et al. 1981). Plants are regenerated from undifferentiated cultured cells via *in vitro* organogenesis and embryogenesis (Ammirato 1983, Evans et al. 1981, Flick et al. 1983).

In vitro organogenesis is the formation of organs (such as roots, shoots, and flowers) *de novo* by cultured plant tissues (Thorpe 1980). The first reports of organogenesis were shoot formation on callus of *Nicotiana* species and root formation from carrot callus (Thorpe 1980). Regeneration of plants via *in vitro* organogenesis has been reported for a variety of plant species from different families (Flick et al. 1983). Successful organogenesis involves the selection of suitable explant material and cell culture media and conditions, which can be different for various plant species and even cultivars within a species (Thorpe 1980).

The initiation and maturation of embryos from somatic cells (termed embryoids) in culture were first observed with carrot cell cultures (Ammirato 1983). Since then, somatic embryogenesis has been reported

for a number of plant species (Ammirato 1983, Evans et al. 1981). Somatic embryoids appear to go through the same development stages as zygotic embryos, becoming polarized with cotyledon initials at one end and radical at the other (Ammirato 1983). Auxins, and in a few cases cytokinins, are vital for embryoid induction, but the hormone levels need to be reduced or eliminated for embryoid maturation and plantlet development (Ammirato 1983, Evans et al. 1981).

Somaclone is the general term given to a plant regenerated from any type of cell culture. Larkin and Scrowcroft (1981) defined somalconal variation as variation detected in plants derived from any form of cell culture, but Evans et al. (1984) narrowed the definition to include only cell cultures which originated from somatic tissues. Gametoclones and gametoclonal variation were terms for plants regenerated from cell cultures originating from gametic tissues (Evans et al. 1984). Protoclones were used to describe plants regenerated from protoplasts (Shepard et al. 1980).

Much of the variability observed in regenerated plants was of genetic origin and was transmitted to self-fertilized progeny in sexually propagated crops (Evans et al. 1984, Larkin and Scowcroft 1981). Somaclonal variation appeared to result from both preexisting genetic mosaics in the explant donor tissue and from genetic changes induced by the cell culture environment (Evans et al. 1984). Kinetin and the synthetic auxin 2,4-dichlorophenoxy acetic acid (2,4-D), the two plant hormones most commonly used in tissue culture media, can cause chromosome aberrations and alterations in chromosome number at the concentration used for tissue culture (Kallak and Vapper 1985). Single

gene mutations, chromosome and gene rearrangements, transposable elements, gene amplification and deletion, and cryptic virus elimination are other mechanisms proposed for somaclonal variation (Evans et al. 1984, Larkin and Scowcroft 1981, Maliga 1980). Genetic changes in mitochondrial genes of regenerants were also reported (Dixon et al. 1982, Gengenbach et al. 1981). Aneuploidy and polyploidy were also observed in cultured cells and, less frequently, in regenerated somaclonal variants (Maliga 1980).

In some cases, selective culturing conditions favoring the growth of a specific mutant type have successfully produced desired phenotypes in regenerated plants (Chaleff 1983, Daub 1986, Maliga 1980). Mutants resistant to herbicides were regenerated from cells or callus grown in the presence of the herbicide (Chaleff and Keil 1981, Chaleff and Parsons 1978, Thomas and Pratt 1982). Host-specific and host-nonspecific toxins and elicitors from different pathogens were also used at the cellular level to select for desired genotypes (Behnke 1979, Behnke 1980, Buiatti et al. 1987, Carlson 1983, Daub 1986, Rines and Luke 1985, Shahin and Spivey 1986, Witsenboer et al. 1988).

Somaclones were also regenerated with no selective pressure and then screened for desired phenotypes (Daub 1986). Somaclones were then screened for increased resistance to a particular pathogen while still in culture conditions, using either the pathogen (Dunbar and Stevens 1988, Heath-Pagiluso et al. 1988, Ostry and Skilling 1988) or toxins (Daub 1986); or after transfer to the greenhouse (Miller at al. 1985) or field (Taylor et al. 1988) with pathogens. These procedures have proven

to be just as effective in providing pathogen-resistant variants as when a selective agent is used at the cellular level (Daub 1986).

Regeneration of plants from callus or cell suspension cultures was first initiated for vegetative propagation of celery for breeding purposes (Chen 1976, Williams and Collin 1976a, 1976b). Somatic embryoids were observed in callus and cell suspension cultures from celery (Al-Abta and Collin 1978a, 1978b, 1979, Chen 1976, Dunstan et al. 1982, Williams and Collin 1976a, Wright 1985), leading researchers to propose using embryoids as planting stock (Orton 1985). Although much variability in chromosome number and structure was observed in cultured celery cells (Browers and Orton 1982a, 1982b, Murata and Orton 1983), cells with gross chromosomal changes rarely regenerated into plants (Browers and Orton 1982-H). Several kinds of somaclonal variants were observed in celery (Orton 1983, Orton 1985), including variants with increased resistance to *F. oxysporum* f. sp. *apii* race 2 (Heath-Pagliuso et al. 1988, Wright 1985, Wright and Lacy 1988), and to *Septoria apiicola*, *Cercospora apii*, and *Pseudomonas cichorii* (Wright 1985, Wright and Lacy 1988).

Tissue culture techniques have provided novel sources of resistance for a variety of other plant species. Six of 370 somaclones regenerated from a tomato cultivar fully susceptible to tobacco mosaic virus were resistant to the virus (Barden et al. 1986). The resistance was passed on to the progeny of the six somaclones. Somaclone-derived resistance in tomatoes to tomato mosaic virus involved multiple nuclear genes and maternally inherited factors (Smith and Murakishi 1987, 1988). Somaclonal variants with a single dominant gene for resistance to *F.*

oxysporum f. sp. *lycopersici* race 2 were regenerated from susceptible tomato lines (Miller et al. 1985, Shahin and Spivey 1986). An increased phytoalexin response was observed in somaclones of tomato when inoculated with *F. oxysporum* f. sp. *lycopersici* and *Phytophthora infestans* (Buiatti et al. 1987).

Cells and protoplasts of tobacco screened for resistance to methionine sulfoximine, a structural analog of the toxin produced by *Pseudomonas syringae* pv. *tabaci*, regenerated plants which were resistant to methionine sulfoximine and to *P. syringae* pv. *tabaci* toxin (Carlson 1973). Protoplast-derived callus screened for resistance to toxins of *P. syringae* pv. *tabaci* and *Alternaria alternata* regenerated tobacco plants resistant to both pathogens (Thanutong et al. 1983). The resistance was stable and transmitted to the sexual progeny. Likewise, tobacco somaclones transmitted their increased resistance to *Phytophthora nicotianae* var. *nicotianae* to their sexual progeny (Daub and Jenns 1988).

Soybean somaclones and their progeny expressed resistance to races of *Phytophthora megasperma* f. sp. *glycinea* to which the parental cultivars were susceptible (Olah and Schmitthenner 1988). Somaclones regenerated from Texas male-sterile cytoplasm corn were resistant to *Drechslera maydis* (= *Helminthosporum maydis*) race T and were male fertile (Brettell and Ingram 1979, Brettell et al. 1980, Gengenbach et al. 1977). Regenerants from potatoes were resistant to *P. infestans* (Behnke, 1979, Behnke 1980, Shepard et al. 1980), *A. solani* (Shepard et al. 1980), and *Erwinia carotovora* subsp. *carotovora* (Taylor et al. 1988).

Tissue culture has provided resistance to Fiji disease (Krish. and Tlaskal 1974, Nickell 1977), to *H. sacchari* (Nickell 1977, Larkin and Scowcroft 1981), *Sclerospora sacchari* (Nickell 1977), and to *Ustilago scitaminea* (Liu 1981, Liu et al. 1983) in sugarcane. Increased resistance was reported in somaclones of oats to victorin toxin from *H. victoriae* (Rines and Luke 1985); in somaclones of alfalfa to *Verticillium albo-atrum* (Latunde-Dada and Lucas 1983) and to *F. oxysporum* f. sp. *medicaginis* (Hartman et al. 1984); and in somaclones of rice to *Rhizoctonia solani* (Xie et al. 1987). Tissue culture was also used to obtain *Populus* species and *Larix* species resistant to *Septoria musiva* and *Gremmeniella abietina*, respectively (Ostry and Skilling 1988).

Variability in several other characteristics was observed in plants regenerated from tissue cultures. Somaclones are frequently male-sterile (Edallo et al. 1981, Evans and Sharp 1983, Prat 1983), female-sterile (Pratt 1983), or self-sterile (Daub and Jenns 1988). Regenerants with altered amino acid composition of enzymes (Brettell et al. 1986), deficiency in ribosomal RNA genes (Landsmann and Uhrig 1985), or containing unique polypeptides (Dixon et al. 1982) and distinct mtDNA organization (Gengenbach et al. 1981) were also reported. Variability in morphology, pigmentation, yield, and other traits under simple and quantitative genetic control (Buiatti et al. 1985, Engler and Grogan 1984, Evans and Sharp 1983, Larkin et al. 1984, Larkin and Scowcroft 1981, Liu 1981, Orton 1980, Shepard et al. 1980, Taylor et al. 1988), and even loss of resistance to a pathogen (Olah and Schmitthenner 1988) were all observed in regenerants of a variety of plant species.

This study was undertaken to determine whether somaclones highly resistant to *F. oxysporum* f. sp. *apii* race 2 could be regenerated from suspensions of celery cells started from callus cultures of a moderately resistant cultivar of celery. After regeneration of whole plants, resistant somaclones were forced to flower, were self-fertilized, and their progeny analyzed for resistance to *F. oxysporum* f. sp. *apii* race 2 to determine if genetically stable resistance was induced. Somaclone progeny were also put through a primary screen in the greenhouse for resistance to the foliar pathogens *Septoria apiicola* Speg., the causal agent of late blight, and *Pseudomonas cichorii* (Swing.)Stapp, the bacterial blight pathogen.

MATERIALS AND METHODS

I. GENETIC TERMINOLOGY

The term somaclone is applied here to plants regenerated from cell cultures which originated from somatic tissues (Evans et al. 1984). Somaclonal variation was defined as variation detected in plants derived from cell culture (Larkin and Scowcroft 1981). The progeny from self-fertilization of somaclones were referred to as the R_1 generation (Chaleff 1981); subsequent generations produced by self-fertilization are termed R_2, R_3, and so on.

II. PLANT GROWTH CONDITIONS

Unless otherwise noted, plants were grown in Baccto® professional planting mix (Michigan Peat Company, Houston, Texas) in the greenhouse. The fertilizer used was 20-20-20 (N-P-K) (Peters® soluble fertilizer). Plants were kept in the greenhouse under sodium vapor or fluorescent lights set for a 16 hr photoperiod.

Hoagland's solution was made by combining 5% (v/v) stock solution A, 8% stock solution B, 0.1% A-Z solution and 86.9% distilled water. Stock A contained 1.928 M $Ca(NO_3)_2 \cdot 4H_2O$. Stock B consisted of 1 M KNO_3, 0.5 M KH_2PO_4, 1 M $MgSO_4 \cdot 7H_2O$, and 2.5 M NaCl. Both solutions were autoclaved and then stored at 4 C. The A-Z solution was made by autoclaving separately a solution of 0.037 M $FeCl_3 \cdot 6H_2O$ and a solution containing

0.091 M H_3BO_3, 0.47 mM $CuCl_2 \cdot 2H_2O$, 0.44 mM $ZnCl_2$, and 3.94 mM $MnCl_2 \cdot 4H_2O$. These two solutions were combined after autoclaving and stored at 4° C.

III. TISSUE CULTURE MEDIA

The media and procedures used followed those of Wright and Lacy (1988) with the following modifications. The duration between establishing cell suspensions and regenerating plants from the suspensions was 7 weeks longer than previously allowed, and the somaclones reported herein were grown to a height 4-7 cm taller under culture conditions than previous somaclones, before being transferred to the greenhouse (Wright and Lacy 1988).

Murashige and Skoog medium (MS) (1962) was used in these studies. The mineral stock solution contained 0.206 M NH_4NO_3, 0.188 M KNO_3, 0.03 M $CaCl_2 \cdot 2H_2O$, 0.015 M $MgSO_4 \cdot 7H_2O$, 12.5 mM KH_2PO_4, 1.00 mM H_3BO_3, 1.12 mM $MnSO_4 \cdot H_2O$, 0.369 mM $ZnSO_4 \cdot 7H_2O$, and 0.05 mM KI in glass distilled H_2O. The MS iron stock solution contained 2.00 mM $FeSO_4 \cdot 7H_2O$ and 2.21 mM Na_2EDTA in glass distilled H_2O. These solutions were stored at 4° C.

The MS-Orton stock solutions consisted of 0.270 mM glycine, 5.55 mM myo-inositol, 41 μM nicotinic acid, 24.3 μM pyridoxine HCl, and 2.97 μM thiamine HCl in glass distilled water. The MS-Orton stock solution for callus initiation also contained 45.3 μM 2,4-dichlorophenoxy acetic acid (2,4-D) and 89 μM 6-benzyladenine, and that for callus maintenance contained 22.6 μM 2,4-D and 4.65 μM kinetin. All MS-Orton stock solutions were stored in 10 ml lots at -18° C.

Culture medium used to initiate callus tissue (CI) contained 0.5% (v/v) MS stock iron solution, 10% MS stock minerals, 1% MS-Orton stock solution for callus initiation, 0.88 M sucrose, and 88.5% glass distilled water. The pH was adjusted to 5.8 with NaOH or HCl, and 9.5 g Difco® agar (Difco Laboratories, Detroit, Michigan) per liter of medium added prior to autoclaving.

Callus maintenance medium (CM) was prepared by mixing 0.5% (v/v) MS stock iron solution, 10% MS stock minerals, 1% MS-Orton stock solution for callus maintenance, 0.88 M sucrose, and 88.5% glass distilled water. The pH was adjusted to 5.8. For agar media, 9.5 g agar per liter of medium was added.

Celery regeneration medium (CRM) was made with 0.5% (v/v) MS stock iron, 10% MS stock minerals, 1% MS-Orton stock solution without hormones, 0.058 M sucrose, 88.5% glass distilled water, and 0.5 g activated charcoal per liter of medium. Again the pH was adjusted to 5.8, and 9.5 g agar per liter was added before autoclaving.

IV. CALLUS INITIATION

Celery cultivar Tall Utah 52-70 HK, moderately resistant to *F. oxysporum* f. sp. *apii* race 2 (Elmer et al. 1986), was grown in the greenhouse for 11 weeks. The axillary buds from the plants were excised, surface sterilized with 10% commercial chlorine bleach for 10 min followed by two rinses with sterile distilled water, placed on CI agar, and kept at 22° C. After 9 days, buds with callus formation were transferred to CM agar. Six weeks later the callus was transferred to

250 ml flasks containing 50 ml CM broth, and these cultures were incubated on a rotary shaker at 100 rpm in the dark.

After 2 months and then monthly, the cell suspension cultures were supplied with fresh CM broth. Cell suspensions were diluted 1:1 (v/v) with fresh CM broth, and 50 ml of the diluted cell suspension were poured into a sterile 250 ml flask and placed back on the shaker.

V. SOMACLONE REGENERATION

Fifteen to 20 weeks after the callus was transferred to CM broth, 2 ml aliquots from cell suspensions containing cell aggregates were plated onto CRM. Two weeks later green plantlets were observed on the CRM plates. The plantlets (somaclones) were transferred every 2 to 4 weeks to new CRM in either Petri plates (100 X 15 mm) or culturing boxes (7.5 X 7.5 X 10 cm) (GA7 vessels, Magenta Corporation, Chicago, Illinois). Somaclones were planted into potting mix when they were 8 to 10 cm tall and had a set of secondary leaflets (4 to 8 months after regeneration).

The somaclones were planted individually in styrofoam cups containing potting mix which was previously autoclaved for 30 min. The transplants were heavily watered and quickly placed in plastic bags to minimize moisture stress. The somaclones were kept in the dark for 2 days, then placed under fluorescent lights (16 hr photoperiod) in the laboratory for 1 to 3 weeks, and then transferred to a mist chamber in the greenhouse. The plastic bags were removed and the plants kept under intermittent mist (15 min on, 15 min off) for 5 days. By this time, the somaclones could be placed on a greenhouse bench under fluorescent lights without wilting.

VI. SCREENING SOMACLONES FOR RESISTANCE TO *F. OXYSPORUM* F. SP. *APII* RACE 2.

Somaclones were transplanted into muck soil naturally infested with *F. oxysporum* f. sp. *apii* race 2 either in the greenhouse or in the field 1 to 2 months after being planted in the potting mix. Somaclones screened in the greenhouse were kept under sodium vapor lamps for 15 to 18 weeks and then rated for disease severity. Somaclones screened for resistance in the field were rated for disease severity after 11 to 13 weeks.

Somaclones screened in the greenhouse, and which had no vascular discoloration in the root or crown area, were replanted, placed back into the greenhouse for 3.5 to 4 months, and then vernalized. Somaclones screened for resistance in the field were uprooted with a shovel and their crowns cut in half. Somaclones with no, or a trace of, vascular discoloration were placed in plastic bags and taken back to the greenhouse. These somaclones were trimmed back to just a few inner petioles, replanted into 25 cm pots and kept under a greenhouse bench for 5 days to reduce tranplanting shock. The somaclones were fertilized and placed under sodium vapor or fluorescent lights in the greenhouse for 5 to 6 weeks before vernalization.

VII. VERNALIZATION AND SELF POLLINATION

Somaclones were vernalized at 4° to 6° C under a 12 hr photoperiod for 8 weeks in a large controlled-temperature chamber, then placed back into the greenhouse under a 16 hr photoperiod to induce flowering (Honma and Lacy 1980). During and after vernalization, the somaclones were

sprayed 3 times per week with approximately 5 ml of a solution of 0.13 M $CaCl_2$ to prevent black heart disease (Lacy and Grafius 1980). Vernalized somaclones were fertilized weekly, and were also given 100 ml of Hoagland's solution every two weeks.

When a somaclone started to flower (at least 2 months after vernalization), the foliar parts were placed under a cage of cheese cloth suspended from a wire ring to prevent insect cross-pollination, and shaken daily to release pollen for self-pollination. The seed was harvested when the pedicel had turned yellow and the seed was brown. The seed was cleaned of debris and stored at 4° C.

VIII. SCREENING SOMACLONE PROGENY FOR RESISTANCE

F. oxysporum f. sp. *apii* race 2. To hasten germination, seeds collected from somaclones were soaked in aerated water for 7 days on a laboratory bench prior to sowing. Seeds were then germinated in potting mix and transplanted into flats 3 to 4 weeks later. Thirty 8-week old R_1 plants from individual somaclones were transplanted 15 cm apart into a 37.5 cm row in a muck field naturally infested with *F. oxysporum* f. sp. *apii* race 2. Transplants of Tall Utah 52-70 HK grown from commercial seed were the controls. After 76 days of growth in the field, 6 to 7 plants per line were uprooted, the crowns cut open, and the plants rated for disease severity. Ratings were on a 1 to 5 scale as follows: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50%

crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant. Plants which had no vascular discoloration were saved for seed production.

When the plants were brought back into the greenhouse their roots were washed in a dilute commercial chlorine bleach solution in an attempt to control the secondary rotting organisms, and rooting hormone (RooTone®, Pratt-Gabriel, Hanover, Pennsylvania) was sprinkled on the cut area prior to planting in the potting mix. The plants were vernalized and cared for as described above.

Foliar pathogens. The following procedure was used to determine whether somaclones resistant to *F. oxysporum* f. sp. *apii* race 2 might also have resistance to two important foliar pathogens. Side shoots from R_1 plants saved for seed production were removed, dipped in rooting hormone, and planted in the greenhouse. The shoots were kept under a greenhouse bench for 2 weeks and then placed under fluorescent or sodium vapor lights. The cuttings were allowed to grow for several months, then cut back and new petioles (1 to 2 weeks old) were inoculated with *Septoria apiicola* or *Pseudomonas cichorii* in the greenhouse. A Tall Utah 52-70 R plant grown from seed and a rooted cutting inoculated with distilled water were the controls.

Dried celery leaves containing pycnidia of *S. apiicola* were soaked in distilled water for 30 min and the resulting spore suspension diluted to 5×10^5 spores/ml (Wright 1985, Wright and Lacy 1988). Three ml of the spore suspension was sprayed onto leaflets on one petiole per plant. The inoculated plants were kept under intermittent mist for 2 days, then placed back on a greenhouse bench under fluorescent lights. The plants

were rated for disease severity after 3 weeks using a 1 to 5 rating scale: 1 = no disease; 2 = 1 to 5% of leaflet surface had necrotic lesions with chlorosis extending beyond the lesions; 3 = 6 to 25% leaf surface diseased; 4 = 26 to 50% leaf surface diseased; 5 = > 50% leaf surface had symptoms (Wright 1985, Wright and Lacy 1988).

P. cichorii was isolated from symptomatic celery leaves collected in the field. Leaves with symptomatic lesions were surface sterilized in 10% commercial chlorine bleach for 4 min followed by 2 rinses with sterile distilled water. The lesion and surrounding green leaf tissue were excised and chopped up in 1 to 2 drops of sterile distilled water which was then streaked onto Difco® Pseudomonas agar F (PsF) (Difco laboratories, Detroit, Michigan). The cultures were incubated at 25° C for 4 days. Colonies on PsF which were fluorescent under UV light were streaked onto fresh PsF and Nutrient broth-yeast extract agar (NBY). Colonies on NBY which were oxidase positive (Schaad 1980), and tested positive to the KOH test (= gram negative) (Schaad 1980) (Kreig and Holt 1984) were used to inoculate celery plants in the greenhouse to determine pathogenicity. A drop of concentrated solution of bacteria was put on a leaflet, and 4 holes pricked in the leaflet under the drop with a sterile needle (Wright and Lacy 1988). The plant was kept under intermittent mist for 4 days, then kept in the greenhouse until symptoms developed.

One isolate which was pathogenic on inoculated plants above was used to inoculate R_1 cuttings. One drop of a bacterial suspension (2×10^3 cells/ml) was placed on each of two leaflets on the same petiole, and 4 holes immediately pricked in the leaf surface under the drop. The

plants were left in the mist chamber for 4 days, then kept in the greenhouse under fluorescent lights until rated for symptoms 3 weeks later. The plants were rated based on a 1 to 5 scale: 1 = no visible necrosis around the inoculated wounds; 2 = necrosis surrounding less than 50% of inoculated wounds; 3 = more than 50% wounds with necrosis; 4 = necrosis and chlorosis spreading from less than 50% of wounds; 5 = necrosis and chlorosis spreading from more than 50% of the inoculations (Wright 1985).

RESULTS

I. SOMACLONE REGENERATION AND SCREENING FOR RESISTANCE TO *F. OXYSPORUM* F. SP. *APII* RACE 2.

The results of previous research from our laboratory showed that the higher the level of resistance present in a cultivar, the higher the frequency of increased resistance in somaclones regenerated from that cultivar (Wright 1985, Wright and Lacy 1988). For this reason, celery cultivar Tall Utah 52-70 HK, which was moderately resistant to *F. oxysporum* f. sp. *apii* race 2 (Ireland et al. 1987), was chosen as the cell donor for the tissue culture procedure.

Callus produced from buds of Tall Utah 52-70 HK plants was very friable and easily broken up in broth culture. Plants were regenerated from 5 of a total of 9 cell suspensions established from individual callus cultures. The rest of the suspensions either became contaminated or did not produce cell aggregates indicative of embryoid production. Somaclones from 3 of the cell suspensions expressed a high level of resistance to *F. oxysporum* f. sp. *apii* race 2.

When activated charcoal was deleted from the regeneration medium, the embryoids either stopped developing or regenerated only shoots. On regeneration medium with activated charcoal, root growth was observed prior to shoot development. Activated charcoal is necessary for somatic

embryoid development in a number of species, probably because it absorbs a number of compounds, including hormones, which inhibit embryogenesis or embroyid maturation (Ammirato 1983).

The first group of somaclones, which was transplanted when 3 to 5 cm in height, all died during the hardening-off process in the greenhouse. It appeared that they were not large enough to survive the shock of transplant. Wright (1985) transplanted celery somclones which were only 3 to 4 cm in height, but had only a 47% survival rate during greenhouse hardening. In contrast, by allowing the somaclones regenerated in this study to grow longer under axenic culture conditions, survivability was increased to 64%.

Much variability was observed in the somaclones. Some were stunted and did not grow past 1 to 2 cm tall. Others had very abnormal morphology and many of these died within a few weeks. A few even began to form callus on the regeneration medium. Many of the somaclones had very different leaf morphologies or growth habits in the greenhouse than parental Tall Utah 52-70 HK plants from commercial seed. Four percent of the somaclones screened for resistance in the field had a complete loss of resistance to *F. oxysporum* f. sp. *apii* race 2.

The somaclones were given a letter and number designation based on where they were screened for resistance. Somaclones with prefix of G were screened for resistance in the greenhouse. Those with prefixes of H were screened in a field trial in near Hudsonville, Michigan, and those with a D were screened in the field at Decatur, Michigan.

A total of 544 somaclones were transplanted into potting mix. Of the 350 which survived the transfer from culture to the greenhouse, 160

were screened for resistance to *F. oxysporum* f. sp. *apii* race 2 in the greenhouse, and 59 of them had no vascular discoloration after 15 to 18 weeks. Twenty three of these somaclones had an open growth habit which more resembled celeraic than celery, and were discarded prior to vernalization.

One hundred ninety somaclones were screened for resistance to *F. oxysporum* f. sp. *apii* race 2 in the field. Twenty two had no disease or just a trace of discoloration when rated and were replanted in the greenhouse. Two somaclones developed soft rot before or during vernalization and were discarded.

A total of 35 somaclones set enough seed for a progeny screen. Some somaclones either did not flower or did not produce enough viable seed to screen. Five of the 35 flowered and set seed too late for the subsequent summer's field trial.

Unique variants were observed in the R_1 progeny from two somaclones. Four of seventeen seedlings from one line were dwarf mutants. In the second line, 7 of 56 seedlings were albino mutants. The albino seedlings were completely white, and did not grow past the cotyledon stage in the greenhouse. Chlorophyll deficient and dwarf mutants were also reported in somaclonal progeny for other plant species (Buiatti et al. 1985, Evans et al. 1983, Prat 1983).

II. SCREENING SOMACLONE PROGENY FOR RESISTANCE.

F. oxysporum f. sp. apii race 2. Most of the R_1 lines screened for resistance to *F. oxysporum* f. sp. *apii* race 2 were uniform in height and appearance in the field, and resembled parental Tall Utah 52-70 HK

plants in gross appearance and growth. A few lines had plants of variable heights, but all these lines had uniform disease ratings, so the stunting of some plants was not the result of increased vascular disease. Plants from line D 157 had abnormally thin petioles, and line M 50 had a very different leaf morphology than Tall Utah 52-70 HK plants.

A total of 29 plants from 11 lines (Table 1) were saved for vernalization and seed set. Nine of these R_1 lines, D 44, G 33(2), G 128, H 37, G 26, G 16, H 33, G 108, and D 128, had mean disease ratings significantly lower than the 2.2 rating for Tall Utah 52-70 HK plants from commercial seed (Table 1) ($P = 0.05$). Lines G 26 and H 33 were the two most vigorous lines, and were uniform in field appearance. Lines G 108, D 128, and M 37 were also fairly uniform in field appearance and disease reaction, but G 16 contained plants of variable sizes. The other two lines, G 129(2) and G 134, had disease ratings not significantly different from Tall Utah 52-70 plants (Table 1).

All 11 lines selected for seed production had disease ratings which were less variable than Tall Utah 52-70 HK plants from commercial seed. Plants from the 11 somaclonal lines all fell within the disease rating classes of 1 or 2. Tall Utah 52-70 HK control plants fell into disease rating classes of 1, 2 or 3. Since celery cultivars are open-pollinated, and the somaclones were self-pollinated, less variability in some characteristics might be expected. All the R_1 plants brought back to the greenhouse survived, and their R_2 progeny will also be screened for resistance to *F. oxysporum* f. sp. *apii* race 2 in the field.

Table 1. Mean disease ratings for somaclone R_1 progenies screened for resistance to *F. oxysporum* f. sp. *apii* race 2 in the field.

R_1 progeny[1]	Disease rating[2]	R_1 progeny	Disease rating
H 54	4.0	G 150	3.0
G 112	3.0	G 34	2.8
H 19	2.6	G 13	2.5
H 55	2.5	G 28	2.4
G 26(2)	2.4	G 56	2.3
Tall Utah 52-70 HK[3]	2.2	H 13	2.2
G 120	2.2	G 151	2.0
G 130	2.0	G 39	2.0
G 129(2) **	2.0	H 50	2.0
D 12	2.0	G 8	2.0
D 157	2.0	G 134 **	1.9
D 44 **	1.8 *	G 33(2) **	1.8 *
G 128 **	1.8 *	H 37 **	1.7 *
G 26 **	1.6 *	G 16 **	1.5 *
H 33 **	1.5 *	G 108 **	1.4 *
D 128 **	1.3 *		

[1] Letter prefix of somaclone lines designates where somaclone parent was screened for resistance to *F. oxysporum* f. sp. *apii* race 2. Lines with G or HK prefixes were screened in the greenhouse, and those with H and D prefixes were screened in the field near Hudsonville and Decatur, Michigan, respectively.

[2] Disease rating was based on a 1 to 5 scale: 1 = no vascular discoloration; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant. Data presented is the mean of 6 to 7 plants/line.

[3] Parental cultivar somaclones were derived from. Seeds of Tall Utah 52-70 HK were from a commercial source.

** Lines from which plants were saved for seed production.

* Mean disease ratings significantly lower than that for Tall Utah 52-70 HK, the cultivar from which the somaclones were produced; LSD = .36 (P = 0.05).

Foliar pathogens. Most of the side shoots from the R_1 plants excised and treated with rooting hormone produced roots and grew well in the greenhouse. Disease reactions ranging from 2 to 5 were observed in the somaclonal lines in response to the *S. apiicola* inoculations (Table 2). Individual plants within somaclonal lines G 33, G 16, and H 33 were uniform in disease expression, but plants from lines lines H 37, G 108, and D 128 had a variety of disease reactions (Table 2). The low disease rating in the Tall Utah 52-70 R plant was unexpected since most commercial varieties are considered susceptible to late blight. Since this was the first plant inoculated, it may not have received an adequate level of inoculum. The somaclonal lines which had disease ratings of 2 will be screened for resistance to late blight in the field. No conclusions regarding resistance to late blight can be made until after the field screening.

All of the plants inoculated in the greenhouse with *P. cichorii* had disease ratings ≤ 3 (Table 2), and no secondary spread of chlorosis or necrosis was observed. A bacterial dilution from ground up surface-sterilized leaflets from one inoculated plant showed that the inoculum was of a single bacterial type (a fluorescent Pseudomonad). Possibly it was an isolate with low virulence. Most of the somaclone progeny had bacterial blight ratings less than that of the Tall Utah 52-70 R check plant (Table 2), but since only one plant per genotype was inoculated, statistical analysis could not be performed to determine if the differences were significant. No conclusions on possible resistance in the somaclonal lines to bacterial blight can be determined from these data.

Table 2. Disease ratings for somaclonal R_1 progeny screened for resistance to *Septoria apiicola* and *Pseudomonas cichorii* in the greenhouse.

Line (plant number)[2]	Disease rating[1]	
	S. apiicola	*P. cichorii*
G 129(2) (2)	4.0	2.0
G 134 (1)	5.0	1.0
D 44 (1)	2.0	1.0
G 33(2) (1)	4.0	1.5
G 33(2) (2)	5.0	2.5
G 128 (1)	3.0	3.0
H 37 (1)	2.0	2.0
H 37 (2)	4.0	1.5
G 26 (1)	2.0	1.0
G 16 (2)	4.0	1.0
G 16 (4)	4.0	1.5
G 16 (5)	5.0	1.0
H 33 (1)	5.0	1.0
H 33 (2)	5.0	2.0
H 33 (3)	5.0	2.0
G 108 (1)	3.0	1.0
G 108 (2)	5.0	1.0
G 108 (3)	4.0	2.5
G 108 (4)	3.0	3.0
D 128 (1)	2.0	1.0
D 128 (2)	4.0	1.0
D 128 (3)	3.0	2.0
D 128 (4)	3.0	1.5
D 128 (5)	2.0	1.0
Tall Utah 52-70 R	2.0	3.0
Uninoculated check	1.0	1.0

[1] Ratings for both diseases were on a 1 to 5 scale. For *S. apiicola*: 1 = no disease; 2 = 1 to 5% of leaflet surface had necrotic lesions with

Table 2 continued.

chlorosis extending beyond the lesions; 3 = 6 to 25% leaf surface diseased; 4 = 26 to 50% leaf surface diseased; 5 = > 50% leaf surface had symptoms.
One petiole/ plant was inoculated, and a visual rating was given to the whole petiole.

For *P. cichorii*: 1 = no visible necrosis around the inoculated wounds; 2 = necrosis surrounding less than 50% of inoculated wounds; 3 = more than 50% wounds with necrosis; 4 = necrosis and chlorosis spreading from less than 50% of wounds; 5 = necrosis and chlorosis spreading from more than 50% of the inoculations. Each of two leaflets on one petiole were inoculated, rated separately, and the mean recorded.

[2] Line refers to the parental somaclone and plant number in parenthesis refers to individual R_1 plants.

DISCUSSION

A successful tissue culture program involves the establishment and proliferation of essentially undifferentiated cells, followed by the regeneration of whole plants, and the selection of desired phenotypic variants which are capable of passing on the genetic changes to their sexual progeny (Evans et al. 1984, Larkin and Scowcroft 1981). We were successful with celery in regenerating somaclones which expressed a higher level of resistance to *F. oxysporum* f. sp. *apii* race 2, and which transmitted that resistance to their progeny through self-fertilization.

Resistance in Tall Utah 52-70 HK is coded for by at least two loci, one dominant gene with a strong effect and at least one gene with a supplemental effect on resistance (Quiros et al. 1988). Somaclonal variation could affect this resistance in a number of ways. The resistance could be increased by amplification of either gene, or by a mutation adding another gene to further supplement the two pre-existing genes. Likewise, one or both of the genes could be deleteriously affected by the tissue culture procedure, resulting in somaclones with a decreased or total lack of resistance. This was in fact observed in 4 percent of the somaclones screened for resistance in the field. Genetic and cytological studies would be needed to determine the nature of the somaclonal variation observed.

One benefit of somaclonal variation is its use in the creation of useful genetic variation without hybridization, thus allowing

maintenance of the desirable characteristics of a cultivar while one characteristic (for example disease resistance) is added. Celery somaclones produced herein were also selected for gross morphology and growth habits resembling the parental cultivar, Tall Utah 52-70 HK. Most R_1 plants from the somalconal lines resembled Tall Utah 52-70 HK plants in the field, but future generations will have to be examined in more detail for variabiltiy in other characteristics, such as petiole width, height, or tendency to form side shoots, which would affect yield.

Tissue culture techniques do, however, have some draw-backs. A researcher has little or no control over the level of variability induced. We observed a number of different morphological variants in somaclonal populations, and even had variants with an increased level of susceptibility to *F. oxysporum* f. sp. *apii* race 2. Tissue culture, especially without any selection pressure at the cellular level, is similar to other types of mutation breeding in that the desired genotypes may not be produced at all, or a large number of plants may have to be screened in order to find one of the desired type (Daub 1986).

We were unable to select for resistance to *F. oxysporum* f. sp. *apii* race 2 at the cellular level. No host-specific toxin has been implicated in the infection of celery by *F. oxysporum* f. sp. *apii* race 2. Heath-Pagliuso et al. (1988) screened somaclones in culture by growing them on a mat of *F. oxysporum* f. sp. *apii* race 2, but all the somaclones eventually died from the disease. Wright (1985) was able to find resistant somaclones when regenerants were screened in the

greenhouse. Likewise, by screening regenerants in the field and greenhouse, we were able to identify lines with a high level of resistance to *F. oxysporum* f. sp. *apii* race 2.

Somaclonal lines described here could provide a source of increased resistance to *F. oxysporum* f. sp. *apii* race 2 for celery breeding programs. Lines which are horticulturally similar or superior to Tall Utah 52-70 HK could be developed into new celery cultivars. Somclonal variation could be taken further. Resistance might be increased further by placing first or second generation progeny from highly resistant somaclonal lines into a tissue culture cycle, and regenerating whole plants from them.

Tissue culture would provide a means of increasing resistance to *F. oxysporum* f. sp. *apii* race 2 and other diseases in cultivars favored by celery growers, and likewise, also has potential for changing other characteristics of celery plants, even quantitative characteristics to increase yield. The limits of somaclonal variation have not been reached, or even realized, to date. Tissue culture is one of many techniques plant scientists can employ for modifying plants to better meet our needs.

LITERATURE CITED

Al-Abta, S. and Collin, H. A. 1978. Control of embryoid development in tissue cultures of celery. Ann. Bot. 42:773-782.

Al-Abta, S. and Collin H. A. 1978. Cell differentiation in embryoids and plantlets of celery tissue cultures. New Phytol. 80:517-521.

Al-Abta, S. and Collin, H. A. 1979. Endogenous auxin and cytokinin changes during embryoid development in celery tissue cultures. New Phytol. 82:29-35.

Ammirato, P. V. 1983. Embryogenesis. pgs. 82-123. In: Handbook of Plant Cell Culture: Vol 1. Techniques for Propagation and Breeding. D. A. Evans, W. R. Sharp, P. V. Ammirato, and Y. Yamada (eds.). Macmillan Publishing Co. New York. 970 pp.

Awuah, R. T., Lorbeer, J. W., and Ellerbrock, L. A. 1986. Occurrence of Fusarium yellows of celery caused by *Fusarium oxysporum* f. sp. *apii* race 2 in New York and its control. Plant Dis. 70:1154-1158.

Barden, K. A., Schiller Smith, S., and Murakishi, H. H. 1986. Regeneration and screening of tomato somaclones for resistance to tobacco mosaic virus. Plant Science 45:209-213.

Behnke, M. 1979. Selection of potato callus for resistance to culture filtrates of *Phytophthora infestans* and regeneration of resistant plants. Theor. Appl. Genet. 55:69-71.

Behnke, M. 1980. General resistance to late blight of *Solanum tuberosum* plants regenerated from callus resistant to culture filtrates of *Phytophthora infestans*. Theor. Appl. Genet. 56:151-152.

Biondi, S. and Thorpe, T. A. 1981. Requirements for a tissue culture facility. pgs. 1-20. In: Plant Tissue Culture- Methods and Applications in Agriculture. T. A. Thorpe (ed.). Academic Press, New York. 379 pp.

Brettell, R. I. S., Dennis, E. S., Scowcroft, W. R., and Peacock, W. J. 1986. Molecular analysis of a somaclonal mutant of maize alcohol dehydrogenase. Mol. Gen. Genet. 202:235-239.

Brettell, R. I. S. and Ingram, D. S. 1979. Tissue culture in the production of novel disease-resistant crop plants. Biol. Rev. 54:329-345.

Brettell, R. I. S., Thomas, E., and Ingram D. S. 1980. Reversion of Texas male-sterile cytoplasm maize in culture to give fertile, T-toxin resistant plants. Theor. Appl. Genet. 58:55-58.

Browers, M. A., and Orton, T. J. 1982a. Transmission of gross chromosomal variability from suspension cultures into regenerated celery plants. J. of Heredity 73:159-162.

Browers, M. A., and Orton, T. J. 1982b. A factoral study of chromosomal variability in callus cultures of celery (*Apium graveolens*). Plant Sci. Lett. 26:65-73.

Buiatti, M., Marcheschi, G., Tognoni, F., Lipucci Di Paola, M., Collina Grenci, F., and Martini, G. 1985. Genetic variability induced by tissue culture in the tomato (*Lycopersicon esculentum*). Phytopath. Z. 94:162-165.

Buiatti, M., Simeti, C., Vannini, S., Marcheschi, G., Scala, A., Bettini, P., Bogani, P., and Pellegrini, M. G. 1987. Isolation of tomato cell lines with altered response to *Fusarium* cell wall components. Theor. Appl. Genet. 75:37-40.

Carlson, P. S. 1973. Methionine sulfoximine-resistant mutants of tobacco. Science 180:1366-1368.

Chaleff, R. S. 1981. Genetics of Higher Plants: Applications of Cell Cultures. Cambridge University Press, New York. 184 pp.

Chaleff, R. S. 1983. Isolation of agronomically useful mutants from plant cell cultures. Science 219:676-682.

Chaleff, R. S. and Keil, R. L. 1981. Genetic and physiological variability among cultured cells and regenerated plants of *Nicotiana tabacum*. Mol. Gen. Genet. 181:254-258.

Chaleff, R. S. and Parsons, M. F. 1978. Direct selection *in vitro* for herbicide-resistant mutants of *Nicotiana tabacum*. Proc. Natl. Acad. Sci. USA. 75:5104-5107.

Chen, C. H. 1976. Vegetative propagation of the celery plant by tissue culture. Proc. S. D. Acad. Sci. 55:44-48.

Daub, M. E. 1986. Tissue culture and the selection of resistance to pathogens. Ann. Rev. Phytopathol. 24:159-186.

Daub, M. E. and Jenns, A. E. 1988. Disease resistance of tobacco somaclones. (Abstr.) Phytopathology 78:1546.

Dixon, L. K., Leaver, C. J., Brettell, R. I. S., and Gengenbach, B. G. 1982. Mitochondrial sensitivity to *Drechslera maydis* T-toxin and the synthesis of a variant mitochondrial polypeptide in plants derived from maize tissue cultures with Texas male-sterile cytoplasm. Theor. Appl. Genet. 63:75-80.

Dunbar, K. B. and Stephens, C. T. 1988. In vitro screening of gernaium somaclones for resistance to bacterial blight. (Abstr.) Phytopathology 78:1565.

Dunstan, D. I., Short, K. C., Merrick, M. A., and Collin, H. A. 1982. Origin and early growth of celery embryoids. New. Phytol. 91:121-128.

Edallo, S., Zucchinali, C., Perenzin, M., and Salamini, F. 1981. Chromosomal variation and frequency of spontaneous mutation associated with *in vitro* culture and plant regeneration in maize. Maydica 26:39-56.

Elmer, W. H. and Lacy M. L. 1984. Fusarium yellows (*Fusarium oxysporum* f. sp. *apii* race 2) of celery in Michigan. Plant Dis. 68:537.

Elmer, W. H., Lacy, M. L., and Honma, S. 1986. Evaluations of celery germ plasm for resistance to *Fusarium oxysporum* f. sp. *apii* race 2 in Michigan. Plant Dis. 70:416-419.

Engler, D. E. and Grogan R. G. 1984. Variation in lettuce plants regenerated from protoplasts. J. of Heredity 75:426-430.

Evans, D. A. and Sharp, W. R. 1983. Single gene mutations in tomato plants regenerated from tissue culture. Science 221:949-951.

Evans, D. A., Sharp, W. R., and Flick, C. E. 1981. Growth and behavior of cell cultures: embryogenesis and organogenesis. pgs. 45-113. In: Plant Tissue Culture- Methods and Applications in Agriculture. T. A. Thorpe (ed.). Academic Press, New York. 379 pp.

Evans, D. A., Sharp, W. R., and Medina-Filho, H. P. 1984. Somaclonal and gametoclonal variation. Amer. J. Bot. 71:759-774.

Flick, C. E., Evans, D. A., and Sharp, W. R. 1983. Organogenesis. pgs. 13-81. In: Handbook of Plant Cell Culture: Vol 1. Techniques for Propagation and Breeding. D. A. Evans, W. R. Sharp, P. V. Ammirato, and Y. Yamada (eds.). Macmillan Publishing Co. New York. 970 pp.

Gamborg, O. L. and Shyluk, J. P. 1981. Nutrition, media and characteristics of plant cell and tissue cultures. pgs. 21-44. In: Plant Tissue Culture- Methods and Applications in Agriculture. T. A. Thorpe (ed.). Academic Press, New York. 379 pp.

Gengenbach, B. G., Connelly, J. A., Pring, D. R., and Conde, M. F. 1981. Mitochondrial DNA variation in maize plants regenerated during tissue culture selection. Theor. Appl. Genet. 59:161-167.

Gengenbach, B. G., Green, C. E., and Donovan, C. M. 1977. Inheritance of selected pathotoxin resistance in maize plants regenerated from cell cultures. Proc. Natl. Acad. Sci. USA 74:5113-5117.

Hart, L. P. and Endo R. M. 1978. The reappearance of Fusarium yellows of celery in California. Plant Dis. Reptr. 62:138-142.

Hartman, C. L., McCoy, T. J., and Knous, T. R. 1984. Selection of alfalfa (*Medicago sativa*) cell lines and regeneration of plants resistant to the toxin(s) produced by *Fusarium oxysporum* f. sp. *medicaginis*. Plant Sci. Lett. 34:183-194.

Heath-Pagliuso, S., Pullman, J., and Rappaport, L. 1988. Somaclonal variation in celery: screening for resistance to *Fusarium oxysporum* f. sp. *apii*. Theor. Appl. Genet. 75:446-451.

Honma, S. 1959. A method for celery hybridization. Proc. Amer. Soc. Hort. Sci. 73:345-348.

Honma, S. and Lacy, M. L. 1980. Hybridization between pascal celery and parsley. Euphytica 29:801-805.

Ireland, K. F., Elmer, W. H., and Lacy, M. L. 1987. Field evaluation of celery germ plasm for resistance to *Fusarium oxysporum* f. sp. *apii* race 2. (Abstr.) Phytopathology 77:1712.

Kallak, H. I. and Vapper, M. A. 1985. Plant tissue culture as a model system for mutagenicity testing of chemicals. Mutat. Res. 147:51-57.

Kreig, M. R., and Holt, J. G. (eds). 1984. Bergey's Manual of Systemic Bacteriology Vol 1. Williams and Wilkins, Maryland. 964 pp.

Krishnamurthi, M. and Tlaskal, J. 1974. Fiji disease resistant *Saccharum officinarum* var. Pindar sub-clones from tissue culture. Proc. Intern. Soc. Sugarcane Technologists 15:130-137.

Lacy, M. L. and Grafius, E. J. 1980. Disease and Insedt Pests of Celery. Extension Bulletin E-1427. Cooperative Extension Service, Michigan State University. 8 pp.

Landsmann, J. and Uhrig, H. 1985. Somaclonal variation in *Solanum tuberosum* detected at the molecular level. Theor. Appl. Genet. 71:500-505.

Larkin, P. J., Ryan, S. A., Brettell, R. I. S., and Scowcroft, W. R. 1984. Heritable somaclonal variation in wheat. Theor. Appl. Genet. 67:443-455.

Larkin, P. J. and Scowcroft, W. R. 1981. Somaclonal variation- a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60:197-214.

Latunde-Dada, A. O. and Lucas, J. A. 1983. Somaclonal variation and reaction to Verticillium wilt in *Medicago sativa* plants regenerated from protoplasts. Plant Sci. Lett. 32:205-212.

Liu, M. C. 1981. *In vitro* methods applied to sugarcane improvement. pp 299-323. In: Plant Tissue Culture: Methods and Applications in Agriculture. T. A. Thorpe (ed.). Academic Press, Inc. New York. 379 pp.

Liu, L. J., Marquez, E. R., and Biascoechea, M. L. 1983. Variation in degree of rust resistance among plantlets derived from callus cultures of sugarcane in Puerto Rico. (Abstr.) Phytopathology 73:797.

Maliga, P. 1980. Isolation, characterization, and utilization of mutant cell lines in higher plants. pgs. 225-250. In: International Review of Cytology, Supplement 11A: Perspectives in Plant Cell and Tissue Culture. I. K. Vasil (ed.). Academic Press, New York. 253 pp.

Martyn, R. D. 1987. Fusarium yellows of celery in Texas. Plant Dis. 71:651.

Miller, S. A., Williams, G. R., Medina-Filho, H., and Evans, D. A. 1985. A somaclonal variant of tomato resistant to race 2 of *Fusarium oxysporum* f. sp. *lycopersici*. (Abstr.) Phytopathology 75:1354.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Planta. 15:473-497.

Murata, M. and Orton, T. J. 1983. Chromosome structural changes in cultured celery cells. In Vitro 19:83-89.

Nickell, L. G. 1977. Crop improvement in sugarcane: studies using *in vitro* methods. Crop Sci. 17:717-719.

Olah, A. F. and Schmitthenner, A. F. 1988. Phytophthora disease reactions in somatic embryo regenerant soybean lines. (Abstr.) Phytopathology 78:1543.

Opgenorth, D. C. and Endo, R. M. 1979. Sources of resistance to Fusarium yellows of celery in California. Plant Dis. Reptr. 63:165-169.

Opgenorth, D. C., and Endo, R. M. 1985. Additional sources of resistance to race 2 of *Fusarium oxysporum* f. sp. *apii*. Plant Dis. 69:882-884.

Orton, T. J., Hulbert, S. H., Durgan, M. E., and Quiros, C. F. 1984. UC1, Fusarium yellows-resistant celery breeding line. HortScience 19:594.

Orton, T. J. 1980. Chromosomal variability in tissue cultures and regenerated plants of *Hordeum*. Theor. Appl. Genet. 56:101-112.

Orton, T. J. 1983. Spontaneous electrophoretic and chromosomal variability in callus cultures and regenerated plants of celery. Theor. Appl. Genet. 67:17-24.

Orton, T. J. 1985. Genetic instability during embryogenic cloning of celery. Plant Cell Tissue Organ Cult. 4:159-169.

Ostry, M. E. and Skilling, D. D. 1988. Use of tissue culture and *in vitro* bioassays for the development and selection of disease-resistant trees. (Abstr.) Phytopathology 78:1608.

Prat, D. 1983. Genetic variability induced in *Nicotiana sylvestris* by protoplast culture. Theor. Appl. Genet. 64:223-230.

Quiros, C. F., D'Antonio, V., and Ochoa, O. 1988. Breeding celery for disease resistance and improved quality. Annual Report, California Celery Research Advisory Board,, Dinuba. CA. 2:1-3.

Rines, H. W. and Luke, H. H. 1985. Selection and regeneration of toxin-insensitive plants from tissue cultures of oats (*Avena sativa*) susceptible to *Helminthosporium victoriae*. Theor. Appl. Genet. 71:16-21.

Schaad, N. W. 1980. Laboratory Guide for Identification of Plant Pathogenic Bacteria. The American Phytopathological Society, St. Paul, Minnesota. 72 pp.

Shahin, E. A., and Spivey, R. 1986. A single dominant gene for *Fusarium* wilt resistance in protoplast-derived tomato plants. Theor. Appl. Genet. 73:164-169.

Shepard, J. F., Bidney, D., and Shahin, E. 1980. Potato protoplasts in crop improvement. Science 208:17-24.

Smith, S. S. and Murakishi, H. H. 1987. Genetic characteristics of somaclonal resistance to tomato mosaic virus. (Abstr.) Phytopathology 77:1705.

Smith, S. S. and Murakishi, H. H. 1988. Nature of resistance of tomato somaclones to tomato mosaic virus. (Abstr.) Phytopathology 78:1536.

Taylor, R. J., Ruby, C. L., and Secor. G. A. 1988. Assessment of field performance and soft rot resistance in a population of protoplast-derived potato clones. (Abstr.) Phytopathology 78:1595.

Thanutong, P., Furusawa, I., and Yamamoto, M. 1983. Resistant tobacco plants from protoplast-derived calluses selected for their resistance to *Pseudomonas* and *Alternaria* toxins. Theor. Appl. Genet. 66:209-215.

Thomas, B. R. and Pratt, D. 1982. Isolation of paraquat-tolerant mutants from tomato cell cultures. Theor. Appl. Genet. 63:169-176.

Thorpe, T. A. 1980. Organogenesis *in vitro*: structural, physiological, and biochemical aspects. pgs. 71-111. In: International Review of Cytology, Supplement 11A: Perspectives in Plant Cell and Tissue Culture. I. K. Vasil (ed.). Academic Press, New York. 253 pp.

Williams, L. and Collin H. A. 1976. Embryogenesis and plantlet formation in tissue cultures of celery. Ann. Bot. 40:325-332.

Williams, L. and Collin H. A. 1976. Growth and cytology of celery plants derived from tissue cultures. Ann. Bot. 40:333-338.

Wright, J. C. 1985. Somaclonal Variation Occurring in Disease Response of Regenerated Celery Plants from Cell Suspension and Callus Cultures. M. S. Thesis. Michigan State University. 90 pp.

Wright, J. C. and Lacy M. L. 1988. Increase of disease resistance in celery cultivars by regeneration of whole plants from cell suspension cultures. Plant Dis. 72:256-259.

Xie, Q. J., Rush, M. C., Massaquoi, R., and Cao, J. 1987. Potential for rice improvement through somaclonal variation for disease resistance. (Abstr.) Phytopathology 77:1723.

CHAPTER 4

EVALUATION OF CELERY GERM PLASM FOR RESISTANCE TO *FUSARIUM OXYSPORUM* F. SP. *APII* RACE 2, AND THE EFFECT OF HIGH SOIL TEMPERATURES ON THE EXPRESSION OF THE RESISTANCE.

INTRODUCTION

Fusarium oxysporum f. sp. *apii* (R. Nels. & Sherb.) Snyd. and Hans., the causal agent of Fusarium yellows of celery (*Apium graveolens* L. var *dulce* (Mill.) Pers.), was the limiting factor in production of yellow and self- blanching celery cultivars grown during the first half of this century (Nelson et al. 1937, Ryker 1935), but the disease vanished after the introduction of highly resistant green celery cultivars in the 1950's (Opgenorth and Endo 1985). In 1978, Fusarium yellows of celery reappeared in California due to a new race of the fungus (race 2) (Hart and Endo 1978). Race 2 attacked previously resistant green cultivars as well as yellow and self-blanching celery cultivars, whereas race 1 attacked only the yellow and self-blanching cultivars (Schneider and Norelli 1981).

F. oxysporum f. sp. *apii* race 2 had spread to Michigan by 1981 (Elmer and Lacy 1984), and was identified in New York in 1982 (Awuah et al. 1986). Today, *F. oxysporum* f. sp. *apii* race 2 is also an important pathogen of celery in Ohio (R. Rowe, personal communication), Wisconsin (Elmer and Lacy 1984), and Texas (Martyn 1987). Fusarium yellows was observed on celery grown in soil from Florida (Toth unpublished), but has not caused important losses in that state.

The most common diagnostic symptom of Fusarium yellows is a red to brown discoloration in the vascular tissue of the roots and crowns of infected plants (Awuah et al. 1986, Hart and Endo 1978, Lacy and Elmer

1985). The discoloration may extend into the petioles in severely infected plants, and be accompanied by rotting of the central crown area. As the disease progresses, plants become stunted and chlorotic, and severely infected plants wilt and die as the crown rots. Even slightly infected plants are of low quality because they are often stunted and bitter tasting (Awuah et al. 1986, Toth unpublished).

I. HOST RESISTANCE

Host resistance is the most practical method of controlling *F. oxysporum* f. sp. *apii* race 2 (Lorenz and Maynard 1988, Opgenorth and Endo 1985). Celery is generally monocropped, and since few vegetables are as profitable as celery to grow (Lorenz and Maynard 1988, Ryder 1979, Ware and McCollum 1975), commercial growers are reluctant to rotate fields out of celery. Soil fumigation is expensive, and has not yet proven to be a viable means of controlling *F. oxysporum* f. sp. *apii* race 2 (Awuah et al. 1986, Greathead 1988, Otto et al. 1976). Likewise, no commercially available fungicides have controlled this pathogen (Awuah et al. 1986).

Field trials in California (Hart and Endo 1978, Opgenorth and Endo, 1979, 1985), Michigan (Elmer et al. 1986), and New York (Awuah et al. 1986) demonstrated that most yellow and green celery cultivars were susceptible to *F. oxysporum* f. sp. *apii* race 2. The green celery cultivars grown today were all derived from a single plant designated number 52-70 selected from the cultivar Crystal Jumbo for resistance to *F. oxysporum* f. sp. *apii* race 1 (Opgenorth and Endo 1985). This

narrowing of the genetic pool in celery might have been the reason for the nearly uniform susceptibility of celery cultivars to *F. oxysporum* f. sp. *apii* race 2.

Cultivars with moderate resistance to *F. oxysporum* f. sp. *apii* race 2 were discovered (Awuah et al. 1986, Elmer et al. 1986, Hart and Endo 1978, Opgenorth and Endo, 1979, 1985). Tall Utah 52-70 HK and Deacon are two cultivars with moderate resistance to *F. oxysporum* f. sp. *apii* race 2 which are recommended for lightly infested fields in Michigan (Elmer et al. 1986). Unfortunately, neither of these cultivars possesses the high level of resistance necessary for growing celery in heavily infested fields.

Potential sources of resistance to *F. oxysporum* f. sp. *apii* race 2 include celeriac (*A. graveolens* var. *rapaceum*) (Awuah et al. 1986, Elmer et al. 1986, Opgenorth and Endo 1979, 1985, Quiros et al. 1988), parsley (*Petroselinum cripsum*) (Elmer et al. 1986), smallage (*A. graveolens* var. *secalinum*) (Opgenorth and Endo 1985), and *Apium* species from other countries (Opgenorth and Endo 1979, 1985). When these plants are crossed with domestic celery cultivars, many undesirable phenotypic characteristics may be inherited along with resistance (Honma and Lacy 1980, Orton et al. 1984, Opgenorth and Endo 1985). Several rounds of backcrossing and selection are necessary to produce a highly resistant cultivar which is horticulturally acceptable to celery growers.

Tissue culture techniques offer an alternative way to induce heritable changes such as disease resistance into existing cultivars (Brettell and Ingram 1979, Daub 1986, Evans et al. 1984, Larkin and Scowcroft 1981, Thorpe 1981). Applying tissue culture to increase

disease resistance involves *in vitro* cultivation of plant cells under aseptic conditions with the subsequent regeneration of plants (Thorpe 1981). With tissue culture techniques, the preferred characteristics of a cultivar may be maintained while a desired trait is added by mutation, protoplast fusion, or gene transfer (Evans et al. 1984, Larkin and Scowcroft 1981, Thorpe 1981).

II. TEMPERATURE EFFECTS ON RESISTANCE TO FUSARIUM

Environmental factors have a role in mediating the effectiveness of host resistance by placing stress on plants and limiting the energy available for resistance mechanisms (Bruhel 1987). In 1987, an increase in Fusarium yellows severity was observed in some moderately resistant celery cultivars and lines grown in field trials in Michigan. This breakdown in resistance occurred concurrently with higher temperatures during the growing season (Figure 1).

Temperature can profoundly affect the expression of host resistance to pathogens, especially in cool weather crops like celery (Ware and McCollum 1975). Michigan celery growers often report a higher incidence of Fusarium yellows in celery harvested during July and August than in earlier and later plantings. In California, moderately resistant celery cultivars are more susceptible to Fusarium yellows when grown during the hotter summer months than during the winter months (Otto et al. 1976). Likewise, moderately resistant celery cultivars Tall Utah 52-70 HK and Tendercrisp produced marketable yields when temperatures were $\leq 15^{\circ}$ C

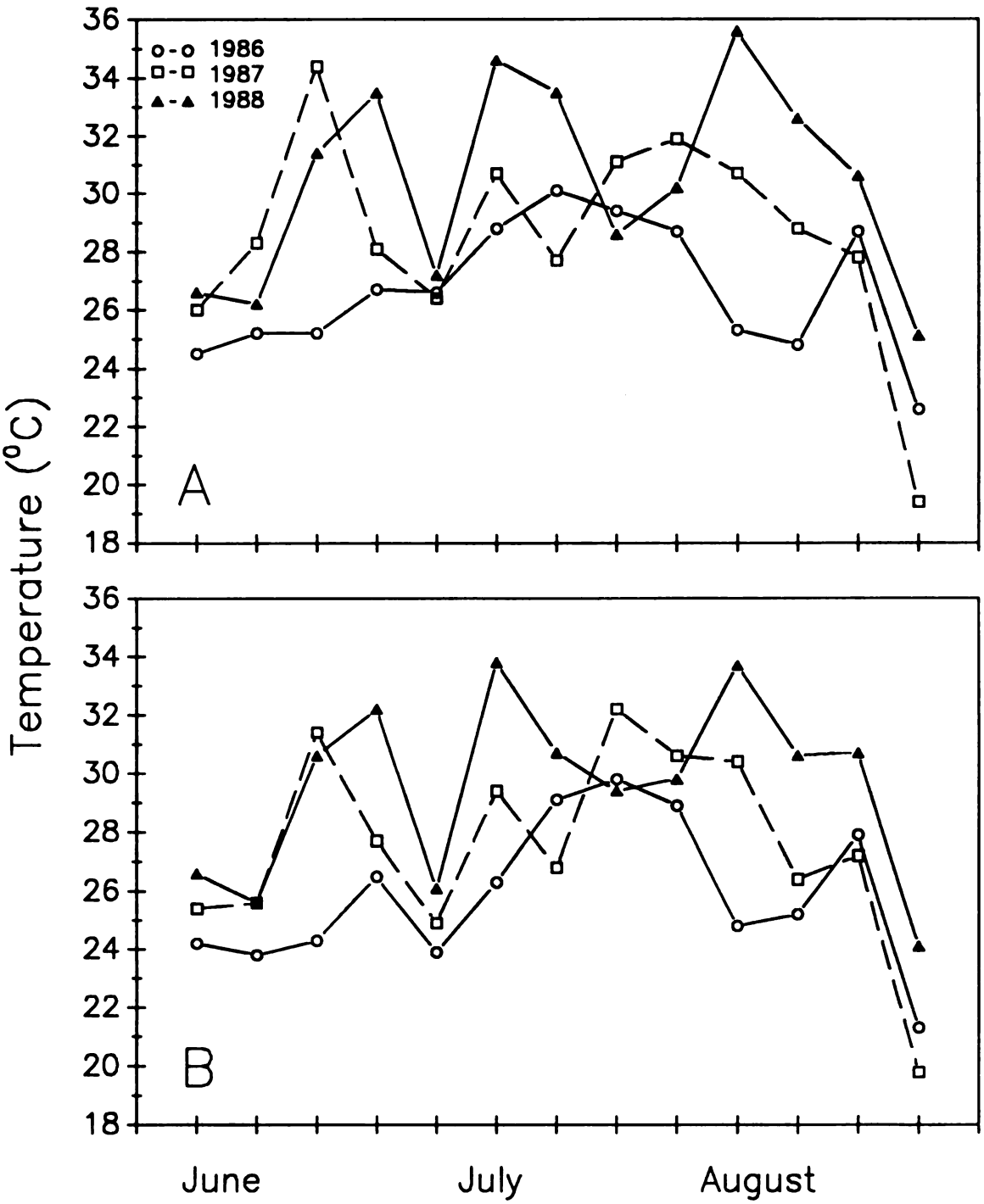

Figure 1. Mean weekly temperatures for June, July and August during 1986 to 1988 for (A) Paw Paw - Decatur, Michigan, and (B) Hudsonville, Michigan.

despite Fusarium yellows infection, but when soil temperatures averaged above 15° C, no plants were harvestable from either cultivar (Endo et al. 1978).

Fusarium wilts are generally warm soil diseases, being most destructive at soil temperatures near 28° C, which is the optimum temperature for *F. oxysporum* growth in culture (Bosland et al. 1988, Bruhel 1987, Clayton 1923, Tisdale 1923, Walker 1941). *F. oxysporum* f. sp. *apii* race 1 grew fastest in culture at 28° C (Ryker 1935), and was most destructive on susceptible self-blanching celery cultivars at 28° C (Nelson et al. 1937, Ryker 1935). *F. oxysporum* f. sp. *lycopersici* incited the severest disease on tomatoes at 28° C, and no disease symptoms were observed below 20° C or above 35° C (Clayton 1923). Likewise, soil temperatures near 27° C were most favorable for wilt of watermelons by *F. oxysporum* f. sp. *niveum* (Walker 1941), and 24° to 28° C for wilt of flax by *F. oxysporum* f. sp. *lini* (Tisdale 1923). Resistance in cabbage to *F. oxysporum* f. sp. *conglutinans* race 2 was progressively less effective as soil temperatures increased from 14° to 24° C (Bosland et al. 1988). The amount of disease caused by *F. oxysporum* f. sp. *chrysanthemi* on chrysanthemum cultivars steadily increased as temperatures increased from 24° to 35° C (Gardiner et al. 1987).

In this investigation, commercial celery cultivars and experimental lines derived from highly resistant breeding lines, parsley X celery crosses, and somaclones were screened for resistance to *F. oxysporum* f. sp. *apii* race 2 in field trials in Michigan. Their Fusarium yellows disease reaction was compared with those of known susceptible and

moderately resistant cultivars. A greenhouse study was also undertaken to examine the effects of soil temperature on Fusarium yellows severity in susceptible and moderately resistant celery cultivars.

MATERIALS AND METHODS

I. FIELD SCREENING

Seven celery cultivars and 21 experimental lines were screened for resistance to *F. oxysporum* f. sp. *apii* race 2 in replicated field trails at two locations in Michigan from 1986 to 1988. Field trials were held on Willbrandt Farms near Decatur, Michigan in 1986-88, and on Gordon Martinie's farm near Hudsonville, Michigan in 1987-88. All plots were in fields naturally infested with *F. oxysporum* f. sp. *apii* race 2.

Celery somaclones previously regenerated from cell cultures by Wright and Lacy (1988) were screened for resistance to *F. oxysporum* f. sp. *apii* race 2 in the greenhouse in 1986 (Chapter 3). Those somaclones which expressed a high level of resistance to *F. oxysporum* f. sp. *apii* race 2 were self-fertilized (Chapter 3) and their progeny screened for resistance in a nonreplicated field trial during 1987. In 1988, 28 breeding lines derived from parsley X celery crosses made by Honma and Lacy (1980) were screened for resistance to *F. oxysporum* f. sp. *apii* race 2 in a nonreplicated trial at Hudsonville.

Seeds were germinated in potting mix (Baccto® professional planting mix, Michigan Peat Company, Houston, Texas), and then transplanted into flats when 3 to 4 weeks old. Seedlings were grown in the greenhouse under a 16 hr photoperiod and fertilized with 20-20-20 (N-P-K) (Peter's® soluble fertilizer). Plants were trimmed back to 15 cm the day prior to

transplanting into field plots to reduce the leaf area for transpiration so the plants would better survive the shock of transplant.

Eight-week-old bare-root celery seedlings were transplanted into the field from June 1 to July 2 using equipment supplied by growers. Replicated plots were set up in a complete randomized block design with 4 replications. Each plot consisted of a 4.5 meter row containing 30 plants. Plants were cared for by the growers during the growing season, receiving the same fertilizer, calcium, and pesticide applications as celery grown for production.

When plants were mature, 75 to 90 days after transplanting into the field, they were uprooted, crowns cut open, and rated for disease severity. Ten plants from each line and cultivar per replication were rated on a 1 to 5 scale (Figure 2): 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant. Mean disease ratings were interpreted as follows: highly resistant = mean disease rating $\leq$ 1.9; moderately resistant = 2.0-2.5; moderately susceptible = 2.6-3.5; highly susceptible = mean disease rating > 3.5,

Weights of plants from some of the more resistant lines and cultivars were measured to get an approximation of yield. The 10 plants rated for disease severity were trimmed for packing and weighed in the field. The weights were converted to tonnes/hectare (t/ha).

Eight of the more promising lines from the 1986 field trial were also rated for some horticultural characteristics. Height from ground level to the tallest point of an average size plant in each replication

Figure 2. Fusarium yellows severity rating scale. From left to right: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant.

for the 8 lines was measured. The width at the top (first node) and base, and the length from base to first node of 2 petioles on each of 5 plants/line/replication were measured. The width of the widest point at the base of the 5 plants was also recorded. The plants were given a visual rating for the relative abundance of side shoots: 1 = few, 2 = medium amount, 3 = above average amount. These are characteristics used by other celery researchers to determine horticultural acceptability and superiority of different lines (B. Zangstra personal communication).

II. FUNGAL POPULATION STUDY

Soil samples were collected from areas in the fields at the Decatur and Hudsonville locations where the trials were planted. Each sample was forced through a sieve with a 2 mm diameter pore size. Two 30 gram portions from each soil sample were air-dried for 2 days. Five grams of air-dried soil samples were stirred into 500 ml of a 0.5% (w/v) solution of carboxymethyl cellulose for 30 min. One ml aliquots from the soil suspensions were diluted with 9 ml sterile distilled water. A total of 11 1-ml samples were taken from the two soil suspensions from each field. A 5 ml aliquot from each dilution was added to 50 ml molten (50° C) Komada's medium (Komada 1975) and immediately poured into 5 Petri dishes (100 X 15 mm). Plates were examined for *Fusarium* colonies after 7 days.

After 7 days, a few aerial hyphal fragments from colonies which had white aerial mycelia with pink, purple, or orange pigment on Komada's medium were transferred to potato carrot agar (PCA) (Chapter 2). Forty three to 100% of selected colonies on Komada's medium were transferred

to PCA, depending on the total number of colonies encountered. Colonies on PCA were examined microscopically to determine which were not *F. oxysporum* (Nelson et al. 1983).

To distinguish *F. oxysporum* f. sp. *apii* race 2 colonies from nonpathogenic *F. oxysporum* colonies, nitrate nonutilizing (*nit*) mutants were induced for unknown isolates using a technique described by Puhalla (Puhalla 1985). *Nit* mutants were produced on a potato-sucrose medium containing 15 g $KClO_3$/ liter of medium (Correll et al. 1987, Puhalla 1985). *Nit* mutants were identified by their thin, but normally expansive, growth and lack of aerial mycelium on a minimal medium containing sodium nitrate as sole nitrogen source (MM) (Puhalla 1985). One to 2 *nit* mutants from each isolate were paired for 3 weeks on MM with *nit* mutants from 2 known *F. oxysporum* f. sp. *apii* race 2 isolates which consistently pair with all other *F. oxysporum* f. sp. *apii* race 2 isolates. Isolates which formed a nitrate-utilizing heterokaryon with either of the *F. oxysporum* f. sp. *apii* race 2 *nits* were identified as *F. oxysporum* f. sp. *apii* race 2 (Correll et al. 1986, Ireland and Lacy 1986). Nitrate-utilizing heterokaryons were identified by the presence of wild-type growth with aerial mycelium and pigmentation where the mycelium from two *nit* mutants intergrew (Correll et al. 1987, Puhalla 1985).

II. TEMPERATURE EFFECTS ON DISEASE RESISTANCE

Eight week old plants of celery cultivars Tall Utah 52-70 R and Pilgrim (Peto) (susceptible to *F. oxysporum* f. sp. *apii* race 2), and Tall Utah 52-70 HK and Pilgrim (MSU) (moderately resistant to *F.*

oxysporum f. sp. *apii* race 2) were transplanted into containers 19 cm high and 21.5 cm in diameter. Coarse sand was put in the bottom 3 cm of containers which were then filled with muck soil naturally infested with *F. oxysporum* f. sp. *apii* race 2. Three 4-week- old seedlings of a single cultivar were transplanted into each container. Four containers (12 plants) were used for each cultivar and temperature. Containers filled with sterile muck were the controls.

The plants were placed in temperature tanks at 20°, 25°, 30°, and 35° C under sodium vapor lights set for a 16 hr photoperiod. After 9 to 10 weeks in the temperature tanks, the plants were uprooted and rated for disease severity using the same scale as above. Shoot heights, in cm, of individual plants and total weight, in grams, of all plants were measured. The experiment was repeated once.

RESULTS

I. FIELD TRIALS

The field in which the trials were held near Decatur had a *F. oxysporum* f. sp. *apii* race 2 population of approximately 198 propagules per gram of soil (ppg). The field near Hudsonville had a lower population of 126 ppg. This could account for the higher disease ratings obtained at the Decatur site than at the Hudsonville site (Tables 1, 2). Both populations were high enough to cause serious disease in susceptible plants (Elmer and Lacy 1987).

Average yields for celery are 60 t/ha, and good yields would be 77 to 79 t/ha (Lorenz and Maynard 1988). The yields in our plots varied from below average to well above average (Tables 3, 4). Since the weight of only 10 plants per replication was converted to yield (t/ha), discrepancies in trimming compared to commercial growers could incorrectly estimate commercial yield. Thus, yield data within the same year and plot can be compared, but care should be taken when comparing yields from different years or plots, or comparing our yield data with commercial celery crops.

Celery cultivars Tall Utah 52-70 R and Florida 683 were included as race 2 susceptible checks (Elmer et al. 1986). Both cultivars had highly susceptible disease ratings in all trials (Tables 1, 2). Yields were not measured for these two cultivars since all of the plants were severely stunted or dead. Tall Utah 52-70 HK and Deacon were included

Table 1. Disease ratings of celery in *F. oxysporum* f. sp. *apii* race 2 field trials in Michigan during 1986 and 1987.

	Mean disease rating[1]		
	1986	1987	
Line	Decatur	Hudsonville	Decatur
MSU 71-28B	5.0 a[2]	---	---
Florida 683	4.6 ab	4.0 a	3.9 b
MSU 79-75	4.5 ab	---	---
Pilgrim (Peto)	---	3.8 b	3.7 c
Tall Utah 52-70 R	4.4 abc	3.7 c	3.6 c
MSU 75-93	4.1 bc	---	---
MSU 77-170	4.0 bc	---	---
FM 1218	---	3.8 bc	4.1 a
Napoleon	3.7 cd	---	---
MSU 79-27	3.0 de	---	---
MSU 77-144	2.9 de	---	---
Tall Utah 52-70 HK	2.9 de	2.5 e	2.9 f
MSU 77-7	2.7 ef	---	---
MSU 77-106	2.6 ef	3.0 d	3.3 d
MSU 63-69	2.6 ef	2.4 f	2.7 g
Deacon	2.3 efg	2.5 e	3.0 e
Companion	1.9 fg	---	---
Pilgrim (MSU)	1.6 g	---	---
FM 1217	1.6 g	2.9 d	2.8 fg
MSU 74-70	1.5 g	1.9 g	2.4 h
UC 1	---	1.8 g	2.2 i

[1] Disease ratings were based on a 1 to 5 scale: 1 = no vascular discoloration; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored, plant stunted; 5 = dead or nearly dead plant.

[2] Mean of 10 plants from each of 4 replications. Within columns, all means followed by the same letter were not significantly different (P = 0.05) according to Duncan's multiple range test. Lines with disease ratings < 2.5 were considered resistant; those with ratings > 2.5 susceptible.

Table 2. Disease ratings of celery in *F. oxysporum* f. sp. *apii* race 2 field trials in Michigan during 1988.

Line	Mean disease rating[1]	
	Hudsonville	Decatur
Florida 683	4.1 a[2]	4.3 a
Tall Utah 52-70 R	---	4.3 a
Pilgrim (Peto)	3.4 b	---
Deacon	2.7 c	3.1 b
SS 7083	2.2 d	2.2 d
Tall Utah 52-70 HK	2.2 d	3.1 b
Pilgrim (MSU)	2.0 e	---
Bosco	1.9 e	1.7 ef
SS 7074	1.8 e	1.7 ef
SS 7073	1.5 f	1.7 ef
SS 7078	1.5 f	1.6 f
SS 7068	1.4 f	2.3 c
MSU 74-70	1.4 f	1.8 e
SS 7067	1.4 f	1.8 e
SS 7076	1.4 f	---

[1] Disease ratings were based on a 1 to 5 scale: 1 = no vascular discoloration; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored, plant stunted; 5 = dead or nearly dead plant.

[2] Mean of 10 plants from each of 4 replications. Within a column, all means followed by the same letter were not significantly different (P = 0.05) according to Duncan's multiple range test. Lines with mean disease ratings less than 2.5 are considered resistant; those with ratings greater than 2.5 susceptible.

Table 3. Yields of celery lines in 1986 and 1987 Michigan celery field trials screening for resistance to *F. oxysporum* f. sp. *apii* race 2.

	Yield (t/ ha)[1]		
	1986	1987	
Line	Decatur	Hudsonville	Decatur
Tall Utah 52-70 HK	51.5[2] bc	55.2 a	97.0 a
Companion	47.8 c	----	----
Pilgrim (MSU)	67.4 a	----	----
Deacon	50.6 bc	47.0 b	91.3 a
MSU 63-69	52.0 bc	40.8 bc	83.9 a
MSU 74-70	57.5 b	46.1 b	92.4 a
FM 1217	59.4 b	34.9 c	85.4 a
MSU 77-7	48.3 c	----	----
UC-1	----	55.7 a	105.2 a

[1] Mean yield of 4 replications converted to tonnes per hectare.

[2] Trimmed weights for 10 plants per replication. Within a column, all means followed by the same letter were not significantly different (P = 0.05) according to Duncan's multiple range test.

Table 4. Yields of celery lines in 1988 Michigan celery field trials screening for resistance to *F. oxysporum* f. sp. *apii* race 2.

Line	Yield (t/ ha)[1]	
	Hudsonville	Decatur
Tall Utah 52-70 HK	54.4 [2] bcd	28.5 c
Deacon	54.0 bcd	51.7 a
Pilgrim (MSU)	63.8 abc	----
MSU 74-70	63.8 abc	52.2 a
Bosco	64.0 abc	50.6 a
SS 7067	64.5 ab	46.6 ab
SS 7068	69.9 a	48.9 ab
SS 7073	57.2 bc	46.2 ab
SS 7074	56.2 bc	42.8 ab
SS 7076	44.9 d	----
SS 7078	54.3 bcd	37.2 bc
SS 7083	53.4 cd	43.3 ab

[1] Mean yield of 4 replications converted to tonnes per hectare.

[2] Yield of 10 plants/ replication. Within a column, all means followed by the same letter were not significantly different (P = 0.05) according to Duncan's multiple range test.

as moderately resistant controls (Elmer et al. 1986). The mean disease ratings for Deacon planted at Decatur increased from moderately resistant in 1986 to moderately susceptible for 1987 and 1988 (Tables 1, 2). Mean disease ratings for Tall Utah 52-70 HK planted in Decatur were consistently in the susceptible range from 1986 to 1988 (Tables 1, 2). Both cultivars had lower disease ratings in the Hudsonville trials than in the Decatur trials (Tables 1, 2). Deacon and Tall Utah 52-70 HK had significantly lower disease ratings than susceptible Florida 683 and Tall Utah 52-70 R in all trials ($P = 0.05$) (Tables 1, 2).

Deacon and Tall Utah 52-70 HK had average to good yields compared to other lines within the same plots and years (Tables 3, 4). The low yield for Tall Utah 52-70 HK at Decatur in 1988 (Table 4) corresponded with a higher disease rating (Table 2). Since stunting is one symptom of Fusarium yellows, this would not be unexpected. Deacon and Tall Utah 52-70 HK were not as tall as some of the other lines tested, but they had wider petioles and less side shoot development (Table 5)

Pilgrim (MSU) and Companion cultivars were developed by Honma et al. (1986) (Michigan State University) for slow flowering and resistance to *Cercospora apii*, respectively. Both cultivars contained celeriac parents in their pedigrees. In the 1986 Decatur trial Pilgrim (MSU) and Companion had disease ratings significantly lower than that for Tall Utah 52-70 HK, but not lower than Deacon ($P = 0.05$) (Table 1). Although Pilgrim (MSU) and Companion had similar ratings for horticultural characteristics (Table 5), Pilgrim (MSU) had a significantly higher yield than Companion (Table 3). Pilgrim (MSU) also had a better field appearance with a full heart and tall uniform petioles.

Table 5. Results of ratings for horticultural characteristics for celery cultivar trials in Decatur, Michigan in 1986.

Line	Plant[1] height	Petiole Width Top	Petiole Width Bottom	Petiole length	Base diameter	Shoot[2] rating
Tall Utah 52-70 HK	65.8[3]	2.0	3.3	23.3	6.5	1.3
Pilgrim	68.8	1.5	2.5	30.8	7.8	2.8
Companion	63.3	1.5	2.5	27.0	6.3	2.8
Deacon	68.8	1.8	2.8	24.5	6.5	2.0
Ferry Morse 1217	72.0	1.5	2.3	24.0	6.8	2.7
74-70	79.5	1.8	2.8	31.0	7.0	3.0
63-69	68.3	1.8	2.5	27.0	6.5	2.8
77-7	66.3	1.8	2.8	27.0	6.5	3.0
LSD (P = 0.05)	6.3	0.3	NS	3.3	0.8	0.7

[1] Plant height, petiole width, petiole length, and base diameter are expressed in centimeters. Mean of 5 plants/line for each of 4 replications.

[2]Side shoot rating: 1= few, 2= medium amount, 3= above average amount.

Pilgrim (Peto) was a seed increase of Pilgrim (MSU) which was first available for the 1987 growing season. Pilgrim (Peto) had a highly susceptible disease rating in both trials in 1987 (Table 1). Pilgrim (Peto) was a mixture of horticultural types; plants had different leaflet shapes and growth habits not observed in Pilgrim (MSU). Both lines were included in the 1988 field trial at Hudsonville, and Pilgrim (MSU) had a significantly lower disease rating than Pilgrim (Peto) (Table 2).

FM 1217 and FM 1218 were experimental lines developed by Ferry Morse Seed Company (San Juan Bautista, California). In 1986, FM 1217 was significantly more resistant than Tall Utah 52-70 HK, but in 1987, FM 1217 had a disease rating in Hudsonville which was significantly higher than for Tall Utah 52-70 HK and Deacon, and a rating in Decatur not different from Tall Utah 52-70 HK and Deacon (Table 1). FM 1218, which was released in 1988 under the name Hercules (Anon. 1988), was rated as highly susceptible to *F. oxysporum* f. sp. *apii* race 2 in the 1987 field trials (Table 1). Napoleon was released by Scattini Seeds (Salinas, California) for commercial production in 1986, and was highly susceptible disease in the 1986 field trial at Decatur (Table 1).

Of the 11 experimental lines (MSU number lines in Table 1) developed by Honma and screened for resistance in 1986, only MSU 74-70 had a highly resistant disease rating (Table 1). The other 10 MSU lines had susceptible disease ratings and were segregating for disease resistance, height, and other horticultural characteristics. MSU 74-70 was also included in the 1987 and 1988 field trials, where it continued to have highly to moderately resistant disease ratings (Tables 1, 2). MSU 74-70

had average to good yields in each plot compared to other lines measured (Tables 3, 4). MSU 74-70 is a tall line with average petiole widths, but has a tendency towards numerous side shoots (Table 5).

Line UC-1 was a breeding line released by Orton et al. (1984) (University of California at Davis) because of its high level of resistance to *F. oxysporum* f. sp. *apii* race 2. The parentage of UC-1 includes celeriac accessions, celery cultivars Tall Utah 52-70 R and Tendercrisp, and resistant introductions from China. UC-1 had the lowest mean disease rating for all the lines tested at both locations (Table 1) and the highest yields in both trials for 1987 (Table 3). UC-1 was a tall vigorous line, but with thin, hollow petioles and numerous side shoots.

Bosco, an experimental line from Brinker Oresetti Seed Company (Watsonville, California), had mean disease ratings of 1.92 and 1.70 in the 1988 Hudsonville and Decatur trials, respectively (Table 2). Bosco was a good line horticulturally, except for its tendency to flower early. Bosco produced one of the higher yields in Hudsonville and Decatur in 1988 (Table 4).

The SS lines screened in 1988 (Table 2) were experimental lines from SunSeeds (Hollister, California). Six of the 7 lines had moderately resistant disease ratings (Table 2). Although yields of 6 SS lines were not significantly different from the yields of Tall Utah 52-70 HK and Deacon in the Hudsonville plot, and Deacon in the Decatur trial (Table 4), they were horticulturally unacceptable lines. The plants were

taller than average, but with thin petioles which were hollow and brittle. The plants also had an open growth habit which looked wild and made them difficult to harvest.

The celery X parsley crosses were an attempt to transfer resistance to late blight caused by *Septoria apiicola* from parsley into celery (Honma and Lacy 1980). Parsley is immune to *S. apiicola* and to *F. oxysporum* f. sp. *apii* race 2. Twenty of the 28 lines derived from celery X parsley crosses had highly susceptible disease ratings (Table 6). Only 3 lines (803, 808, and 835) had moderately resistant disease ratings (Table 6). Of these, line 808 most resembled celery in morphology and growth habit. Lines 803 and 835 resembled the parsley parentage more. These three lines will be examined again in future field trials.

The somaclones whose progenies were screened for resistance were regenerated from cell cultures of Tall Utah 52-70 HK (Wright and Lacy 1988). None of the 15 lines screened in 1987 had mean disease ratings significantly less than the rating for Tall Utah 52-70 HK plants from commercial seed (Table 7). Lines J 4, J 7, J 8, J 9, J 12, J 20 and J 21 had individual plants which had no vascular discoloration (rating of 1.0). These plants were saved for seed production. None of the plants of Tall Utah 52-70 HK from commercial seed had disease ratings < 2.0. The progenies from two plants of the J 12 line were tested in a nonreplicated plot in 1988, but the lines were only moderately resistant. The progenies from the rest of the plants will be screened in a field trial during 1989.

Table 6. Disease ratings for lines derived from celery X parsley crosses and screened in a nonreplicated trial for resistance to *F. oxysporum* f. sp. *apii* race 2 at Hudsonville, Michigan in 1988.

Line	Disease rating[1]	Line	Disease rating
801	4.5	802	3.3
803	2.3	804	4.0
805	4.5	806	4.0
808	2.3	809	3.4
810	2.6	811	2.5
813	4.0	814	5.0
815	5.0	816	5.0
817	4.5	818	5.0
820	5.0	821	5.0
822	5.0	824	5.0
825	5.0	826	5.0
827	5.0	828	4.5
829	5.0	834	3.3
835	2.0	837	5.0

[1] Plants were rated on a 1 to 5 class scale: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant. Mean rating for 10 plants/line.

Table 7. Disease ratings for somaclonal progenies screened for resistance to *F. oxysporum* f. sp. *apii* race 2 at Decatur, Michigan in 1987.

Line	Disease rating[1]	Line	Disease rating
J 10	3.8 *	J 1	3.4 *
J 19	3.3 *	J 14	3.3
J 22	3.3	J 13	3.1
J 21 **	3.1	J 9 **	3.0
J 17	2.9	J 8 **	2.9
J 5	2.9	J 20 **	2.8
J 4 **	2.8	J 7 **	2.7
J 12 **	2.6	Tall Utah 52-70 HK	2.9
LSD (P = 0.05)	0.42		

[1] Plants were rated on a 1 to 5 class scale: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant. Mean rating of 20 plants/line.

* Mean disease rating significantly different from Tall Utah 52-70 HK, the cultivar somaclone parents were derived from.

** Lines from which plants were saved for seed production.

II. TEMPERATURE EFFECTS ON RESISTANCE

The greenhouse temperature study was an attempt to determine if the increased severity of disease observed in moderately resistant lines in the 1987 field trials were the result of higher temperatures experienced during 1987 compared to 1986 (Figure 1). Fusarium yellows severity did not change significantly as temperatures increased from 20° to 35° C for all cultivars tested ($P = 0.05$) (Figures 3, 4). A trend towards increased disease ratings in Tall Utah 52-70 HK at 35° C and decreased disease ratings in Tall Utah 52-70 R at 30° and 35° C in the first study could not be repeated in the second study (Figure 3).

Inoculated and uninoculated plants from celery cultivars Pilgrim (Peto), Tall Utah 52-70 HK, and Tall Utah 52-70 R grew tallest at 20° and 25° C, and growth significantly declined between 25° and 30° C ($P = 0.05$) (Figure 5). Although the slopes of the regression lines for all cultivars tested in the first test were significantly different from zero ($P = 0.01$), the relationships were nonlinear ($P = 0.01$). The weights for both inoculated and uninoculated plants of all three cultivars reflected the decrease in shoot height (Figure 6). The same trend in height and weight was observed in the second test, although data were not collected.

Root growth corresponded to shoot growth for all cultivars tested. Plants grown at 20° C had roots which grew to the bottom of the containers (approximately 15 cm from the soil surface). Roots at 25° C had grown at least into the sand (within 3 cm of the container bottom). At 30° C some, but not all, roots were near the sand, and at 35° C the roots were only within the top 4 cm of the soil. The roots of plants

Figure 3. Mean Fusarium yellows severity ratings for moderately resistant celery cultivar Tall Utah 52-70 HK and susceptible cultivar Tall Utah 52-70 R grown at different soil temperatures. Plants were rated on a 1 to 5 class scale: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant.

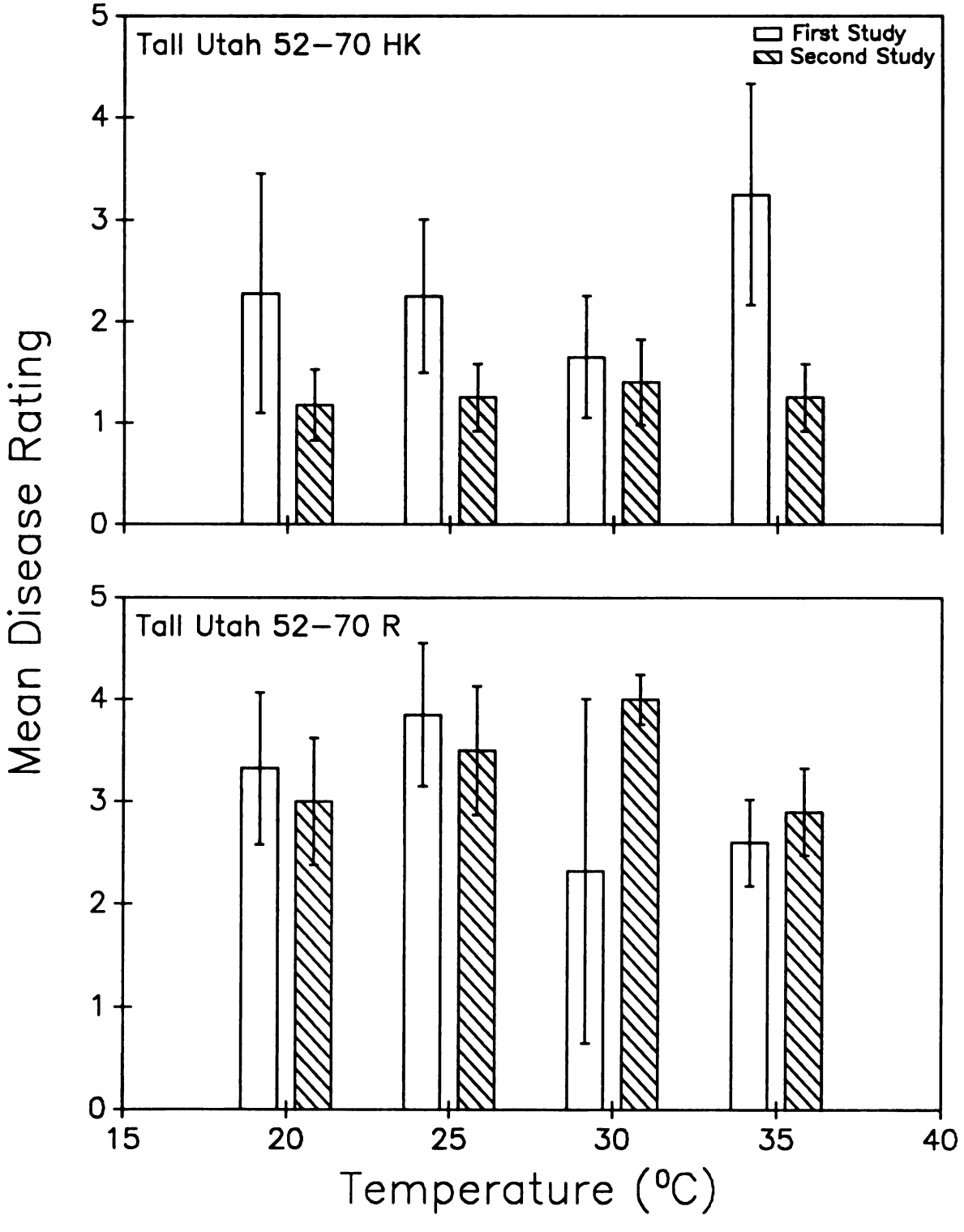
Tall Utah 52–70 HK
First Study
Second Study
Tall Utah 52–70 R
Mean Disease Rating
Temperature (°C)
0
1
2
3
4
5
15
20
25
30
35
40

Figure 3.

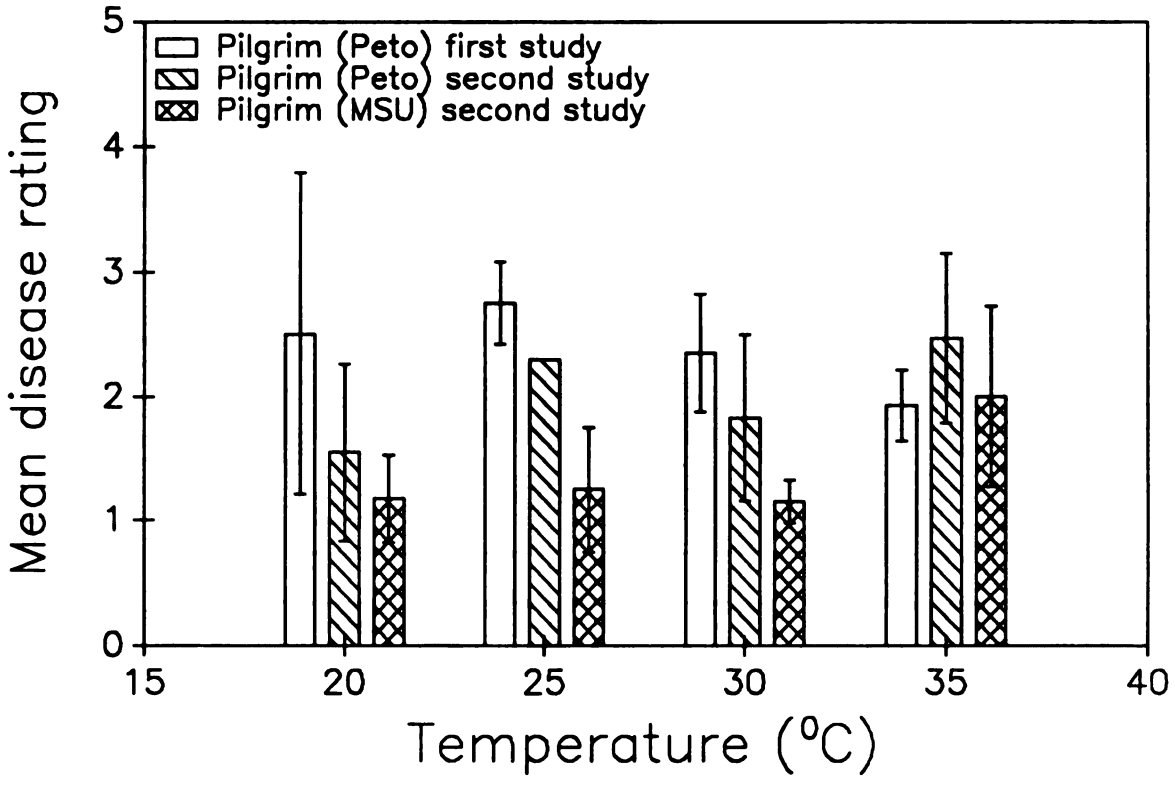

Figure 4. Mean Fusarium yellows severity ratings for celery cultivars Pilgrim (Peto) (susceptible) and Pilgrim (MSU) (moderately resistant) grown at different soil temperatures. Plants were rated on a 1 to 5 class scale: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant.

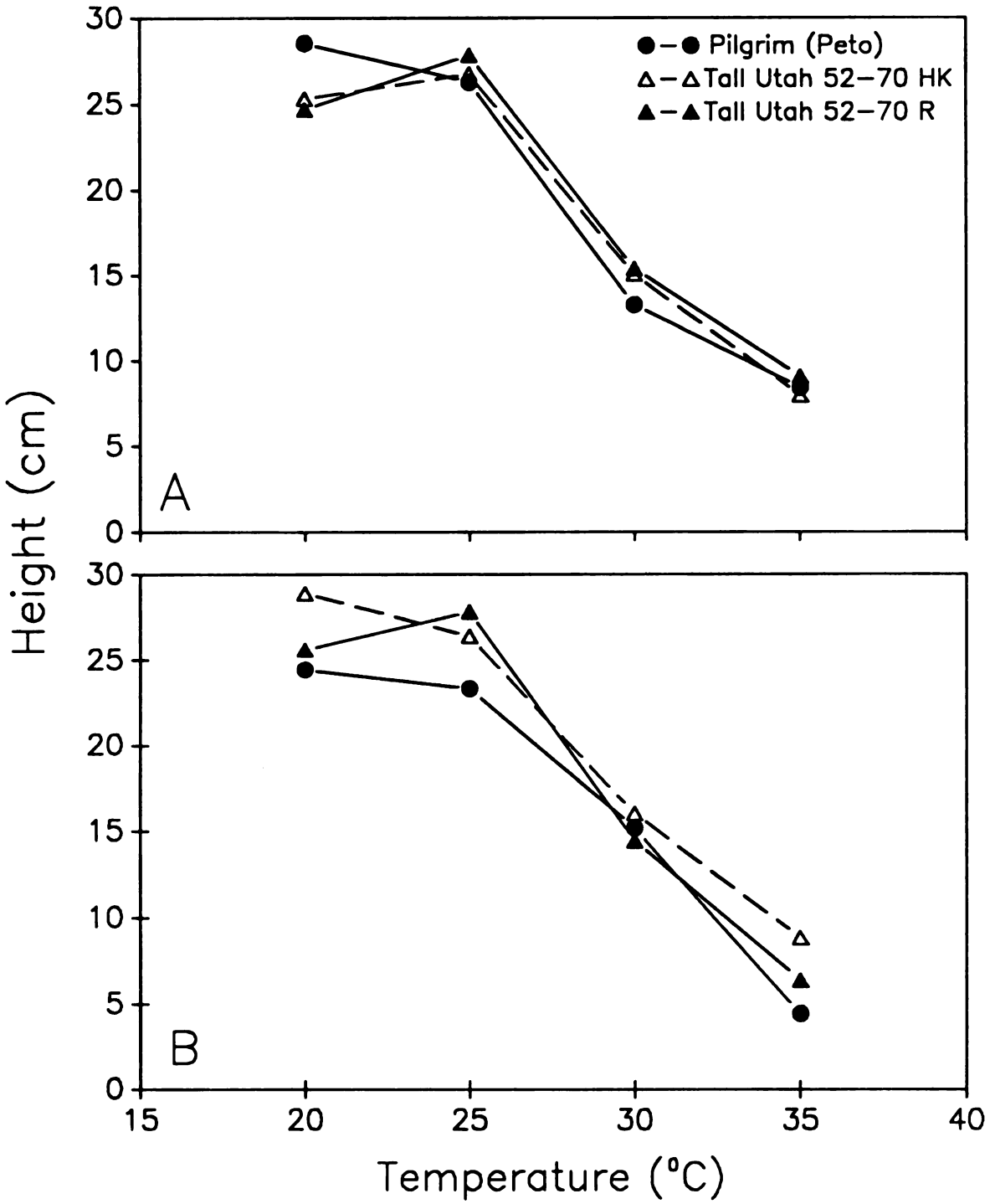

Figure 5. Mean height of plants from susceptible celery cultivars Pilgrim (Peto) and Tall Utah 52-70 R, and moderately resistant cultivar Tall Utah 52-70 HK grown at different temperatures in (A) muck soil naturally infested with *F. oxysporum* f. sp. *apii* race 2 and (B) steamed muck soil.

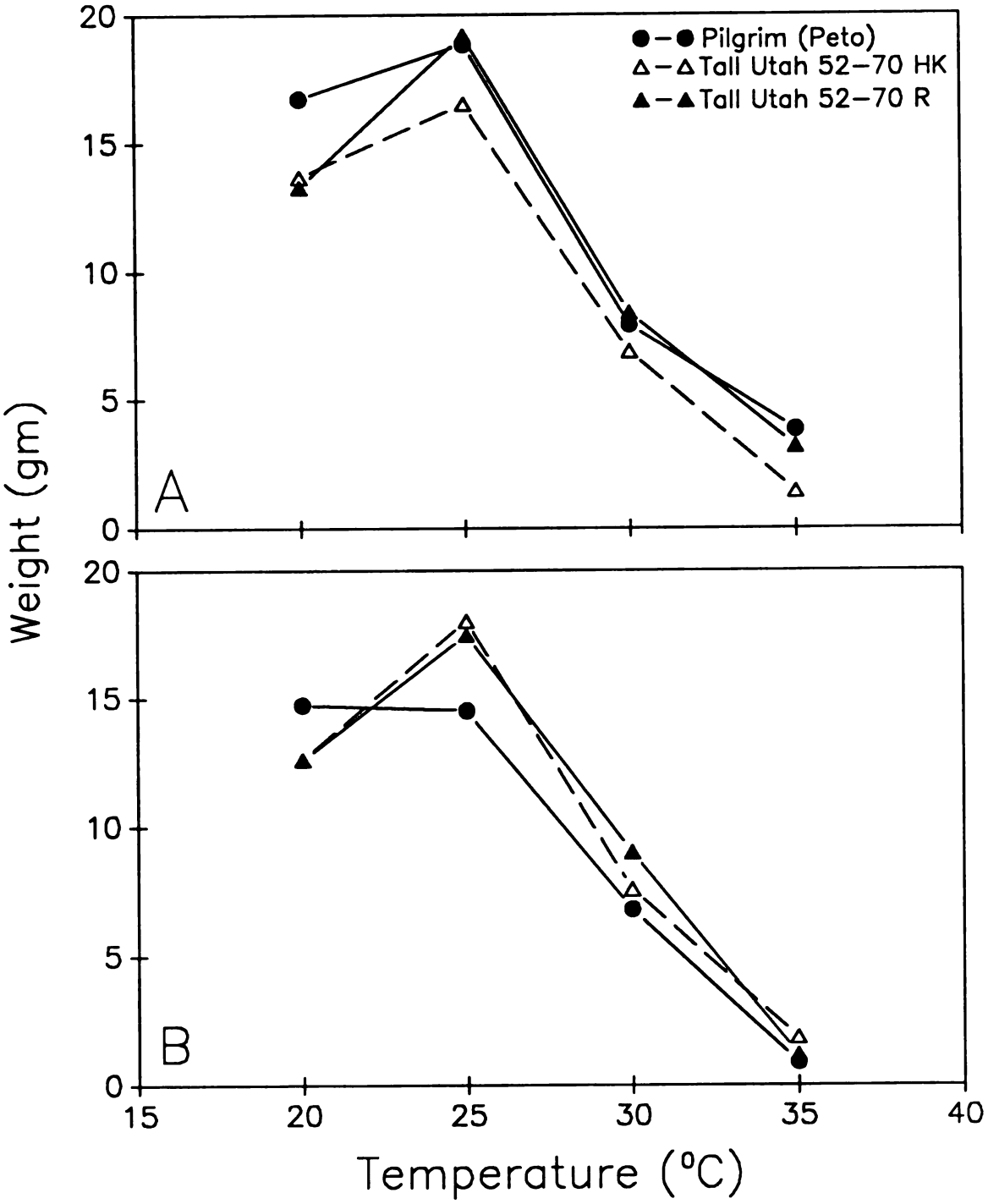

Figure 6. Mean weight of plants from susceptible celery cultivars Pilgrim (Peto) and Tall Utah 52-70 R, and moderately resistant cultivar Tall Utah 52-70 HK grown at different soil temperatures in (A) muck soil infested with *F. oxysporum* f. sp. *apii* race 2 and (B) steamed muck soil.

grown at 30° and 35° C would have come in contact with less soil and, thus, fewer *F. oxysporum* f. sp. *apii* race 2 propagules than roots grown at the lower temperatures.

DISCUSSION

Some celery lines with high resistance to *F. oxysporum* f. sp. *apii* race 2 were identified in field trials in Michigan. Most of the highly resistant lines were horticulturally inferior to susceptible celery cultivars, but are steadily being improved. Experimental lines UC-1 and MSU 74-70 show promise as sources of resistance for celery breeding programs.

Results from celery trials in Michigan were consistent with those in Ohio (R. Rowe, personal communication) and California (Greathead 1987, 1988). Tall Utah 52-70 R, Florida 683, and FM 1218 were highly susceptible in all trials. Tall Utah 52-70 HK and Deacon were rated moderately resistant to slightly susceptible in Ohio and California. Likewise, MSU 74-70 was highly resistant in Ohio, and both MSU 74-70 and UC-1 were highly resistant in California (Greathead 1987, 1988). Bosco had a moderate level of resistance in California (Greathead 1988)

Not all of the lines tested reacted in the same way to *F. oxysporum* f. sp. *apii* race 2 under the growing conditions in Ohio and California. Pilgrim (MSU) and Companion were rated as susceptible in Ohio (R. Rowe, personal communication) and California (Greathead 1987). Napoleon, which was rated as susceptible in Michigan, had a moderate level of resistance under California conditions (Greathead 1987, 1988).

Like other hosts of *F. oxysporum* (Douglas 1970, Mace et al. 1981, Martyn and McLaughlin 1983, Smith and Snyder 1971), the level of

resistance in some moderately and highly resistant celery lines appears to be affected by inoculum concentrations. Tall Utah 52-70 HK and Deacon had higher disease ratings under the *F. oxysporum* f. sp. *apii* race 2 populations found at the locations used in this study (126 ppg for Hudsonville and 198 ppg for Decatur) than in previous trials in a Michigan celery field with a race 2 population of 70 to 100 ppg (Elmer et al. 1986). Likewise, most moderately to highly resistant lines tested had higher disease ratings at Decatur than Hudsonville.

The results of these studies indirectly indicate an effect of high temperatures on Fusarium yellows severity. As in most greenhouse studies with celery and *F. oxysporum* f. sp. *apii* race 2, uniform disease is rarely achieved (Correll et al. 1986, Hart and Endo 1985) and so a lot of variability is obtained within treatments, affecting the precision of the results within and between replications of experiments. The first experiment was run in the early fall, and the second one during the winter months. Even though all treatments were under sodium vapor lights set at a 16 hr photoperiod, the lower light intensity during the winter could also account for the differences in disease reaction observed between the two studies for Tall Utah 52-70 HK and Pilgrim (Peto).

Although the disease severity as measured by vascular discoloration in the greenhouse did not change as temperatures increased from 20° to 35° C, plant growth was severely retarded, indicating a slowing of the metabolic processes and/or increase in catabolic processes at the higher temperatures. It is probable that defense reactions would also be slowed at these higher temperatures. Root growth was less at 30° and

35° C, so plants grown at those temperatures would have come into contact with fewer fungal propagules. Data in the literature provide evidence that the growth or virulence of race 2 may also be reduced at high temperatures (Elmer 1985, Endo et al. 1978, Endo and Jordan 1985). Thus, the same amount of disease developed at all temperatures tested, even though plants grown at 30° and 35° C were exposed to less inoculum and less virulent inoculum, which supports the idea that resistance responses were suppressed at the higher temperatures.

The other data which indicated that high temperatures affect the severity of Fusarium yellows comes from the field plots. Most of the moderately to highly resistant lines had higher disease ratings in 1987 and 1988 when temperatures were higher than in 1986 when temperatures were lower (Figure 1). FM 1217 was one of the best examples. The FM 1217 plants used in 1986 and 1987 came from the same seed lot, so the genotypes were identical, yet FM 1217 went from highly resistant in 1986 to susceptible in 1987.

Pilgrim (MSU) was another cultivar which at first appeared to totally lose resistance as temperatures increased. Pilgrim (MSU) was rated as moderately resistant as an experimental line (Honma et al. 1986, Elmer et al. 1986) and in the 1986 field trial. In 1987, the Pilgrim line planted was highly susceptible to *F. oxysporum* f. sp. *apii* race 2. This Pilgrim line (designated Pilgrim (Peto) in this report) was a seed increase of Pilgrim (MSU). It was also observed that Pilgrim (Peto) contained plants with different growth habits and leaf morphologies not observed in Pilgrim (MSU). Pilgrim (Peto) was highly susceptible in 1988 also, but plants of Pilgrim (MSU) from the seed lot

used in 1986 continued to have a moderate level of resistance during 1988, the hottest of the three years. This indicates that the genotype of Pilgrim (MSU) probably became contaminated prior to or during the seed increase cycle which produced Pilgrim (Peto) and which resulted in the genetic changes affecting the appearance and disease resistance of the cultivar.

Disease resistance appears difficult to transfer to celery from parsley during sexual crosses between the two species. Total resistance to *F. oxysporum* f. sp. *apii* race 2 was not transferred during celery X parsley crosses, and only partial resistance occurred in three of the 28 lines tested from these crosses. High resistance to *Septoria apiicola* was also not obtained by these crosses (Honma and Lacy 1980).

A high level of resistance to *F. oxysporum* f. sp. *apii* race 2 which was stable and heritable was not obtained in the somaclonal lines in trials reported here (Table 7). This by no means disproves the potential of tissue culture techniques for increasing resistance in celery to pathogens. The progenies from only 15 somaclones were examined in this trial. The somaclones were all screened for resistance in the greenhouse, where severity to Fusarium yellows is often less than in field screening, and escapes are more frequent. Furthermore, the progenies were screened in a highly infested site during a year of unusually stressful temperatures. Possibly the somaclones were in fact not highly resistant to *F. oxysporum* f. sp. *apii* race 2, or the phenotypic changes induced by cell culturing were not genetically stable (Orton 1983). It is also possible they may express a more resistant reaction under less stressful, more normal conditions.

Heath-Pagliuso et al. (1988, 1989) released celery germ plasm derived via tissue culture from cells of susceptible celery cultivar Tall Utah 52-70 R. The germ plasm had a high level of resistance to *F. oxysporum* f. sp. *apii* race 2 Likewise, further research in our laboratory has resulted in somaclonal lines with a higher level of resistance to *F. oxysporum* f. sp. *apii* race 2 than the parent cultivar (Tall Utah 52-70 HK) (Chapter 3).

Host resistance is the most practical method for controlling *F. oxysporum* f. sp. *apii* race 2 on celery. Highly resistant cultivars which are horticulturally as good or superior to susceptible cultivars are not yet available. Identifying sources of resistance and using them in breeding superior cultivars with high resistance should remain a priority of celery research programs nationwide.

LITERATURE CITED

Anonymous. 1988. New vegetable varieties from Ferry-Morse for 1989. The Great Lakes Vegetable Growers News 22(12):8

Awuah, R. T., Lorbeer, J. W., and Ellerbrock, L. A. 1986. Occurrence of Fusarium yellows of celery caused by *Fusarium oxysporum* f. sp. *apii* race 2 in New York and its control. Plant Dis. 70:1154-1158.

Bosland, P. W., Williams, P. H., and Morrison, R. H. 1988. Influence of soil temperature on the expression of yellows and wilt of crucifers by *Fusarium oxysporum*. Plant Dis. 72:777-780.

Brettell, R. I. S. and Ingram, D. S. 1979. Tissue culture in the production of novel disease-resistant crop plants. Biol. Rev. 54:329-345.

Bruehl, G. W. 1987. Soilborne Plant Pathogens. Macmillan Publishing Company, New York. 368 pp.

Clayton, E. E. 1923. The relation of temperature to the Fusarium wilt of tomato. Am. J. Bot. 10:71-88.

Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77:1640-1646.

Correll, J. C., Puhalla, J. E., and Schneider, R. W. 1986. Identification of *Fusarium oxysporum* f. sp. *apii* on the basis of colony size, virulence, and vegetative compatibility. Phytopathology 76:396-400.

Daub, M. E. 1986. Tissue culture and the selection of resistance to pathogens. Ann. Rev. Phytopathol. 24:159-186.

Douglas, D. 1970. The effect of inoculum concentration on the apparent resistance of muskmelons to *Fusarium oxysporum* f. sp. *melonis*. Can. J. Bot. 48:687-693.

Endo, R. M., Hart, L. P., and Opgenorth, D. C. 1978. Fusarium yellows. 1977-1978 Annual Report of California Celery Research Advisory Board, Dinuba, California. 8-14.

Endo, R. M. and Jordan, C. 1985. The biology and control of the Fusarium yellows disease of celery. 1985 Annual Report of California Celery Research Advisory Board, Dinuba, California. 4-5.

Elmer, W. H. and Lacy M. L. 1984. Fusarium yellows (*F. oxysporum* f. sp. *apii* race 2) of celery in Michigan. Plant Dis. 68:537.

Elmer, W. H. and Lacy, M. L. 1987. Effects of inoculum densities of *F. oxysporum* f. sp. *apii* in organic soil on disease expression in celery. Plant Dis. 71:1086-1089.

Elmer, W. H., Lacy M. L., and Honma, S. 1986. Evaluations of celery germ plasm for resistance to *Fusarium oxysporum* f. sp. *apii* race 2 in Michigan. Plant Dis. 70:416-419.

Evans, D. A., Sharp, W. R., and Medina-Filho, H. P. 1984. Somaclonal and gametoclonal variation. Amer. J. Bot. 71:759-774.

Gardiner, D. C., Horst, R. K., and Nelson, P. E. 1987. Symptom enhancement of Fusarium wilt of chrysanthemum by high temperatures. Plant Dis. 71:1106-1109.

Greathead, A. 1987. Monterey county fusarium trials results reported. California Celery. California Celery Research Advisory Board, Dinuba, California. 1:1-4.

Greathead, A. 1988. Control of Fusarium yellows. California Celery. California Celery Research Advisory Board, Dinuba, California. 2:3-5.

Hart, L. P. and Endo R. M. 1978. The reappearance of Fusarium yellows of celery in California. Plant Dis. Reptr. 62:138-142.

Heath-Pagliuso, S., Pullman, J., and Rappaport, L. 1988. Somaclonal variation in celery: screening for resistance to *Fusarium oxysporum* f. sp. *apii*. Theor. Appl. Genet. 75:446-451.

Heath-Pagliuso, S., Pullman, J., and Rappaport, L. 1989. UC-T3 somaclone: celery somaclone resistant to *Fusarium oxysporum* f. sp. *apii* race 2. HortScience 24:(In press).

Honma, S. and Lacy, M. L. 1980. Hybridization between pascal celery and parsley. Euphytica 29:801-805.

Honma, S., Lacy M. L., and Murakishi, H. H. 1986. 'Advantage', 'Pilgrim', and 'Companion' celery. HortScience 21:1073-1074.

Ireland, K. F. and Lacy M. L. 1986. Laboratory identification of pathogenic isolates of *Fusarium oxysporum* f. sp. *apii* race 2. (Abstr.) Phytopathology 76:956.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-124.

Lacy, M. L. and Elmer, W. H. 1985. Fusarium yellows of celery in Michigan. Michigan Extension Bulletin E-1823. 2 pg.

Larkin, P. J. and Scowcroft, W. R. 1981. Somaclonal variation- a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60:197-214.

Lorenz, O. A. and Maynard, D. N. 1988. Knott's Handbook for Vegetable Growers. Third edition. John Wiley and Sons, New York. 456 pp.

Mace, M. E., Bell, A. A., and Beckman, C. H. 1981. Fungal Wilt Diseases of Plants. Academic Press, New York. 640 pp.

Martyn, R. D. 1987. Fusarium yellows of celery in Texas. Plant Dis. 71:651.

Martyn, R. D. and McLaughlin, R. J. 1983. Effects of inoculum concentration on the apparent resistance of watermelons to *Fusarium oxysporum* f. sp. *niveum*. Plant Dis. 67:493-495.

Nelson, R., Coons, G. H., and Cochran, L. C. 1937. The Fusarium yellows disease of celery. Mich. Agric. Exp. Sta. Tech. Bull. #155. 74 pp.

Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* species: An Illustrated Manual for Identification. The Pennsylvania State University Press. University Park, Pennsylvania. 193 pp.

Opgenorth, D. C. and Endo, R. M. 1979. Sources of resistance to Fusarium yellows of celery in California. Plant Dis. Reptr. 63:165-169.

Opgenorth, D. C., and Endo, R. M. 1985. Additional sources of resistance to race 2 of *Fusarium oxysporum* f. sp. *apii*. Plant Dis. 69:882-884.

Orton, T. J. 1983. Experimental approaches to the study of somaclonal variation. Plant Molec. Biol. Reptr. 1:67-76.

Orton, T. J., Hulbert, S. H., Durgan, M. E., and Quiros, C. F. 1984. UC1, Fusarium yellows-resistant celery breeding line. HortScience 19:594.

Otto, H. W., Paulus, A. O., Snyder, M. J., Endo, R. M., Hart, L. P., and Nelson, J. 1976. A crown rot of celery. Calif. Agric. 30:10-11.

Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.

Quiros, C. F., D'Antonio, V., and Ochoa, O. 1988. Breeding celery for disease resistance and improved quality. Annual Report of California Celery Research Advisory Board, Dinuba. California. 2:1-3.

Ryker, T. C. 1935. Fusarium yellows of celery. Phytopathology 25:578-600.

Schneider, R. W. and Norelli, J. L. 1981. A new race of *Fusarium oxysporum* f. sp. *apii* in California. (Abstr.) Phytopathology 71:108.

Smith, S. N. and Snyder, W. C. 1971. Relationship of inoculum density and soil types to severity of Fusarium wilt of sweet potato. Phytopathology 61:1049-1051.

Thorpe, T. A. 1981. Plant Tissue Culture- Methods and Applications in Agriculture. Academic Press, New York. 379 pp.

Tisdale, W. B. 1923. Influence of soil temperature and soil moisture upon the *Fusarium* disease of cabbage seedlings. J. Agric. Res. 24:55-86.

Walker, M. N. 1941. Fusarium wilt of watermelons. I. Effect of soil temperature on the wilt disease and the growth of watermelon seedlings. Fla. Agric. Exp. Stn. Bull. # 363. 29 pp.

Ware, G. W. and McCollum, J. P. 1975. Producing Vegetable Crops. The Interstate Printers and Publishers, Inc. Danville, Illnois. 599 pp.

Wright, J. C. and Lacy M. L. 1988. Increase of disease resistance in celery cultivars by regeneration of whole plants from cell suspension cultures. Plant Dis. 72:256-259.

CHAPTER 5

BIOLOGICAL CONTROL OF *FUSARIUM OXYSPORUM* F. SP. *APII* RACE 2.

INTRODUCTION

Fusarium oxysporum f. sp. *apii* race 2, the cause of Fusarium yellows of celery, was first identified in Michigan in 1981 (Elmer and Lacy 1984). By 1987 *F. oxysporum* f. sp. *apii* race 2 was isolated from all celery-growing regions in Michigan (Toth unpublished). *F. oxysporum* f. sp. *apii* race 2 is currently limiting celery production in Michigan because highly resistant cultivars are not widely available, experimental celery lines which are highly resistant are horticulturally inferior to susceptible cultivars (Chapter 4), and no other effective method of control has been found. The *F. oxysporum* f. sp. *apii* race 2 populations are building up in celery fields in Michigan to levels where moderately resistant celery cultivars are severely infected. Alternate control strategies need to be examined, particularly ones which would limit or reduce the *F. oxysporum* f. sp. *apii* race 2 populations in muck soils.

Biological control (biocontrol) as defined by Baker and Cook (1974) is "... the reduction of inoculum density or disease-producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonist, or by mass introduction of one or more antagonists." Nutrients are the factor limiting most microbial populations in soil, and an extensive and active competition occurs

between soil microbes for available nutrients (Baker 1968). Antagonism develops between microbes as they compete for available nutrients.

Soil-borne fungi are usually in a state of dormancy induced by lack of nutrients. Called fungistasis, this phenomenon can be overcome by the addition of appropriate nutrients. Root exudates and crop residues can provide the nutrients necessary for germination and growth of fungal propagules.

Competition between microbes leads to control of soil-borne pathogenic fungi in different ways. Adding nutrients which increase soil microbial populations ultimately enhances competition, fungistasis, and microparasitism as the nutrients are depleted (Lockwood 1977). As microbial activity increases around a propagule of a pathogen, it expends more energy and may weaken to the point where it cannot germinate or infect its host (Baker and Cook 1974). Non-pathogens and weak pathogens may take possession of infection courts on roots and prevent the pathogen from invading the tissue (Cook 1977). Other rhizosphere and rhizoplane organisms protect infection courts by utilizing oxygen and nutrients, by modifying their environment to one unfavorable to the pathogen, and by the production of antibiotics (Cook 1977).

Soil saprophytes are able to use a greater range of nutrients than plant pathogens (Baker and Cook 1974). Organisms which germinate and grow through the soil fastest have a competitive advantage because organisms which occupy organic matter or host tissue first usually retain possession of it (Baker 1968). Soil bacteria are effective scavengers and so are important in competition. Some bacteria, notably

Bacillus and *Pseudomonas* species, produce antibiotics which have localized effects. Actinomycetes are poor competitors due to their slow growth, but are excellent antibiotic producers. Fungi are effective in competition, hyperparasitism and antibiosis. Bacteria are the most effective antagonists in the rhizosphere, and bacteria, fungi and actinomycetes are effective on organic debris (Baker and Cook 1974).

Complex microbiological interactions which provide biological control are influenced by the physical and chemical (including organic matter) makeup of the soil. Biocontrol techniques for soil-borne pathogens rely on the manipulation of the soil environment to produce a habitat where microbes antagonistic toward a particular pathogen can proliferate and the pathogen cannot (Baker and Cook 1974, Cook 1977). This can be done by changing the quality and quantity of nutrients either by crop rotations or by adding organic or inorganic amendments. A *F. oxysporum* f. sp. *apii* race 2-suppressive soil could provide antagonistic organisms which might have use as biocontrol agents.

I. ROTATION CROPS AND ORGANIC AMENDMENT EFFECTS ON SOIL MICROBIAL COMPETITION

Crop rotation, the oldest form of biological control, generally lowers inoculum density (Baker and Cook 1974). Crop rotation starves pathogens, so they are eventually consumed by associated microbes. Root exudates from nonhosts may promote germination and subsequent lysis of pathogenic fungal propagules as available nutrients are consumed.

Rotation crop residues, and likewise organic amendments, affect pathogenic survival. Residues and amendments act as a food sources,

almost instantaneously increasing certain fungal and bacterial populations (Baker and Cook 1974, Cook 1977, Curl 1963). If the pathogen is readily able to compete for the residue, it may increase its numbers on the food base (Baker and Cook 1977, Lewis and Papavizas 1975). If other microbes colonize the tissue first, fungal propagules could undergo germination followed by lysis, remain dormant due to increased fungistasis as the food base is depleted, or be affected by fungitoxic substances produced by the increased microbial activity (Baker and Cook 1977, Lewis and Papavizas 1975). Likewise, volatiles arising from decomposing residues may stimulate germination of propagules or be fungitoxic (Lewis and Papvizas 1975). Thus, the effect of a particular crop residue or organic amendment on a particular disease depends on the plant species, the pathogen, and the other microorganisms present in that field (Baker and Cook 1974, Cook 1977, Lewis and Papavizas 1975).

II. SUPPRESSIVE SOILS

Pathogen-suppressive soils are soils in which a particular pathogen cannot become established, becomes established but fails to produce disease, or becomes established and causes disease at first, but then later declines (Baker and Cook 1974). Suppressive soils are a natural habitat where specific antagonists proliferate and the pathogen sensitive to these antagonists does not. Suppressive soils act principally against a particular pathogen, and a particular soil may be suppressive to one pathogen and conducive to others (Baker and Cook 1974).

Fusarium wilt-suppressive soils are found throughout the world, and have been noted for a number of form species of *F. oxysporum* (Baker and Cook 1974, Toussoun 1975). The study of Fusarium-suppressive soils began in the 1920's with the examination of soils in which Fusarium wilt of banana progressed significantly slower than in other fields (Toussoun 1975). Early investigators found a distinct relationship between soil type and Fusarium wilt severity. Dryer sandy soils had a higher incidence and severity of wilt than did wetter clay soils. Later work with Fusarium wilts of peas and muskmelons established the same relationship between soil type and disease severity (Toussoun 1975).

Further studies determined that the suppressiveness of Fusarium suppressive fields was microbial in origin (Baker and Cook 1974, Hopkins et al. 1987, Opgenorth and Endo 1983, Scher and Baker 1980, Schneider 1984, Sneh et al. 1987, Toussoun 1975, Yuen et al. 1985). Sandy soils were more Fusarium conducive because bacteria antagonistic to Fusaria remained viable and multiplied to a greater degree in moister clay soil than in dryer sandy soils. Siderophore-producing bacteria which tie up soil iron (Elad and Baker 1985, Sneh et al. 1984), bacteria and fungi which compete with pathogens for soil nutrients (especially root exudates which chlamydospores need to germinate and infect host roots) (Elad and Baker 1985, Paulitz and Baker 1987, Sivan et al. 1987, Sneh et al. 1984), and nonpathogenic *Fusaria* which may occupy infection sites (Garibaldi and Gullino 1987, Paulitz and Baker 1987, Schneider 1984) were implicated as Fusarium-suppressive agents.

The objectives of this study were to examine the effects of rotation crops and crop amendments on Fusarium yellows severity or *F. oxysporum*

f. sp. *apii* race 2 populations in natural muck soils. In 1988, a celery field suppressive to Fusarium yellows was identified on Willbrandt Farms near Decatur, Michigan. Preliminary studies were done to determine the nature of its suppressiveness.

MATERIALS AND METHODS

I. MEDIA

Potato carrot agar (PCA) was prepared by autoclaving 20 g carrots and 20 g potatoes in 500 ml distilled water for 30 min. The autoclaved broth was filtered through cheesecloth and brought to a final volume of 1.0 l with distilled water. 20 grams of agar were added, the medium was autoclaved for 20 min, and then dispensed into Petri plates.

Minimal medium (MM) (Puhalla 1985) contained 0.9 M sucrose, 0.2 M $NaNO_3$, 7.3 mM KH_2PO_4, 2.0 mM $MgSO_4 \cdot 7H_2O$, 6.7 mM KCl, 0.0002% sterile trace elements solution, and 20 grams agar per liter medium. Sterile trace elements solution contained 0.3 M citric acid, 0.2 M $ZnSO_4 \cdot 7H_2O$, 0.2 M $FeSO_4 \cdot 7H_2O$, 0.03 M $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 1.0 mM $CuSO_4 \cdot 5H_2O$, 2.1 mM $MnSO_4 \cdot H_2O$, 8.5 mM H_3BO_3, and 2.5 mM $Na_2MoO_4 \cdot H_2O$. The trace elements solution was stored at 4° C.

II. CROPS

All the celery cultivars used in these experiments were tested in field trials for their reaction to *F. oxysporum* f. sp. *apii* race 2 (Elmer et al. 1986, Ireland et al. 1987). Tall Utah 52-70 HK is moderately resistant to *F. oxysporum* f. sp. *apii* race 2. Florida 683 and Tall Utah 52-70 R are highly susceptible to *F. oxysporum* f. sp. *apii* race 2.

Rye (*Secale cereale*), barley (*Hordeum vulgare*), and oat (*Avena sativa*) seed was obtained from J. Clayton (Michigan State University). Certified Kirby mustard (*Sinapis alba*), Cutlass oriental mustard (*Brassica juncea*), and brown mustard (*B. juncea*) were compliments of Minn-Dak Growers Association (Grand Forks, North Dakota). Onion (*Allium cepa*) bulbs and cabbage (*B. oleracea* var. *capitata*) were purchased from Michigan State University Food Stores. Onion leaves were collected from bulbs planted in the greenhouse. Mint foliage was collected from variety plots at the Michigan State University Muck Farm near Bath, Michigan.

III. SOIL POPULATIONS

Soil dilutions. Muck field soil was forced through a sieve with a 2-mm-diameter pore size (#10 from US Standard Sieve Series, W. S. Tyler Company, Cleveland, Ohio). A 30-g sample was air-dried for 2 days. Five g of air dried soil were stirred into 500 ml of a 0.5% (w/v) carboxymethylcellulose solution for 30 min. One ml of the soil suspension was diluted with 9 ml sterile distilled water, and 5 ml of the dilution were added to 50 ml molten (50° C) Komada's medium (Komada 1975) and immediately poured into 5 Petri plates (100 X 15 mm). Three samples were taken from each soil suspension. All cultures were kept in an incubator at 25-28° C under a 12 hr photoperiod.

After 7 days, a few aerial hyphal fragments from colonies which had white aerial mycelium with pink, purple, or orange pigment on Komada's medium were transferred to PCA. Twenty five to 100% of these colonies were transferred from each Komada's medium plate, depending on the total

number of colonies present. Colonies on PCA were examined microscopically to determine which were not *F. oxysporum* (Nelson et al. 1983).

Identification of isolates. *F. oxysporum* f. sp. *apii* race 2 isolates were identified using a technique developed by Puhalla (Puhalla 1985) and based on vegetative compatibility between nitrate nonutilizing (*nit*) mutants (Correll et al. 1987, Puhalla 1985). *Nit* mutants were produced for unknown isolates on a potato sucrose medium containing 15 g $KClO_3$/liter of medium (Puhalla 1985). Mycelia from fast-growing chlorate resistant sectors on KPS were transferred to MM. *Nit* mutants were identified by their thin, but normally expansive, growth and a lack of aerial mycelium on MM (Correll et al. 1987, Puhalla 1985). One to 2 *nit* mutants from each isolate were placed on the same MM plate with *nit* mutants from two known *F. oxysporum* f. sp. *apii* race 2 isolates and allowed to grow together for 3 weeks. The *nit* mutants from the two known isolates consistently anastomosed with all other *F. oxysporum* f. sp. *apii* race 2 isolates in previous experiments (Ireland and Lacy 1986). Unknown isolates which formed a nitrate utilizing heterokaryon with either of the *F. oxysporum* f. sp. *apii* race 2 tester isolates were identified as *F. oxysporum* f. sp. *apii* race 2 (Correll et al. 1986, Ireland and Lacy 1986). Nitrate-utilizing heterokaryons were identified by the presence of wild-type growth with aerial mycelium and pigmentation where the mycelia from two *nit* mutants intergrew (Correll et al. 1987, Puhalla 1985).

IV. FUSARIUM YELLOWS SEVERITY RATING SCALE

Disease severity was rated on a 1 to 5 scale for all experiments. A rating of 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant. Individual plants were uprooted, and their crowns and roots were cut open and rated for disease severity.

V. ROTATIONAL CROPS

Onions and other vegetables. *F. oxysporum* f. sp. *apii* race 2 populations in two fields on Willbrandt Farms near Muskegon, Michigan were followed from 1986 to 1987. The two fields were divided by a paved street, and were designated as the East and West field based on their direction from the street.

Both fields were diagnosed as being infested with *F. oxysporum* f. sp. *apii* race 2 in 1981 (Elmer and Lacy 1984). The West field was in continuous celery, but the East field was rotated out of celery and into a variety of vegetables, including cole crops, sweet corn, and melons, for 4 growing seasons prior to the first soil sample in the Spring of 1986. During the summer of 1986, onions were grown in the West field, and a variety of vegetables were again grown in the East field. Celery cultivars moderately resistant to *F. oxysporum* f. sp. *apii* race 2 were grown in both fields during the summer of 1987.

Ten soil samples were collected in the spring after planting and in the fall after harvest from each field. The *F. oxysporum* f. sp. *apii* race 2 population was measured for each sample using the vegetative

compatibility test described above. The data from the 10 samples from each field were combined to provide an estimate of the fungal population in the whole field.

Leeks. Soil samples were taken from two adjacent areas in a celery field naturally infested with *F. oxysporum* f. sp. *apii* race 2 near Hudsonville, Michigan. One area was in continual celery production. The second area was rotated to leeks for one growing season. Four-week-old plants of celery cultivars Tall Utah 52-70 HK and Florida 683 were planted into 10-cm pots containing the soil samples (2 plants per cultivar and soil sample). Soil from each sample which was steamed for 1 hr was also tested. The plants were grown in the greenhouse for 15 weeks and then rated for disease severity.

VI. CROP RESIDUES

These studies were carried out in a muck field highly infested with *F. oxysporum* f. sp. *apii* race 2 near Decatur, Michigan. The plot was on a celery grower-cooperator's field. Dried and green amendments from various crops were incorporated into muck fields during 1987 and 1988, respectively. Unamended, fallow plots were included as untreated checks.

Dried amendments. Air-dried mint stems and a mixture of oven-dried onion bulbs and leaves (75% (w/w) bulbs and 25% (w/w) leaves) were incorporated into the field plots using a rototiller. Three rates of residues corresponding to 0.5, 1.0, and 2.0 g residue per liter soil (Elmer and Lacy 1987b) were incorporated into rows 4.5 m long by 60 cm wide by 15 cm deep. One week later, seedlings of celery cultivar Tall

Utah 52-70 HK were transplanted into the plot. The plants were harvested after 90 days of growth in the field and rated for disease severity. The experiment was a randomized block design with 3 replications.

Green amendments. Green foliage of rye, brown mustard, and Kirby mustard, onion bulbs or cabbage was incorporated into an area 2.4 m wide by 4.5 m long by 15 cm deep with a rototiller. The experiment was a randomized block design with 2 replications.

The amount of residue used differed for each crop. Plots 2.4 m by 4.5 m were established on the Michigan State University muck farm (not infested with *F. oxysporum* f. sp. *apii* race 2) and seeded with rye at the rate used for cover crop plantings of (90 kg/ha), and with brown and Kirby mustard based on the rate recommended for soil improvement (22.5 kg/ha) (Lorenz and Maynard 1988). The crops were grown for 4 weeks, then the foliage was harvested and was cut by hand into smaller pieces 2 days prior to incorporation into the infested muck field.

The onions and cabbage were cut by hand into smaller pieces to facilitate incorporation. Onions and cabbage were incorporated at rates based on 20% residue from average yields of 20.5 t/ha for onions and 22.5 t/ha for cabbage (Lorenz and Maynard 1988). Approximately 5400 g of cabbage or 4860 g onions were incorporated into a test plot.

Three weeks after the plant residues were incorporated, seedlings of Tall Utah 72-70 HK and Florida 683 were transplanted into two 4.5-m rows in each plot. After 11 weeks, 10 plants from each cultivar and

treatment per replication were rated for disease severity. The Tall Utah 52-70 HK plants were trimmed and weighed in the field; Florida 683 did not provide enough plant material to weigh.

VII. COVER CROPS

Muck soil naturally infested with *F. oxysporum* f. sp. *apii* race 2 was collected from celery fields in Muskegon and Decatur, Michigan, sieved to remove stones and other debris, and mixed for 20 min in a concrete mixer. Seeds of barley, rye, oats, Kirby mustard, brown mustard, oriental mustard, or celery cultivars Tall Utah 52-70 HK and Tall Utah 52-70 R were planted in 25-cm clay pots filled with the soil mixture (1 pot per crop). One pot of soil left unplanted served as a fallow treatment.

The disease potential of the soil mixture was measured. Four 10-cm pots were filled with the soil mixture, and two 4-week-old plants of celery cultivar Tall Utah 52-70 R were planted in each pot. After 10 weeks, the Tall Utah 52-70 R plants were rated for disease severity.

After emergence, the crops were thinned to 15 to 20 plants per pot. Barley, rye, oats and the mustards were grown for 1 month, trimmed at the soil line, weighed, and cut into pieces approximately 5 cm long. Twenty g of rye, barley, and oat foliage; and 40 g of Kirby, oriental, and brown mustard foliage were incorporated back into the soil they grew in. The roots were left in the soil. The soil-residue mixture was placed back into the original pots and kept in the greenhouse. The celery cultivars were grown for 2 months, then 40 g of foliage incorporated back into the soil.

The residues were allowed to decompose for 4 weeks, then soil from individual pots was mixed for 10 min in a concrete mixer and used to fill ten 10-cm pots. Two 3-week-old plants of celery cultivar Tall Utah 52-70 R were planted into each pot. The plants were placed in the greenhouse, and were fertilized with Hoagland's solution and 20-20-20 (N-P-K) (Peter's® soluble fertilizer) 1 to 2 days after transplanting, and then once every 2 weeks for the duration of the experiment. Ten weeks after transplanting, the Tall Utah 52-70 R plants were uprooted and rated for disease severity.

VIII. SUPPRESSIVE SOIL

Muck soil was collected from two fields on Willbrandt Farms near Decatur, Michigan. One field had a low incidence of Fusarium yellows, where the second field had a high incidence of the disease, as did all other fields on the farm. Soils from the two fields were mixed in varying proportions in a cement mixer for 20 min and dispensed into 8 16-cm clay pots. Soil from the second (diseased) field was also mixed in varying proportions with muck soil previously steamed for 1 hr. Pots of steamed soil from each field served as controls. One 4-week-old seedling of celery cultivar Tall Utah 52-70 HK or Florida 683 was planted into each pot (4 plants per cultivar and treatment). The plants were grown in the greenhouse for 13 weeks, then rated for disease severity. The *F. oxysporum* f. sp. *apii* race 2 populations for both soils were also measured using the procedure described previously. The pH, phosphorus, potassium, calcium, magnesium, zinc, manganese, copper, iron, and organic content of both soils was analyzed by the Michigan State University Soil Testing Laboratory (East Lansing, Michigan).

RESULTS

I. EFFECT OF CROP ROTATION ON FUSARIUM YELLOWS

Onions and other vegetables. In spring 1986, the West field at Muskegon had a *F. oxysporum* f. sp. *apii* race 2 population of 80 propagules per gram of soil (ppg) (Figure 1). The East field, which had been rotated out of celery for 4 years, had a *F. oxysporum* f. sp. *apii* race 2 population of 40 ppg (Figure 1). Both populations are large enough to cause a significant amount of disease in susceptible celery (Elmer and Lacy 1987a). After onions were grown in the West field for one year, the population remained high in the fall (94 ppg), but was reduced to 26 ppg by the next spring (Figure 1). One growing season of celery following onions in the West field brought the population up to 160 ppg by fall (Figure 1). After the 5th growing season out of celery, the *F. oxysporum* f. sp. *apii* race 2 population in the East field had decreased below a level detectable by the procedure used, and was only 10 ppg after celery was grown for 1 year (Figure 1).

Leeks. Tall Utah 52-70 HK and Florida 683 celery had mean disease ratings of 2.5, and 4.0 respectively when grown in the greenhouse in soil collected from a muck field in continuous celery production (Table 1). These ratings were significantly higher than those for plants grown in soil from an area rotated to leeks for one year ($P = 0.05$) (Table 1). The disease ratings for the celery in the leek soil were not significantly different from those in steamed muck soil (Table 1).

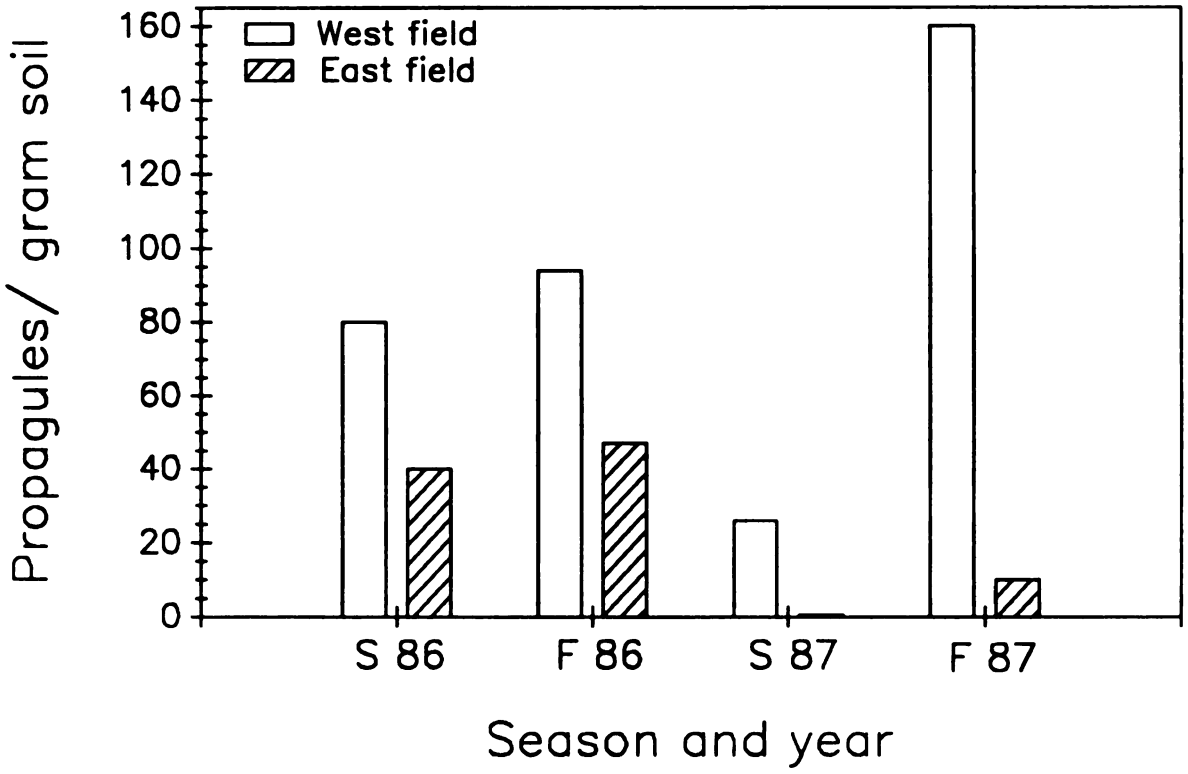

Figure 1. *F. oxysporum* f. sp. *apii* race 2 populations in two fields from a Willbrandt Farms near Muskegon, Michigan from the spring (S) of 1986 through the fall (F) of 1987. Prior to the first sample in the spring of 1986, the West field was in continuous celery, but the East field was rotated out of celery and into a variety of vegetables, including cole crops, sweet corn, and melons, for 4 growing seasons. During the summer of 1986, onions were grown in the West field, and a variety of vegetables again in the East field. Celery cultivars moderately resistant to *F. oxysporum* f. sp. *apii* race 2 were grown in both fields during the summer of 1987.

Table 1. Fusarium yellows disease ratings for celery planted in muck soil collected from a field naturally infested with *Fusarium oxysporum* f. sp. *apii* race 2 and cropped continuously to celery or rotated to leeks for one year.

	Celery mean disease rating[1]	
Previous crop[2]	Tall Utah 52-70 HK[3]	Florida 683
Celery	2.5 a	4.0 a
Leek	1.0 b	2.0 b
Celery (steamed)	1.0 b	1.0 b
Leek (steamed)	1.0 b	1.0 b

[1] Plants were rated on a 1 to 5 class scale: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50 % crown area discolored; 4 = more than 50 % crown area discolored; 5 = dead or nearly dead plant.

[2] Samples were from adjacent areas in the field. Celery refers to soil from area continuously cropped celery, and leeks to soil from area rotated to leeks for 1 year. Steamed soils were autoclaved for 1 hour.

[3] Tall Utah 52-70 was moderately resistant to *F. oxysporum* f. sp. *apii* race 2. Florida 683 was highly susceptible to *F. oxysporum* f. sp. *apii* race 2. Mean of 4 plants per cultivar for celery and leek soil samples. Mean of 2 plants per cultivar for streamed soil samples. Numbers for individual cultivars followed by the same letters are not significantly different according to Duncan's multiple range test (P = 0.05).

II. EFFECT OF COVER CROPS ON FUSARIUM YELLOWS SEVERITY IN THE GREENHOUSE

Tall Utah 52-70 R plants grown in the original muck soil mixture used in the cover crops study had a mean disease rating of 3.0. The disease rating for Tall Utah 52-70 R plants was 2.0 following oats, 2.2 following oriental mustard, and 2.2 following brown mustard (Table 2). These disease ratings were all significantly lower than the 2.8 rating for Tall Utah 52-70 R plants grown in the fallow treatment (LSD = 0.47) (P = 0.05) (Table 2). The disease ratings for celery following rye, barley, and Kirby mustard were not significantly different from the fallow treatment (Table 2). The disease ratings for celery following both Tall Utah 52-70 HK and Tall Utah 52-70 R celery were all 5.0, a rating significantly higher than the fallow treatment rating (Table 2).

III. EFFECT OF CROP RESIDUES ON FUSARIUM YELLOWS SEVERITY IN THE FIELD

Dried onions and mint. Tall Utah 52-70 HK plants had similar disease ratings following onion residues incorporated at rates corresponding to 0.5, 1.0, and 2.0 g residue per liter of soil, respectively (Table 3). Similar results were obtained with mint residues (Table 3). These ratings were not significantly different from the mean disease rating for Tall Utah 52-70 HK plants grown in soil left fallow (P = 0.05).

Green amendments. Disease ratings of Florida 683 plants when grown in *F. oxysporum* f. sp. *apii* race 2 infested muck following incorporation of brown mustard foliage, Kirby mustard, cabbage, rye, or onions were not significantly different from disease ratings for Florida 683 plants grown in the fallow treatment (P = 0.05) (Table 4). Tall Utah 52-70 HK

Table 2. Effect of cover crops on Fusarium yellows disease potential of muck soil in the greenhouse.

Treatment	Disease rating[1]
Fallow	2.8
Rye	2.5
Barley	2.3
Oats	2.0
Kirby mustard	2.4
Oriental mustard	2.2
Brown mustard	2.2
Tall Utah 52-70 HK	5.0
Tall Utah 52-70 R	5.0
LSD (P = 0.05)	0.47

[1] Mean disease ratings of 20 plants per treatment for susceptible celery cultivar Tall Utah 52-70 R. Plants were rated on a 1 to 5 class scale: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant.

Table 3. Disease ratings for moderately resistant celery cultivar Tall Utah 52-70 HK plants grown in *F. oxysporum* f. sp. *apii* race 2 infested soil amended with dried onion and mint residues.

Treatment[1]	Disease rating[2]
Onions low	2.9 [3]
Onions medium	2.9
Onions high	2.8
Mint low	3.2
Mint medium	2.8
Mint high	2.8
Fallow	3.0

[1] Low level is based on a rate of 0.5 grams residue per liter of soil, medium based on 1 g residue per liter, and high based on 2 g per liter.

[2] Plants were rated on a 1 to 5 class scale: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant.

[3] Mean of 10 plants per treatment and replication. Two-way analysis of variance indicated differences were nonsignificant (P = 0.05).

Table 4. Effect of green crop amendments on Fusarium yellows disease potential of muck soil in the field.

Treatment	Disease rating[1]		Yield (t/ha)[3]
	Florida 683[2]	Tall Utah 52-70 HK	
Brown mustard	3.4	2.3	41.4
Kirby mustard	3.0	1.7	39.4
Cabbage	3.3	2.1	36.2
Rye	3.1	1.7	30.6
Onions	2.9	1.6	39.6
Fallow	3.3	1.6	33.3
LSD (P = 0.05)	NS	0.2	NS

[1] Mean for 10 plants per cultivar and replication. Plants were rated on a 1 to 5 class scale: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = less than 50% crown area discolored; 4 = more than 50% crown area discolored; 5 = dead or nearly dead plant.

[2] Florida 683 and Tall Utah 52-70 HK are celery cultivars highly susceptible and moderately resistant to *F. oxysporum* f. sp. *apii* race 2, respectively.

[3] Yield for the 10 Tall Utah 52-70 HK rated for disease severity averaged for the two replications and converted to metric tonnes/hectare.

plants following brown mustard and cabbage residues had mean disease ratings which were significantly greater than the ratings for Tall Utah 52-70 HK plants in the fallow treatment (LSD = 0.23) (P= 0.05) (Table 4). After incorporation of Kirby mustard, rye, and onion residues, Tall Utah 52-70 HK plants had mean disease ratings which were not significantly different from the fallow treatment (Table 4). The yields for Tall Utah 52-70 HK plants were not significantly different in any of the treatments (P = 0.05) (Table 4).

IV. SUPPRESSIVE SOIL

A slight decrease in Fusarium yellows severity on susceptible and moderately resistant celery was observed as the proportion of suppressive soil to conducive soil was increased (Figure 2), although the slopes of the regression were not significantly different from zero (P = 0.05). The same trend was obtained as the proportion of autoclaved muck to conducive soil increased (Figure 3), and again, slopes of the regression were not significantly different from zero (P = 0.05). The *F. oxysporum* f. sp. *apii* race 2 population for the conducive soil was 120 ppg, whereas the suppressive soil had a population which was too low to detect with the procedure used in this study. The suppressive and conducive soils contained different amounts of calcium, copper, magnesium, manganese, phosphorus, potassium, and zinc (Table 5). The suppressive soil had a pH of 7.0, whereas the pH of the conducive soil was only 6.4 (Table 5).

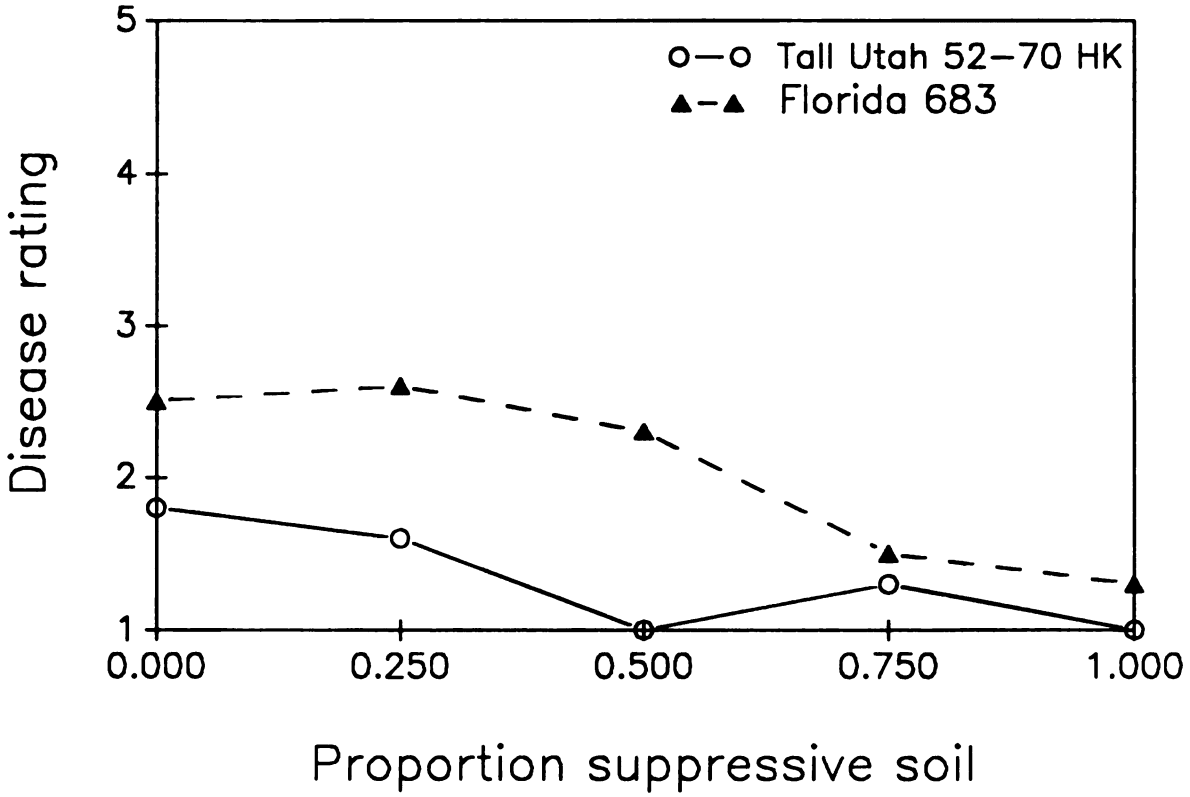

Figure 2. Mean disease ratings for celery cultivars Florida 683 (susceptible to *F. oxysporum* f. sp. *apii* race 2) and Tall Utah 52-70 HK (moderately resistant) grown in various ratios of Fusarium yellows-conducive and Fusarium yellows-suppressive muck field soil.

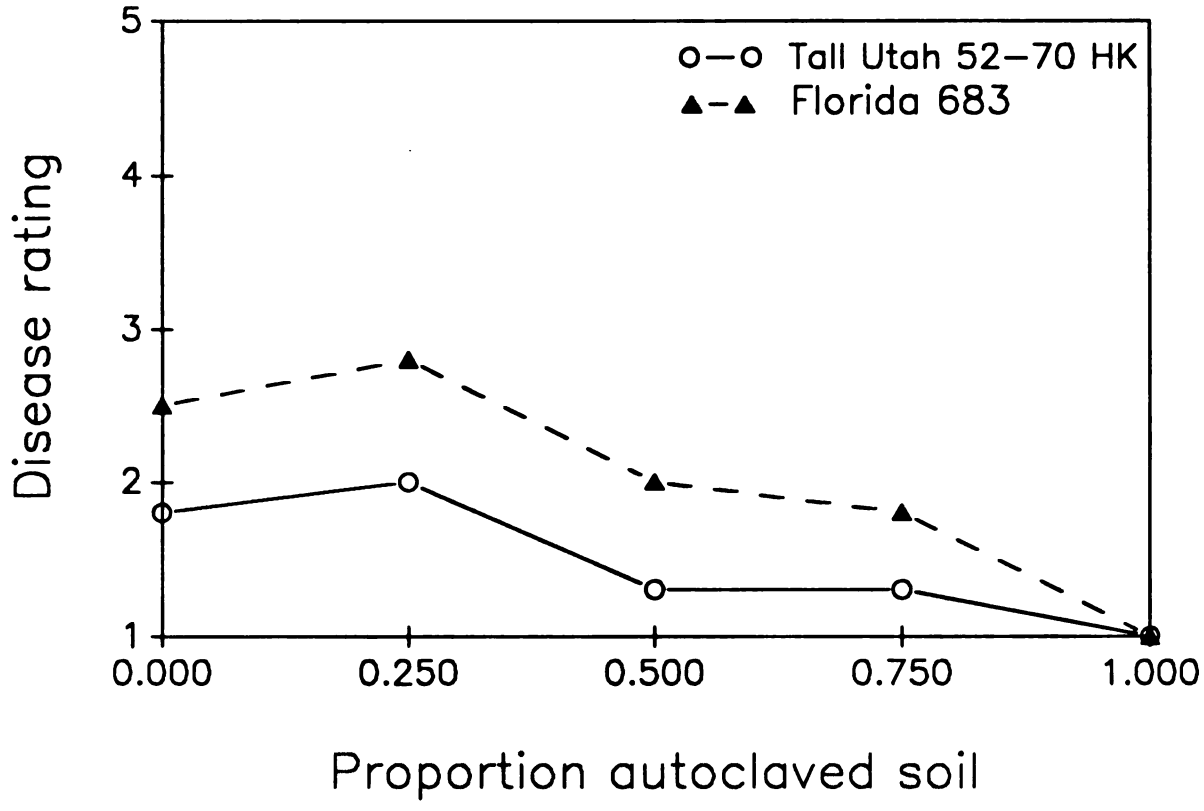

Figure 3. Mean disease ratings for celery cultivars Florida 683 (susceptible to *F. oxysporum* f. sp. *apii* race 2) and Tall Utah 52-70 HK (moderately resistant) grown in various ratios of Fusarium yellows-conducive soil and autoclaved muck field soil.

Table 5. Properties of a soil suppressive and a soil conducive to *F. oxysporum* f. sp. *apii* race 2.

Property	Conducive soil	Suppressive soil
Calcium	10105 lbs/acre	5760 lbs/acre
Copper	74.0 ppm	112.0 ppm
Iron	24.0 ppm	21.0 ppm
Magnesium	1299 lbs/acre	593 lbs/acre
Manganese	10.9 ppm	38.0 ppm
Phosphorus	160 lbs/acre	285 lbs/acre
Potassium	552 lbs/acre	185 lbs/acre
Zinc	15.3 ppm	32.1 ppm
Organic matter	56.5 %	73.5 %
pH	6.4	7.0

DISCUSSION

Since few vegetables are as profitable to grow as celery (Lorenz and Maynard 1988, Ryder 1979, Ware and McCollum 1975), commercial growers are reluctant to rotate fields out of celery. Crop rotation may become necessary to maintain *F. oxysporum* f. sp. *apii* race 2 populations at levels low enough for celery to be profitable. It has been demonstrated that monocropping celery increases *F. oxysporum* f. sp. *apii* race 2 populations, especially when celery trimmings are incorporated back into celery fields (Elmer 1985), and data presented in this report support this.

Little research has been done on the effects of crop rotation on Fusarium yellows, but data presented elsewhere (Elmer 1985, Elmer and Lacy 1987b) and in this report provide some evidence that rotating celery fields to onions or relatives of onions may help control Fusarium yellows. Previous evidence suggested that onions may help decrease *F. oxysporum* f. sp. *apii* race 2 populations (Elmer 1985, Elmer and Lacy 1987b). Indeed, rotating a celery field to onions for one year reduced the *F. oxysporum* f. sp. *apii* race 2 population in that field from 96 ppg in the fall to 26 ppg the following spring (Figure 1). Likewise, rotating a celery field to leeks for one growing season significantly reduced the Fusarium yellows disease potential of a muck field compared to monocropping celery in the same field (Table 1).

Onion bulbs have a high sugar content, and contain compounds toxic to fungi and bacteria (Jones and Mann 1963). The high sugar content would

stimulate the growth of a number of microorganisms. This would increase competitiveness in the soil, which might deleteriously effect *F. oxysporum* f. sp. *apii* race 2 populations. Also, fungitoxic substances released as onion bulbs decompose could be toxic to *F. oxysporum* f. sp. *apii* race 2 propagules (Elmer 1985). Leeks are in the genus *Allium* and may contain some of the same fungitoxic compounds as onions. Unfortunately, neither oven-dried nor fresh chopped onion bulbs reduced the Fusarium yellows severity on moderately resistant and susceptible celery when incorporated into a celery field naturally infested with *F. oxysporum* f. sp. *apii* race 2 (Table 3). However, it is possible that root exudates may have some fungitoxic effects, or that they may stimulate microbial antagonists.

Other crops may make good rotational crops for celery in controlling Fusarium yellows. A five year rotation out of celery and into several different vegetables was able to reduce the *F. oxysporum* f. sp. *apii* race 2 populations to a level which could not be detected with the procedure used in this study. After growing celery again in this field for one season, the *F. oxysporum* f. sp. *apii* race 2 population remained low.

Celery is grown in Michigan on valuable organic soils. These fields are usually not left fallow during the winter, but are planted to cover crops (usually rye) to prevent wind erosion. Cover crops are disked under in the spring before celery is transplanted into the fields. Living plant roots and crop residues greatly influence both parasitic and saprophytic microbial behavior (Baker and Cook 1974, Cook 1977, Curl 1963, Lewis and Papavizas 1975). *F. oxysporum* f. sp. *apii* race 2 readily colonizes the roots of a number of plant genera without causing obvious symptoms (Elmer and Lacy 1987b). The roots of some monocots tested (sweet corn and

barnyard grass) were colonized to a greater degree by *F. oxysporum* f. sp. *apii* race 2 than were susceptible celery roots. Since rye is the most common cover crop used in celery fields, it seemed important to examine the effects of its growth and residue decomposition of Fusarium yellows severity, and compare rye to other cover crops for muck soils.

In a greenhouse study, rye, oats, barley, and three mustard cultivars did not increase *F. oxysporum* f. sp. *apii* race 2 populations (as measured by disease severity on susceptible celery) in naturally infested muck soil. In fact, susceptible celery grown in muck soil following oats, Oriental mustard, and brown mustard had a significantly lower level of Fusarium yellows than celery grown in fallow soil. Rye, barley and Kirby mustard did not reduce Fusarium yellows severity in susceptible celery.

In field studies, the incorporation of green residues of brown mustard did not decrease Fusarium yellows on susceptible celery, and actually significantly increased severity on a moderately resistant celery cultivar. As in the greenhouse study, rye and Kirby mustard did not affect Fusarium yellows severity on susceptible or moderately resistant celery compared to the fallow treatment. Researchers with other soil-borne pathogens have noted difficulty in reproducing greenhouse experiments using amendments in field situations (Papavizas and Lewis 1971).

While determining the effect of residues on pathogens, possible phytotoxicity of the residues must also be assessed. Decomposing residues can injure plants, allowing otherwise weak pathogens to cause disease

(Curl 1963). None of the crops tested appeared phytotoxic to celery because the yields of celery grown following incorporation of amendments did not differ significantly from the fallow control.

There could be a number of reasons why crop amendments in this study did not reduce Fusarium yellows in the field. Cabbage residues contain a number of volatile compounds which can kill fungi (Lewis and Papavizas 1971), but the effect is not significant unless the fields are covered with a tarp to keep the compounds in the soil (Ramirez-Vallipuda and Munnecke 1987). The plots in this study were not tarped. Since mustards are in the same family as cabbage, they may contain some of the same compounds, although tarping would seem necessary for full effectiveness of volatiles also.

Many successful experiments using amendments for disease control utilized a prohibitively large amount of amendments, often as much as to 5-10 tons/acre (11-22 tonnes/hectare) (Papavizas 1975) in the field. The amounts of amendments used here were based on those which would occur under normal cropping situations.

It is possible that too much time elapsed between celery transplantation and incorporation of amendments. Amendments almost instantaneously increase soil microbe populations (Baker and Cook 1974, Cook 1977, Curl 1963). By waiting three weeks after incorporation of the green amendments before transplanting celery into the plots, any organisms which could have protected the infection courts were dormant again, or at too low a level again to offer enough protection. Chlamydospores which germinated in response to added amendments most likely formed replacement chlamydospores before the mycelia underwent lysis (Cook 1977).

Since the field used in the amendment studies is highly conducive to *F. oxysporum* f. sp. *apii* race 2, it seems logical that organisms antagonistic to *F. oxysporum* f. sp. *apii* race 2 would either not exist or be present at very low levels. It is possible that antagonists were stimulated, but since the original population was low, they still could not get to the critical population level needed to control *F. oxysporum* f. sp. *apii* race 2. If this is the case, another strategy would be necessary for control.

The next control strategy could well come from suppressive soil. Suppressive soils are naturally high in antagonistic organisms, which if isolated and identified could have potential for control when added to conducive soils (Baker and Cook 1974). Organisms which work best for this seem to be ones which grow near or on the root surface or colonize the roots without damaging the plants (Baker and Cook 1974). These organisms often can be introduced on seedling roots, and if they can grow and colonize new roots faster than the pathogen can infect them, may offer protection from infection.

No significant reduction in Fusarium yellows severity was observed when soil from a possible *F. oxysporum* f. sp. *apii* race 2-suppressive field or autoclaved soil was mixed with soil from a field with a high incidence of Fusarium yellows. The major confounding factor in this study is that a high level of disease was not obtained in any of the treatments, even in highly susceptible celery plants grown in 100% conducive soil. Obtaining a high level of disease is often a problem in greenhouse experiments with this disease (Hart and Endo 1978, Opgenorth and Endo 1979), but the reasons are not known. Light intensity appears to affect

Fusarium yellows severity (P. Hart, personal communication), and for that reason, all plants were grown under sodium vapor lights for a 16 hr photoperiod.

These data do not disprove the hypothesis that the soil is suppressive to Fusarium yellows. Some factor is keeping the *F. oxysporum* f. sp. *apii* race 2 population low in this particular field. Schneider (1984) observed a significant linear decline in Fusarium yellows severity as the proportion of suppressive soil mixed with a conducive soil was increased. The mixtures were kept fallow for 10 days after mixing to allow the microbial populations to stabilize. In this study, celery plants were transplanted into the soil blends immediately after they were mixed. A fallow period might have given the antagonistic organisms an opportunity to multiply and proliferate throughout the mixture. Nonpathogenic *F. oxysporum* isolates which colonize celery roots were implicated as biocontrol agents for *F. oxysporum* f. sp. *apii* race 2 (Schneider 1984). Their populations would increases relatively slowly in the soil mixtures and so would need time for their levels to increase to one high enough for effective control.

Bacteria, which proliferate more rapidly than fungi, are more affected by the soil environment, particularly soil pH. The suppressive soil examined here had a pH of 7.0, whereas the conducive soil had a pH of 6.4. It is possible that mixtures of suppressive and conducive soil had a lower pH than is optimal for antagonistic bacteria to proliferate. Opgenorth and Endo (1983) found that bacteria antagonistic towards *F. oxysporum* f. sp. *apii* race 2 multiplied at a much higher rate at a pH of 7.4 than at a pH of 6.2. Likewise, Scher and Baker (1980) demonstrated that flax wilt

increased from 31% to 61% as the pH of a soil suppressive to *F. oxysporum* f. sp. *lini* was lowered from 8.0 to 7.0, and a disease incidence of 87% when the pH was reduced to 6.0.

The environment in conducive soils is often not conducive to antagonists, or they would be there in high numbers already (Baker and Cook 1974). Amendments (both organic and inorganic) may play a role in these soils in the control strategy for *F. oxysporum* f. sp. *apii* race 2. If the right combination of amendments could be found which would change the soil environment to one in which added antagonists would survive and proliferate, *F. oxysporum* f. sp. *apii* race 2 populations might be kept at low levels. This may provide the level of control necessary for continued economic production of celery in *F. oxysporum* f. sp. *apii* race 2-infested fields in Michigan.

Biological control methods do not completely eradicate pathogens. Many pathogens, including *Fusariums*, are able to colonize the roots of a number of weeds, and survive for a number of years as dormant structures, especially in soil layers below cultivation where microbial activity and competition is reduced (Curl 1963). The object of these control methods is to reduce the pathogen population to a level which permits economic production of a host crop for one or more years. This level of control would depend on the cost of the control and the return on the increased production (Baker and Cook 1974).

LITERATURE CITED

Baker, R. 1968. Mechanisms of biological control of soil-borne pathogens. Ann. Rev. Phytopathol. 6:263-294.

Baker, K. F. and Cook R. J. 1974. Biological Control of Plant Pathogens. W. H. Freeman and Company, San Francisco. 433 pp.

Cook, J. R. 1977. Management of the associated microbiota. Chapter 8 In: Plant Disease An Advanced Treatise Vol I. J. G. Horsfall and E. B. Cowling (eds.) Academic Press, New York. 465 pp.

Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77:1640-1646.

Correll, J. C., Puhalla, J. E., and Schneider, R. W. 1986. Identification of *Fusarium oxysporum* f. sp. *apii* on the basis of colony size, virulence, and vegetative compatibility. Phytopathology 79:396-400.

Curl, E. A. 1963. Control of plant diseases by crop rotation. Botan. Review 29:413-479.

Elad, Y. and Baker, R. 1985. The role of competition for iron and carbon in suppression of chlamydospore germination of *Fusarium* spp. by *Pseudomonas* spp. Phytopathology 75:1053-1059.

Elmer, W. H. 1985. The Ecology and Control of Fusarium Yellows of Celery in Michigan. Doctoral Dissertation, Michigan State University. 146 pp.

Elmer, W. H. and Lacy M. L. 1984. Fusarium yellows (*Fusarium oxysporum* f. sp. *apii* race 2) of celery in Michigan. Plant Dis. 68:537.

Elmer, W. H. and Lacy, M. L. 1987a. Effects of inoculum densities of *Fusarium oxysporum* f. sp. *apii* in organic soil on disease expression in celery. Plant Dis. 71:1086-1089.

Elmer, W. H. and Lacy, M. L. 1987b. Effects of crop residues and colonization of plant tissues on propagule survival and soil populations of *Fusarium oxysporum* f. sp. *apii* race 2. Phytopathology 77:381-387.

Elmer, W. H., Lacy M. L., and Honma, S. 1986. Evaluations of celery germ plasm for resistance to *Fusarium oxysporum* f. sp. *apii* race 2 in Michigan. Plant Dis. 70:416-419.

Garibaldi, A. and Gullino, M. L. 1987. Attempts of biocontrol of *Fusarium* wilt of carnation in Italy. (Abstr.) Phytopathology 77:1721.

Hart, L. P. and Endo, R. M. 1978. The reappearance of Fusarium yellows of celery in California. Plant Dis. Reptr. 62:138-142.

Hopkins, D. L., Larkin, R. P., and Elmstrom, G. W. 1987. Cultivar-specific induction of soil suppressiveness to Fusarium wilt of watermelon. Phytopathology 77:607-611.

Ireland, K. F., Elmer, W. H., and Lacy, M. L. 1987. Field evaluation of celery germ plasm for resistance to *Fusarium oxysporum* f. sp. *apii* race 2. (Abstr.) Phytopathology 77:1712.

Ireland, K. F. and Lacy, M. L. 1986. Laboratory identification of pathogenic isolates of *Fusarium oxysporum* f. sp. *apii* race 2. (Abstr.) Phytopathology 76:956.

Jones, H. A., and Mann, L. K. 1963. Onions and Their Allies. Leonard Hill Books Limited, London. 286 pp.

Komada. H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-124.

Lewis, J. A. and Papavizas, G. C. 1971. Effect of sulfur-containing volatile compounds and vapors from cabbage decomposition on *Aphanomyces euteiches*. Phytopathology 61:208-214.

Lewis, J. A. and Papavizas, G. C. 1975. Survival and multiplication of soil-borne plant pathogens as affected by plant tissue amendments. In: Biology and Control of Soil-Borne Plant Pathogens. G. W. Bruehl (ed.) The American Phytopathological Society, St. Paul. 216 pp.

Lockwood, J. L. 1977. Fungistasis in soils. Biol. Rev. 52:1-43.

Lorenz, O. A. and Maynard, D. N. 1988. Knott's Handbook for Vegetable Growers. Third edition. John Wiley and Sons, New York. 456 pp.

Nelson, P. E., Toussoun, T. A., and Marasas, W. F. 1983. *Fusarium* species: An Illustrated Manual for Identification. The Pennsylvania State University Press. University Park, PA. 193 pp.

Opgenorth, D. C. and Endo, R. M. 1979. Sources of resistance to Fusarium yellows of celery in California. Plant Dis. Reptr. 63:165-169.

Opgenorth, D. C. and Endo, R. M. 1983. Evidence that antagonistic bacteria suppress Fusarium wilt of celery in neutral and alkaline soils. Phytopathology 73:703-708.

Papavizas, G. C. 1975. Crop residues and amendments in relation to survival and control of root-infecting fungi: an introduction. In: Biology and Control of Soil-Borne Plant Pathogens. G. W. Bruehl (ed.) The American Phytopathological Society, St. Paul. 216 pp.

Papavizas, G. C. and Lewis, J. A. 1971. Effect of amendments and fungicides on Aphanomyces root rot of peas. Phytopathology 61:215-220.

Paulitz, T. C. and Baker R. 1987. Biological control of Fusarium wilt of cucumber with nonpathogenic isolates of *Fusarium oxysporum* and strains of *Pseudomonas putida*. (Abstr.) Phytopathology 77:1707.

Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.

Ramirez-Villapudua, J. and Munnecke, D. E. 1987. Control of cabbage yellows (*Fusarium oxysporum* f. sp. *conglutinans*) by solar heating of field soils amended with dry cabbage residues. Plant Dis. 71:217-221.

Ryder, E. J. 1979. Leafy Salad Vegatables. Avi Publishing Company, Inc., Westport, Connecticut. 266 pp.

Scher, F. M. and Baker, R. 1980. Mechanisms of biological control in a Fusarium-suppressive soil. Phytopathology 70:412-417.

Schneider, R. W. 1984. Effects of nonpathogenic strains of *Fusarium oxysporum* on celery root infection by *F. oxysporum* f. sp. *apii* and a novel use of the Lineweaver-Burk double reciprocal plot technique. Phytopathology 74:646-653.

Sivan, A., Ucko, O., and Chet, I. 1987. Biological control of Fusarium crown rot of tomato by *Trichoderma harzianum* under field conditions. Plant Dis. 71:587-592.

Sneh, B., Dupler, M., Elad, Y., and Baker, R. 1984. Chlamydospore germination of *Fusarium oxysporum* f. sp. *cucumerinum* as affected by fluorescent and lytic bacteria from a Fusarium-suppressive soil. Phytopathology 74:1115-1124.

Toussoun, T. A. 1975. Fusarium-suppressive soil. In: Biology and Control of Soil-Borne Plant Pathogens. G. W. Bruehl (ed.). The American Phytopathological Society. St. Paul. 216 pp.

Ware, G. W. and McCollum, J. P. 1975. Producing Vegetable Crops. The Interstate Printers and Publishers, Inc. Danville, Illnois. 599 pp.

Yuen, G. Y., Schroth, M. N., and McCain, A. H. 1985. Reduction of Fusarium wilt of carnation with suppressive soils and antagonistic bacteria. Plant Dis. 69:1071-1075.