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DISSERTATION

FUSARIUM OXYSPORUM F. SP. *CEPAE*, THE CAUSAL AGENT OF FUSARIUM BASAL ROT OF ONION: BIOLOGY, EPIDEMIOLOGY AND MANAGEMENT.

Submitted by

Curtis E. Swift

Department of Bioagricultural Sciences and Pest Management

In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

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COLORADO STATE UNIVERSITY

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY CURTIS E. SWIFT, ENTITLED "*FUSARIUM OXYSPORUM* F. SP. *CEPAE*, THE CAUSAL AGENT OF FUSARIUM BASAL ROT OF ONION: BIOLOGY, EPIDEMIOLOGY AND MANAGEMENT.

Committee on Graduate Work

Michael E. Bartolo
Outside Committee Member

Committee Member

Howard F. Schwartz
Coadviser

William M. Brown
Coadviser

Thomas O. Holtzer
Department Head

ABSTRACT OF DISSERTATION

FUSARIUM OXYSPORUM F. SP. *CEPAE*, THE CAUSAL AGENT OF FUSARIUM BASAL ROT OF ONION: BIOLOGY, EPIDEMIOLOGY AND MANAGEMENT.

The bulb onion (*Allium cepae* L.) is an important food crop having been used for over 5000 years. *Fusarium oxysporum* Schlecht. f. sp. *cepae* (Hanzawa) Snyder & Hansen (*F. o. cepae*), the causal agent of Fusarium basal rot of onion bulbs, is present in most parts of the world where onions are grown and is a major disease problem of this crop. This pathogen causes up to 90% loss of seedlings, and 30% loss of stored bulbs. This dissertation reports research conducted on the biology, epidemiology and management of *Fusarium oxysporum* f. sp. *cepae* in Colorado.

Three Vegetatively Compatible Groups of *Fusarium oxysporum* f. sp. *cepae* were identified in Colorado. One VCG has a wide ranging presence in Colorado, one is restricted to Delta County, and the third is present in Mesa County. Two additional VCGs identified are single isolate VCGs.

Up to one hundred and thirty cfu's of Fusarium were present in each milliliter of

irrigation water. Fusarium contaminated irrigation water can infest fields previously free of *F. o. cepae*. Care must be taken to prevent tailwater from infested fields from entering irrigation water supplies.

Total *Fusarium* and *F. o. cepae* populations varied between year and location in Fusarium contaminated fields. Propagule numbers were greatest in fields planted to onions than fields planted to pinto beans. Total *Fusarium* populations was as high as 7900 cfu's gram^{-1} of native soil.

Greenhouse trials of metalaxyl, imazalil, fosetyl-aluminum/iprodione, and thiophanate methyl, resulted in significantly fewer living plants than untreated checks. This may have been due to phytotoxic effects of these materials when applied to steam-sterilized soil.

Seedling emergence was significantly greater with metam-sodium treatments than other treatments evaluated. There was no significant difference between band-applied rates of 280.7 and 327.4 liters hectare^{-1}. Post-emergence treatments of thiophanate-methyl, metalaxyl, and fosetyl-aluminum/iprodione are ineffective in reducing Fusarium basal rot or increasing yield.

Metam-sodium, band-applied at 223.8 and 280.7 liters hectare^{-1}, and broadcast-applied at 701 liters hectare^{-1}, resulted in significant increased yields of marketable onions.

Metalaxyl consistently resulted in reduced marketable yields due to reduced emergence and seedling survival.

Curtis Elmer Swift
Department of Bioagricultural Sciences and Pest Management
Colorado State University
Fort Collins, CO 80523
Spring 2001

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Literature Review

The Genus *Fusarium* Link:Fr. with Special Emphasis on *Fusarium oxysporum* Schlect. end.:Fr.

Basics

Members of the genus Fusarium are some of the most important plant pathogens in the world (Nelson et al., 1983). They attack a wide range of major food and fiber crops which include wheat (Burgess et al., 1975), grain sorghum (Burgess and Trimboli, 1986; Nelson et al., 1987), millet (Marasas et al., 1987), sweet corn (Lawrence et al., 1981), field corn, rice, banana, potato, and cotton (Burgess et al., 1982; Nelson et al., 1981). The surfaces of roots of both host and nonhost plants can be colonized (Bell and Mace, 1981). Fusarium are well adapted to life on earth and can be found in soil, water and air, on subterranean and aerial plant parts, as well as in plant debris and other organic substrates (Burgess, 1981; Burgess et al., 1985; Marasas et al., 1985; Snyder, 1981).

Gilman found that more than half of the recorded soil-inhabiting species of fungi belong

to some ten genera, of which *Penicillium*, *Fusarium*, *Mucor*, and *Aspergillus* contain the major part of these species (Burges, 1965). When economic plant species are evaluated, wilt diseases caused by species of the genera *Fusarium* and *Verticillium* are the most numerous. In some cases, losses due to vascular wilt diseases are often such that it is no longer profitable, or sometime even possible, to continue to grow the crop (Green, 1981).

Fusarium species are even found in extreme environments ranging from the desert to the arctic (Joffe and Palti, 1977; Nelson, 1990). The Fusarium consist of both soil-borne and air-borne species. Some soil-borne species are even adapted to dispersal in the atmosphere (Burgess, 1981). The Fusarium consist of four main groups: plant pathogens (including mycoparasites), insect pathogens, saprophytes, and soil inhabitants (Booth, 1971). A few species bridge the gap attacking both plants and insects (Price, 1984). None are obligate parasites (Burnett, 1984).

members of the genus *Fusarium* have no known sexual stage. Those that do have a sexual stage include *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. [sexual stage *Nectria haematococca* Berk. & Br.], *Fusarium graminearum* Schwabe [sexual stage *Gibberella zeae* (Schw.) Petch], and *Fusarium moniliforme* (Sheld.) emend. Snyd. & Hans. [sexual stage *Gibberella fujikuroi* (Sawada) Wr.] (Booth, 1981; Nelson et al., 1983).

Fusarium oxysporum Schlecht. emend. Snyd. & Hans.[1], a common soil-borne[2] Fusarium with a worldwide distribution, has no known sexual stage. This organism probably causes more economic damage than any other plant pathogen (Correll, 1991), and has been shown to constitute as much as 80 - 90% of the total fungal microflora in the rhizosphere of several agricultural crops (Elias et al., 1991). It is responsible for wilt and cortical rot diseases of more than 100 economically important plant hosts (Booth, 1971). While it has one of the broadest host ranges of any known fungal plant pathogen, the ability of individual *Fusarium oxysporum* strains to cause vascular wilt diseases are host specific and are limited to only one or a few kinds of plants (Kistler and Momol, 1990). Wilt Fusarium also may invade plants without causing external symptoms[3] of disease (Armstrong and Armstrong, 1948).

The phrases 'without symptoms' and 'symptomless carrier' were discussed by Price in 1984. The common definition used with these two phrases is 'a lack of external symptoms'. His discussion, however, indicates two possible meanings. First, a plant

---

[1] Based on Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1983. *Fusarium* Species: An Illustrated Manual for Identification. Pennsylvania State Univ. Press, University Park.

[2] Burgess thought the term 'Soil-based' more appropriate. Typical representatives of these 'soil-based' Fusarium include *Fusarium oxysporum, F. solani, F. roseum (culmorum)* and *F. roseum* 'Graminearum' Group 1 and *F. roseum* 'Equiseti' (Burgess, 1981).

[3] Symptoms - the qualitative changes that distinguish diseased from healthy plants (Bald, 1970).

which is actively invaded but does not exhibit symptoms. Second, a plant or plant part which carries the fungus externally.

Fusarium oxysporum has the ability to colonize many substrates (Burgess, 1981) and over 120 *formae speciales*[4] and races[5] have been identified (Armstrong and Armstrong, 1981) with each *forma specialis* having a limited host range (Vazquez et al., 1993). *F. oxysporum* is highly variable with some very pathogenic strains causing wilts and crown and root rots. Others are vigorous saprophytes and common colonizers of senescent or damaged plant tissue (Armstrong and Armstrong, 1975; Jarvis and Shoemaker, 1978; Kommedahl et al., 1975) and are well adapted for survival in soil (Paulitz et al., 1987). The propagules of *F. oxysporum* f. sp. *raphani* are able to pass through the alimentary canals of earthworms despite enzymatic activity. Propagules were found in the gut of earthworms as well as in 26 out of 28 worm casts (Toyota and Kimura, 1994).

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[4] *Forma specialis* - (pl) *formae speciales* - "a taxon characterised from a physiological (especially host adoption) standpoint but scarcely or not at all from a morphological standpoint" (Federation of British Plant Pathologists, 1973).

[5] Race - variants which differentially attack specific cultivars of a crop (Mount and Lacy, 1982, page 10).

Methods of Classifying *Fusarium*

The classification[6] of *Fusarium* began in 1809 when Link proposed *Fusarium* as a genus containing fungal species with fusiform, nonseptate spores borne on a stroma (Booth, 1971). In 1821, Fries included *Fusarium* in the order Tuberculariaceae based on the International Botanical Code (Booth, 1971).

In 1910, Appel and Wollenweber divided the genus into sections or groups consisting of 16 groups (sections), 6 subgroups and 142 species, varieties and forms. They used the words 'section' and 'group' indiscriminately. Their paper "Grundlagen einer Monographie der Gattung *Fusarium* (Link)" (Appel and Wollenweber, 1910) established them as the founders of the present system of classification of *Fusarium* (Booth, 1971).

Wollenweber (1913) excluded all fungi having straight macroconidia from the genus *Fusarium*, placing such fungi in the genera *Ramularia* and *Cylindrocarpon* (Wollenweber, 1913). In 1915, Sherbakoff described macroconidia of the genus "as sickle-shaped septate (with usually 3 or more septa) apically pointed, mostly pedicellate".

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[6] "Taxonomy should endeavor to reflect phylogeny but with the virtual absence of a fossil record of microorganisms, fungal taxonomists are working on speculation. However, supplementing morphological knowledge with the results of nutritional and biochemical studies, it is possible to devise arrangements which correspond to the current beliefs of possible evolutionary sequences" (Ainsworth, 1973).

Wollenweber et al. (1925), and Wollenweber and Reinking (1935) further revised the Appel-Wollenweber taxonomy system. They began with 1000 species named up to that time and reorganized them into 16 sections consisting of 65 species, 55 varieties and 22 forms (Joffe, 1986).

The following set of characteristics was used to separate each group/section (Nelson, 1991):

1. Presence or absence of microconidia
2. Shape of microconidia
3. Presence or absence of chlamydospores
4. Whether chlamydospores were intercalary or terminal
5. Shape of macroconidia[7]
6. Shape of the basal or foot cells of macroconidia

The sections were further divided into species, varieties and forms on the basis of:

1. Color of stroma
2. Presence or absence of sclerotia
3. Number of septations in macroconidia
4. Length and width of macroconidia (Nelson, 1990).

[7] Macroconidia may be 0-10 or more septate, and straight to curved (Booth, 1971).

Wollenweber and Reinking's 1935 publication is now considered the standard reference work on this genus with all 'new' or 'modern' systems for the taxonomy of *Fusarium* based on this publication (Nelson, 1990).

The 16 sections of the Wollenweber and Reinking system are (Nelson, 1990):

Eupionnotes

Macroconia

Spicarioides

Submicrocera

Pseudomicrocera

Arachnites

Sporotrichiella

Roseum

Arthrosporiella

Gibbosum

Discolor

Lateritium

Liseola

Elegans

Martiella

Ventricosum

Wollenweber and Reinking (1935) may not have recognized cultural mutations[8] when developing their system of taxonomy as their cultures were not derived from single spores and many of their forms and species may have been cultural mutations. In addition, some of their species may have been based on only one or two cultures (Nelson, 1990). Mutations are common to all fungi and ultimately underlie most of the observed variation. This extensive genetic diversity is characteristic of most, if not all, root-infecting fungi (Parmeter, 1970).

Snyder and Hansen (1940) revised the Wollenweber and Reinking classification lumping many of their species together. They lumped all species and varieties from the Elegans[9] section into the species *F. oxysporum* (Snyder and Hansen, 1940), and later reduced all *Fusarium* to nine species (Snyder and Hansen, 1941 and 1945). Wollenweber and Reinking's Roseum, Arthrosporiella, Gibbosum and Discolor sections were combined into the species *Fusarium roseum*. Sections Martiella and Ventricosum were combined into *Fusarium solani* (Nelson, 1990). According to Booth (1971 and 1984) there was no justification for lumping so many species into one. Due to their propensity to group species together, Snyder and Hansen are considered to be the ultimate "lumpers" (Nelson, 1990).

---

[8] Mutation - "any heritable change in the genetic material" (Hartl, 1980, page 170).

[9] Hansford (1926) was the first to express the idea of combining all species and varieties of the section Elegans into the species *Fusarium oxysporum* (Armstrong and Armstrong, 1981).

Snyder and Hansen's system, known as the nine species system, introduced the term *formae speciales* to differentiate differences in pathogenicity[10] within species. The concept of limited host specificity of the 25 *formae speciales* listed by Snyder and Hansen in their revision of section Elegans was established on the basis of limited or highly selective pathogenicity (Snyder and Hansen, 1940). Initially it was believed that a *forma specialis* was specific to one host and hence the name was taken from the host, e.g. *betae, callistephi, apii,* etc. (Booth, 1971). Differences in two or more hosts in the degree of virulence[11] of the pathogen, rate of disease development and type of symptoms produced have been the main criteria used to separate *formae speciales* and races since the term was first proposed by Snyder and Hansen (Armstrong and Armstrong, 1966).

Though the use of *forma specialis* by Snyder and Hansen implies pathogenicity to a specific plant or related groups of plants (i.e., f. sp. *cerealis*) this is not necessarily correct (Tammen, 1958). Even strains in pure culture can change in pathogenicity (Kistler and Miao, 1992). While limited pathogenic tests were initially conducted and used in defining *formae speciales* and races, some f. sp. of *Fusarium* are pathogenic on 50 different genera, species, or cultivars of plants (Armstrong and Armstrong, 1975). When the concept of *forma specialis* was first devised and applied to *Fusarium* species, narrow

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[10] Pathogenicity - the ability of a microorganism to cause disease. Virulence is a component of pathogenicity (Shaner et al., 1992).

[11] Virulence - the ability of a pathogen to overcome the resistance of its host (Bell and Mace, 1981).

host ranges were implied or imagined. In some respects, this is correct (or at this moment is correct) for a few *formae speciales* (Price, 1984). In reality, host specificity occurs for some but not all *formae speciales*. Some *formae speciales* are host specific while others have an extremely wide range of hosts (Armstrong and Armstrong, 1975 and 1981). Armstrong and Armstrong (1975) found the host range of the cotton wilt Fusarium, *Fusarium oxysporum* f. sp. *vasinfectum* encompassed plants in the families Malvacea, Solanacea, and Leguminosae. Problems with symptomless hosts further compounds the problem of how the term *forma specialis* should be used (Armstrong and Armstrong, 1981).

In 1957, Snyder et al. proposed the adoption of the non-botanical concept of cultivar based on differing conidial morphology. Morphologically different strains of *F. roseum* were given cultivar names such as *Fusarium roseum* 'Graminearum' or, if pathogenic, *F. roseum* f. sp. *cerealis* cultivar such as 'Graminearum' and 'Culmorum'. Nash and Snyder (1965) converted three species named by Wollenweber into *F. roseum* cultivars accordingly:

> *F. heterosporium* was converted into *F. roseum* cultivar 'Heterosporium'. This cultivar was described by Nash and Snyder as bearing numerous large sclerotia on a white mycelial mat.
>
> *F. sambucinum* was converted into *F. roseum* cultivar 'Sambucinum'. This was described as having small conidia with numerous yellow sporodochial masses on

a non-carmine colony.

F. reticulatum was converted into *F. roseum* cultivar 'Reticulatum'. This was described as a small-conidial type having carmine pigmentation.

Nash and Snyder (1965) also named a new cultivar ('Redolens') for *F. oxysporum*. The concept of cultivar, however, was never fully explained or finalized (Nelson, 1990).

Other systems have been proposed by Raillo (1936 and 1950), Bilai (1978), Gerlach (1981), Gordon (1956), Booth (1971), Matuo (1972) and Joffe (1986). Gordon's system was a compromise between that of Wollenweber and Reinking, and Snyder and Hansen. Booth modified Gordon's system and added information based on his own studies (Nelson, 1990). Joffe (1986) developed his taxonomic system based on morphological, cultural, physiological and toxicological characteristics. According to Nelson et al. (1983), the continued proliferation of "new" or "modern" systems for the taxonomy of *Fusarium* species will not solve the problem but unfortunately only add to the current state of confusion.

Nelson et al. (1983) combined the best parts of each system with the results of their own research to produce what Nelson (1990) reports is a compromise system. These researchers utilized *Fusarium oxysporum* and *Fusarium solani* as described by Snyder and Hansen (1940 and 1941), as well as information regarding conidiophore and conidiogenous spores as provided by Booth (1971).

In the 'Current Status of *Fusarium* Taxonomy', Windles (1991) discussed gaps and problems with the current system of Fusarium taxonomy covering an anamorph-teleomorph connection, section relationships, species delimitation, mutational variants, and subgroup identification. She indicates the nomenclature is further confused by the fact that some species have characteristics of two different sections. *Fusarium nygamai* Burgess and Trimboli forms short chains of microconidia which places it in the section *Liseola*; it also forms chlamydospores which places this species in section *Elegans*. Section, species and variety within the genus *Fusarium* are recognized by the International Code of Botanical Nomenclature (based on morphology). *Formae speciales* and races (based on physiology) and vegetative compatibility grouping (based on genetics) are not recognized by the code (Windels, 1991). Nelson's historical account (1991) provides additional details on the history of the nomenclature of *Fusarium*.

Identification of Fusarium Species

All *Fusarium* species have one taxonomic feature in common: production of a distinctly shaped macroconidia, usually with a foot-shaped basal cell, when produced in sporodochia (Booth, 1984). The presence of fusoid macroconidia with a foot cell bearing some kind of heel is still accepted as the most definitive character of this genus.

Perithecia states, where known, belong to the Hypocreales[12]. The teleomorphs[13] include the genera *Gibberella* and *Nectria* (Gams and Nirenberg, 1989), as well as *Hyphomyces* and *Calonectria* (Messiaen and Cassini, 1968). It has been assumed that *Fusarium* spores are phialospores; phialospores[14] and blastospores[15] occur within the genus. Even in species with only phialidic conidiogenous cells, the structure is often used to separate the species. Thus the microconidiophore structure provides the easiest means of separating *F. oxysporum* from *F. solani*, and the polyphialides of *F. moniliforme* var *subglutinans* readily separate it from *F. oxysporum* without having to wait for chlamydospore development. The blastospores of *F. sporotrichioides* readily separate this species from *F. tricinctum* (Booth, 1971).

Microconidia of *F. oxysporum* form on short conidiophores with no septum, while the microconidia of *F. solani* develop on long conidiophores which have a septum (Matuo, 1972). *F. oxysporum* produces microconidia in false heads on monophialides (Marasas et al., 1985). The conidiophores of *Fusarium solani* can be as tall as the length of the

---

[12] Hypocreales - an order within the subdivision Ascomycotina having perithecia which are light-colored, red or blue (Agrios, 1997).

[13] Teleomorph - the name used to describe the sexual stage of some fungi (VanDemark and Batzing, 1986).

[14] Phialospores - these conidia form from an open apex of a conidiophore or conidiogenous cell (phialide), which ordinarily does not increase in length. These conidia often collect in drops of slime or mucilage or form chains of conidia.

[15] Blastospores - conidia developing as buds from simple or branched conidiophores, or directly from vegetative cells or previous conidia (Barnett and Hunter, 1998).

macroconidia by a few times (Watanabe, 1994).

Microconidia and terminal or intercalary chlamydospores[16] may be present or absent. In some cases, single spore cultures may form chlamydospores only after 4 to 6 weeks. This late development may result in misidentification (Klotz et al., 1988). In addition to producing a chain of microconidia, *F. moniliforme* lacks the chlamydospores of *F. oxysporum* and *F. solani* (Booth, 1971). Chlamydospores produced by *F. oxysporum* are usually borne singly (Marasas et al., 1985).

Physiological and Pathological Variants

Adaptation to drastic environmental changes is necessary for survival, thus a source of genetic variability in fungi is essential (Klittch and Leslie, 1988). *Fusarium oxysporum* f. sp. *dianthi*, like other *Fusarium*, has the ability to produce new physiological races as well as pathogenic variants (Scovel, 1987). Little is known about the spontaneous origin of new races of *Fusarium* in nature except that they arise irregularly, unpredictably and appear to be discrete events (Burnett, 1984). Abawi and Lorbeer (1971a), however,

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[16] Schippers and van Eck (1981) feel the 'double wall' should not be considered as an identifying criteria as the primary wall often quickly disappears leaving the newly excreted cell wall. They therefore define a chlamydospore as follows: " a viable, asexually produced accessory spore resulting from the structural modification of a vegetative hyphal segment(s) or conidial cell possessing a thick wall mainly consisting of newly synthesized cell wall material; its function is primarily survival in soil".

observed hyphal anastomosis[17] of *Fusarium oxysporum* f. sp. *cepae* in onion tissue and felt this may partially account for the presence and isolation of cultural variants from infected tissue. Because of this capacity for rapid morphological and physiological change, species identification presents problems. Snyder and Hansen (1940) reported that *Fusarium* is known the world over as the most difficult fungus genus to identify down to species.

When maintained on synthetic media there is a general degradation of growth and morphology (Booth, 1971). Resulting isolates may differ from the original colony in morphology, virulence, and other characteristics (Burnett, 1984; Puhalla, 1981). The sporodochial type was most virulent; ropy and pionnotal types were intermediate in virulence; and the mycelial type was least virulent (Abawi, 1971). Patch mutants, described as areas of abnormal growth in otherwise uniform colonies, may appear after a few weeks of growth on Potato Dextrose Agar (PDA) (Lawrence et al., 1986).

The majority of *Fusarium* species, when first isolated, produce macroconidia on sporodochia. Nelson reported that in culture these mutate to either the mycelial[18] or

---

[17] Anastomosis - the union of hypha resulting in the intercommunication of their contents (Agrios, 1997).

[18] Mycelial - produce abundant mycelium but few macroconidia, and are often white with no distinguishing features (Windels, 1991).

pionnotal[19] type. In both cases, these variants may lose virulence and the ability to produce toxins (Nelson, 1990).

Abawi (1971) reported three morphological types developing in culture from the sporodochial form; ropy, pionnotal and mycelial. The isolation of only the sporodochial type of *F. oxysporum* f. sp. *cepae* from naturally infested field soil led Abawi to the conclusion that these cultural variants either do not exist in field soil or exist in the soil at extremely low levels.

Mycelial cultures are less stable than pionnotal cultures and produce fewer macroconidia on CPDA[20] and chlamydospores in soil culture (Awuah and Lorbeer, 1988). The percentage of mutants was medium dependent with the highest percentage of mutations occurring when PDA was used as the medium (Lawrence et al., 1986). Cormack (1951) found that up to 12% of single spore cultures derived from a sporodochial colony were pionnotal. The mutational forms have not been known to revert to the sporodochial (wild parental) type (Abawi, 1971; Windels, 1991).

Mutations increase when *Fusarium* species are grown on PDA and similar media.

---

[19] Pionnotal - produce little or no aerial mycelium but abundant macroconidia on a slimy effuse sporodochium of simple conidiophores; the macroconidia may be distorted (Windels, 1991).

[20] CPDA - chloroamphenicol-amended potato dextrose agar (Awuah and Lorbeer, 1988).

Transfers made from such cultures gradually or suddenly sector and mutate (Nelson et al., 1983). On the whole, the higher the C and N content of the medium, the higher the frequency of sectoring (Burnett, 1984). The spontaneous appearance of sectors in cultures of *Fusarium* species was referred to by early researchers as 'saltations' rather than mutations, as the genetic basis of the altered phenotype[21] was unknown (Leslie, 1990).

A medium with a low C/N ratio is reported to produce short spores with low septation. Conversely a high C/N ratio results in long spores with increased septation. Neutral phosphate in the medium increases sporulation and diminishes aerial mycelium, whereas acid phosphate is known to have the opposite effect (Brown, 1922; Brown and Horne, 1926). Brown (1922) found spores formed short stunted germ tubes when acid medium conditions developed due to high carbon dioxide levels.

Mycelial cultures of *Fusarium oxysporum* f. sp. *apii* were inhibited when incubated under a 12 to 24 hour photoperiod, while darkness and diffuse sunlight favored the development of mycelial cultures. Under these conditions, pionnotal cultures were favored. Awuah and Lorbeer (1989) also found the transfer of mycelial cultures from a dark to light condition resulted in the development of an inner mycelial zone and an outer pionnotal zone.

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[21] Phenotype refers to the physical appearance of an individual (Hartl, 1980, page 8).

Isolates adapt both their form and color in response to the presence of the cultural environment to include the pH of the medium used (Brown and Horne, 1926), therefore culture medium must be standardized and environmental conditions must be kept uniform for identification proposes (Awuah and Lorbeer, 1989).

Cultural mutations are kept to a minimum by transferring cultures using either the single-spore or hyphal-tip method, using media low in carbohydrate, and limiting the number of transfers (Nelson, 1990; Nelson et al., 1983). The most stable colonies are those propagated either by hyphal tips transferred at 4-day intervals, or by microconidia at about 20-day intervals (Burnett, 1984).

The identification of *Fusarium* species should be based on pure cultures from single spores (Booth, 1971) or from a hyphal tip (Nelson et al., 1983). Both Carnation Leaf Agar (CLA) and potato-dextrose agar (PDA) prepared from fresh potatoes, are necessary for identifying *Fusarium* cultures (Windels, 1991). Fisher et al. (1982), however, report that PDA may delay the formation of sporodochia and result in atypical, nonuniform, misshapen conidia creating problems with species identification. Carnation leaf agar (CLA) promotes good growth, sporulation , and maintenance of the original cultural type of *Fusarium* isolates. CLA promotes sporulation in 5-10 days (Tio et al., 1977; Toussoun and Nelson, 1976).

Major Soil-borne Fungal Pathogens of Onions

Root diseases are a major source of loss of *Allium* crops throughout the world (Entwistle, 1990). Fungal root pathogens of onion include but are not limited to [22]:

> Pink root caused by *Phoma terrestris* E.M. Hans. [syn. *Pyrenochaeta terrestris* (E.M. Hans.) Gorenz, J.C. Walker, & R.H. Larson],
>
> Fusarium basal rot caused by *Fusarium oxysporum* (Schlectend.:Fr. f. sp. *cepae* (H.N. Hans.) W.C. Snyder & H.N. Hans.,
>
> White rot caused by *Sclerotium cepivorum* Berk,
>
> Smut caused by *Urocystis colchici* (Schlechtend.) Rabenh.,
>
> Damping off caused by various *Pythium* species,
>
> Damping off caused by *Rhizoctonia solani* Kühn,
>
> Smudge caused by *Colletotrichum circinans* (Berk.) Voglino (syn. *Vermicularia circinans* Berk. and *C. dematium* (Pers.) Grove f. *circinans* (Berk.) Arx),
>
> Southern blight caused by *Sclerotium rolfsii* Sacc.,
>
> and Charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goidanich (syn. *Sclerotium bataticola* Taubenhaus).

Schwartz and Mohan (1995) also include Sclerotinia rot caused by *Sclerotinia sclerotiorum* (lib.) De Bary as a root pathogen of onions. This disease, however, is not important enough on onion to justify that control measures be implemented.

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[22] The complete names are in accordance with Schwartz and Mohan. 1995. Compendium of Onion and Garlic Diseases. APS Press.

Fusarium Basal Rot - *Fusarium oxysporum* f. sp. *cepae*

Introduction

The current approved name for the causal agent of Fusarium Basal Rot of onions is *Fusarium oxysporum* Schlecht. emend.:Fr. f. sp. *cepae* (H.N. Hans.) W.C. Snyder & H.N. Hans.[23]. The complexity of *Fusarium* taxonomy, however, has resulted in a variety of synonyms for the same pathogen (Entwistle, 1990). The following names have been used for this organism:

F. cepae Hanz. (Snyder and Hansen, 1940)

F. cepae (Hanz.) emend. Link and Bailey (Link and Bailey, 1926)

F. cepae (Hanz.) emend. Walker and Tims (Walker and Tims, 1924)

F. oxysporum Schlect. forma 7 Wollenweber (Snyder and Hansen, 1940)

F. oxysporum Schlect. emend. Snyder and Hansen f. sp. *cepae* (Hanzawa) (Entwistle, 1990)

F. vasinfectum Atkinson v. *zonatum* (Sherbakoff) f.1 (Link and Bail.) Wollenw. (Snyder and Hansen, 1940)

F. vasinfectum Atk. v. *zonatum* (Sherbakoff) f.2 (Link and Bail.) Wollenw. (Snyder and Hansen, 1940)

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[23] According to the Common Names of Plant Diseases, APSNet - The American Phytopathological Society. This name is also used on page 10, Compendium of Onion and Garlic Diseases. 1995. Schwartz, H.F., and Mohan, S.K., eds. APS Press, St. Paul, MN.

F. zonatum (Sherb.) Wollenw. (Sherf and McNab, 1986)

F. zonatum (Sherb.) Wollenw. forma 1 (Davis and Henderson, 1937)

F. zonatum (Sherb.) Wollenw. f.2 Link and Bail. (Link and Bailey, 1926)

Reports of basal rot on onions was reported by Selby in Ohio in 1910 (Selby, 1910), and in Connecticut in 1915 (Clinton, 1916). In 1920, Link found serious losses in onion fields near Grand Junction and Delta, Colorado. During the preceding winter, Link reported very heavy losses of stored onions from these basal rot-infected Colorado fields (Link and Bailey, 1926).

Fusarium oxysporum Schlecht. f. sp. *cepae* (Hanzawa) Snyder & Hansen (*F. o. cepae*) is present in most parts of the world where onions are grown (Everts et al., 1985). It has been reported as causing substantial onion losses in South Africa (Holz and Knox-Davies, 1974), Greece (Aganostopoulus, 1932), Tanganyika (Anonymous, 1958), Australia (Pugsley, 1938), Ireland (McKay, 1953), the Philippines (Palo, 1928-1929) and Canada (Woolliams, 1928). Up to 90% loss of seedlings (Davis and Henderson, 1937) and 30% loss of stored bulbs have been reported (Entwistle, 1990).

In the Rocky Mountain region, *Fusarium* basal rot is widespread but rarely causes severe losses except in irrigated valleys (Walker, 1919). Losses in British Columbia range from 10 to 60 percent (Woolliams, 1928).

The bulb onion (*Allium cepae* L.) is an important food crop consumed in most areas of the world (Maude, 1990). Of the 600 species of *Allium*, the bulb onion is the most widely known edible *Allium* having been in use for at least 5000 years (Hanelt, 1990; Jones, 1990). Over 25 million metric tons were harvested around the world in 1988 with the United States reported as the fourth highest producer behind China, India, and the USSR (Rabinowitch and Brewster, 1990). International trade in bulb onions approaches 2 million metric tons annually worth (US) $400 million (Brewster, 1994). Colorado's 1998 onion harvest was valued at $77.8 million (Colorado Ag Stats, 1999).

Colorado plants more than 5,000 hectares each year making this state one of the largest onion producing states in the United States (Schwartz et al., 1990). Most of the Colorado acreage is devoted to storage onions valued between 40 and 50 million dollars annually. Yields average approximately 450 kgs hectare^{-1} unless damaged by pathogens or environmental factors (Schwartz et al., 1991). Over 6250 hectares of onions were harvested in Colorado in 1997. Onions represented 50% of the total acreage of vegetables for which statistics are maintained and 46% of the value of the vegetable crops reported by the Colorado Department of Agriculture in 1997 (Colorado Ag Stats, 1998). In 1998, 6,680 hectares of onions were planted in Colorado of which 6,478 hectares were harvested (Colorado Ag Stats, 1999). Diseases which infect this crop are obviously of major importance.

Symptoms of Bulb Decay

F. o. cepae causes a wilt in the field and dry rot in storage (Holz and Knox-Davies, 1974). The fungus attacks seedlings causing pre- and post-emergence damping off as well as root rot of older plants, stem plate discoloration and basal rot of onions in the field, as well as in storage (Abawi, 1971). *Fusarium* was reported to attack onions only as wound parasites (Link and Bailey, 1926; Woolliams, 1928). The Armstrongs were the first to report wilt *Fusarium* may invade plants without causing external symptoms of disease (Armstrong and Armstrong, 1948). Other researchers have reported direct parasitism of onion by *Fusarium* spp. without prior damage (Palo, 1928-1929), from infected roots (Abawi, 1971; Abawi and Lorbeer, 1971a), as well as through wounds and in the vicinity of old root scars at the base of the bulb (Davis et al., 1998). *F. o. cepae* isolates used by Everts et al. (1985) caused disease on wounded as well as unwounded bulbs. Isolates are reported to exist which infect only through wounded tissue (Link and Bailey, 1926). Davis and Henderson (1937) state that *Fusarium zonatum forma 1* , a synonym for *F. o. c.*, "will not ... attack the roots or bulbs, except following injury or after the initial invasion by another pathogen, such as *Phoma terrestris*."

Onion root and stem plate infections can occur under field conditions throughout the season (Abawi and Lorbeer, 1971c). *F. o. cepae* usually invades roots, stem plate (modified stem), and fleshy leaf bases, in that order (Abawi and Lorbeer, 1971a). Root and stem plate infections can reach as high as 62.5% by mid to late July in New York

(Abawi, 1971).

Movement of the fungus from stem plate to bulb scale tissue is essential for development of the basal rot stage of the disease (Abawi and Lorbeer, 1971c). Occasional invasion of bulb scales in contact with soil occurs when propagule levels in the soil are high (Abawi and Lorbeer, 1971a).

The percentage of discoloration of the stem plate prior to and at harvest does not correlate with the percentage of bulb decay in storage (Abawi and Lorbeer, 1971c). Apparently healthy onions in Tempe, Arizona developed Fusarium basal rot when stored for 3 months at 23.9 to 29.4° C. (Marlatt, 1958). Davis and Henderson (1937) reported that 30% of bulbs which matured in an Iowa field showed evidence of dry-rot lesions, while another 15% of the apparently healthy bulbs developed Fusarium bulb rot in storage. Sumner et al. (1991) reported less than 3% of bulbs going into controlled atmospheric storage exhibited symptoms of internal decay, while 22 percent of these bulbs were infected with the fungus. *F. oxysporum* and *F. moniliforme* accounted for 12 and 3 percent of the total fungi in infected bulbs, respectively. The other fungi infecting these bulbs were *Penicillium digitatum* (53.8%), *Aspergillus niger* (30.9%), *Trichoderma* spp. (1.3%), and *Epicoccum* spp. (1.3%). Onion samples analyzed after storage indicated a significant decrease in *A. niger* but no difference occurred with the other fungi. In addition, the percentage of bulbs with internal discoloration before and after storage was equivalent. This supports Maude's conviction (1990) that there is no evidence the disease

spreads from bulb to bulb in storage.

Initial infection of onion by *F. o. cepae* in all commercial onion varieties in New York under field conditions occurs 4 to 8 weeks after planting. This delayed seedling infection by the fungus under normal New York field conditions is believed to be regulated by temperature control of pathogen growth (Abawi and Lorbeer, 1971c). Davis et al. (1998) report *F. o. cepae* is favored by soil temperatures in the range of 13.9° to 32.2° C., with optimal temperatures being 26° to 27.8° C. No growth occurred on petri plates incubated at 0, 3, 6, 9 or 36°C (Abawi, 1971).

Onion Resistance/Susceptibility

Onion varieties vary considerably in their resistance to infection (Holz and Knox-Davies, 1974) but susceptibility tends to change from year to year (Lorbeer and Stone, 1965). Differences in the percentage of bulb decay among varieties may occur after several months of storage (Abawi and Lorbeer, 1971c).

Holtz and Knox-Davies (1974) found resistance to *Pyrenochaeta* (= *Phoma*) *terrestris*, the causal agent of Pink Root of onion, provided adequate protection against invasion by *F. o. cepae*. Research in 1962 (Kehr et al., 1962), 1965 (Lorbeer and Stone, 1965), and 1990 and 1991 (Thornton et al., 1992a; Thornton et al., 1992b) found Pink Root and *Fusarium* basal rot were independent and no cause and effect relationship between these

two disease pathogens existed. Onions resistant to both pathogens singularly are resistant to them in combination. The combined occurrence of both pathogens generally produces more injury and loss than attack by either organism alone (Kehr et al., 1962).

Nonpathogenic or less virulent *Fusarium* spp. may suppress several *formae speciales* of *F. oxysporum* in different plants (Postma and Rattink, 1992). Cuttings of susceptible tomatoes exposed to suspensions of *F. o.* f. sp. *lycopersici* and *F. o.* f. sp. *pisi* before planting were less susceptible to *F. o. lycopersici* as the concentration of *F. o. pisi* was increased (Langton, 1969). Similar results were obtained with muskmelon inoculated with virulent and nonvirulent strains of *F. oxysporum* f. sp. *melonis* (Meyer and Maraite, 1971). Previous research with non-pathogenic *Fusarium* isolates resulted in different degrees of disease suppression (Postma and Rattink, 1991). Further research in 1992, however, found systemic resistance did not develop when nonpathogenic *Fusarium* were used (Postma and Rattink, 1992).

Disease resistance may differ depending on the stage of growth. Research with Asiatic hybrid lilies demonstrated that different developmental stages of the bulb determined the plant's resistance to *Fusarium oxysporum* (Straathof and Löffler, 1994). Claudius and Mehrotra (1973) found that *F. oxysporum* f. sp. *lentis* was inhibited by exudates from lentil (*Les culinaris medic*) roots. These exudates (glycine and phenylalinine) were produced as the plant aged. Young plants did not produce these inhibitory substances.

Abawi and Lorbeer (1971c) speculated that tolerant onion cultivars differ from susceptible ones by possessing factors which restrict further advance of the fungus. Tolerant cultivars and others which were intermediately susceptible to the fungus were either lines of *A. fistulosum* or an interspecific hybrid of *A. cepa* and *A. fistulosum*. All commercial onion varieties tested (lines of *A. cepae*) were susceptible. No anatomical differences existed between susceptible and resistant varieties (Abawi and Lorbeer, 1971a).

Chapter 1: Population Variability of *F. oxysporum* f. sp. *cepae*

Abstract

The objective of the current study was to determine the diversity among Colorado isolates of the Fusarium basal rot organism, *F. o. cepae* based on Vegetatively Compatible Groups (VCG). In addition, studies were done to determine the most effective chlorate media for generation of *nit* mutants.

Twenty four fungal isolates of *F. o. cepae* from onion producing areas throughout Colorado were plated on various chlorate media. Potato dextrose agar, and minimal medium (MM) supplemented with L-asparagine and L-threonine were tested. Each medium was supplemented with 1.5 and 3.0% potassium chlorate ($KClO_3$) (w/v) to encourage the development of nitrate nonutilizing mutants (nit mutants). Thirty three percent of the isolates examined did not generate NitM mutants, and consequently could not be compared in further testing. An additional 16% (4 isolates) produced only 1 NitM mutant each. Five percent (16) of the 343 mutants generated were *nit3*.

Chlorate PDA and 3% chlorate MM/L-threonine are ineffective in producing the nit mutants necessary for complementation pairings. 1.5% chlorate MM/L-asparagine

medium is as effective in generating the required nit mutants as 3% chlorate MM/L-asparagine medium. 1.5% chlorate MM/L-threonine medium produces a higher percentage of NitM mutants than *nit1* mutants and is acceptable as a medium for generating mutants for complementation pairings.

Pairings of complementary mutants were made from isolates collected from various onion producing areas of Colorado. Three Vegetatively Compatible Groups of *F. o. cepae* were identified. One of the VCGs identified has a wide ranging presence in Colorado, one VCG is restricted to Delta, and the third VCG is present in Fruita and LaSalle.

The VCGs identified in this dissertation need to be compared with the *F. o. cepae* VCG reported from Japan by Yoo in 1993. Additional isolates from Colorado and other onion growing areas need to be compared with the VCGs reported in this dissertation to determine the true extent of the different genetic (VCG) populations. Additional genetic evaluation techniques need to be used to determine the origin and diversity of the respective isolates.

Literature Review

The form species *Fusarium oxysporum* Schlect. emend. Snyd. & Hans. is a complex collection of fungi (Kistler and Momol, 1990), with pathogenic and nonpathogenic strains of *F. oxysporum* being morphologically indistinguishable (Elias et al., 1991). Subspecific

subdivision is usually based upon pathogenic or physiological race reactions on a set of differential cultivars (Woo et al., 1996). Pathogenicity tests to determine race based on host resistance/susceptibility are time consuming and give varying results depending on conditions such as temperature, age of hosts and inoculation methods. Developing techniques to quickly distinguish pathogenic from nonpathogenic isolates has, therefore, been important (Kondo et al., 1997).

Enzyme-linked immunosorbent assays (ELISA) (Linfield, 1993), restriction fragment length polymorphism (RFLP) (Woo et al., 1996), polymorphisms in mitochondrial DNA (mtDNA) (Appel and Gordon, 1994), pulsed field electrophoresis (Kistler et al., 1995), and the ability to produce volatile organic compounds in culture (Moore et al., 1991 and 1993; Stover, 1959), are used to identify *formae speciales*. Pathogenic strains also are subdivided into *formae speciales* and race by pathogenicity tests using appropriate host differential cultivars (Snyder and Hansen, 1940). Each *forma specialis* is characterized by its ability to cause a vascular wilt disease on a limited taxonomic range of plant hosts (Booth, 1971).

In the 1970's, in research with *Aspergillus nidulans*, Cove used various levels of chlorate to produce mutants, which were then plated on medium when ammonium, nitrate, nitrite, and hypoxanthine were the only sources of nitrogen (Cove, 1976). In the mid-1980's, Puhalla proposed a method by which various strains (*formae speciales*) of *F. oxysporum*

could be identified and classified based on Vegetative Compatibility Grouping (VCG) (Puhalla, 1985). Puhalla's modification of the technique developed by Cove is a reliable technique to determine vegetative compatibility among *Fusarium oxysporum formae speciales* (Ploetz, 1990).

The Concept of Vegetative Compatibility

F. oxysporum is fragmented into dozens of genetically distinct groups or subpopulations. These are known as Vegetatively Compatible Groups (VCGs) (Kistler and Momol, 1990).

In many soil-borne fungal pathogens, the apparent absence of a sexual stage may be compensated by other processes to ensure genetic exchange (Fernandez et al., 1994). Genetic exchange in the *Fusarium*, with no sexual stage such as *F. oxysporum*, can be accomplished only through the parasexual cycle, one-way exchanges of one or a few genes, and exchange of cytoplasmically inherited particles, e.g. dsRNA (Leslie, 1990). The capacity for this genetic recombination or nuclear reassortment in root-infecting fungi rests entirely with anastomosis (Parmeter, 1970).

Heterokaryosis, a step in the parasexual cycle, is a means by which normally haploid fungi may enjoy the benefits of functional diplody (Leslie, 1993). Heterokaryosis permits the segregation and recombination of genetic factors outside the sexual stage

(Buxton, 1956). For genetic exchange to occur in the parasexual cycle, a heterokaryon[24] must be formed. The interaction of alleles at these loci[25] govern the formation of a heterokaryon between two different isolates (Leslie, 1990). Such mycelial interactions have been recognized in the genus *Fusarium* for at least 100 years (Leslie, 1993). The spontaneous appearance of sectors in cultures of *Fusarium* species was referred to by early researchers as 'saltations' rather than mutations, as the genetic basis of the altered phenotype was unknown (Leslie, 1990).

Researchers use heterokaryosis[26] and parasexuality[27] in their genetic examinations of *F. oxysporum* (Puhalla, 1984). The vegetative compatibility approach provides a means of characterizing subspecific groups based on the genetics of the fungus rather than on the host-pathogen interaction (Correll, 1991). Puhalla used heterokaryon tests to determine that the *F. oxysporum* f. sp. *apii* affecting celery in New York was Race 2 (Lorbeer, 1982).

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[24] Het*ero*kary*on (noun): a cell (as in the mycelium of a fungus) that contains two or more genetically unlike nuclei (Parmeter et al., 1963).

[25] Loci (locus, single) - the position of a gene on a chromosome (Hartl, 1980, page 11).

[26] het*ero*kary*o*sis (noun): this term first appeared in 1912: the condition of having cells that are heterokaryons (Parmeter et al., 1963).

[27] Parasexuality - (Gr. Para = beside + sexuality): a process in which plasmogamy, karyogamy, and haploidization take place in sequence, but not at specified points in the life cycle of an individual; of significance in heterokaryotic individuals that derive some of the benefits of sexuality from a parasexual cycle (Alexopoulos et al., 1996).

Determination of vegetative compatibility groups (VCGs) is obtained from heterokaryon formation between anastomosing nitrate nonutilizing (*nit*) mutants (Puhalla, 1985) which have lost their ability to catabolize nitrate (Leslie, 1990). Isolates that share identical alleles at the loci governing heterokaryon incompatibility, commonly referred to as *het*[28] or *vic*[29], are vegetatively compatible (Leslie, 1993).

All filamentous fungi possess a system of regulating heterokaryon formation with most having multinucleate cells in which the heterokaryotic nuclei divide and migrate throughout the mycelium (Glass and Kuldau, 1992). Heterokaryosis may be confined to the anastomosed cells (Puhalla and Spieth, 1985). In heterokaryons of *Gibberella fujikuroi* (anamorph = *Fusarium moniliforme*), heterokaryotic nuclei do not migrate between cells and, as a result, the extent of heterokaryosis is limited (Glass and Kuldau, 1992).

Anastomosis does not appear to occur at the hyphal tips but rather 2-3 mm behind the tips (Puhalla, 1984). The hyphal tip cells at the periphery remain homokaryotic (Puhalla and Spieth, 1985), even though they are multinucleate (Puhalla and Spieth, 1983). With *F. moniliforme*, Puhalla and Spieth (1983) found continued growth of the heterokaryotic

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[28] *Het* - heterokaryon incompatibility (Correll, 1991).

[29] *Vic* - for vegetative incompatibility (Leslie, 1993).

colony requires continued hyphal anastomoses[30].

Isolates in a VCG often share pathological and physiological attributes, as well as geographic origins. Consequently, vegetative compatibility has been used to study the origins of, and relatedness among plant pathogenic fungi (Ploetz and Shepard, 1989). Pathogenic isolates of *F. oxysporum* in the same VCG are presumed to be clones of one another even if they are geographically isolated from each other. Isolates with similar pathogenic capabilities that are vegetatively incompatible are assumed not to be clones (Leslie, 1990). At their simplest, each VCG can be thought of as a series of clones of a single parental strain, although in reality each VCG is composed of isolates that possess the same set of *vic* alleles (Leslie, 1990 and 1993). Two isolates may belong to the same VCG but yet not be asexual clones of a common parent (Campbell et al., 1992). A mutation at a single *vic* locus could result in closely related isolates becoming vegetatively incompatible[31], or it is possible that distantly related isolates have the same *vic* loci and are vegetatively compatible even though they are not otherwise genetically similar (Bentley et al., 1995).

The diversity of vegetative compatibility groups of *F. subglutinans* f. sp. *pini*, the causal

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[30] Anastomoses - hyphal fusions: these can occur at some distance from the hyphal tip. In principle they allow exchange of cytoplasm and also nuclei between hyphae of different origin leading to heterokaryons (Bos, 1996).

[31] Incompatibility is triggered when two strains that contain different alleles at one or more of the *het* loci fuse (Glass and Kuldau, 1992).

agent of pine pitch canker, is greater in Florida (45 distinct VCGs) than in California (5 VCGs). Seventy percent of the California population, where the organism was first reported in 1986, consists of a single VCG. The organism has been established since the mid 1970s in Florida. Correll et al. (1992) report the lack of diversity of the VCG population in California is indicative of an asexually reproducing pathogen and/or one that has recently been introduced. They surmise that when a single VCG predominates in the epidemic front, the disease has been only recently introduced. Several years after establishment, the VCG population becomes more diverse. A California survey of pine pitch canker conducted between 1993 and 1995 resulted in the identification of three new VCGs. A large infestation in San Luis Obispo County was in the same VCG as an isolated infestation near Santa Barbara and at a tree nursery in Los Angeles County. This implies that movement of infected trees from the nursery might have contributed to the spread of the disease (Gordon et al., 1996).

The loci and alleles that define VCGs are presumed to be selectively neutral with respect to traits such as pathogenicity[32] and vegetative viability[33] (Leslie, 1990). In 1985, Puhalla suggested that when the sexual stage and meiotic recombination of *F. oxysporum* were

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[32] Pathogenicity - the disease producing capability of a group of microbes (VanDemark and Batzing, 1987).

[33] Viability - "probability of survival to reproductive age" (Hartl, 1980, page 201).

lost, the loci that determine vegetative incompatibility and virulence[34] became closely linked. Ahn et al. (1998) found the virulence of *F. oxysporum* f. sp. *cucumerinum* (Owen) Snyder & Hansen, was significantly different in one VCG tested when compared with 5 other VCGs of this same *forma specialis*. Virulence tests of 78 isolates collected from various provinces in Korea were performed on a susceptible cucumber variety.

Vegetative compatibility is controlled by multiple vegetative incompatibility loci (alternately called *vic*, *vc*, *tol*, or *het* loci). Mutations of *nit* phenotypes[35] occur at a number of different loci (at least 7) and occur frequently (Leslie, 1990). In *Fusarium moniliforme*, 10 nuclear *vic* or *het* loci have been identified that control a homogenic vegetative compatibility system (Kedera et al., 1994; Puhalla and Spieth, 1983). *Fusarium solani* is reported to have 7 or 8 loci responsible for vegetative compatibility (Hawthorne and Rees-George, 1996).

Two fungal isolates are vegetatively compatible only if the alleles[36] at each of their corresponding *vic* loci are identical (homogenic) (Correll et al., 1987). Differences at the *vic* loci, limit the exchange of genetic information to individuals which belong to the

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[34] Virulence - the relative capacity of a pathogen to overcome host defenses; the degree or measure of pathogenicity (Ulloa and Hanlin, 2000).

[35] Phenotype; genetics - the appearance or other characteristics of an organism resulting from the interaction of its genetic constitution with the environment, as opposed to its underlying hereditary determinants, or genotype (Morris, 1996).

[36] Allele - an alternative form of gene (Hartl, 1980, page 3).

same VCG (Leslie, 1990). Where two compatible mutant colonies touch, heterokaryon formation occurs as indicated by dense mycelial growth (Puhalla, 1985).

A killing reaction occurs after the anastomosis of two heterokaryon-incompatible isolates (Leslie, 1990). Cell-membrane components or materials in the space between the cell membrane and cell wall are believed to be responsible for at least some of the killing reaction. This reaction restricts the transfer of nuclear and cytoplasmic elements between the incompatible isolates (Leslie, 1993). This is referred to as a barrage reaction, and is characterized by degenerating, dying hyphae and aborted heterokaryotic cells (Glass and Kuldau, 1992). The mycelium remains very thin at the point where two incompatible isolates meet and a barrage reaction occurs. Vegetative incompatibility reduces the spread of cytoplasmic genetic elements, including viruses (Anagnostakis, 1977).

Mutants from the same isolate, with the inability to fuse properly to form a heterokaryon, have been described and termed heterokaryon self-incompatible (Correl et al., 1989; Jacobson and Gordon, 1988). It is important to identify such strains to prevent the overestimation of the number of VCGs within a population (Leslie, 1993). No self-incompatibility was observed with *F. oxysporum* f. sp. *cucumerinum* (Ahn et al., 1998).

Self-incompatibility has been reported for *F. oxysporum* (Correll et al., 1987) and *Fusarium moniliforme* (Correl et al., 1989). Self-incompatibility has been demonstrated with *F. oxysporum* f. sp. *melonis* (Jacobson and Gordon, 1988). Some isolates of *F.*

oxysporum f. sp. *lilii* are self-incompatible (Löffler and Rumine, 1991).

If an isolate is self-compatible but does not complement with any other isolate, it is considered as a single member of a VCG (Woo et al., 1996).

The limitations of the VCG technique depend on the particular *forma specialis* being examined. Limitations of this technique include:

1. recovering *nit* mutants on chlorate-containing medium
2. Weak heterokaryon reactions between *nit* mutants
3. Cross-compatibility reactions between certain isolates of different VCGs
4. The presence of heterokaryotic, or vegetatively, self-incompatible isolates (Correll, 1991).

Producing Mutants

Researchers previously used ultraviolet light irradiation (Bos and Stadler,1996; Buxton, 1956), or nitrosoguanidines (NG) (Bos, 1987) to induce mutagens. UV-light irradiation can in principle induce mutations in any gene, while NG-compounds can result in a sequence of mutations (Bos, 1987). Puhalla (1985) and Puhalla and Spieth (1985) reported strains of *F. oxysporum* and *F. moniliforme* sectored when cultured on minimal medium containing 1.5% $KClO_3$.

Proteins and nucleic acids are especially rich in nitrogen, with major control systems regulating nitrogen metabolism, which in turn ensure a constant nitrogen supply for growth (Marzluf, 1981). When placed on chlorate medium, isolates mutate at different loci associated with the nitrate-assimilation pathway. *Nit1* mutants are mutated in a structural locus of the enzyme nitrate reductase. Such non-nitrate utilizing mutants will grow in the presence of nitrite or hypoxanthine. *Nit3* mutants mutate in a locus involved with the regulation of both nitrate reductase and nitrite reductase. Such mutants cannot utilize nitrate or nitrite as the nitrogen source, but will grow in the presence of hypoxanthine. NitM mutants mutate in one of the loci associated with the synthesis of a co-factor[37]. This co-factor is necessary for both nitrate reduction and hydroxylation[38] of hypoxanthine. Those mutants cannot grow on nitrate or hypoxanthine, but will grow on nitrite (Löffler and Rumine, 1991). The phenotypes of mutants recovered from the various chlorate media, classified as *nit1*, *nit 3*, and NitM mutants based on growth patterns on various phenotyping media, are indicated in Table 1.

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[37] This is a molybdenum-containing cofactor necessary for nitrate reductase activity (Baayen and Kleijn, 1989).

[38] Hydroxylation reaction - an oxidation reaction that introduces one or more hydroxyl groups into an organic compound (Morris, 1996).

Table 1. Nit Mutants as Phenotyped by Minimal Medium containing Nitrate, Nitrite, Ammonium and Hypoxanthine

Phenotype	NO_3^-	NH_4^+	NO_2^-	Hypoxanthine
nit1	-	+	+	+
nit3	-	+	-	+
NitM	-	+	+	-
Crn	+	+	+	+

Nit1 - unable to utilize nitrate
Nit3 - unable to utilize nitrate or nitrite
*Nit*M - unable to utilize nitrate, hypoxanthine or uric acid (Hawthorne and Rees-George, 1996).
Crn - chlorate-resistant, nitrate-utilizing or revertant

Different media augmented with potassium chlorate are used to produce *nit* mutants. These media include potato-dextrose chlorate (PDC) (Alves-Santos et al., 1999; Hopkins et al., 1992; Katan et al., 1994; Woo et al., 1996), potato-sucrose chlorate (KPS) (Clark et al., 1995), Czapek Dox chlorate (Blok and Bollen, 1997; Löffler and Rumine, 1991), and minimal medium chlorate (MMC) (Puhalla and Spieth, 1983). Bosland and Williams (1987) used PS (potato-sucrose), cornmeal agar with dextrose (CMDA), PDA, and potato-carrot-dextrose agar (PCDA) media, each with 20 g $liter^{-1}$ of potassium chlorate added. Their research indicated 2% chlorate PDA and CMDA, generated more sectors than chlorate PCDA. CMDA was equal to KPS, with isolates on the latter producing 53% of complementary *nit* mutants.

Fusarium oxysporum f. sp. *niveum*[39] mutants were easily obtained on potato-dextrose agar containing 1.5% $KClO_3$ (Hopkins et al., 1992). Fusarium *subglutinans* f. sp. *pini* isolates from California, however, are not restricted on 1.5% MMC, but chlorate-resistant sectors are readily recovered from these isolates on 3% MMC. In contrast, the Florida isolates are readily recovered on 1.5% MMC (Correll et al., 1992).

In most instances, L-asparagine at 1.6 g liter^{-1}, was added to the medium. L-threonine at 2.3 g liter^{-1} is used (Hawthorne and Rees-George, 1996), as has 1.6 g l^{-1} L-glutamine (Löffler and Rumine, 1991). Bos and Stadler (1996) used urea in lieu of ammonium tartrate when isolating chlorate-resistant mutants of *Aspergillus niger*. They treated spores with a low dose of UV, and then compared growth on different N-sources to classify the mutants (Table 1a). Nitrate, nitrite, hypoxanthine and urea were the sources of nitrogen used.

Klittich and Leslie (1988) used phenotyping media containing NH_4^+, NO_3^-, NO_2^-, hypoxanthine and uric acid to produce *nit* mutants 1-7. NitM is not produced with this procedure.

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[39] *Fusarium oxysporum* Schlechtend.:Fr. *niveum* (E.F. Sm.) Synd. & Hans., the cause of Fusarium wilt of watermelon [*Citrullus lanatus* (Thyunb.) Matsum. & Nakai] (Hopkins et al., 1992.

Table 1a. Nit Mutants as Phenotyped by the use of Minimal Medium containing Nitrate, Nitrite, Hypoxanthine and Urea as the Sole Source of Nitrogen

Boss and Stadler classified mutants from their research (1996) as follows:

Phenotype	NO_3^-	NO_2^-	Hypoxanthine	Urea
Nia	-	+	+	+
Nir	-	-	+	+
Cnx	-	+	-	+
Crn	+	+	+	+

Nil - unable to utilize nitrate, but able to utilize hypoxanthine and urea
Nir - unable to utilize nitrate or nitrite, but able to utilize hypoxanthine and urea
Cnx - unable to utilize nitrate and hypoxanthine but able to utilize nitrite and urea
Crn - chlorate-resistant, nitrate utilizing or revertant

Nitrate non-utilizing (*nit1* and *nit3*) mutants (95% - 89 out of a sample of 94) were obtained from isolates of *Fusarium solani* (Teleomorph = *Nectria haematococca*) collected from Japan, New Zealand, and North America on a minimal medium containing 30 g liter^{-1} $KClO_3$, but NitM mutants were only obtained from 53 isolates (56%) on this medium. Modified MMC containing L-threonine instead of L-asparagine enabled recovery of NitM from an additional 11 isolates for a total of 64 (68%) NitM mutants from the 89 isolates tested (Hawthorne and Rees-George, 1996).

Various chlorate supplemented media may be required to produce mutants. Most *Fusarium moniliforme* mutants produced on MMC are NitM, while most recovered from PDC are *nit1* or *nit3* (Munkvold et al., 1997). On chlorate minimal medium (1.5%

$KClO_3$), strains of *F. moniliforme* sectored 91% of the time, and produced *nit1* mutants 39-66% of the time, and *nit3* mutations 23 - 42% of the time, depending on strain (Klittich and Leslie, 1988). On Czapek Dox agar (1.5% chlorate), 87% of *Fusarium oxysporum* f. sp. *lilii* isolates yielded mutants. Of those 51% were *nit1* , 26% were *nit3*, and 23% were NitM (Löffler and Rumine, 1991). Woo et al. (1996) found the relative frequencies of *nit* mutants of *Fusarium oxysporum* f. sp. *phaseoli* varied based on the chlorate media used. Nit mutants recovered from potato-dextrose agar supplemented with 1.5% potassium chlorate, were as follows:

nit1 - 60 to 86%,

nit3 - 4 to 23 %,

nitM - 0 to 15% .

On minimal medium supplemented with 1.5% potassium chlorate, the following percentages of mutants were recovered:

nit1 - 58 to 62%,

nit3 - 5 to 25 %,

NitM - 0 to 25%.

Correll et al. (1987) compared a series of *Fusarium oxysporum formae speciales*. They generated *nit* mutants on MM and PDA to which 15 grams of $KClO_3$ had been added to each liter. They placed a 2-mm^3 piece of agar (CM) of the isolate in the center of a 10 x 60 mm petri dish. They found this procedure was sufficient to generate a NitM mutant.

Their examination was conducted on *F. o. apii*, *F. o. chrysanthemi*, *F. o. conglutinans*, *F. o. cubense*, *F. o. dianthi*, *F. o. lycopersici*, *F. o. medicaginis*, *F. o. melonis*, *F. o. niveum*, *F. o. pisi*, *F. o. radicis-lycopersici*,*F. o. tracheiphilum*, and *F. o. vasinfectum*. Mutants of *F. oxysporum* f. sp. *apii* (race 2) are easily produced on potato-sucrose agar containing 1.5% potassium chlorate. After 5-10 days, fast-growing sectors emerge from the restricted colonies (Correll et al., 1986a).

The yield of mutants, in general, increases with increasing doses of the mutagen. Bos (1987) found the relationship between mutagen dose and mutant yield was not a linear relationship. He found that as UV-treatment levels increased, the number of *Aspergillus nidulans* mutants also increased but the effect leveled off and even decreased at high doses. Researchers found the various *forma specialis* and even isolates within the *forma specialis* respond differently to various levels of chlorate, requiring several chlorate levels to be utilized until a mutant is produced. Several isolates of *Fusarium oxysporum* f. sp. *asparagi* did not produce restricted growth on CDA with 15 g l^{-1} of $KClO_3$ and required an increased concentration of 5% $KClO_3$ (Blok and Bollen, 1997).

Mycelium from the leading edge of any fast-growing sector that appears on the chlorate media is transferred to minimal medium (MM) containing nitrate as the sole source of nitrogen[40]. Those that have a thin expansive growth on MM with no aerial mycelium are

[40] Czapek Dox agar (CDA) has been used in lieu of minimal medium (Blok and Bollen, 1997; Löffler and Rumine, 1991).

considered *nit* mutants (Alves-Santos et al., 1999; Clark et al., 1995).

Isolates having a wild-type growth habit on MM are considered chlorate-resistant, nitrate-utilizing (*crn*) mutants and are discarded (Klittich and Leslie, 1989). Growth of wild-type isolates is restricted on chlorate, presumably because chlorate is reduced by nitrate reductase to highly toxic chlorite. Nitrate nonutilizing mutants are unable to reduce chlorate to chlorite, and hence are chlorate-resistant (Correll et al., 1987). Puhalla (1985) reported 2 to 4% of *nit* mutants grown on MM for an extended period of time developed patches of heavy growth.

Daboussi et al. (1992), discovered a transposable[41] element in *Fusarium oxysporum* associated with the nitrate reductase structural gene. They feel this transposon-like element is responsible for the mutability and instability associated with the species. Cross pathogenicity between different host plants has been demonstrated (Elmer et al., 1999). The concept of transposable elements provides a plausible explanation for the alteration of host specificity within clonal lineages (Gordon and Martyn, 1997).

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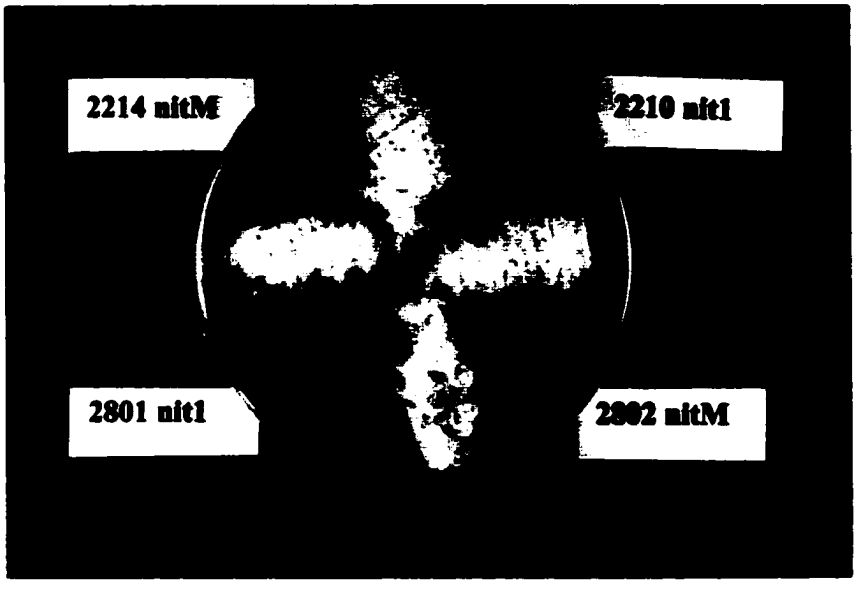
[41] Transposon or transposable element - DNA sequences that can change position from one place in a DNA molecule to another place (Hartl, 1980, page 365).

Complementation of Mutants

When two genetically different *nit* mutants of the same strain are paired on minimal medium (MM), they form a line of dense, wild-type growth where they come in contact with each other (Puhalla, 1985). *Nit* mutations are located on different sites in the genome, consequently, combinations of certain *nit* mutants can complement each other in the heterokaryons (Baayen and Kleijn, 1989). The development of this dense aerial growth is where mycelia of the *nit* mutant colonies anastomose to form a heterokaryon. Isolates are considered to be vegetatively compatible if the complementation of the *nit* mutants result in the formation of this dense aerial growth at the zone of the anastomosing mycelia (Leslie, 1990). Figure 1 is an example in which a NitM and *nit1* of two different isolates (22 and 28) collected in two different counties in Western Colorado are compared. The NitM and *nit1* of isolate 22 (2214 and 2210, respectively) have formed a heterokaryon where the hypha of both mutants meet. This indicates the mutants of isolate 22 are self-compatible. The mutants from isolate 28 are also self-compatible as seen by the formation of a heterokaryon. In addition, the *nit1* and NitM produced from both isolates (e.g., 2214 NitM and 2801 *nit1*, and 2210 *nit1* and 2802 NitM) have formed heterokaryons indicating the two isolates are compatible, and hence are from the same clonal parent.

Figure 1. Photograph Showing Heterokaryosis



Not all combinations of different mutant types of vegetatively compatible mutants lead to mycelial growth (heterokaryosis). The strength of the pairing reaction is, in some cases, dependent on the particular combination of *nit* mutants used (Gordon and Okamoto, 1991). *Nit1* and *nit3* mutants may form weak, slow-growing heterokaryons, giving ambiguous results (Klittich and Leslie, 1988). Löffler and Rumine (1991) found the combination of *nit1* x *nit3* was positive 50% of the time, *nit1* x nitM was positive 90% of the time, and the combination of *nit3* x nitM was positive 78% of the time. Baayen and Kleijn (1989) found positive complementations for *F. oxysporum* f. sp. *dianthi* occurred 44, 94, and 73% of the time, respectively, for *nit1* x *nit3*, *nit1* x NitM, and *nit3* x NitM.

Two isolates might be vegetatively incompatible even though each is compatible with a third isolate (Gordon and Okamoto, 1991).

Some isolates may be compatible with tester strains of more than one VCG. This is referred to as 'bridging' and appears to be widespread (Lodwig et al., 1999). Members of such 'bridging' isolates may in actuality form a single clonal population (Koenig et al., 1997).

The Practicality of the VCG Procedure

The VCG technique determines vegetative incompatible phenotypes and genetic diversity within the field population of *F. oxysporum* (Gordon and Okamoto, 1991; Kistler et al., 1998). The use of *nit* mutants enables researchers to compare isolates of *F. oxysporum* (*forma specialis*, races[42] and nonpathogens) for vegetable compatibility on a global basis. This provides details on genetic diversity (Leslie, 1990). *Forma specialis* is a useful grouping of importance to plant pathologists. However, in many cases, a *forma specialis* appears to be a grouping of genetically diverse isolates possibly polyphyletic[43] in origin. There are also instances where different VCGs within a forma specialis are

[42] Race; a group of biotypes with a similar virulence-avirulence pattern on a particular group of plants (Ulloa and Hanlin, 2000).

[43] Polyphyletic; Genetics - of a species or other taxonomic group, displaying hereditary characters from two or more distinct ancestral lineages (Morris, 1996).

monophyletic[44] in origin (Correll, 1991). Australian isolates of *Fusarium oxysporum* f. sp. *vasinfectum* are not vegetatively compatible with any foreign isolates, and belong to a unique vegetatively compatible group. Davis et al. (1996a) speculate that the Australian strain arose locally, perhaps from a minor population and became prominent due to wide-scale planting of susceptible cotton cultivars.

A large percentage of the pathogenic isolates of *F. proliferatum* sampled from asparagus in Australia belong to VCGs common on asparagus in the United States. The pathogen is probably disseminated between countries on crowns and seeds (Elmer et al., 1999).

The VCG process is used to differentiate pathogenic isolates cultured from green celery (Correll et al., 1985a), peas (Correll et al., 1985b), beans (Woo et al., 1996), cotton (Fernandez et al., 1994), and other crops. Vegetative compatibility tests can be used to effectively identify pathogenic isolates of *F. oxysporum* f. sp. *adzukicola*[45] from nonpathogenic ones within a heterogeneous population of *F. oxysporum*. Physiological races of *F. oxysporum* f. sp. *adzukicola*, however, could not be distinguished using VCG tests as several races were located in the same vegetatively compatible group (Kondo et al., 1997).

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[44] Monophyletic; Evolution - of or relating to any group sharing a single ancestral form (Morris, 1996).

[45] Causal agent of Fusarium wilt of adzuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi] (Kondo et al., 1997)

Some non-pathogenic isolates from field soil in the San Joaquin Valley of California are in the same VCG as the melon wilt pathogen *Fusarium oxysporum* f. sp. *melonis*. This may indicate that either the pathogen was a recent derivative of non-pathogenic strains at the same location or avirulent strains were derived from the pathogen (Gordon and Okamoto, 1992b).

Aloi and Baayen (1993) tested vegetative compatibility as a means to identify races of *F. o.* f. sp. *dianthi*. They report VCG tests are accurate in placement of new isolates to race. In addition, VCG classification correctly indicated that the identities previously ascribed to two old isolates was incorrect. Wright et al. (1996) collected isolates of *Fusarium oxysporum* from symptomless cuttings of carnations. Of 69 tested for pathogenicity, 24 were pathogenic to carnations and the remaining 45 were non-pathogenic. The pathogenic isolates belonged to two VCGs. Baayen et al. (1997) compared *Fusarium oxysporum* VCG isolates collected from diseased *Dianthus* spp. from around the world, and were able to classify them into *F. o.* f. sp. *dianthi* and *F. redolens* f. sp. *dianthi*, based on differences in the internal transcribed spacer region of the ribosomal DNA. They feel the significant differences in VCGs indicate they originated from different ancestors. Research conducted on *F. o.* f. sp. *lilii* by Baayen et al. (1998) found 24 isolates from different locations in the world [The Netherlands (18 isolates), Italy (4 isolates), Poland and the United States (1 isolate each)]. These isolates all belonged to the same VCG and had a single clonal lineage. *F. oxysporum* f. sp. *gladioli* and f. sp. *tulipae* had separate VCGs and thus do not share a common ancestry with *F. oxysporum* f. sp. *lilii*.

Vegetatively compatible fungal strains may be considered to be members of unique groups and represent clonal populations genetically isolated from strains in other VCGs (Elias et al., 1991; Wright et al., 1996).

The inoculation of differentially resistant varieties of carnation was used to determine there were four pathological variants within race 2 of *F. oxysporum* f. sp. *dianthi* (Arbelaez and Calderon, 1992). Pathogenicity also can be determined by Vegetative Compatibility Grouping (VCG) tests (Correll et al., 1986a). Vegetative compatibility grouping can more rapidly and more accurately identify race 2 of *F. o.* f. sp. *apii* than greenhouse pathogenicity tests (Toth and Lacy, 1991).

Genetic variations among *Fusarium oxysporum* f. sp. *betae* (FOB) isolates from sugar beets, as determined by vegetative compatibility, were found to represent distinct isolated populations indigenous to their respective areas (Harveson and Rush, 1997). They collected 160 isolates from symptomatic sugarbeet and red root pigweed (*Amaranthus retroflexus* L.) from seven counties in Texas between 1992 and 1994. Isolates from other states were included in their analysis. Their results suggest that substantial variation exists among sugar beet isolates of FOB from the United States and that these populations of FOB are apparently distinct and endemic to their respective areas.

Among the pathogenic isolates of *F. oxysporum* f. sp. *phaseoli*, there is a weak relationship of VCG to race and geographic origin (Woo et al., 1996). With a global

collection of 123 isolates of *Fusarium oxysporum* from crucifers, geographic origin was not found to be associated with vegetative compatibility (Bosland and Williams, 1987).

One hundred and twenty eight isolates of *F. oxysporum* f. sp. *radicis-lycopersici* from Israel, Belgium, Canada, Greece, France, Italy, Japan, and the United States were placed in seven VCGs. Approximately 25% of the isolates did not form heterokaryons with any other strain, but were self-compatible (Schneider et al., 1992). Isolates of this same *forma specialis* from central and southern Florida were studied by Rosewich et al. (1999). One VCG (0094), previously known only from northwestern Europe, was predominant among Florida isolates. The European population was less diverse than the Florida population. As a result the authors make the case that the European VCG 0094 originated from Florida.

Fusarium oxysporum Schlectend.:Fr. f. sp. *albedinis* (Killian & Maire) Gordon, the causal agent of Bayoud, the fungal vascular wilt of date palm (*Phoenix dactylifera* L.), was studied by Tantaoui et al. (1996). They studied isolates from Morocco and Algeria using vegetative compatibility grouping, RFLP, and RAPD[46]. They report the Moroccan isolates may belong to a single clonal lineage that originated in Moroccan palm groves and eventually reached the Algerian oases.

[46] RAPD - random amplified polymorphic DNA assay (Wright et al., 1996).

In a worldwide collection of *F. o.* f. sp. *vasinfectum*, Fernandez et al. (1994) found isolates belonging to distinct races were never in the same VCG. Race A isolates were separated into eight VCGs, whereas isolates of race 3 were classified in the same VCG. Within Australian populations of *F. o.* f. sp. *cubense*, there is good correlation between race and VCG, as only one race was found to occur with each VCG (Brake et al., 1990; Moore et al., 1993).

With isolates of *F. lateritium*, the causal agent of chlorotic leaf distortion of sweet potato *[Ipomoea batatas* (L.) Lam.], no relationship between VCG and pathotype *(forma specialis*) was noted. There was, however, an apparent relationship between VCG and geographic origin of the isolates (Clark et al., 1995). Hopkins et al. (1992) were able to differentiate between race 1 and race 2 of *F. o.* f. sp. *niveum*[47], the cause of Fusarium wilt of watermelon[48], using VCG techniques. Over 250 isolates of *Fusarium oxysporum* collected from infected watermelon plants, soil samples from a pathogen-infested field, and samples from various locations around the world, were readily segregated into pathogenic and non-pathogenic isolates. All isolates of *F. o. niveum* tested belong to one of three distinct VCGs, and are incompatible with non-pathogenic isolates. The researchers in this study report a direct relationship between VCG and race (Larkin et al., 1990).

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[47] *Fusarium oxysporum* Schlecht. f. sp. *niveum* (E.F. Sm.) Snyd. & Hans (Larkin et al., 1990).

[48] [*Citrulus lanatus* (Thunb.) Matsum. And Nakai] (Hopkins et al., 1992).

Ninety seven isolates of *F. o.* f. sp. *asparagi* from a collection from the United States, Europe, and Taiwan, had a minimum of 42 vegetative compatibility groups (Elmer and Stephens, 1989). Dutch *F. o.* f. sp. *asparagi*, is equally diverse genetically, with twenty-four isolates assigned to 18 different VCGs (Blok and Bollen, 1997). One hundred eighty-seven isolates of *F. o.* f. sp. *melonis* consisted of only eight VCGs (Jacobson and Gordon, 1990a). Based on pairing complementary *nit* mutants, Appel and Gordon (1994), identified 56 VCGs among 197 isolates of *F. o.* collected from two fields in Maryland known to be contaminated with *F. o.* f. sp. *melonis*, a wilt pathogen of muskmelon (*Cucumis melo* L.). Their study also included an examination for polymorphisms in mtDNA and a pathogenicity test using a root-dip assay. The two largest VCGs represented *F. o.* f. sp. *melonis*. Sixty percent of the 197 isolates collected were pathogenic to muskmelon, but nonpathogenic isolates accounted for 92% of the VCG diversity and 86% of the diversity in mtDNA haplotypes. Distinct mtDNA restriction fragment patterns correspond to each of six vegetative compatibility groups of *F. o. melonis* tested (Jacobson and Gordon, 1990b). Between VCGs (and *formae speciales*), mtDNA patterns are quite distinct (Kistler and Momol, 1990). California isolates of *F. o.* f. sp. *melonis*, in the same vegetative compatibility group, were always associated with the same mitochondrial DNA haplotype (Gordon and Okamoto, 1992b). Gordon and Okamoto (1992a) surmise the identical mtDNA among isolates with different nuclear DNA may be due to the transfer of mtDNA through hyphal anastomosis. The nonpathogenic strains that co-occur with *F. o. melonis* might have been derived from an

indigenous population present in the soil prior to its cultivation, or they may have been introduced to the fields through agricultural practices (Gordon and Okamoto, 1991). Nonpathogenic forms are often associated with roots and may colonize them without any apparent effect on the plant (Kistler and Momol, 1990). A collection of 122 isolates of *F. o. melonis* collected in Israel belonged to two VCGs and four physiologic races (root-dip assay) (Katan et al., 1994).

Within certain *formae speciales*, there are cases where more than one race may occur within a single VCG, and others where isolates of a single race may belong to several different VCGs (Correll, 1991). For example, Koenig et al. (1997) report *F. o.* f. sp. *cubense*, the causal agent of Panama disease of banana, may have several races in one VCG. Races are not defined genetically, but grouped based on pathogenicity to host cultivars in the field. A collection of 208 isolates of *F. o.* f. sp. *cubense*, represented 4 physiological races and 20 vegetative compatibility groups. Isolates within each VCG generally produced an identical DNA fingerprint and were therefore closely related, regardless of geographic origin or cultivar source (Bentley et al., 1998). The relationship between race and VCG is rather simple, where all isolates of a given race, even from a widespread geographical area, belong to the same VCG (Correll, 1991).

The appearance of a race in a new location may represent the independent origin of that race or movement from another location (Gordon and Martyn, 1997; Gordon and Okamoto, 1992b). The close relationship between pathogenic and nonpathogenic strains

of *F. o.* indicates that the *forma specialis* designation is not entirely congruent with genetic relationships in *F. o. melonis*. The occurrence of a race in a new location is most likely the result of introductions of previously recognized pathogenic races from other locations or possibly the derivation of a new race from a pre-existing race of the same *forma specialis* (Appel and Gordon, 1994). In some cases, a pathogenic race is associated with more than one clonal lineage. This suggests independent origin. Some evidence suggests one pathogenic race may give rise to another, by relatively simple genetic changes, but the derivation of a pathogen from a nonpathogen has not been documented (Gordon and Martyn,1997).

VCGs are reported to correlate with *formae speciales* and the method is proposed as a fast and easy way to distinguish pathotypes of *F. o.* (Puhalla, 1985). Woo et al. (1996), however, indicates this technique is time consuming, labor intensive, and subject to varying environmental or cultural growth conditions during the experiments.

VCG is also used to determine genetic differences among nonpathogenic isolates. One hundred and ten *F. o.* nonpathogenic isolates of celery isolated from celery roots fell within 14 VCGs (Correl et al., 1986b). Further tests conducted by Correll et al. (1986a), showed the VCG technique would have correctly identified 197 of 199 *F. o.* isolates collected from celery roots as either pathogenic or nonpathogenic without resorting to pathogenicity tests. Heterokaryon formation never occurred between pathogenic and nonpathogenic isolates of *F. o.* f. sp. *phaseoli* (Woo et al., 1996).

The VCG procedure can be used to determine the effect of plant residue on the longevity of *Fusarium* spp. in soil. Cotten and Munkvold (1998) studied the survival of *F. moniliforme*, *F. subglutinans*, and *F. proliferatum* in maize residue under field conditions. Pieces of maize stalk were inoculated with spore suspension of the test *Fusarium*, and placed on the soil surface or buried in field soil. Vegetative compatibility tests confirmed these *Fusarium* can survive at least 630 days in surface or buried maize residue. Consistent isolations of these three species of *Fusarium* from symptomatic and asymptomatic maize plant tissue suggests the fungus can colonize kernels, their respective glumes, and their cob tissue. Comparisons of *nit* mutants determined the fungal isolates in all three tissues belonged to the same VCG (Kedera et al., 1994).

Numerous investigators have classified isolates of *F. oxysporum* using Puhalla's numerical system (Puhalla, 1985). According to Kistler et al's 1998 paper, over 125 VCG plant pathogenic isolates of *F. oxysporum* were identified.[49]. They indicated the overall classification process is confusing and duplications have occurred. In Puhalla's system (1985), vegetatively compatible isolates are given a VCG code consisting of a four- (or five-) digit number with the first three digits corresponding to host specialization, or *forma specialis*, and the last digit(s) corresponding to individual VCGs

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[49] *F. o. cepae* is conspicuously absent from Kistler's list. Yoo et al., 1993, makes mention of *F. o. cepae*. The latter publication, however, is mostly in Japanese. The abstract (in English), however, mentions an isolate identified as *F .o. cepae* was placed in its own VCG. This isolate was not compatible with other isolates from different *formae speciales* of *F. oxysporum*.

with that *forma specialis* (Puhalla, 1985).

Kistler et al. (1998) recommended changes to help standardize the procedure and reduce confusion. Their suggestions are to assign VCG numbers when:

1. Isolates belonging to the same VCG are recovered from multiple locations,
2. Isolates belonging to the same VCG compose over 5% of the population at a single site (sample size > 100 independent isolates),
3. Isolates belonging to the same VCG are of particular interest (e.g. biological control isolates).

Katan (1999) published a list of 38 *formae speciales* (ff. sp.), along with their 3-digit numerical codes and assigned VCG numbers. Katan and Di Primo (1999) updated that list adding five newly characterized *formae speciales*. Numerical codes were given to four ff. spp., to include *F. o. cepae*. The f. sp. code assigned to *F. o. cepae* is 042. At the time of the Katan and Di Primo publication, only one VCG[50] had been characterized, with the assigned VCG number 0420.

The Relationship of VCG to Other Methods of Classification

The close relationship between pathogenic races implied by their association with the

[50] Yoo et al., 1993.

same VCG, is corroborated by other measures of genetic relationship (Gordon and Martyn, 1997). Edel et al. (1997), used polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis of ribosomal DNA (rDNA[51]) to differentiate between strains of *Fusarium* species. Their study of 18 Fusarium species (92 strains), including *F. oxysporum*, found the PCR-RFLP method provided a rapid tool for differentiation of *Fusarium* strains at the species level. Nineteen strains were found in *F. oxysporum* alone. RFLP of intergenic spacer (IGS) haplotypes is well correlated with the PCR-RFLP method. Bentley et al. (1995) found RAPD-PCR banding patterns were generally VCG specific, validating the use of vegetative compatibility to identify genetically isolated populations of *F. o.* f. sp. *cubense*. Lodwig et al. (1999) found PCR fingerprinting of *F. o. cubense* isolates produced banding patterns that correlated with clonal lineage (VCG). Fusarium wilt of cyclamen (*Cyclamen persicum* Mill.) caused by *F. o.* Schlechtend.:Fr. *aurantiacum* (Link) Wr., was subdivided into three clonal lineages (VCGs) each with similar IGS organization and DNA fingerprints (Woudt et al., 1995).

Intergenic spacer haplotypes which separate rDNA repeat units, evolve rapidly with closely related species showing considerable differences in these units (Hillis and Dixon, 1991). Distinctive IGS haplotypes 'hitch-hike' along with unique combinations of vegetative compatibility genes as well as the gene or genes which confer virulence to muskmelon (Appel and Gordon, 1995). RFLP analyses of intergenic spacer (IGS)

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[51] rDNA - ribosomal DNA (Alves-Santos et al., 1999).

haplotypes of rDNA by Appel and Gordon (1995), strengthened the view that within *F. o.* f. sp. *melonis*, the VCG's define discrete clonally propagating lineages (Jacobson and Gordon, 1991). VCG 0134 contains all four known races of *F. o.* f. sp. *melonis*. All have identical mtDNA and nuclear DNA haplotypes (Gordon and Martyn,1997).

Woo et al. (1996) also report RFLP patterns correspond to VCGs. Isolates of *F. o.* f. sp. *phaseoli* recovered by Alves-Santos et al. (1999) in Spain, differed from isolates from the America's in VCG, IGS haplotype and electrophoretic karyotype, indicating a different origin. Authors of the Spanish research report the importance of such research to the development of plant resistance. Electrophoretic technology used to estimate genetic diversity within and among VCGs and races of *F. o. lycopersici* isolates within a VCG, were more genetically similar than those in different VCGs. Elias and Schneider (1992) propose that vegetative compatibility genes are very stable, and evolutionary events such as mutations led to the occurrence of multiple races within VCGs. They also propose that mutations at vegetatively compatible loci led to the development of single-member VCGs.

Moore et al. (1991 and 1993) analyzed the production of volatiles and found specific VCGs produced characteristic volatile profiles, while other isolates tested did not. These isolates were previously classified as variety 'odoratum' and 'inodoratum', respectively (Brandes, 1919). The Australian population of *F. o.* f. sp. *vasinfectum* produces a distinctive odor when grown on a starch substrate (Davis et al., 1996a).

Methods and Materials

The media used in this study have appeared in other publications, but for the sake of completeness, all are described in the appendices.

Samples of diseased onions were collected in the fall of 1999 in Fruita (Mesa County Gobbo Farm), Delta County (Hines Farm) and Montrose County (Homewood Farm). These counties represent the onion producing areas in Western Colorado. *F. o. cepae* isolates from the collection of Dr. Howard F. Schwartz, Extension Plant Pathology Specialist, Colorado State University, were included in this study to determine if isolates from other onion production areas of Colorado were related to isolates of Western Colorado.

Yellow Sweet Spanish onions from a local store were surface sterilized with 90% methanol, the outer two scales removed and the bulbs sliced to a thickness of 2 mm using a Progressive Multi-slicer (Progressive International Corp., Seattle, WA.). A 10% bleach solution [1 part bleach (5.25% sodium hypochlorite) to 9 parts sterile distilled water] was used to sterilize the Multi-slicer. Onion slices were placed in 100x15 mm sterile plastic petri plates. This process was accomplished under sterile conditions using an Air Clean ™ Systems Workstation (laminar flow hood).

Dried tissue collected from individual onions collected in Western Colorado was

pulverized under aseptic conditions. This inoculum was diluted with sterile distilled water and a 1 ml aliquot was evenly spread over onion slices prepared as described above. Colonies which developed on onion slices were transferred to complete medium (CM) and grown for 4 days at which time hyphal tips were transferred to chlorate media.

Initially isolates were transferred to chlorate media [MM supplemented with either 1.5 or 3% potassium chlorate ($KClO_3$)]. Inadequate sectoring and lack of complementary *nit* mutants resulted in some cases. As a result, several chlorate media were included in further trials to determine which was most effective in generating the required complementary *nit* mutants from *F. o. cepae* isolates.

The chlorate media used were potato-dextrose agar (PDA) and minimal medium (MM). MM was supplemented with 1.6 g L-asparagine or 2.3 g L-threonine $liter^{-1}$. All media was supplemented with 1.5% or 3.0% $KClO_3$ (w/v). Three thin pieces of agar containing hypha of the isolate were equally spaced on each 100x15 mm plastic disposable petri plate. Three plates of each medium/chlorate level were used per isolate for a total of 18 plates per isolate. Inoculated plates were grown for a minimum of 2 days under continuous fluorescent lights at 25° C.

Chlorate plates were checked every other day for sectoring. If a thin colony without (or with very sparse) aerial mycelium developed, a hyphal tip from the sector was transferred to a 60x15 mm petri plate containing minimal medium (MM), and grown for a minimum

of two days under continuous fluorescent lights at 25° C. Isolates which retained their sparse characteristics were considered *nit* mutants and phenotyped. Isolates which reverted to a dense aerial mycelium on MM were considered chlorate-resistant, nitrate-utilizing isolates (crn) and discarded.

Phenotyping media consisted of basal medium (MM) supplemented with ammonium tartrate (NH_4), nitrate (NO_3), nitrite (NO_2), and hypoxanthine (HX). Four mutants were placed on each 100x15 mm phenotyping plate and grown at 25° C. Isolates could normally be read within 3 to 4 days. Fluffy colonies were considered as having a "+" growth; and sparse, transparent growth was considered as having a "-" growth. *Nit* mutants were characterized as *nit1*, *nit3*, or NitM as indicated previously in Table 1.

Fusarium oxysporum isolates representing other onion producing areas in Colorado were selected from the cryogenic collection maintained by Dr. Howard F. Schwartz. Nit mutants for these isolates were generated by Erin Wickliff, Research Assistant, or in Western Colorado by the author. *Nit* mutants provided by Dr. Howard F. Schwartz and Research Assistant Erin Wickliff, Colorado State University, were produced from single spore isolates. Nit mutants produced by the author were from hyphal tips due to the difficulty to locate and see individual spores. When complementary pairs of *nit1* and NitM, or *nit1* and *nit3* mutants could not be produced, the isolate was not considered for further examination.

Vegetative Compatibility Testing

Nit mutants were paired in all possible combinations on 100x15 mm MM petri plates (1st pairings) and 96 well tissue culture plates (2nd pairings) to determine the isolates compatibility and classification to a Vegetatively Compatible Group. A total of 21 and 25 pairs of mutants were compared in this study, respectively. Complementation pairings were made between *nit1* and NitM mutants, or *nit1* and *nit3* mutants. All possible pairings were made with the other isolates..

Pairings on 100x15 mm petri plates containing minimal medium (MM), were made by transferring tiny pieces of MM agar containing hypha of the mutant. Pairings on the 96 well plate was accomplished as follows:

1. A tiny piece of MM agar containing the mutant was transferred to a 60x15 mm petri dish containing complete medium (CM).

2. The CM plates were maintained at 25° C under fluorescent lights until the surface of the each plate was covered with aerial mycelium of the mutant.

3. Two mls of a 2% solution of Tween 60® was added to the plate and the surface of the plate scraped with a sterile scalpel.

4. A drop of Tween 60® containing propagules of the mutant were placed in the respective wells of the 96-well tissue culture plate (Table 1i).

Plates were incubated in the light at 25°C and scored as early as 5 days after inoculation.

Isolates were considered to be vegetatively compatible if the complementation of the *nit* mutants resulted in the formation of a heterokaryon with dense aerial growth at the zone of the anastomosing mycelia (100x15 mm petri plate) or the combination of aliquots resulted in dense mycelial growth (tissue culture plates).

Results and Discussion

Twenty four isolates from various onion producing areas in Colorado were plated on different types of chlorate media to determine which media was most effective in producing the mutants required (NitM and *nit1*, or *nit3* and *nit1*) for comparison tests. A total of 358 mutants were generated. The relative frequency of mutants produced on various chlorate media was 66% for *nit1*, 4% for *nit3* and 30% for *nitM* (Table 1e).

PDA medium, minimal medium (MM) supplemented with 1.6 g L-asparagine liter^{-1}, and MM supplemented with 2.3 g L-threonine liter^{-1} were used in this comparison. Each was further supplemented with $KClO_3$ to 1.5% and 3% (w/v).

One hundred eighty five mutants (52% of the total) were generated on various media supplemented at the 3% chlorate rate, while the 1.5% chlorate media generated 173 mutants (48%). Sixty six percent of the mutants were *nit1*, 4% were *nit3*, and 30% were NitM. While the 3% and 1.5% PDA chlorate media produced the greatest number of mutants (91 and 62, respectively), they also produced the lowest percentage of NitM

mutants (10% and 15%, respectively).

The *nit1* and NitM mutants generated on the MM/L-asparagine chlorate medium were roughly the same. The 3% chlorate generated 55% *nit1* mutants and 43% NitM mutants. The 1.5% chlorate generated 50% and 46% *nit1* and NitM mutants, respectively. The respective percentage of *nit1* and NitM mutants generated on the MM/L-threonine chlorate medium were 69% and 25%, respectively, for the 3% chlorate, while 35% of the mutants generated on the 1.5% chlorate were *nit1* and 45% were NitM. When a higher percentage of *nit1* and NitM mutants are produced, a greater number of complementations should be expected. However, 8 (33%) of the 24 isolates examined (19, 20, 21, 22, 24, 43, 45, and 49) on the different chlorate media did not generate NitM mutants. Four other isolates (23, 31, 44, and 47) produced only one NitM mutant each.

Pairing #1

The first set of complementary pairings were made on 100x15 mm petri plates containing minimal medium as previously described. Twenty one isolates were compared in this trial (Table 1h). Three VCGs were identified from these pairings as follows:

Table 1b: VCGs from 1st Pairing on 100x15 mm Petri Plates of Colorado Isolates of *F. o. cepae.*

VCG #1	VCG #2	VCG #3
22 - Montrose	31 - Delta	41 - Fruita
25 - Delta	32 - Delta	42 - Fruita
27 - Delta		47 - Fruita
28 - Delta		48 - Fruita
FOC1 - Sakata		49 - Fruita
FOC4 - Rocky Ford		50 - Fruita
FOC8 - Western Slope		FOC201A - LaSalle
FOC201A - LaSalle		
FOC201D - LaSalle		
FOC204A - Gilcrest		

In this pairing, isolates 21 and 23 were incompatible with all other isolates. Isolate 26 was compatible with FOC1, but no other isolate. Isolate 204A, *nit1* and NitM, were incompatible and therefore were disqualified. Isolate 49 NitM reverted to the wild state and thus comparisons with this isolate were not valid. All other isolates tested in this trial were compatible with at least two other isolates in the same VCG, with the exception of VCG #2 which contains only two isolates. FOC201A of VCG #1 was cross-compatible with isolate 47 of VCG #3.

VCG #2 consists of 2 isolates, 31 and 32. Technically, due to the limited number of isolates examined, this would not be classified as a VCG.

Pairing #2

The second set of complementary pairings used 96 well tissue culture plates containing minimal medium as previously described. In this case 25 isolates[52] were compared (Table 1i). Three VCGs were identified from the 96 well procedure as follows:

Table 1c: VCGs from 2nd Pairing on 96 Well Tissue Culture Plates of Colorado Isolates of *F. o. cepae.*

VCG #1	VCG #2	VCG #3
22 - Montrose	31 - Delta	41 - Fruita
25 - Delta	26 - Delta	42 - Fruita
27 - Delta	27 - Delta	44 - Fruita
28 - Delta	32 - Delta	48 - Fruita
FOC1 - Weld County		
FOC4 - Rocky Ford		
FOC8 - West Slope		
FOC201A - LaSalle		
FOC201D - LaSalle		
FOC204A - Gilcrest		

Isolates 21 and 23 were incompatible with all other isolates. Isolate 27 (VCG #1) was cross-compatible with isolate 31 of VCG #2. Isolate 49 was not included due to reversion.

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[52] The difference in isolates between trial one and trial two is a result of the addition of isolates over the course of time. Isolate 20, 24 and 202B were included in trial #2; isolate 49, included in trial #1 was not included in trial #2 as it reverted to a wild strain on minimal medium.

Table 1d: Final Vegetative Compatible Groups Resulting from Two or More Pairings of Colorado Isolates of *F. o. cepae.*

VCG #1	VCG #2	VCG #3
22 - Montrose	31 - Delta	41 - Fruita
25 - Delta	32 - Delta	42 - Fruita
27 - Delta		47 - Fruita
28 - Delta		48 - Fruita
FOC1 - Weld County		50 - Fruita
FOC4 - Rocky Ford		
FOC8 - West Slope		
FOC201A - LaSalle		
FOC201D - LaSalle		
FOC204A - Gilcrest		

Isolates in each VCG share the same clonal lineage. In some cases, however, cross-compatibility does occur. VCG #1 is present in Delta, Montrose and 5 onion growing areas in Eastern Colorado (4 counties). It would be of interest to conduct a survey to determine if the spread of this VCG is the result of equipment sharing or field contamination from the boots of visiting growers, crop consultants, seed company representatives, or other visitors. Genetic testing would help determine the origin of this VCG and the variability in each of the fields infected. The more diverse the genetic variability, the longer the VCG would have been in the respective field VCG #2 is present only in Delta and represents a clonal population genetically isolated from the other two VCGs identified. VCG #3 is located only in Fruita (Mesa County). Further genetic testing would determine the origin as discussed in the above paragraph.

Complementary pairings on 100x15 mm petri plates is time consuming, however, the results are more distinct and thus easier to read. Complementary pairings on 96 well tissue culture plates is less precise due to the inability to observe the actual heterokaryon formation. Growing the isolate on complete medium, as recommended for this process, is a problem due to the greater potential to produce reverts than when the isolate is grown on minimal medium. Isolate 7, 47, 50 and 201A reverted on complete medium and consequently were not included in the vegetative compatible groups for trial #2.

Chapter 2: Irrigation Water as a Source of Reinfestation of Onion Fields

Abstract

Irrigation water samples collected in Western Colorado in the summer of 1994 carried chlamydospores of *Fusarium* species and *F. o. cepae* . Up to 110 colony forming units (cfu) of *Fusarium* spp. were contained in each ml of irrigation water entering the field; 22 cfu's were *F. o. cepae*. Results revealed up to one hundred and thirty cfu's of *Fusarium* spp. were carried in each ml of water leaving the field as tailwater; up to seventy nine (60%) of these cfu's were *F. o. cepae*. The results of this analysis demonstrate that irrigation water can infest fields previously free of *F. o. cepae*. Care therefore must be taken to avoid over irrigation and the tailwater which runs off *Fusarium* contaminated fields. Irrigation techniques which reduce tailwater are important, especially in new areas being planted to susceptible hosts. Irrigation systems should be designed which prevent tailwater from returning to the main irrigation system. These results support the findings of previous researchers.

Literature Review

The dissemination of *Fusarium* occurs principally by movement of infected plants, plant debris, or contaminated soil (Green, 1981). The main source of Fusarium infestation in asparagus plantings is infected one-year old crowns (Knaflewski, 1990). Celery transplants from Florida are assumed to be responsible for the introduction of *F. oxysporum* f. sp. *apii* race 2 into Orange County , New York (Awuah and Lorbeer, 1982). Research by Ramsey and Lorbeer (1980) found up to 84% of onion seed harvested in 1978 in Orange County, New York was infected internally with *Fusarium*. *F. oxysporum* and *F. moniliforme* were the most prevalent *Fusarium* species in several seed lots. Seed lots with the highest percentage of internal *Fusarium* infection produced the lowest seedling stands when planted in either natural organic soil or autoclaved soil.

Reports of various *formae speciales* of *Fusarium oxysporum* have been reported as being found in fields never previously cropped to the host (Price, 1984). Whether the organism was brought in on a symptomless host (or non-host), arrived on wind-blown soil or in irrigation water is unknown.

The conidia which form on sporodochia are not suited to wind dispersal in dry weather as they usually adhere to the substrate (Hirst, 1965). At the same time, microconidia of pathogenic isolates of *F. oxysporum* have been trapped from air suggesting contamination of treated soil may be due to airborne microconidia of this pathogen (Rowe et al., 1977).

Dust storms may be responsible for long distance air dispersal of *Fusarium* conidia for several hundred kilometers (Ooka and Kommedahl, 1977). Unlike conidia, chlamydospores are not readily disseminated in air. *F. solani* chlamydospores have an adhesive spore surface (Griffin, 1981). Movement of chlamydospores through irrigation water is, however, possible. Plant pathogens have been found in irrigation canal water, and irrigation pond water (Klotz et al., 1959; Shokes and McCarter, 1979). Furrow irrigation water in Israel is reported to be responsible for the dissemination of *F. o.* f. sp. *vasinfectum*, the cotton wilt pathogen (Grinstein et al., 1983), and is suspected to be a major source of reinfesting fumigated soil (Shokes and McCarter, 1979).

While chlamydospores are reported to survive for up to five years in sterile distilled water (Abawi, 1971) and *F. o.* f. sp. *lycopersici* is known to sporulate profusely in saline irrigation water (Price, 1984), excessive irrigation does not necessarily promote an increase in Fusarium root rot. Knaflewski et al. (1990) found that neither irrigation nor plant density had any significant influence on the occurrence of Fusarium root rot on asparagus.

Materials and Methods

Irrigation water samples [500 milliliters (mls)] were collected in 500 ml sterile bottles in the summer of 1994 from the irrigation ditch just prior to being syphoned into two fields near Delta, Colorado. These two fields were being used in soil inoculum studies (Chapter

3). The irrigation water applied to these fields entered through a common irrigation ditch. Field #1 was cropped to pinto beans at the time samples were collected. This field had been used to grow onions in 1993. Field #2 was cropped to onions at the time the samples were collected. Pinto beans had been grown in this field the preceding year (1993). Five hundred mls of water were also collected from the tailwater leaving each field.

A serial dilution of the collected samples was used to determine the colony forming units per ml of water. A 1 ml subsample of each agitated sample was drawn and suspended in 10 ml of sterile distilled water in a 15x150 mm vial. The water suspension in each vial was shaken using a Vortex-Genie, (Scientific Industries, Inc., Bohemia, N.Y.). A 1 ml aliquot of the suspension was collected during agitation and placed in another vial containing 10 ml of sterile distilled water. This vial was likewise agitated, a 1 ml aliquot taken and added to the next vial containing 10 ml of sterile distilled water. This process was repeated until a 10 tube serial dilution resulted. A 1 ml aliquot of each suspension was drawn and spread over the surface of a 100x15 mm style sterile plastic petri plate containing Komada's selective medium (Komada, 1975). Assay plates were prepared by pouring 12-15 ml of the medium into each plastic disposable petri plate.

Five petri plates were inoculated for each dilution. Each aliquot was drawn while the suspension was being agitated. The inoculated petri plates were incubated at room temperature (22 ± 3^0 C) with light. Five to seven days later, well-separated colonies,

tentatively identified as *Fusarium*, based on colony morphology, were transferred to Carnation Leaf Agar (Nelson et al., 1983). Individual colonies which developed on Carnation Leaf Agar (CLA) were identified under a compound microscope as *Fusarium oxysporum* based on the presence of macroconidia, microconidia, chlamydospore and length of microconidiophore. Colonies which formed on CLA were characterized by a very compact center with a loose uniform margin.

All colonies tentatively identified as *F. oxysporum* were transferred to onion slices[53] to determine pathogenicity. Suspect *F. oxysporum* colonies from one petri plate were transferred to one onion slice. Petri plates containing onion slices were marked with the corresponding number of the CLA petri plate from which the colonies were transferred for identification and control purposes.

The onion slices were prepared using yellow sweet Spanish onions provided by local growers or purchased from local grocery stores. These were surface sterilized for 2 minutes in 90% ethanol. The outer 3 scales were removed, and the onion bulbs was using a Progressive Multi-slicer. The Multi-slicer was sterilized with 90% ethanol. This process was accomplished under sterile conditions using an Air Clean ™ Systems Workstation (laminar hood) (AirClean Systems, Raleigh, NC.). Onion slices were

[53] Abawi (1971) compared several methods to differentiate the pathogenicity of *F. o. cepae*: pot, test tube culture, root dip, injection and onion slices. The onion slice method was found adequate for that purpose (Abawi, 1965; Abawi and Lorbeer, 1965).

placed in sterile plastic petri plates, inoculated and incubated in the dark at 25 ° C. Slices were two mm thick. Onion slices were inspected 4 to 5 days after inoculation. The presence of abundant mycelial growth, typical of *F. o.*, on the onion slice was considered positive for pathogenicity.

Results and Discussion

Insufficient samples were collected to allow a comprehensive analysis of the movement of *Fusarium* via irrigation water and tailwater, however, the following observations were noted.

In June, water sampling revealed 95 cfu's of *Fusarium* spp. entered Field #1 (cropped to onions) ml^{-1} of irrigation water, while a total of 110 cfu's ml^{-1} of water entering Field #2 (cropped to pinto beans) was *Fusarium* spp. When a comparison was made between total *Fusarium* and *F. o. cepae* it was found that an average of 19% of the *Fusarium* population entering the fields in June via irrigation water was *F. o. cepae*

Tailwater collected from these fields in June contained 27 and 45 cfu's ml^{-1} of water of total *Fusarium* spp. from the onion and pinto bean fields, respectively. This equated to a 25% difference in Fusarium propagules being carried off these fields, with the pinto bean field producing the greater number of cfu's ml^{-1} of tailwater. These fields were at the lower end of the irrigation system with upstream fields draining into the irrigation

system.

One could expect the percentage of the population of *F. o. cepae* leaving the field to be higher in a field cropped to onions than a field cropped to beans. More *F. o. cepae* chlamydospores would be produced in a field containing the host plant than would be produced in a field containing a non-host (pinto beans). Forty-eight percent of the total *Fusarium* propagules leaving the onion field were *F. o. cepae,* while only 31 % of the *Fusarium* leaving the pinto bean field were *F. o. cepae*

July data comparing the total *Fusarium* population to *F. o. cepae* for Field #1 (onions) indicates a greater percentage of *F. o. cepae* (61%) left the field via tailwater then entered the field (22%). This may have been due to an increased amount of dead material from the bean crop the previous year, the saprophytic ability of *F. o. c.*, and exudates from the preferred host (onion).

Summation:

1. Propagules of *Fusarium* are carried in irrigation water.
2. *F. o. cepae* can move off an irrigated field via tailwater even during years when the host of this pathogen (*Allium* spp.) is not being grown in the field.
3. A higher level of propagules entered both fields in June than were carried from the fields via tailwater.

4. In July the total *Fusarium* as well as the *F. o. cepae* population carried from the field in tailwater were greater then the number of propagules which entered the field in irrigation water. This is due in part to an increase in the number of propagules in the field as the season progressed.

5. Efforts need to be made to avoid the use of tailwater to irrigate fields which eventually will be planted to onions.

6. Additional research is needed to determine the change in cfu's ml^{-1} of *F. o. c.* in tailwater throughout the growing season and how this change is affected by host and non-host environments.

Table 2a. Fusarium Population Counts Recovered from Irrigation Water at the Clarence Wallace Farm, 1600 Road, Delta in June and July, 1994; Field #1 Planted with Onions.

Month	Dilution	CLA Plate # [3]					Onion Slices Plate # [3]					Carnation Leaf Agar Total *Fusarium* MPN [4] cfu ml^{-1} H_2O	Onion Slices *F. o. cepae* MPN [4] cfu ml^{-1} H_2O
		1	2	3	4	5	1	2	3	4	5		
June	10^1	+	+	+	+	+	+	+	+	-	+		
Water	10^2	+	-	-	+	-	-	-	-	+	-		
In [1]	10^3	+	+	-	-	-	-	-	-	-	-		
	10^4	-	-	-	-	-	-	-	-	-	-	95	17
June	10^1	+	+	+	-	+	+	+	+	-	+		
Water	10^2	-	-	+	+	+	-	-	-	-	-		
Out [2]	10^3	-	-	-	-	-	-	-	-	-	-		
	10^4	-	-	-	-	-	-	-	-	-	-	27	13
July	10^1	+	+	+	+	+	-	+	+	+	+		
Water	10^2	-	+	-	+	+	-	-	-	+	-		
In [1]	10^3	-	-	-	-	+	-	-	-	-	+		
	10^4	-	-	-	-	-	-	-	-	-	-	79	17
July	10^1	+	+	+	+	+	+	+	+	+	+		
Water	10^2	+	-	+	+	+	+	-	-	+	+		
Out [2]	10^3	-	-	-	-	-	-	-	-	-	-		
	10^4	-	-	-	-	-	-	-	-	-	-	130	79

[1] 500 ml of water was collected just prior to the point where water was siphoned into the field.

[2] 500 ml of water was collected from 5 water furrows where the water leaves the field.

[3] Colony development; If colony formation occurred the plate/onion slice was given a (+); if no colony formation occurred, the plate/onion slice was awarded a (-).

[4] The number of colony forming units $gram^{-1}$ of soil was determined by using the Most Probable Number method as described by Alexander, 1982.

Table 2b. Fusarium Population Counts Recovered from Irrigation Water at the Clarence Wallace Farm, 1600 Road, Delta in June, 1994; Field #2 Planted with Pinto Beans.

Month	Dilution	CLA Plate # [3]					Onion Slices Plate # [3]					Carnation Leaf Agar Total *Fusarium* MPN [4] cfu ml^{-1} H_2O	Onion Slices *F. o. cepae* MPN [4] cfu ml^{-1} H_2O
		1	2	3	4	5	1	2	3	4	5		
June	10^1	+	+	+	+	+	+	+	-	+	+		
Water	10^2	+	-	+	+	-	+	-	-	+	-		
In [1]	10^3	-	-	+	-	-	-	-	-	-	-		
	10^4	-	-	-	-	-	-	-	-	-	-	110	22
June	10^1	+	+	-	+	+	+	+	-	-	+		
Water	10^2	-	+	+	+	+	-	+	-	-	-		
Out [2]	10^3	-	+	+	-	-	-	+	-	-	-		
	10^4	-	-	-	-	-	-	-	-	-	-	45	14

[1] 500 ml of water was collected just prior to the point where water was siphoned into the field.

[2] 500 ml of water was collected from 5 water furrows at the point where the water left the field at the end of the furrow.

[3] Colony development; If colony formation occurred the plate/onion slice was given a (+); if no colony formation occurred, the plate/onion slice was awarded a (-)

[4] The number of colony forming units $gram^{-1}$ soil was determined using the Most Probable Number method as described by Alexander, 1982

Chapter 3: *F. oxysporum* f. sp. *cepae* Inoculum Density in Soil

Abstract

Soil samples were collected over a five year period from two fields in Delta, Colorado consisting of a Billings silty clay loam soil. These field were alternately planted to pinto beans and onions. Samples were collected from a location 15.24, 30.50, 45.75 and 61.00 meters from the top of each field. Samples were collected over a four month period in 1991, three months in 1994, and in August in 1992, 1993 and 1995. Samples were collected at approximately the middle of each month.

In 1991, no significant differences in the total *Fusarium, F. o. cepae*, or non-*F. o. cepae* populations occurred in the field planted to pinto beans for months or locations in the field where samples were collected. The *F. o. cepae* population dropped from 343 to 69 cfu g^{-1} of soil between August and September. A similar drop occurred in the total *Fusarium* population from 875 to 168 cfu's g^{-1} of soil, as well as with the non-*F. o. cepae* population. The latter drop was from 533 cfu's g^{-1} in August to 99 cfu's g^{-1} of soil in September. This drop most likely occurred as the result of the mature growth stage of the pinto beans and reduced exudates being released from the root system to

stimulate the pathogen.

In the field planted to onions in 1991, no significant differences in total *Fusarium*, *F. o. cepae* , or non-*F. o. cepae* populations occurred during the months samples were collected or among locations in the field where samples were taken. In this field a drop in propagules occurred in August. All three populations recovered by the September collection date when temperatures had dropped. No significant differences existed in 1991 for either month or site when data from both fields were combined.

When data from both fields were analyzed over five years (1991 - 1995; August of each year), the total *Fusarium* population ranged from 419 (1994) to 1625 (1993) cfu's g^{-1} of soil. The number of cfu's at the 45.75 meter location in the field (1339 cfu's) was greater than that found at the 61.00 meter location (529 cfu's). The *F. o. cepae* population ranged from 587 (1993) to 73 (1992) cfu's g^{-1} of soil (P=0.10). The non-*F. o. cepae* population ranged from 254 to 1039 cfu's g^{-1} of soil for 1994 and 1993, respectively. The fields planted to pinto beans during the five year study did not significantly differ.

The total *Fusarium* population in the fields planted to onions ranged from a low of 87 in 1991 to a high of 2183 cfu's g^{-1} of soil in 1993. The *F. o. cepae* population ranged from 54 to 670 cfu's g^{-1} of soil yr^{-1}. No significant differences were noted with any analysis conducted. The lowest mean maximum temperature in August during the study occurred in 1993 which correlates with the maximum propagule count.

Data collected in the field planted to onions in 1994 revealed a significantly higher (P=0.10) level of *Fusarium* propagules in July when compared to June and August. The *F. o. cepae* population in this field was significantly greater (P=0.10) in July when compared with June. No significant differences were found in the different locations where samples were collected. Comparisons for non-*F. o. cepae* populations were not significant.

Literature Review

Fungal Location in the Soil Profile

The majority of *Fusarium* are normally found in or on soil, where they exist as colonizers of living plant parts or plant residues within the top 100 centimeters (cm) of the soil or adjacent to the soil surface (Gordon, 1956; Nash and Snyder, 1965). They often form sporodochia on substrates at or just above the soil surface; the resulting spores can be spread by rain splash directly or after being washed to the soil surface (Gregory et al., 1959; Hirst, 1965). The presence of *Fusarium* spores on aerial parts is due to passive dispersal in the atmosphere (Gregory et al., 1959; Hirst, 1965; Horst et al., 1970; Rowe et al., 1977). Airborne macroconidia of *F. o.* f. sp. *lycopersici* were trapped on selective medium by Katan et al. (1997) in tomato greenhouses.

In greenhouse carnation soil, *F. o.* f. sp. *dianthi* and other *F. o.* f. sp. were concentrated

mainly in the 0-20 cm soil layer (Reuven et al., 1992). *F. o.* f. sp. *lycopersici* race 2 was recovered from greenhouse soil at depths to 90 cm (Farley et al., 1974). The vertical distribution of *F. o.* f. sp. *apii* race 2 was 1125 and 900 propagules g^{-1} of air-dried soil in the top 0-20 and 20-40 cm, respectively. The latter pathogen was not found at a soil depth below 40 cm (Awuah and Lorbeer, 1986a).

Baker (1970) reported that inoculum in cultivated soil appears at times to be uniformly distributed. However, in organic soils in New York, research clearly showed an uneven distribution of propagules (Abawi and Lorbeer, 1971b). Similar patterns of uneven distribution have also been reported for *F. o.* f. sp. *cubense*, *F.o.* f. sp. *melonis*, *F.o.* f. sp. *lini*, and *F. solani* f. sp. *cucurbitae* and *F. solani* f. sp. *pisi* (Burke et al., 1970; Houston and Knowles, 1949; Nash and Alexander, 1965; Trujillo and Snyder, 1963; Wensley and McKeen, 1962).

Studies in bean fields have shown counts of *F. solani* f. sp. *phaseoli* as high as 1000 - 3000 propagules g^{-1} of soil spread rather uniformly throughout the plow depth of 15 - 20 centimeters. "In a field in which the average count is 1000 propagules g^{-1} of soil, we might expect that 7.5 centimeters of the underground bean stem alone would come in contact with 10 propagules of the pathogen. Further, an average of 1500 propagules would be within 1 mm of the stem " (Nash and Snyder, 1962).

Woltz's (1974) research with gladiolus found that the presence of fusarium in soil did

not equate with symptom development in the plant. The disease reactions of various cultivars was not uniform. This may be in part due to variation in plant nutrition.

In cultivated soils, Paulitz et al. (1987) found the total population[54] densities of *Fusarium* spp. ranged from 11000 to 27000 colony forming units (cfu) g^{-1} of soil with *F. o.* being a major component of this fungal population. Onion fields in New York with long histories of Fusarium basal rot had from 660 to 13000 propagules g^{-1} of oven dry soil in the top 15 centimeters of this organic soil (Abawi, 1971).

Abawi et al. (1971c) reported one New York farm with a long history of basal rot having an average *F. o. cepae* population of 3766 (670-7300) propagules g^{-1} of soil. One hundred propagules of *F. o. cepae* g^{-1} of soil caused extensive disease development (80%) in a steam-sterilized soil, while a population of 5×10^4 or more propagules g^{-1} of oven-dry soil was needed before significant (30%) disease development could be detected (Abawi, 1971; Abawi and Lorbeer, 1972).

The correlation between total *F. oxysporum* populations and pathogenic species, however, is not linear. Abawi (1971) reported there was no correlation between the population of *F. oxysporum* and those of *F. o. cepae*. Reuven et al. (1992) likewise

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[54] The population of soil-borne plant pathogens is expressed in the mass or number of propagules per unit of soil. This is referred to as "inoculum density" and "inoculum intensity" (Menzies, 1970).

found it was not possible to predict the population of *F. o.* f. sp. *dianthi* from the total *F. oxysporum* population in greenhouse soil. Propagules of *F. oxysporum* were detected in all soil samples tested, whereas *F. o.* f. sp. *dianthi* was found in only two-thirds of them.

Soil and Pathogen Interactions

It seems safe to generalize that soil-borne pathogens characteristically overproduce inoculum (Menzies, 1970). The propagules of most soil-borne plant pathogenic fungi exist in soil in a quiescent state and do not develop unless provided with some external stimulus (Short and Lacy, 1974) or exogenous nutrients (Paulitz, 1990). These nutrients are released from plants as root exudates, mucigels (Cook and Snyder, 1965) and from decaying plant parts (Baker, R., 1981).

When the host is present, root exudates provide the stimulus for spore germination (Baker and Scher, 1987). Menzies and Gilbert (1967) found that plant residues give off volatile compounds which have a stimulating effect on germination and respiration of soil organisms. These include several low-molecular-weight aldehydes and alcohols which stimulate spore gemination *in vitro*[55] (Owens et al., 1969; Sussman, 1965).

Water, oxygen, nitrogen, sulfur and various micronutrients were reviewed by Clark

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[55] *In vitro* - "within an artificial environment, as a test tube" (Stein and Urdang, 1967).

(1965) in his discussion of competition in microbial ecology. He concluded that nutrient limitations were critical for soil-borne pathogenic organisms. Goyal et al. (1973) used different rates of glucose in research on chlamydospore production and found different concentrations of glucose and incubation time had a direct influence on chlamydospore production. They suggest the conditions necessary for chlamydospore development must be determined for each species of *Fusarium*.

In 1969, Baker suggested the nutritional factors potentially being in short supply in soil include carbon and nitrogen. That same year, Griffin (1969) reported germination of chlamydospores of *Fusarium oxysporum* increased the closer the spores were to tips of roots. His findings suggest the exogenous substances which are necessary for germination increase in concentration as the young root is approached. He found germination began an average of 9.5 mm from the tips of roots of 3 day-old peanut (*Arachis hypopaea* L.) and increased to a high level of germination (>73%) as the root surface of young roots was approached. Most germ tubes exhibited growth toward the root surface (Griffin, 1969). Griffin (1964) previously reported pre-germinated conidia produced chlamydospores after 4.5 days when placed in a solution of KNO_3 solution, distilled water or 0.5 M phosphate buffer. In unbuffered solutions containing only glucose, chlamydospore germination was inhibited. The pre-germination solution consisted of 0.5% glucose plus 500 ppm KNO_3. The pre-germinated spores were washed before being placed in the final germination solution.

Early research on the nutritional requirements for germination of *Fusarium* macroconidia indicated there were absolute requirements for exogenous carbon and nitrogen sources. These findings, however, appear to have resulted mainly from using high conidial densities (10^5 to 10^{6+} conidia ml^{-1}) in germination assays (Cochran et al., 1963; Griffin, 1969; Griffin and Pass, 1969; Marchant and White, 1966; Sisler and Cox, 1954).

In the 1970's, Griffin did extensive research on the effects of carbon and nitrogen on the germination of Fusarium spores (Griffin, 1973). He found macroconidial germination of *Fusarium solani* was fully independent of exogenous carbon at 3×10^3 conidia ml^{-1} or below. Full dependency on exogenous carbon was required when conidial density reached 3×10^5 spores ml^{-1} or higher (Griffin, 1970a). He later reported the exogenous carbon and nitrogen requirements for chlamydospore gemination depended on conidial density as well as nutritional condition of the chlamydospores. He demonstrated the effect of nutritional status of chlamydospores on germination by providing macroconidia with two levels of nutrients and using the resulting chlamydospores in his research project (Griffin, 1970b).

Not all exogenous sources of carbon and nitrogen are utilized by Fusarium. In 1976, Griffin reviewed various sources of carbon to determine their effects on Fusarium macroconidial germination. His research suggests that while a carbon compound promotes germination of macroconidia, it may also inhibit the formation of chlamydospores, especially in the absence of nitrogen (Griffin, 1976).

Washed macroconidia have been reported capable of rapid and complete germination (within 7 hours) in the absence of exogenous organic carbon at low conidial densities (Cappellini and Peterson, 1971; Griffin, 1970a; Hsu and Lockwood, 1973). Chlamydospore germination, however, appears to be dependent on exogenous energy sources (Cook and Schroth, 1965; Griffin, 1969). *Fusarium solani* f. sp. *phaseoli* chlamydospores have germinated as far as 10-12 mm from germinating bean seeds (Short and Lacy, 1974). Germination, however, is highest along the actively growing parts of roots (Schippers and Voetberg, 1969).

Carbon sources such as D-glucose ($C^6H^{12}O^6$), maltose ($C^{12}H^{22}O^{11}$), and sucrose ($C^{12}H^{22}O^{11}$) have been effective for most macroconidia and chlamydospore germination studies (Cochran et al., 1963; Griffin, 1970a; Griffin, 1970b; Griffin and Pass, 1969; Marchant and White, 1966; Sisler and Cox, 1954). Organic acids such as citrate, malate, succinate and acetate have been less satisfactory possibly due to decreased permeability of the spore to these organic acids (Cochran et al., 1963; Griffin, 1976; Marchant and White, 1966). R. Baker (1981) reported that germination and penetration by *Fusarium* required carbon as well as nitrogen.

In vitro studies by Griffin (1970b) demonstrated that *F. solani* required nitrogen for germination and host penetration. His research concluded that exogenous nitrogen is required for a high percentage or complete germination of chlamydospores at high spore

densities (10^5) in axenic[56] culture and in soil. This was not true, however, at low spore density (10^3) (Cook and Schroth, 1965; Griffin, 1969; Griffin, 1970b). Spore density appears to be the single most important factor affecting nutritional requirements for germination of conidia and chlamydospores in pure culture (Griffin, 1981).

The type of nitrogen fertilizer applied influences Fusarium problems (Duffy and Defago, 1999; Woltz and Jones, 1973). *F. o.* f. sp. *niveum* utilizes nitrate nitrogen sources more efficiently than ammonium nitrogen. Maximum growth was obtained on potassium nitrate followed by calcium nitrate, sodium nitrate, ammonium nitrate, ammonium oxalate, ammonium sulphate and ammonium phosphate (Jhamaria, 1972). Macroconidial germination of the bean Fusarium (*F. solani* f. sp. *phaseoli*), on the other hand, was highest when supplemented with ammonium (NH_4-N) rather than the nitrate (NO_3-N) form of nitrogen (Byther, 1965). In research with *F. solani* and *F. roseum*, Byther found both fungi utilized NH_4-N more rapidly than NO_3-N.

The fact that nitrogen fertilization inhibits one pathogen but favors another is an unfortunate complication when attempting to understand soil factors and disease (Menzies, 1970). Woltz and Jones (1973) found that as NO_3-N was increased, pH increased and Fusarium wilt of tomato was reduced; while the use of NH_4-N decreased pH values and increased disease. The use of ammoniacal nitrogen in chrysanthemum

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[56] Axenic - [Gk. Axenos, inhospitable] without, or deprived of any commensals, symbionts or parasites (Kenneth, 1963).

production [*Chrysanthemum moriflorium* (Ramat.) Hemsl.] increases Fusarium wilt diseases (Fisher and Toussoun, 1983), while the use of nitrate nitrogen reduced the incidence of bean root rot (Maurer and Baker, 1965). Raising the soil pH from 6.0 to 7.5 and fertilizing with nitrate nitrogen successfully controlled Fusarium wilt of tomato, chrysanthemum and other crops (Jones et al., 1989). Wensley and McKeen (1964) found an increase in fertilizer caused wilt of melons due to *Fusarium oxysporum* f. sp. *melonis* to occur sooner and more severely. Stoddard (1947) reported that high levels of available nitrogen is of prime importance to the increase of this muskmelon disease. The various soil nutrients and their affect on soil-borne pathogens is the reason soil fertility should be taken into account during attempts to evaluate soil inoculum potential (Woltz, 1974). Available soil iron levels should also be evaluated due to the fact that iron is a limiting nutrient for microbial growth and development (Paulitz, 1990).

Microbes produce siderophores which capture iron and transport it back to the organism through specific membrane-bound transport systems (Paulitz, 1990). Kawamoto and Lorbeer (1976) added *Pseudomonas cepacia* to onion soil and found a marked reduction in onion seedling loss from damping-off. This pseudomonad colonized the root tip, root-stem zone, and seedcoat yet the mechanism responsible for this protection was open to speculation. Pseudomonads are now known to produce siderophores that sequester iron in the root environment, thus making iron less available to competing microbes (Leong, 1986; Schippers et al., 1987).

Scher and Baker (1982) found that iron was necessary for normal germ-tube development from chlamydospores of *Fusarium* spp. Chlamydospores of *F. oxysporum* require at least 10^{-19} mole iron for germination (Simeoni, 1987). The siderophore that *Fusarium* spp. produce has a stability constant of 10^{29}, while the siderophore produced by fluorescent Pseudomonads has a stability constant of 10^{34}. Consequently, under iron-limiting conditions, *Pseudomonas* spp. out compete *Fusarium* spp. for iron (Paulitz, 1990).

Simeoni (1987) studied the effects of the siderophore-producing *Pseudomonas putida* isolate A12 on the chelation of Fe^{-3}. As iron availability decreased, the siderophore of *P. putida* resulted in even less iron being available. This resulted in reduced germination of chlamydospores of *F. o.* f. sp. *cucumerinum*. The non-siderophore-producing mutant of *P. putida* A1/UV/AB-6 used in this study did not reduce chlamydospore germination. The bacterium *Alcaligenes* also is shown to inhibit microconidial germination and germination-tube elongation of *F. o.* f. sp. *dianthi*, and reduce the severity of fusarium wilt of carnation as the result of its siderophore production (Martinetti and Loper, 1992).

The use of foliar nutrient sprays change the rhizosphere microfloral population. Nutrient sprays, especially potassium chloride, markedly reduce the population of *F. o.* f. sp. *melonis* in the rhizosphere of melons (Kannaiyan and Prasad, 1974).

Soil pH (Jones and Woltz, 1969), moisture content (Hall and MacHardy, 1981) and temperature (Armstrong and Armstrong, 1975) influence Fusarium diseases. Symptom

expression in the florist's chrysanthemum [*Chrysanthemum morifolium* (Ramat.) Hemsl.] caused by *F. o.* f. sp. *chrysanthemi* depends on environmental conditions in addition to the nutritional status of the plant (Fisher and Toussoun, 1983).

Antibiotics produced by ectomycorrhizal fungi were suggested by Zak (1964) as one form of root protection which prevents infection by phytopathogenic fungi. The mycorrhizal fungi have since been investigated as a means of protecting roots from pathogenic infection (Richard et al, 1971; Sinclair et al., 1982). Kope and Fortin (1989) studied seven ectomycorrhizae only one of which (*Pisolithus tinctorius*) inhibited the growth of *Fusarium oxysporum*. Other *Fusarium* antagonists include *Penicillium*, *Trichoderma* (Price, 1984), and bacteria. Larkin et al. (1996) conclude that nonpathogenic isolates of *F. oxysporum* are primary antagonists and responsible for suppression of fusarium watermelon wilt caused by *F. o.* f. sp. *niveum* in certain suppressive soils. *Trichoderma* species stimulate plant growth by the production of a growth-stimulating metabolite as well as by its mycoparasitic abilities (Lynch, 1990). *Pseudomonas* spp. suppress the development of chlamydospores which develop from mycelia and reduce the germination of chlamydospores (Toyota and Kimura, 1993).

Cropping history of the field as well as different climatic circumstances can influence the fungal propagule count (Nash and Snyder, 1962). Wheat plants are known to depress populations of *Fusarium oxysporum*, while beans and tomatoes increase fungal population numbers (Price, 1984). Weeds also influence the survival of Fusarium.

Oxalis corniculata is a symptomless host of *F. o. cepae* (Abawi, 1971). Helbig and Carroll (1984) found that 16 out of 21 dicotyledonous weed species commonly found in soybean fields, were infected with the soybean pathogen *F. o.* f. sp. *glycines* without showing disease symptoms. Of 17 isolates of the pathogen tested, 16 were pathogenic to seedlings of the soybean cultivar 'Essex'. Jenkinson and Parry (1994) screened 15 common broadleaf weed species for *Fusarium* and found 14 of the 15 were hosts for cereal *Fusarium*.

Fungal Survival and Dissemination

The population of resting structures of vascular pathogens in soil is diminished by crop rotation. In fact, crop rotation is man's oldest control measure against soil-borne plant pathogens (Baker, K., 1981), having been used for at least two thousand years (Glynne, 1970). Long term rotation with nonsusceptible crops for 3 to 4 years or longer may help reduce losses to *F. o. cepae* in infested fields (Gabor, 1996; Hoffman et al., 1996; Schwartz and Bartolo, 1995; and Schwartz and Mohan, 1995).

Once established, a *Fusarium* chlamydospore population in the soil does not remain at a constant level, but may rapidly decline. The germination of chlamydospores reduces the population as does the lysis of germlings which cannot find a suitable host (Van Eck, 1976). Yet, Kenneth Baker (1981) reports *Fusarium* can infest non-host plants and thus survive for 8 to 12 years without a host. McKeen and Wensley (1961) reported

chlamydospores of *F. o.* f. sp. *melonis*, *F. o.* f. sp. *niveum*, and *F. o.* f. sp. *lycopersici* were capable of surviving for 10 to 17 years in dry soil tube cultures. Davis et al. (1998) report *F. o. cepae* can survive indefinitely in soil.

Living mycelium of *Fusarium solani* f. sp. *phaseoli* is known to lyse completely when in contact with soil. *Streptomycetes* isolates caused 30 to 70% lysis of living mycelium of *Fusarium solani* within four days, while autolytic enzymes were responsible for the death of remaining active mycelium (Lloyd and Lockwood, 1966).

Fungi may exist in soil both as active mycelium and dormant spores (Garrett, 1951). Hargreaves and Fox (1977) found thickened hyphae on *Fusarium avenaceum* which they assumed were survival structures. *Fusarium moniliforme* which does not produce chlamydospores can survive for several years, presumably as macroconidia (Price, 1984).

A study by Warcup (1965) has shown the majority of *Fusarium* colonies isolated from soil by the dilution plate method originated from spores rather than from active mycelium. *Fusarium* species may exist as chlamydospores, and only if plant debris or roots are available will conidia or mycelial fragments be present (Price, 1984; Schippers and Old, 1974). Schroth and Hendrix (1962) reported that *Fusarium* macroconidia

readily convert to form chlamydospores [57]. Abawi (1971) reported conidia added to non-sterilized organic soils were converted to chlamydospores or lysed within two weeks. The use of soil inoculum containing chlamydospores may more clearly simulate pathogenesis under field conditions (Salgado et al., 1991).

The ability of certain *Fusarium* species to form chlamydospores is important to their long-term survival in soil (Alexander et al., 1966) and is well documented (Qureshi and Page, 1970). Nash et al. (1961) demonstrated that *F. solani* f. sp. *phaseoli* survives in soil as chlamydospores. Warcup (1955) reported that *F. oxysporum* exists in soil as chlamydospores and not as conidia.

There are three major phases in the life cycle of chlamydospores: formation, dormancy, and germination. Chlamydospore formation in pathogenic *Fusarium* commonly takes place in hyphae in infected and decaying host tissue. They also may form from macroconidia that originate from sporodochia at the soil level on lesions on the host plant (Schippers and van Eck, 1981).

In naturally infested soils, *F. o. cepae* chlamydospores generally are globose to subglobose in shape and vary from 7.5 to 10.0 microns in diameter. They are found either free between soil particles or embedded in organic tissue (Abawi, 1971).

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[57] Under low temperature (6° C), macroconidia form germ tubes which in turn produce two- or three-celled macroconidia (Griffin and Pass, 1969).

Chlamydospores of *F. o. cepae* form in the cortex and vascular tissues of the root but not in the basal stem plate (Abawi and Lorbeer, 1971a). They can form by transformation of macroconidial cells or from the germ tubes of chlamydospores stimulated to germinate in soil in response to a temporary supply of nutrients provided by diffusates from seeds, living host plants, non-host plants, or crop residues. The formation of chlamydospores from germ tubes of germinating chlamydospores occurred frequently in the rhizosphere of susceptible and resistant hosts due to exogenous materials exuding from roots (Schippers and Voetberg, 1969).

Many biotic and abiotic soil factors affect the formation of chlamydospores (Schippers and van Eck, 1981). These factors include temperature (Banihashemi and de Zeeuw, 1973), CO_2 content (Newcomb, 1960), and pH (Griffin, 1964). Chlamydospore formation is said to be a response to nutrient starvation occurring concurrently with lysis[58] of macroconidia and hyphae (Schippers and Old, 1974). Sherbakoff (1915) expressed this same sentiment with his findings that an "excess of water in a medium usually leads to comparatively quick degeneration (self-digestion?) of the spores". Research by Alexander et al. (1966) indicates that high nutrient levels had the opposite effect by counteracting the factor(s) which favor production of chlamydospores.

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[58] The production of autolytic enzymes is the result of nutrient deprivation (Ko and Lockwood, 1970).

The Most Probable Numbers (MPN) Technique

The population of soil-borne pathogens is difficult to define as the organisms are found in association with remnants of plant material in various degrees of decomposition, as well as intermingled with soil aggregates (Menzies, 1963). Soil assays, however, have been used to determine inoculum potential[59] and incidence[60] of diseases caused by *Fusarium oxysporum* (Woltz, 1974). Population estimates of *Fusarium* are feasible with plate counts reported as the simplest and most practical method to determine propagules g^{-1} of soil. Verification of such counts, however, and determination of their meaning in terms of the inoculum potential in the field is necessary (Nash and Snyder, 1962).

Reuven et al. (1992) found it necessary to inoculate plants of susceptible carnation cultivars with spore suspensions of *F. oxysporum* to determine which *Fusarium oxysporum* colonies were pathogenic (e.g., *F. o.* f. sp. *dianthi).* He initially identified *F. oxysporum* colonies from serial dilutions of a soil/water suspension on PDS medium[61] .

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[59] Inoculum potential is the result of the action of the environment, vigor of the pathogen to cause infection, host susceptibility, and the amount of inoculum present (Dimond and Horsfall, 1970).

[60] There is evidence that the incidence of disease (severity) correlates well with inoculum density as long as other factors are equal (Baker et al., 1967; Dimond and Horsfall, 1965 and 1970).

[61] Potato dextrose agar supplemented with 250 mg dihydrostreptomycin per liter (Reuven et al., 1992).

Bioassays using serial soil dilutions and the most probable number (MPN) estimates have been used by various authors to quantify inoculum of soil-borne plant pathogens (Adams and Welham, 1995). The MPN method permits the estimation of population density without an actual count of single spores or colonies (Alexander, 1982). These procedures were first developed for quantification of bacteria in drinking water (McCrady, 1915), and subsequently in 1958 to quantify inoculum of various soil-borne pathogens (Maloy and Alexander, 1958).

The MPN technique is based on a determination of the presence or absence of microorganisms in several individual portions of each of several consecutive dilutions of soil. It is called the dilution method as well as the method of ultimate or extinction dilution (Alexander, 1982). A dilution count almost invariably counts only fungal spores; no colonies develop from fungal hyphae (Burges, 1965). These propagules are assumed to be randomly distributed through the initial soil sample as well as in the different steps of the dilution series (Adams and Welham, 1995).

The MPN procedure was used to detect 17 species of mycorrhizal fungi, while wet-sieving of field soils detected only 10 species. Population densities of viable spores of individual species were usually lower than those of total spores, although the differences were not always statistically significant (An et al., 1990).

Materials and Methods

The purpose of this study was to determine the changes in total *Fusarium, F. o. cepae* , and non-*F. o. cepae* populations over time (month to month, between years), and at different locations in fields planted to pinto beans and onions.

Soil samples were collected from nine different fields during the summer of 1991 and evaluated for total *Fusarium* and *F. o. cepae* populations. Two fields near Delta, Colorado, were selected for further sampling based on their high levels of inoculum. These fields consisted of a Billings Silty Clay Loam with an organic matter content below 1% and a pH of 7.5 to 7.8.

Samples were collected in 1991, 1992, 1993, 1994, and 1995 from the 2 fields at four different locations within each field. Samples were collected monthly from June through September in 1991, June, July and August in 1994, and August in 1992 and 1993.

Fields were alternately cropped with onion and pinto bean by the grower/cooperator. Soil samples of 227 grams were collected 15.25, 30.50, 45.75 and 61.00 meters from the head of the row. At each site, five samples were collected from the top 15 centimeters at an equal distance across five beds of onions/beans. Samples were collected at a distance of 5 to 7.5 cm from the base of plants with a clean core-type auger (7.5 cm diam). The five samples collected at an equal distance from the head of the row were blended together to

produce one sample. The samples were transported in a cooler in zip lock bags to the lab for analysis. Samples were air-dried at 22 ±2 C for 2 weeks, gently ground, thoroughly mixed and screened through a 50 mesh brass screen.

A 1-g subsample was taken from each soil sample and suspended in 10 ml of sterile distilled water in a 15x150 mm vial. The water/soil suspension was shaken using a Vortex-Genie, (Cat. # 12-812, Scientific Industries, Inc., Bohemia, N.Y.). A 1 ml aliquot of the suspension was collected during agitation and placed in another vial containing 10 ml of sterile distilled water. This vial was likewise agitated, a 1 ml aliquot taken and added to the next vial containing 10 ml of sterile distilled water. This process was repeated until a 10 tube serial dilution resulted.

A 1 ml aliquot of each suspension was drawn and spread over the surface of a 100x15 mm style sterile plastic petri plate containing Komada's selective medium (Komada, 1975). Assay plates were prepared by pouring 12-15 ml of the medium into each plastic disposable petri plate. Five petri plates were inoculated for each dilution. Each aliquot was drawn while the suspension was being agitated. The infested petri plates were incubated at room temperature (22 ± 3 C) with fluorescent light. Five to seven days later, well-separated colonies, identified as *Fusarium*, based on colony morphology, were transferred to CLA (Nelson et al., 1983).

Individual colonies on CLA were identified 7 to 10 days later as *F. oxysporum* using a

compound microscope based on the presence of macroconidia, microconidia, chlamydospores and length of microconidiophores. Colonies formed were characterized by a very compact center with a loose uniform margin. The colonies were transferred to onion slices[62] to determine pathogenicity.

Yellow sweet Spanish onions provided by local growers or purchased from local grocery stores were surface sterilized with 90% ethanol, the outer 3 scales were removed, and sliced (2 mm thick) using a Progressive Multi-slicer. The Multi-slicer was sterilized with 90% ethanol. This process was accomplished under sterile conditions using a laminar hood. Slices were placed in sterile plastic petri plates and incubated with light at 22 ± 3 C. Onion slices were inspected 4 to 5 days after inoculation. The presence of abundant mycelial growth characteristic of Fusarium on the onion slice was considered positive for pathogenicity.

To calculate the most probable number of organisms in the original sample, the following procedure was followed:

1. The number of CLA plates at each dilution rate were evaluated for the presence of *Fusarium* colonies as previously described. When a colony developed on the CLA plate,

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[62] Abawi (1971) compared several methods to differentiate the pathogenicity of *F. o. cepae*, pot, test tube culture, root dip, injection and onion slices. The onion slice method was adequate for that purpose (Abawi, 1965; Abawi and Lorbeer, 1965).

it was given a (+); if no colony formation occurred, the CLA plate was given a (-)[63] Each of the five dilution plates was evaluated in this manner. These ratings are given in Table 3j - 3j23.

2. The dilution plate series (5 plates) which was the most diluted with the greatest number of positive plates (+), was called p1. The next most diluted 5-plate series was assigned p2; the following 5-plate dilution was assigned p3.

Example 1:

10^{-1} had 5 plates assigned a +
10^{-2} had 4 plates assigned a +
10^{-3} had 4 plates assigned a +
10^{-4} had 3 plates assigned a +
10^{-5} had 2 plates assigned a +

p1 = 10^{-1} as it has the greatest number of positive plates in the five-plate dilution

p2 = 10^{-2} the next lower level of dilution

p3 = 10^{-3} the next lower level of dilution

Thus p1 = 5 at 10^{-1} ; p2 = 4 at 10^{-2}; p3 = 4 at 10^{-3}. According to the MPN table (Alexander, 1982), the most probable number is 3.5. When multiplied by 10^{2} the reciprocal of p2, the final total is 350 cfu's g^{-1} soil.

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[63] Note: If one colony or a dozen colonies developed on the plate it was still given a +.

Example 2:

10^{-1} had 5 plates assigned a +
10^{-2} had 5 plates assigned a +
10^{-3} had 4 plates assigned a +
10^{-4} had 3 plates assigned a +
10^{-5} had 2 plates assigned a +

p1 = 10^{-2} is the most diluted level with the greatest number of positive plates

p2 = 10^{-3} the next lower level of dilution

p3 = 10^{-4} the next lower level of dilution

Thus p1 = 5 at 10^{-2} ; p2 = 4 at 10^{-3} ; p3 = 4 at 10^{-4}. According to the MPN table (Alexander, 1982), the most probable number is 3.5. When multiplied by 10^3 the reciprocal of P2, the final is 3500 cfu's g^{-1} soil. The same procedure was used to determine the MPN of *F. o. cepae* colonies on onion slices.

Analyses of variance (ANOV-2) were carried out with the statistical program MSTAT-C[64]. Least significant differences (LSD) were calculated at a significant level of *P* as indicated in each table.

Results and Discussion (Summary Tables 3ie - 3ig)

[64] Microcomputer Statistical Program developed by Michigan State University.

Field #1, 1991; Tables 3a1 and 3a2; Crop = Pinto Beans

The total *Fusarium* population in the field planted to pinto beans in 1991 ranged from 22 to 1700 propagules (cfu) g^{-1} of soil, while the *F. o. cepae* population was 17 to 1100 . There was a drop in propagule numbers for the total Fusarium and *F. o. cepae* readings taken in September when compared with July and August (Table 3a1). These population differences were not significant . The drop in air temperature in September (Table 3i1) may have been responsible for the decrease in propagules during that month.

Differences in the total *Fusarium*, *F. o. cepae*, and non-*F. o. cepae* at different locations in the field, were not significant. The total population of Fusarium increased the further into the field samples were collected. The third site (45.75) into the field where samples were collected, had the highest level of *F. o. cepae* propagules (359 cfu g^{-1} of soil) while the last location where samples were collected (61 meters) had the lowest level of propagules (141 cfu g^{-1} of soil) (Table 3a2).

Field #2, 1991; Tables 3b1 and 3b2; Crop = Onions

The total *Fusarium* population in the field planted to onions in 1991 ranged from 14 to 2200 propagules (cfu) g^{-1} of soil. As occurred in Field #1 planted to beans (1991), a drop in the number of propagules occurred later in the season in Field #2 , however, this

occurred in August in Field #2 (Table 3b1) and September in Field #1 (Table 3a1). Samples collected in September revealed the propagule numbers of *Fusarium* spp. had increased to 838 cfu g^{-1} of soil.

The range in total *Fusarium* population was from 127 cfu g^{-1} (30.50 meters) and 873 cfu g^{-1} of soil (61.00 meters from the top of the field) (Table 3b2). The 15.24 meter site contained 325 cfu g^{-1} of soil when averaged over the four months in which samples were collected, as compared to 504 cfu g^{-1} of soil 45.75 meters into the field.

The population of *F. o. cepae* was higher during June and September than in July and August (Table 3b1). Propagule levels were 202, 85, 53 and 400 cfu's g^{-1} of soil for June, July, August and September, respectively. This indicates a drop in propagule levels in July and August, with high levels in June and September. This can be explained by the high temperatures which occurred in the area in July and August (Table 3i1). Propagule levels were 131, 80, 106, and 424 cfu's g^{-1} of soil at 15.24, 30.5 45.75 and 61 meters, respectively (Table 3b2). These differences were not statistically significant.

Field #1 and Field #2, 1991 (Combined Data); Tables 3c1 and 3c2

Analyses of Variance for total *Fusarium*, *F. o. cepae* and the difference between total *Fusarium* and *F. o. cepae* populations were conducted as follows:

Month vs. Site

Field vs. Site

Month vs. Field

No significant differences were found for any of these comparisons.

The total *Fusarium*, and *F. o. cepae* populations increased the further into the field samples were collected. The combined monthly data for the two fields ranged from 474 to 514 for total *Fusarium*, 164 to 262 cfu g^{-1} for *F. o. cepae*, and 252 to 310 cfu g^{-1} of soil for non-*F. o. cepae*. The July propagule count was higher for both total *Fusarium* and *F. o. cepae* populations. The non-*F. o. cepae* population, however, was lowest in July (Table 3c1). Field #1 (planted to pinto beans) had a higher population of total *Fusarium*, *F. o. cepae*, and non-*F. o. cepae* than Field #2 planted to onions (Table 3c3).

Field #1 and Field # 2 Combined Data over a Five Year Period; Tables 3d1 and 3d2

This comparison was done to determine the change in population during the month of August over a five year period (1991 -1995) when both fields were averaged. The total Fusarium and *F. o. cepae* populations in this study ranged from 13 to 7900, and 8 to 2200 cfu g^{-1} of soil, respectively. August was selected for this trial as it is the end of the production season with some area growers harvesting their onions during that month. It was felt that analyzing data collected during this month would give a more realistic account of what was occurring in fields throughout Western Colorado.

The population of *F. o. cepae* differed significantly (P=0.10) over the five year study (Table 3d1). The drop in propagules in August, 1994 could be explained by the high air temperature experienced in that month (Table 3i2), but a similar drop should have occurred in August 1995 due to high air temperatures. The latter drop, however, did not occur.

Comparison of Fields Planted with Pinto Beans over a Five Year Period; Tables 3e1 and 3e2

The two fields analyzed in this study were alternately cropped to beans and onions. In this part of the study, the fields planted to beans over the 5 year period (1991 - 1995) were analyzed using August data. In 1991, field #1 was planted to beans; in 1992, field #2 was planted to beans; on an alternating basis. These were the fields used in this comparison. The total *Fusarium*, *F. o. c.*, and non-*F. o. c.* populations were not significantly different between years or location.

Comparison of Fields Planted with Onions over a Five Year Period; Tables 3f 1 and 3f2

In this part of the study, fields planted to onions over the 5 year period (1991 - 1995) were analyzed. In 1991, field #2 was planted to onions; in 1992, field #1 was planted to onions; etc. These were the fields used in this comparison. No significant differences

were found with any of the analyses run.

The total *Fusarium* population ranged from an average of 87 cfu's in 1991 to 2183 cfu's g^{-1} of soil in 1993. The averages for the other years fell within this range. The colony forming units g^{-1} of soil ranged from 409 to 1765 when sites from which the samples were collected were averaged. The 45.75 meter site had a greater number of propagules than the 61 meter site.

The *F. o. cepae* population changed from year to year from 54 to 670 cfu's g^{-1} of soil. 1993 had the greatest number of propagules g^{-1} of soil. The *F. o. cepae* populations at the various locations in the field, varied from 164 cfu's g^{-1} of soil at the 61 meter site to 729 cfu's g^{-1} of soil at the 15.24 meter sampling site. August 1993 had the lowest mean maximum temperature of the years this trial encompassed, possibly explaining the reason 1993 had the highest propagule count (Table 3i2)..

Field #1, 1994; Tables 3g1 and 3g2; Crop = Onions

Samples were collected in the field planted to onions in 1994 (Field #1) in June, July and August. There were no significant differences for location in the field for total *Fusarium* population. The *Fusarium* population in July was significantly higher than the population in either June or August ($p= 0.10$). The cfu's g^{-1} of soil in June was 77, while the cfu's g^{-1} of soil in July was 3398. The August *Fusarium* population was 228 cfu's g^{-1}

^{1} of soil (Table 3g1).

The *F. o. cepae* population in June was significantly lower (P=0.05) than the population in July (Table 3g1). The high air temperatures in July and August would point to lower populations during those months, yet this did not occur (Table 3i4). The *F. o. cepae* population ranged from 50 to 1198 cfu's g^{-1} of soil. There were no significant differences for Total *Fusarium*, *F. o. cepae*, or the non-*F. o. cepae* populations at various locations in the field (Table 3g2).

Field #2, 1994; Tables 3h1 and 3h2; Crop = Pinto Beans

Samples were collected from the field planted to pinto beans in 1994 (Field #2) in June, July and August. Total *Fusarium* populations ranged from 11 to 1700 cfu's g^{-1} of soil, while the *F. o. cepae* populations ranged from 2 to 1100 cfu's g^{-1} of soil. There were no significant differences in any of the analyses conducted.

Summation

1. The total *Fusarium* population often varied from year to year as well as from location to location in the field. This is in agreement with the results of several other researchers as discussed previously.

2. Differences in propagule numbers occurred among different locations in the field where samples were collected. In the field planted to beans in 1991, the total *Fusarium* cfu's increased as the distance from the edge of the field increased. Some of this increase in propagules could be the result of propagules carried in irrigation water (see Chapter 2), while the remaining increase could be the result of in-field disease development as previously discussed. An increase in propagules also occurred with *F. o. cepae* as one moved from 15.24 to 30.5 to 45.75 meters into the bean field. In this instance, however, a reduction in propagules of *F. o. cepae* occurred from 45.75 (359 cfu g^{-1} of soil) to 61 meters (141 cfu's g^{-1} of soil).

The second location (30.5 meters) in the onion field in 1991, had the lowest cfu's of *Fusarium* (127 cfu's), as well as the lowest level of *F. o. cepae* propagules (80 cfu's). The furthest point from which samples were collected (61 meters) had the greatest number of propagules of both total *Fusarium* (873 cfu's) and *F. o. cepae* (424 cfu's).

The bean and onion fields were side by side and the soils appeared to be fairly uniform, presumably having been plowed to a uniform depth over many years. If, however, a strip of soil of a different consistency or different nutrient levels extended on a diagonal through the two fields, this change in population could be explained.

3. Differences in propagule numbers occurred in the months samples were collected. In the onion field in 1991, a drop in *F. o. cepae* cfu's occurred in July (85 cfu's) and August

(54 cfu's) with a rebound of cfu's occurring in September (400 cfu's g^{-1} of soil). This is different than what occurred in the field planted to beans when the cfu's of *F. o. cepae* dropped in September to 70 from the August level of 343. The *F. o. cepae* population drop cannot be explained by a simple change in temperature alone. If temperature was the cause of the drop in cfu's, this drop should have occurred in both fields simultaneously unless there is a physiological change in the exudates given off by the crop due to temperature or crop maturity.

A greater number of propagules developed when the field was planted to onions than when planted to pinto beans possibly due to different exudates produced by the crop.

Average Propagule Count over Five Years at the Clarence Wallace Farm, Delta, Colorado - Colony Forming Units g^{-1} of soil			
Fields Planted to:			
Pinto Beans		**Onions**	
Total *Fusarium*	*F. o. cepae*	Total *Fusarium*	*F. o. cepae*
694	299 (43%)	943	304 (32%)

This increase in colony forming units in the onion fields is likely due to the facultative nature of the pathogen especially when in association with onions. There is, however, no research to support the assumption that onions provide a better substrate for the saprophytic nature of *Fusarium* than is provided by pinto beans.

4. High air temperatures do not necessarily mean a corresponding drop in propagule numbers.

5. The lack of significance when the sites were compared indicate the need for more collection points uniformly spaced from the edge of the field. In addition, the need to collect and analyze more samples at each site is indicated. In this research project, 5 samples were collected from each distance from the top of the field, but these samples were combined to create one composite sample from each site. Significance may have been shown if each of the five samples had been analyzed for total *Fusarium* and *F. o. cepae*.

Soil temperatures need to be collected throughout the test period at each site where samples are collected. This will allow a closer comparison between the change in propagule numbers and soil temperature.

6. In 1991, 46% of the total *Fusarium* population of Field #1 (pinto beans) was *F. o. cepae* while 41% of the *Fusarium* population of Field #2 (onions) was *F. o. cepae*. This points to the possibility that the exudates from pinto beans are a better substrate for *F. o. cepae* than are the exudates from onion. Averages over 5 years, however, do not support this hypothesis.

Table 3a1. Fusarium Population Counts Recovered During the Summer of 1991 from Soil in Field #1, Planted with Pinto Beans, at the Clarence Wallace Farm, 1600 Road, Delta, CO.

Month of Collection	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
June	351.7	125.5	226.2
July	720.0	438.3	281.8
August	875.0	342.5	532.5
September	168.0	69.5	98.5
Probability	0.3558	0.3492	0.5134
C.V.%	111.24	128.54	141.02
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis
** *F. o. cepae* propagules based on onion slice method
*** The difference between total *Fusarium* and *F. o. cepae* populations

Table 3a2. Fusarium Population Counts Recovered During the Summer of 1991 from Soil in Field #1, Planted with Pinto Beans, at the Clarence Wallace Farm, 1600 Road, Delta, CO.

Meters from the edge of the field	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
15.24	318.0	191.0	127.0
30.50	547.5	285.8	261.8
45.75	666.7	358.5	308.2
61.00	683.3	140.5	442.0
Probability	0.8545	0.7666	0.7437
C.V.%	111.24	128.54	141.02
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis
** *F. o. cepae* propagules based on onion slice method
*** The difference between total *Fusarium* and *F. o. cepae* populations

Table 3b1. Fusarium Population Counts Recovered During the Summer of 1991 from Soil in Field #2, Planted with Onions, at the Clarence Wallace Farm, 1600 Road, Delta, CO.

Month of Collection	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
June	596.3	202.3	394.0
July	307.5	85.3	222.3
August	87.3	53.5	33.8
September	837.5	400.0	437.5
Probability	0.1807	0.2223	0.2433
C.V.%	100.87	127.17	104.91
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis

** *F. o. cepae* propagules based on onion slice method

*** The difference between total *Fusarium* and *F. o. cepae* populations

Table 3b2. Fusarium Population Counts Recovered During the Summer of 1991 from Soil in Field #2, Planted with Onions, at the Clarence Wallace Farm, 1600 Road, Delta, CO.

Meters from the edge of the field	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
15.24	325.0	130.5	194.5
30.50	127.3	80.3	47.0
45.75	503.8	106.3	397.5
61.00	872.5	424.0	448.5
Probability	0.2022	0.2074	0.2367
C.V.%	100.87	127.17	104.91
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis

** *F. o. cepae* propagules based on onion slice method

*** The difference between total *Fusarium* and *F. o. cepae* populations

Table 3c1. Fusarium Population Counts Recovered During the Summer of 1991 from Soil in Field #1 & #2 at the Clarence Wallace Farm, 1600 Road, Delta, CO. Comparison. The Fields were Planted with Pinto Beans and Onions, Respectively.

Month of Collection	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
June	474.0	163.9	310.1
July	513.8	261.8	252.0
August	481.1	198.0	283.1
September	502.8	234.8	268.0
Probability	0.9985	0.9107	0.9888
C.V.%	74.38	95.24	89.32
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis.
** *F. o. cepae* propagules based on onion slice method.
*** The difference between total *Fusarium* and *F. o. cepae* populations.

Table 3c2. Fusarium Population Counts Recovered During the Summer of 1991 from Soil in Field #1 and #2 at the Clarence Wallace Farm, 1600 Road, Delta, CO. The Fields were Planted with Pinto Beans and Onions, Respectively.

Meters from the edge of the field	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
15.24	321.5	160.8	160.8
30.50	337.4	183.0	154.4
45.75	585.2	232.4	313.9
61.00	727.5	282.3	445.3
Probability	0.3767	0.8382	0.3185
C.V.%	74.38	95.24	89.32
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis.
** *F. o. cepae* propagules based on onion slice method.
*** The difference between total *Fusarium* and *F. o. cepae* populations.

Table 3c3. Fusarium Population Counts Recovered During the Summer of 1991 from Soil in Field #1 & #2 at the Clarence Wallace Farm, 1600 Road, Delta, CO. The Fields were Planted with Pinto Beans and Onions, Respectively.

Meters from the edge of the field	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
Field #1 (Pinto Beans)	528.7	243.9	284.7
Field #2 (Onions)	457.1	185.3	271.9
Probability	0.6642	0.6612	0.8652
C.V.%	42.78	79.84	35.34
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis.
** *F. o. cepae* propagules based on onion slice method.
*** The difference between total *Fusarium* and *F. o. cepae* populations.

Table 3d1. Fusarium Population Counts Recovered from Soil each August from 1991 through 1995 at the Clarence Wallace Farm, 1600 Road, Delta, CO. A Comparison of Data from Field #1 plus Field #2. The Fields were Alternately Planted with Pinto Beans and Onions.

Year	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
1991	481.1	198.0 c	283.1
1992	544.8	72.7 d	482.1
1993	1625.0	586.5 a	1038.5
1994	419.1	165.3 cd	253.9
1995	1028.8	487.5 b	541.3
Probability	0.5149	0.1056	0.7017
C.V.%	133.64	94.54	163.37
LSD	n.s.	0.10	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis
** *F. o. cepae* propagules based on onion slice method
*** The difference between total *Fusarium* and *F. o. cepae* populations

Table 3d2. Fusarium Population Counts Recovered from soil each August from 1991 through 1995 at the Clarence Wallace Farm, 1600 Road, Delta, CO. A Comparison of Data from Field #1 plus Field #2. The Fields were Alternately Planted with Pinto Beans and Onions.

Meters from the edge of the field	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
15.24	921.0	316.4	604.6
30.50	498.4	252.6	245.8
45.75	1338.6	464.6	874.0
61.00	529.0	174.4	354.6
Probability	0.6004	0.4572	0.6619
C.V.%	133.64	94.54	163.37
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis

** *F. o. cepae* propagules based on onion slice method

*** The difference between total *Fusarium* and *F. o. cepae* populations

Table 3e1. Fusarium Population Counts Recovered each August from 1991 through 1995 from Soil at the Clarence Wallace Farm, 1600 Road, Delta, CO. A Comparison of Data Collected from Fields Planted with Pinto Beans during the Five Year Study.

Years of the Study	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
1991	875.0	342.5	532.5
1992	139.3	41.5	97.8
1993	1067.5	503.3	564.2
1994	610.0	220.8	389.2
1995	810.0	387.5	422.5
Probability	0.3423	0.1256	0.7326
C.V.%	90.36	78.64	129.48
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis.
** *F. o. cepae* propagules based on onion slice method.
*** The difference between total *Fusarium* and *F. o. cepae* populations.

Table 3e2. Fusarium Population Counts Recovered each August from 1991 through 1995 from Soil at the Clarence Wallace Farm, 1600 Road, Delta, CO. A Comparison of Data Collected from Fields Planted with Pinto Beans during the Five Year Study.

Meters from the edge of the field	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
15.24	728.0	318.2	409.8
30.50	587.4	313.4	274.0
45.75	898.0	380.0	518.0
61.00	588.0	184.8	403.2
Probability	0.8449	0.6225	0.9048
C.V.%	90.36	78.64	129.48
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis.
** *F. o. cepae* propagules based on onion slice method.
*** The difference between total *Fusarium* and *F. o. cepae* populations.

Table 3f1. Fusarium Population Counts Recovered each August from 1991 through 1995 from Soil at the Clarence Wallace Farm, 1600 Road, Delta, CO. A Comparison of Fields Planted with Onions during the Five Year Study.

Years of the Study	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
1991	87.3	53.5	33.8
1992	970.3	103.9	866.4
1993	2182.5	669.8	1512.7
1994	228.3	109.8	118.5
1995	1247.5	587.5	660.0
Probability	0.5711	0.2621	0.6394
C.V.%	206.20	159.24	234.71
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis.
** *F. o. cepae* propagules based on onion slice method.
*** The difference between total *Fusarium* and *F. o. cepae* populations.

Table 3f2. Fusarium Population Counts Recovered each August from 1991 through 1995 from Soil at the Clarence Wallace Farm, 1600 Road, Delta, CO. A Comparison of Fields Planted with Onions during the Five Year Study.

Meters from the edge of the field	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
15.24	1114.0	314.6	799.4
30.50	409.4	191.8	217.6
45.75	1779.2	549.2	123.0
61.00	470.0	164.0	306
Probability	0.6607	0.5960	0.6940
C.V.%	206.20	159.24	234.71
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis.
** *F. o. cepae* propagules based on onion slice method.
*** The difference between total *Fusarium* and *F. o. cepae* populations.

Table 3g1. Fusarium Population Counts Recovered during the Summer of 1994 from Soil in Field #1, Planted with Onions at the Clarence Wallace Farm, 1600 Road, Delta, CO.

Month of Collection	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
June	76.75 b	49.5 b	27.25
July	3398.0 a	1198.0 a	2200
August	228.3 b	109.8 ab	118.5
Probability	0.0772	0.0134	0.2374
C.V.%	151.07	92.14	231.41
LSD	3103 $^{0.10}$	937.7 $^{0.05}$	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis
** *F. o. cepae* propagules based on onion slice method
*** The difference between total *Fusarium* and *F. o. cepae* populations

Table 3g2. Fusarium Population Counts Recovered during the Summer of 1994 from Soil in Field #1, Planted with Onions at the Clarence Wallace Farm, 1600 Road, Delta, CO.

Meters from the edge of the field	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
15.24	400.0	268.3	131.7
30.50	2719.0	434.3	2284.3
45.75	509.3	388.7	120.7
61.00	1309.0	717.7	591.0
Probability	0.4597	0.6242	0.4687
C.V.%	151.07	92.14	231.41
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis

** *F. o. cepae* propagules based on onion slice method

*** The difference between total *Fusarium* and *F. o. cepae* populations

Table 3h1. Fusarium Population Counts Recovered during the Summer of 1994 from Soil in Field #2, Planted with Pinto Beans at the Clarence Wallace Farm, 1600 Road, Delta, CO.

Month of Collection	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
June	601.0	318.2	282.8
July	83.4	46.8	36.6
August	610.0	220.8	389.3
Probability	0.4354	0.5727	0.4338
C.V.%	142.78	180.00	156.06
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis
** *F. o. cepae* propagules based on onion slice method
*** The difference between total *Fusarium* and *F. o. cepae* populations

Table 3h2. Fusarium Population Counts Recovered during the Summer of 1994 from Soil in Field #2, Planted with Pinto Beans at the Clarence Wallace Farm, 1600 Road, Delta, CO.

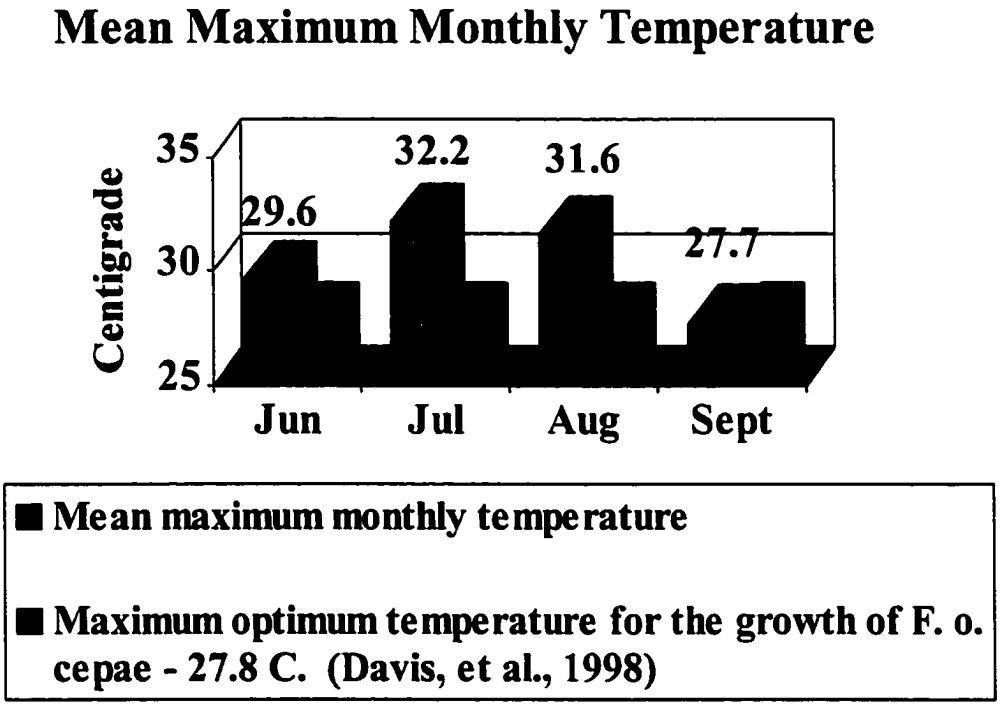
Meters from the edge of the field	Total *Fusarium* * cfu g^{-1} of Soil	*F. o. cepae* ** cfu g^{-1} of Soil	non-*F. o. cepae* *** cfu g^{-1} of Soil
15.24	438.5	76.27	362.2
30.50	649.7	394.0	255.7
45.75	437.0	194.67	242.3
61.00	200.7	116.0	84.7
Probability	0.8478	0.7047	0.8322
C.V.%	142.78	180.00	156.06
LSD	n.s.	n.s.	n.s.

* Total *Fusarium* propagules based on carnation leaf agar analysis

** *F. o. cepae* propagules based on onion slice method

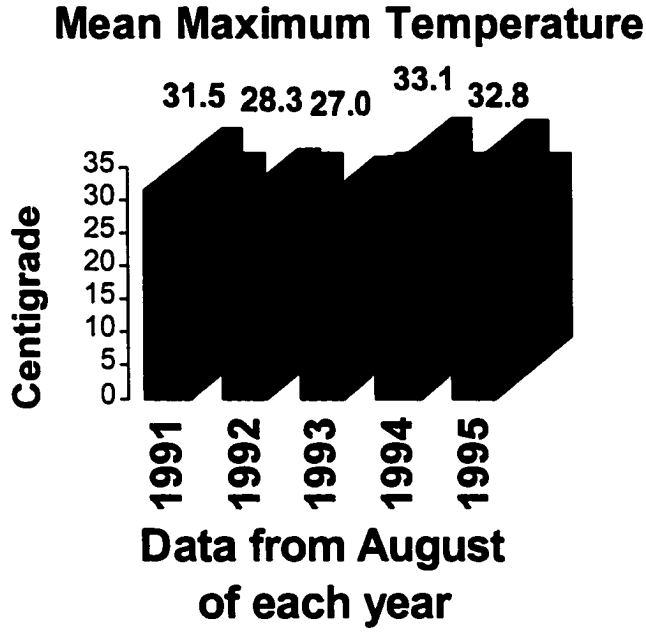
*** The difference between total *Fusarium* and *F. o. cepae* populations

Table 3i1. Mean Maximum Monthly Temperatures in Delta, Colorado During the Summer of 1991.



Data source: CoAgMet Data Access, Weather Station Delta, CO - Colorado State University. The Web Address is http://ccc.atmos.colostate.edu/~coag/

Table 3i2. Mean Maximum Temperature for the Month of August from 1991 through 1995, Delta, Colorado.

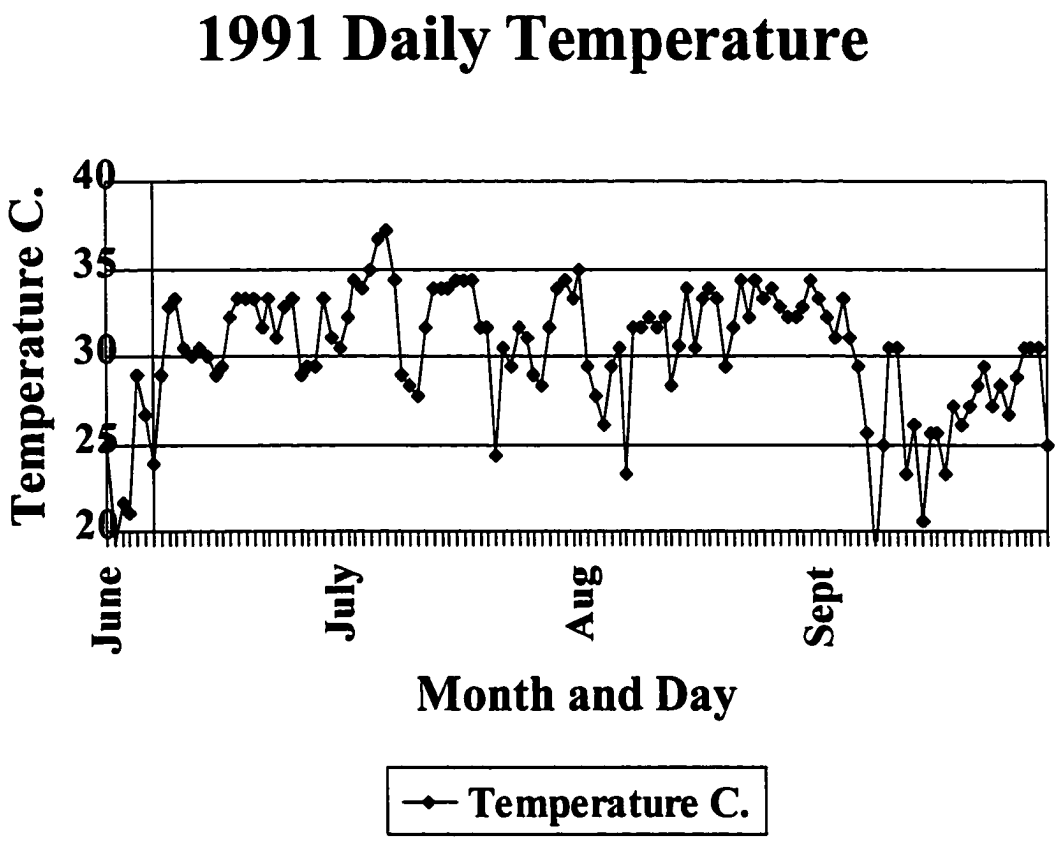


■ mean maximum monthly temperature
■ maximum optimum temperature for growth of F. o. cepae - 27.8 C. (Davis et al., 1998)

Temperature Summary Data for 1991, 1993, 1994 & 1995 is from the Delta weather station located at Lat. 38.45 Long. 108.4. The 1992 data is from Montrose weather station No. 2. Delta, Colorado weather data is unavailable for August 1992.

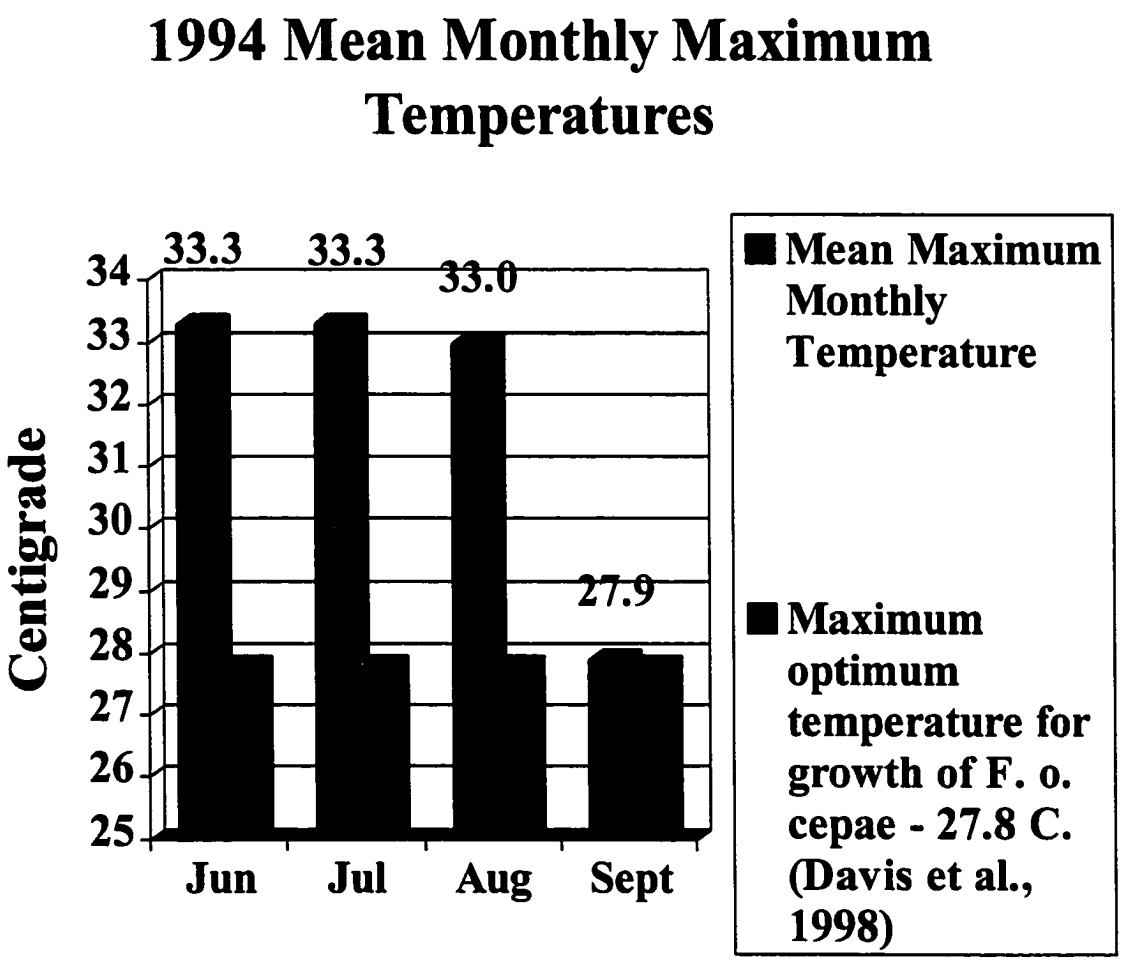
Data source: CoAgMet Data Access, Weather Station Delta, CO - Colorado State University. The Web Address is http://ccc.atmos.colostate.edu/~coag/

Table 3i3. Daily Maximum Temperatures during the Summer of 1991, Delta, Colorado.



Data source: CoAgMet Data Access, Weather Station Delta, CO - Colorado State University. The Web Address is http://ccc.atmos.colostate.edu/~coag/

Table 3i4. Mean Maximum Monthly Temperatures during the Summer of 1991, Delta, Colorado.



Data source: CoAgMet Data Access, weather station Delta, CO - Colorado State University: http://ccc.atmos.colostate.edu/~coag/

Chapter 4: Efficacy of Seed and Soil Treatments against *Fusarium oxysporum* f. sp. *cepae*

Abstract

A series of seed and soil treatments, to include fumigation, were conducted in 1992, 1993 and 1994 to determine the efficacy of selected fungicides. These fungicide trials included carboxin, fosetyl-aluminum/iprodione (Aliette®/Rovral® at 34.5 g 100 m^{-1} of row), imazalil, metalaxyl (Ridomil® at 22.k kg h^{-1}), metam-sodium ((Busan® 1020 - band and broadcast-applied), thiophanate-methyl (Topsin® M at 31.5 kg h^{-1}), and thiram.

Two greenhouse trials were conducted during the spring and early summer of 1993. Soil and seed treatments were applied to uncoated Brown Beauty onion seed and planted in steam pasteurized soil which was subsequently infested with *F. o. cepae* (10,000 cfu's g^{-1} of soil) prepared from a virulent isolate collected from an infested onion bulb collected in a field in Delta, Colorado. Treatments consisted of metalaxyl, imazalil, carboxin/thiram, the combination product fosetyl-aluminum/iprodione, and thiophanate methyl applied in accordance with label rates. Ten pots of ten seeds each were used. This trial was repeated twice.

In 1993 three rates of metam-sodium and post germination soil treatments were compared for their efficacy against *F. o. cepae*, the causal agent of Fusarium basal rot of onions. The treatments of sodium N-methyldithiocarbamate (Busan® 1020 - metam-sodium) were band applied in the fall of 1992 by injecting 223.8, 280.7 and 327.4 liters per hectare. The product was injected to a depth of 15.24 and 30.48 cntimeters in a band under each seedling row. In 1994, three rates of metam-sodium (560.9, 607.7 and 701.2 liters per hectare) were uniformly broadcast and tilled into planting beds the preceding fall. Data on the number of living seedlings were collected for these comparisons.

Post germination soil treatments of metalaxyl, thiophanate methyl, and the combination product fosetyl-aluminum/iprodione were surface applied in 1993 approximately one month after planting. In 1994, these same materials were applied at planting time according to label directions.

In greenhouse trials, no treatments tested provided significant protection from infection. In one study, imazalil, metalaxyl, fosetyl-aluminum/iprodione and thiophanate methyl had significantly (P=0.01) less healthy plants than the untreated control. In the second greenhouse trial, metalaxyl, fosetyl-aluminum/iprodione and thiophanate methyl had significantly fewer healthy plants than the untreated control.

In the 1992 field trials, thiophanate methyl resulted in a significant (P=0.01) yield

increase of marketable onion bulbs when compared with the untreated control. No significant yield increase resulted with fosetyl-Al/iprodione, metalaxyl, imazalil or carboxin/thiram. All products were applied at seeding in accordance with label directions.

In 1993, post germination soil treatments of metalaxyl, thiophanate methyl and fosetyl-aluminum/iprodione did not increase the number of living seedlings when compared with the untreated control, or reduce incidence of *Fusarium* basal rot or increase yield of onion bulbs. When data from both fields were combined, metam-sodium treatments of 223.8, 280.7 and 327.4 l hectare^{-1} (band-applied) had more live seedlings than the untreated control (P=0.05). The 280.7 liter hectare^{-1} rate of metam-sodium significantly increased total yield over the untreated control (P=0.10). A significantly higher number of *Fusarium*-infected and total unmarketable (doubles, *Fusarium*-infected, and other cull onions) onion bulbs (P=0.05 and 0.01, respectively) resulted due to the use of metam-sodium.

1994 stand count data were collected on 29 May, 15 June, 15 July, and 12 August, and on 6 June, 1 July and 29 July at the Hines and English farms, respectively. The metalaxyl treatment had significantly fewer seedlings in both fields over all dates. By the end of the study, the significantly high stand count (P=0.05) for the high rate of metam-sodium (701 l hectare^{-1}) in the Hines field was not significantly different than the untreated control.

Post-germination treatments of fosetyl-aluminum/iprodione (34.5 g 100 m^{-1} of row), metalaxyl (22.5 kg h^{-1}), and thiophanate methyl (31.5 kg $hectare^{-1}$) are not effective in reducing *Fusarium* basal rot or increasing yield of onion bulbs. Applications of fosetyl-aluminum/iprodione, and thiophanate methyl at planting are ineffective in increasing total marketable yield when compared with the untreated control. Fosetyl-aluminum applied at planting in accordance with label directions, is not effective in reducing the number of *Fusarium*-infected onion bulbs at harvest.

The use of metalaxyl as an in-furrow application results in lower total marketable weight, and lower weight of colossal and jumbo sized onion bulbs. The lowest stand counts were in plots treated with metalaxyl.

Metam-sodium applied at 280.7 l $hectare^{-1}$ resulted in a significantly greater total yield than the untreated control. Metam-sodium, band-applied, at the rate of 327.4 liters $hectare^{-1}$, is ineffective in increasing total marketable weight of onion bulbs. Metam-sodium rates of 223.8 and 280.7 liters $hectare^{-1}$ resulted in a greater significant overall weight of marketable onion bulbs (P=0.10) than a metam-sodium rate of 327.4 liters $hectare^{-1}$.

Metam-sodium broadcast-applied at 608 and 701 liters $hectare^{-1}$ had a significantly greater weight of double onion buls than when metam-sodium is applied at 561 liters h^{-1}. Metam-sodium broadcast-applied at 701 l $hectare^{-1}$ produced a significantly higher yield

(P=0.01) than untreated plots.

Fewer unmarketable onion bulbs (double, *Fusarium*-infected, and culls) occurred in untreated control plots than plots treated with metam-sodium (P=0.01). Metam-sodium, band-applied at the rate of 327.4 liters hectare^{-1}, had more unmarketable bulbs than lower rates of metam-sodium. More unmarketable onions (P=0.01) developed when metam-sodium was broadcast-applied at 701 and 608 liters hectare^{-1}, possibly due to residual phytotoxicity. The broad-cast application of metam-sodium at 561 liters hectare^{-1} did not increase the unmarketable onions over what occurred with the untreated control. Fewer jumbo and more prepack onion bulbs developed when metam-sodium was band-applied as compared with untreated plots.

There were no significant differences in total marketable yield, colossal, jumbo, medium or prepack onion bulbs for the Hines farm when all treatments were compared. The weight of double onion bulbs (kg 25 m^2), however, was significantly greater (P=0.10) for the two higher metam-sodium rates (608 and 701 l hectare^{-1}). Thiophanate methyl, fosetyl-aluminum/iprodione, as well as the high and low rates of metam-sodium (701 and 561 liters hectare^{-1}, respectively) had significantly (P=0.10) greater numbers of *Fusarium*-infected bulbs than the untreated control, metalaxyl or medium rate metam-sodium treatment (608 liters hectare^{-1}). The two higher rates of metam-sodium and the fosetyl-aluminum/iprodione treatments had significantly greater numbers (P=0.10) of unmarketable onion bulbs than the treatments of thiophanate methyl, metalaxyl, or

untreated control.

In 1994, significant differences in weight for total marketable, double, colossal, and jumbo onion bulbs, and the number of *Fusarium*-infected, and unmarketable onion bulbs were found at one site when all treatments were compared. Metalaxyl had a significantly lower weight of total marketable (P=0.01), colossal (P=0.05) and jumbo (P=0.10) onion bulbs of any of the treatments, including the untreated control. Metalaxyl also had the fewest *Fusarium*-infected (P=0.01) and total unmarketable (P=0.05) bulbs at harvest. This can be explained by poor germination, early infection and subsequent disintegration of bulbs in those plots. Those bulbs that had been infected earlier were no longer present to be counted. The untreated control had 17 infected bulbs 25 m^2 plot $^{-1}$ compared with an average of 3 infected bulbs for the metalaxyl plots.

When data for the two fields were combined, the two higher rates of metam-sodium (701 and 608 liters hectare^{-1}) produced a significantly greater weight (in kgs) of double onion bulbs (P=0.05). Thiophanate methyl, metalaxyl, metam-sodium at 561 liters hectare^{-1}, and fosetyl-aluminum/iprodione had significantly equal weights of double bulbs as the untreated control plots (P=0.05).

Additional findings in 1994 were:

1. Production of total marketable onion bulbs (by weight) was significantly higher (P=0.01) for the high rate of metam-sodium (701 liters hectare^{-1}) when

compared to fosetyl-aluminum/iprodione, thiophanate methyl, and the untreated control. There were no significant differences in total yield for the three metam-sodium rates. The metalaxyl treatment produced the lowest weight of marketable onion bulbs.

2. The greatest weight of colossal onion bulbs (P=0.05) was produced in the untreated plots. There was no significant difference between untreated plots and the medium rate (608 liters hectare^{-1}) of metam-sodium. The metalaxyl treatment had a significantly lower weight of colossal bulbs than any of the treatments used. The high rate of metam-sodium produced significantly more jumbo onion bulbs (weight in kgs), while the metalaxyl treatment was the lowest producer of jumbo bulbs (P=0.05).

3. The highest number of *Fusarium*-infected onion bulbs was located in the fosetyl-aluminum/iprodione and untreated control plots, while the lowest number was in the metalaxyl plots. There was no significant difference between the various rates of metam-sodium and the thiophanate methyl treatments (P=0.01).

Literature Review

Chemicals may be used to inhibit or destroy soil pathogens, however, the control of Fusarium wilts with fungicides is difficult (Keinath, 1994).

The effective fungicide treated/toxic area, is defined as the "proportion of the crop at any one time in which the fungicide level is higher than the threshold of control of the common fungicide-sensitive genotype[65] of the pathogen" (Wolfe and Barrett, 1986, page 247). Control of soilborne diseases often requires that granular fungicides be applied uniformly with little soil area left untreated. If the soil is left untreated, disease will develop from inoculum that has escaped exposure to the fungicide (Backman, 1978).

The response of a pathogen to a fungicide depends on genetic variation for resistance/sensitivity present in the population (Wolfe and Barrett, 1986). Consequently, the ultimate value of any chemical compound as a plant pathogen control agent depends on the mode of action of the molecule at the physiological level on one or more components of the life cycle of the pathogen (Matheron and Porchas, 2000).

The degree of toxicity of a chemical to a soil microorganism in pure culture is markedly different from that shown against the same organism in its natural habitat (Kreutzer, 1963). Richardson and Munnecke (1964) found that the fungicide dosage required to control soil pathogens is proportional to the inoculum density.

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[65] Genotype refers to the genetic constitution of an individual (Hartl, 1980, page 8).

In vitro[66] tests using contact and systemic fungicides have shown varying levels of efficacy against *F. o. cepae*. Abawi (1971) found *in vitro* trials with Benlate® and Daconil® 2787 resulted in complete inhibition of conidial germination at 1, 10 and 100 ppm formular rates. Many pesticides, however, have no detectable effects on soil microorganisms at field-relevant application rates (Anderson, 1993).

Studies conducted on *Sclerotium cepivorum* by Crowe in the 1970's found excellent fungicide efficacy at low to moderate populations, but partial or total loss of efficacy at high inoculum density (Crowe, 1998). Pathogens can develop resistance to fungicides increasing problems with control. Resistance to benomyl and thiophanate-methyl is reported in a number of foliar fungal plant pathogens (Keinath, 1998).

Soil contains populations of microorganisms that have exceptional degradative capabilities and the ability to adapt to utilize pesticides applied to soil . This microbial ability has resulted in economically significant pest control failures (Racke, 1990).

Methods of Fungicide Application

Seed Treatments and Soil Drenches

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[66] "The majority of protectant fungicides are directly toxic to fungi and so will show up as active against spore germination (during) *in vitro* tests" (Cremlyn, 1991, page 158).

Seed treatment refers to the application of bioactive chemicals, antagonistic or symbiotic microorganisms to the seed prior to sowing. Farmers in Bristol England salvaged seed from the sea and found it to be bunt-free. This started the practice of disinfecting seed in brine (Morton, 1999). Commercial seed treaters view seed treatments as a means to substantially increase the value of seed and to improve plant growth and productivity (Taylor and Harman, 1990).

Morton (1999) lists three types of fungicides currently available for seed treatment:

1. non-systemic (i.e., thiram, captan[67] and PCNB),
2. locally systemic (i.e., fludioxonil and thiabendazole), and
3. systemic (i.e., carboxin, metalaxyl, mefenoxam and triazoles).

Non-systemic Fungicides

The non-systemic protectants must:

1. have very low phytotoxicity; none of the protectant fungicides currently on the market is completely non-phytotoxic,
2. be fungitoxic *per se* or be capable of converting to a substance which is fungitoxic,
3. be able to penetrate the fungal spore and reach the ultimate site of action of the fungicide, and

[67] N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide (Meister, 2000)

4. be capable of resisting weathering (Cremlyn, 1991).

Systemic Fungicides

Systemic treatments can control deep-seated plant pathogens (Taylor and Harman, 1990). These compounds are taken up by and translocated within the plant thus providing protection from pathogenic fungi or limiting an established infection. Reports on the translocation of systemic fungicides applied as foliar treatments conclude that the amount transported within the plant downward into the roots is small and of little significance (Erwin, 1973).

An effective systemic fungicide must be:

1. fungicidal or be converted into an active fungitoxicant within the host plant,

2. possess low phytotoxicity,

3. capable of being absorbed and translocated, at least locally (Cremlyn, 1991). Most systemics move predominately upward with the evapotranspiration stream and thus are considered apoplastic[68] not symplastic[69] (Edgington, 1981).

4. capable of transversing the plasmalemma membrane and entering the plant

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[68] Apoplast - the nonliving part of the plant, i.e. cell walls and cuticle, including xylem vessels and tracheids (Edgington, 1981).

[69] Symplast - the living part of the plant enclosed by membranes, i.e. protoplast and plasmadesmata, including the phloem sieve cells (Edgington, 1981).

protoplast,

5. metabolically stable. Since systemics enter plant protoplasts, they must have a degree of metabolic stability to control the pathogen successfully (Edgington, 1981).

In 1986, there were four classes of systemic fungicides commercially available: carbamates (i.e. prothiocarb, propanocarb), cymoxanil, acylanilides (i.e. metalaxyl) , and alkyl phosphonates (i.e. fosetyl-aluminum) (Cohen and Coffey, 1986). Others are now available.

Ware (1991) provides a more extensive listing of systemic fungicide groups:

Oxathiins
carboxin, oxycarboxin
Benzimidazoles
benomyl, thiabendazole (TBZ), thiophanate methyl (Topsin M®)[70]
Pyrimidines
dimethirimol, ethirimol, bupirimate, fenarimol
Organophosphates
iprobenfos (IBP), fosetyl-aluminum
Phenylamides (Acylalanines)
metalaxyl, furalaxyl
Triazoles
diniconazole (Spotless ®)
triadimefon (Bayleton ®)
propiconazole (Tilt ®)
cyproconazole (Alto ®)
penconazole (Award ®)

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[70] Thiophanate methyl (Topsin M®) is not a benzimidazole, but because it is converted to this group's structure when metabolized, it is placed in this group (Ware, 1991).

tebuconazole (Folicur ®)
myclobutanil (Rally ®)
difenoconazole (Score ®)
triadimerol (Baytan ®)
bitertanol (Baycor ®)

Piperazines
triforine

Imidines
iprodione (Rovral ®), vinclozoline (Ronilan ®), procymidone (Sumilex ®)

Carbamates
propamocarb (Banol ®, Prevex ®)

Soil drenches with 14 fungicides applied at the time of transplanting and four weeks later were unsuccessful when attempting to control Fusarium wilt of celery. The fungicides tested by Awuah and Lorbeer (1986b) in this research included:

benomyl (Benlate ® 50WP),
chlorothalonil (Bravo ® 500, 4.17F),
mancozeb (Dithane ® M-45, 50WP),
vinclozolin (Ronilan ® 50 WP),
captafol (Difolatan ® 4F),
triadimefon (Bayleton ® 50WP),
fenaminsulf (Dexon ® 25WP),
PCNB (Terrachlor ® 75WP),
chloroneb (Demosan ® 65 WP),
and triadimenol (Baytan ® 150 1.25 FS).

Pesticides that reside within or on the soil are subject to abiotic and microbiological transformation processes (Racke, 1990), with these pesticide degrading-abilities being easily spread from one soil to another (Walker and Welch, 1990). The microbial metabolic enzymatic processes involved with pesticide degradation fall within two general categories:

catabolic - when the pesticide is converted into the carbon skeleton feedstock for

microbial metabolism and the energy released in this process is used for growth of the microbe,

cometabolism - when the pesticide is degraded by the microbe but the energy derived is not used to support microbial growth (Racke, 1990).

Fumigants

The primary shortcoming of soil fumigation is too short a residence period for effective control. High rates of product or tarping must be used to overcome this deficiency (Backman, 1978). Fumigation of soil is costly and the high expense generally prohibits such treatment with the majority of crops (Cremlyn, 1991). The effect of fumigation on some soilborne pathogens such as *Pseudomonas solanacearum* is highly variable (Chellemi et al., 1994).

While the control of Fusarium wilts with fungicides is difficult, the efficacy of fumigants are notable exceptions because of their broad biocidal activity (Anderson, 1993; Domsch et al., 1983). Methyl bromide, chloropicrin and metam-sodium reduce populations of *Fusarium* pathogenic to onion seedlings (Jaworski et al., 1978). Fumigation with methyl bromide eliminates *F. o.* f. sp. *apii* in celery seedbed soil (Awuah and Lorbeer, 1986b), and is used as a preplant soil fumigant to control *Fusarium oxysporum* in seed beds of susceptible conifer species (Krelle et al., 1993).

At times fumigation does not work. In carnation greenhouses, *F. o.* f. sp. *dianthi* is a problem even after fumigation reduces propagule counts to below detection levels possibly due to one or more of the following reasons:

1) rapid root colonization by the small number of propagules that survive fumigation as a result of lack of competition (Ben-Yephet et al., 1994),

2) presence of non-exposed propagules in soil 60 cm and deeper (Farley et al., 1974),

3) planting of symptomless *F. o.* f. sp. *dianthi*-infected cuttings (Nelson, 1964),

4) planting of susceptible cultivars (Reuven et al., 1992).

Y. Ben-Yephet et al. (1994) found methyl bromide ineffective in providing protection for even one year against *F. o.* f. sp. *dianthi* on greenhouse carnations. There was no correlation between inoculum level in the soil after fumigation and the level of inoculum or Fusarium wilt incidence at the end of the growing season. Fumigation effects on *F. o.* f. sp. *cepae* is short lived, because low populations of the pathogen can increase rapidly in the presence of onions (Abawi and Lorbeer, 1972).

Fungicides Used in this Study

The following products were used in this study. The active ingredients are discussed alphabetically.

Aliette® /Rovral® 15G premix (AgroEvo)

fosetyl-aluminum
iprodione
Apron® (metalaxyl) - Novatis
Busan ® 1020 (Metam-sodium) - Buckman Laboratories, Inc.
Flo Pro® - Gustafson LLC.
imazalil
Pro-Gro® - Gustafson LLC. - a combination product containing:
carboxin
thiram
Ridomil® PC 11G (metalaxyl) - Novartis
Topsin ® M (thiophanate methyl) - Elf Atochem N.A.

Carboxin

Composition: 5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide (CAS)[71] (Meister, 1999); formerly, 2,3-dihydro-5-carboxanilido-6-methyl-1,4 oxathiin (Erwin, 1973).

Action: Systemic fungicide and seed protectant (Meister, 2000). The breakdown product strongly inhibits glucose and acetate oxidative metabolism[72] and RNA and DNA synthesis (Cremlyn, 1991).

Uses: The Oxathiin group of systemic fungicides are primarily active against the *Basidiomycetes* class of fungi which includes rusts, smuts, bunts and the soil fungus *Rhizoctonia solani* (Raid, 1991).

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[71] Chemical Abstracts Service (Meister, 2000).

[72] "The complete course of metabolism in a plant is not understood for any pesticide in use today" (Shimabukuro et al., 1982 - page 22).

Formulation used in this study: Pro-Gro® - a combination product containing carboxin and thiram.

Literature Review:

Carboxin was introduced in 1966. It is absorbed and translocated by plant roots apoplastically (Cremlyn, 1991) eventually accumulating with no redistribution in the margins of transpiring leaves (Erwin, 1973). In barley seedlings, carboxin degrades within three days to produce oxycarboxin (Edgington, 1981). When it concentrates in the fungal cell, it causes the inhibition of succinic dehydrogenase, an enzyme important for respiration in the mitochondrial system (Ware, 1991). Damage to the mitochondrial system and vacuolar membrane in sensitive fungi may occur within 120 minutes of application of the fungicide (Erwin, 1973).

Carboxin is used as a seed treatment, particularly on seed affected by embryo-infecting smut fungi. It is selectively toxic to smuts, rusts, and *Rhizoctonia* (teleomorph = *Thanatephorus*), organisms belonging to the Basidiomycetes, but not to the host plant (Ware, 1991). Carboxin is rapidly degraded by microorganisms in the soil with 90 percent degraded to sulfoxide within two to three weeks.

Vitavax ® 200 at 2.5 g kg^{-1} used as a seed treatment is ineffective in increasing stand count, or yield of 'scabby' 'Tyler' soft red winter wheat seed internally infected with *Fusarium* spp. (Bergstrom et al., 1989). Buechley et al. (1989) found yield of scabby

wheat seed (*Triticum aestivum* 'Caldwell' and 'Monon') was not significantly increased (P=0.05) when treated with:

Vitavax® 200FF at 2.6 ml kg $^{-1}$ of seed; or

Vitavax ® 34FF at 1.3 ml kg $^{-1}$ plus Mertect® LSP[73] at 0.325 ml kg $^{-1}$ of seed.

The following treatments, however, resulted in a significant difference (P=0.05) for yield for one or both varieties of wheat treated:

Vitavax ® 34FF at 1.3 ml kg $^{-1}$ plus Thiram ® 42S at 2.6 ml kg $^{-1}$

Vitavax ® 200FF at 2.6 ml kg $^{-1}$ plus Mertect ® LSP at 0.325 ml kg $^{-1}$ of seed;

Vitavax ® 200FF at 2.6 ml kg $^{-1}$ plus Mertect ® LSP at 0.65 ml kg $^{-1}$ of seed;

Vitavax ® 34FF at 1.31 ml kg $^{-1}$ plus Captan ® 400 at 1.31 ml kg $^{-1}$ of seed; and

Vitavax ® 34FF at 2.6 ml kg $^{-1}$ of seed (Buechley et al., 1989).

Vitavax® 200 (2.6 ml kg^{-1} of seed) alone and in combination with Prochloraz® [74] 20SD (1.6 ml kg^{-1}) or Rovral® 30F (3.9 ml kg^{-1}), did not increase stand count or yield, or reduce the incidence of foot rot caused by *Fusarium* and *Rhizoctonia* spp. in winter wheat (*Triticum aestivum* 'Houser') planted in contaminated soil. Vitavax® 200 (2.6 ml kg^{-1} of seed) in combination with foliar applications of Benlate® 50 wp (1.12 kg h^{-1}) and Mertect® 340F (1.12 kg h-^{1}) did not produce significant differences in germination, plant height or shoot dry weight when seed of 'Geneva' and 'Houser' were treated for pre- and

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[73] Mertect - thiabendazole (Meister, 2000).

[74] Prochloraz® - an imidazole - N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl] imidazole-1-carboxamide (Meister, 2000).

post-emergence damping-off and root rot complex (Kalb et al., 1988). In Oklahoma, Vitavax® 200 applied as a seed treatment at 2.6 ml kg^{-1} of seed, produced a significant (P=0.05) increase in percent germination at two sites (Williams, 1988).

In laboratory and greenhouse experiments, Kalb et al. (1989), found no significant difference in percent germination, plant height or shoot dry weight when Vitavax® was applied to seed of 'Geneva' or 'Houser' winter wheat (*Triticum aestivum*) when compared with the untreated check. At the rate of material used (2.6 ml kg^{-1} of seed), no phytotoxicity occurs.

There were variations but no significant differences from the untreated control on field emergence or yield of soybean (*Glycine max* 'Williams 82') when Vitavax ® was applied alone or in combination with Apron® (metalaxyl). A significant increase (P=0.05) of *Cercospora kikuchii* resulted due to the application of Vitavax®. Fernandez et al. (1989) were unable to explain the increase of this seedborne pathogen.

Pro-Gro® (carboxin + thiram) applied in combination with methyl cellulose and Dithane® significantly (P=0.05) reduced the incidence of onion smut (causal agent *Urocystis cepulae*) for the first two readings (16 June and 14 July). Significance was not consistent, however, when the third reading for smut (%) was taken on 15 September. No significant difference in yield was noted when compared with the untreated check (McDonald et al., 1998). The same trials conducted in 1999, again gave inconsistent

results when the incidence of onion smut was evaluated. As occurred the previous year, there was no significant (P=0.05) difference in yield (McDonald et al., 1999).

Fosetyl-aluminum

Composition: Aluminum tris (O-ethyl phosphonate).

Action: Bactericide, systemic fungicide.

Uses: Preventive and curative activity against *Alternaria*, Oomycetes, and *Penicillium* on avocado, cacao, citrus, fruits, hops, ornamentals, pineapple, rubber, strawberries, tobacco, vegetables, and vines (Meister, 2000).

Formulation used in this study: Aliette® /Rovral® 15G premix containing fosetyl-aluminum, and iprodione, respectively.

Literature Review:

Aliette® (LS74-783) is a true systemic capable of upward or downward movement in plants with demonstrated activity on *Alternaria*, *Fusarium*, *Pythium*, *Venturia*, *Xanthomonas*, and *Erwinia* as well as other plant pathogens. Edgington (1981) does not consider Aliette® a true fungicide as it is not toxic *in vitro* but works by activating phytoalexins.

The esters of phosphorous and phosphonic acid are termed phosphites and phosphonates,

respectively (Cohen and Coffey, 1986). Fosetyl-aluminum, released by Rhône-Poulenc in 1977 (Cremlyn, 1991; Dercks and Creasy, 1989), an ester of phosphonic acid, is the aluminum salt of tris-o-ethyl phosphonate (Meister, 1999). It was first registered by EPA in 1983. EPA issued a Registration Standard for fosetyl-aluminum in February 1988 (U.S. EPA, 1991).

Fosetyl-aluminum has a narrower usage than metalaxyl (Cohen and Coffey, 1986). Nevertheless, it is used as a foliar spray with several important crops and as a spray or soil drench with field crops and ornamentals (Beach et al., 1979; Williams et al., 1977). Fosetyl's systemic characteristics include local penetration and acropetal[75] as well as basipetal[76] translocation (Schwinn and Urech, 1986).

This systemic fungicide is one of the salts of a phosphorous acid ester with a monoalkyl group (ethyl, isopropyl) all of which display activity *in vivo*[77] against various Oomycetes. Other compounds consist of the Na^{+}, Mg^{++}, Cu^{++}, and Zn^{++} salts. Fosetyl-aluminum is the only alkyl phosphonate commercially available (Lopez-Serrano et al., 1997).

Fosetyl-aluminum is translocated in the symplast and can move downward in plants

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[75] Acropetal - ascending (Kenneth, 1963).

[76] Basipetal - from apex to base (Kenneth, 1963).

[77] *In vivo* - in the host (Agrios, 1997).

(Jabaji-Hare and Kendrick, 1987). It was shown to be effective against Oomycetes (Dercks and Creasy, 1989) reducing the formation of sporangia, zoospores, chlamydospores and oospores of various *Phytophthora* species (Coffey and Joseph, 1985; Duncan, 1985). This chemical is metabolized in the plant to produce an antimicrobial product, phosphonate (Cohen and Coffey, 1986; Fenn and Coffey, 1989). Its mode of action at the biochemical level is unknown (Schwinn and Urech, 1986).

Fosetyl-aluminum breaks down rapidly in soil to H_3PO_3 and CO_2 (Saindrenan et al., 1985) with a half-life from 0.32 to 1.89 days in soils with an organic matter content of 1.7 to 4.7 percent (Cohen and Coffey, 1986). In contrast, the breakdown product of fosetyl-aluminum, H_3PO_3 persists in soil for several months. *In planta* this chemical is rapidly hydrolyzed to phosphorous acid, Al^{3+} and ethanol. It is apparent that H_3PO_3 is the active metabolite of fosetyl-aluminum and most likely of the other alkyl phosphonates as well (Cohen and Coffey, 1986).

Plants respond to infection by producing physical and chemical barriers which function as wall reinforcements, antibiotics or lytic enzymes (Nemestothy and Guest, 1990). The level of peroxidase and phytoalexins strongly increases during the development of local and systemic resistance (Fenn and Coffey,1985; Guest, 1984a; Guest et al., 1989). Certain fragments from both fungal and plant cell walls are very effective elicitors; these induce the *de novo* synthesis of enzymes catalyzing the synthesis of phytoalexins in higher plants. These fragments trigger plant defenses against phytopathogens (Gross,

1991).

An increase in phytoalexin production and early 'hypersensitivity' had been proposed as one means of the control provided by fosetyl-aluminum (Guest, 1984a). However, inadequate evidence supports this hypothesis (Cohen and Coffey, 1986). H_3PO_3 produces a local and systemic induction of peroxidase and chitinase resulting in increased resistance in cucumber (Irving and Kuc, 1990). Al^{3+} is also known to elicit phytoalexin accumulation in fruits of thorn apple (Lopez-Serrano et al., 1994). Research by Lopez-Serrano et al. (1994) suggests that fosetyl-aluminum effects on peroxidase are identical to those of Al^{3+} ions. They further conclude the mode of action of fosetyl-aluminum cannot be due to specific activation of the plant's natural defense mechanisms. Dercks and Creasy (1989) came to this conclusion in their 1989 research with fosetyl-aluminum and grapevines.

According to Cremlyn (1991), fosetyl, or its metabolite phosphonic acid, probably act directly on the target fungus, slowing its growth which allows the plant's natural defenses to kill the fungus. The sequence of events in the host plant is thought to be as follows:

1. a primary direct toxic effect on the pathogen leads to a retardation or even cessation of fungal growth (fungistasis),

2. natural defense reactions of the host plant, normally too weak in susceptible cultivars, become effective and kill the pathogen,

3. thus, the death of the pathogen is due to combined action of fungicide and host

plant (Schwinn and Urech, 1986).

According to Ware (1991) the mode of action of the organophosphates such as fosetyl, is uncertain, but tentatively they are considered to interfere in phospholipid synthesis of the invading organism.

H_3PO_3 provides control equal to fosetyl-aluminum of *Phytophthora cinnamomi* on avocado (Fenn and Coffey,1984; Pegg et al., 1985), and is highly inhibitory to the mycelial growth of several *Phytophthora* spp. in vitro (Bompeix and Saindrenan, 1984; Coffey and Bower, 1984; Coffey and Joseph, 1985; Fenn and Coffey,1984 and 1985). Foliar applications of Aliette® WDG (5.6 kg h^{-1}) applied six times during the season, however, did not reduce the mortality of summer squash (*Cucurbita pepo* 'Supersett') infected with Phytophthora crown rot (*Phytophthora capsici*). One hundred percent plant death resulted from this disease by the end of the season (Holmes et al., 1999).

Lopez-Serrano et al (1994) reported that fosetyl-aluminum reduces the level of peroxidase secreted from roots into the culture medium, thereby reducing Oomycete infection. Their 1997 research suggested Ca^{2+} and Mg^{2+} ions specifically overcome the inhibitory effect of fosetyl-aluminum on peroxidase. Thus the efficacy of fosetyl-aluminum could be significantly reduced in soils with high levels of Ca^{2+} and/or Mg^{2+} (Lopez-Serrano et al., 1997).

Fosetyl-aluminum (Aliette®) foliar applied to Vesicular-Arbuscular Mycorrhiza (VAM) leek resulted in increased colonization by *Glomus intraradices*, the number of intramatrical[78] vesicles, root exudates and plant growth compared to inoculated but untreated plants (Jabaji-Hare and Kendrick, 1985 and 1987). These results support the hypothesis that root exudates are predisposing factors for the colonization and spread of mycorrhizal fungi (Ratnayake et al., 1978).

Foliar applications of fungicotoxins have less effect on the establishment of mycorrhiza in root tissues than seed treatments. Triforine and benomyl treatments have relatively strong inhibitory effects. Benomyl, thiabendazole and ethirimol when applied as seed dressings inhibit the development of VAM even at low concentrations (Jalali and Domsch, 1975).

Fosetyl-aluminum inhibits the growth of wild-types of *Penicillium digitatum* but is less effective against resistant strains (Gutter, 1983). Aliette® is effective in increasing nodulation by Aliette®-resistant strains of *Rhizobium meliloti*, a root-nodule bacteria. The increase in colonization may have been a result of inhibition of rhizosphere bacteria competing with the root-nodule bacteria for root exudates. This toxicant also may reduce the grazing effect of protozoa (Siddiqi and Alexander, 1991).

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[78] Intramatrical - an organism that lives in the interior of the host, within the substrate (Ulloa and Hanlin, 2000).

Aliette® 80 WP is effective in controlling *Pythium ultimum* root rot in geranium. Research by Pfleger et al. (1990) found drenches much more effective in controlling this root rot organism than foliar sprays. Fosetyl-aluminum is effective against non-Oomycetes such as *Penicillium digitatum* and *P. italicum* (Gutter, 1983; Gutter et al., 1981).

Aliette® 80 WP (2.24 kg h^{-1}) alone and in combination with Triton® CS-7 (8.33 EC; 0.7 liter h^{-1}) and Rovral® 4F (1.16 liter h^{-1}) significantly reduced (P=0.05) purple blotch of onion caused by *Alternaria porri*, but had no effect on yield when compared with the untreated check (Miller, 1992b; Miller and Amador, 1991). An Aliette® 80 WG/Dithane® 75DF/potassium carbonate 100WG (2.24 kg /2.24 kg /1.12 kg h^{-1}) combination is significantly effective (P=0.05) in reducing purple blotch and increasing total yield of onion (Miller, 1998).

Imazalil

Composition: ±1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole (Meister, 2000).

Action: Systemic fungicide, used as a curative and protectant (Ware, 1991).

Use: Wheat and barley seed treatment for common (dryland) root rot (*Fusarium*, *Helminthosporium*), and associated seedling diseases. For control of seedborne leaf stripe, net blotch, and *Septoria nodorum* (Meister, 2000).

Imazalil was the first imidazole agricultural fungicide, being introduced in 1969. It is now used mainly as a seed dressing, but can be applied as a spray to control powdery mildews and Fusarium (Cremlyn, 1991). It was developed especially for control of *Helminthosporium* diseases of plants (Büchel, 1986). Imazalil is particularly active on mycelial growth (Bendahmane et al., 1992). This group is quite phytotoxic.

The imidazoles owe their fungitoxicity to their ability to inhibit ergosterol biosynthesis. Ergosterol is a major sterol in many fungi where it plays a vital role in membrane structure and function. Imazalil acts specifically as a C-14 demethylation inhibitor and probably binds to the enzyme cytochrome P 450[79]. Consequently, this material may interfere with the oxidative metabolism of other pesticides (Cremlyn, 1991). Imazalil is related to benomyl and thiabendazole in that it is an imidazole compound without the benzene ring (Ware, 1991). Resistance of fungi to one member of the group such as thiabendazole, however, does not mean those fungi are equally resistant to imazalil. Research by Johanson and Blazquez (1992) found thiabendazole resistant strains of *Fusarium* spp. were susceptible to imazalil treatment.

In laboratory *in vitro* tests, imazalil was the most active non-benzimidazole of all the compounds studied against the *Penicillium* spp. The same was true in *in vivo* tests with

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[79] The Cytochrome P-450 proteins are components of the 'mixed-function oxidases' which are involved in the metabolism of pesticides, pollutants, drugs, steroid hormones, etc. (Wickramasinghe, 1990).

95% of sensitive and TBZ-resistant strains being controlled with 1000 ppm of imazalil. Sporulation was reduced by 50% with 50 ppm, while complete inhibition of sporulation occurred at 2000 ppm (Gutter et al., 1981).

Preplant dips with imazalil (Fungaflor® 75SP) at 500, 750 and 1,000 ppm of active ingredient (a.i.) are ineffective in the control of *Fusarium oxysporum* f. sp. *apii* (Awuah et al., 1986). Robertson et al. (1987b) found that levels of imazalil above 125 mg a.i. liter^{-1} (125 ppm) used as a seed treatment, significantly lowered foot rot (*Fusarium solani* f. sp. *pisi*) of peas, but also resulted in lowered plant emergence. At this level of imazalil, emergence was 33% compared with 84% for the untreated check. When used as a drench, 125 ppm of imazalil resulted in a 33% emergence as compared with 73 % for the untreated control. Severe phytotoxicity occurred above 125 ppm (Robertson et al., 1987a).

Root rot of barley (*Hordeum vulgare* L.) is significantly reduced by seed treatment of imazalil (0.1 g kg^{-1} of seed) (Goss et al., 1989), yet imazalil had no effect on *Fusarium* root rot of winter wheat (*Triticum aestivum* 'Houser') when applied as a seed treatment at 0.14 ml kg^{-1} of seed (Kalb et al., 1988).

Carnegie et al. (1998) studied the effect of imazalil on *Fusarium solani* var. *coeruleum*, the causal agent of dry rot of potatoes, over a six year period. They found that the mixture of thiabendazole and imazalil was more effective than imazalil alone for

controlling this *Fusarium* disease on stored tubers.

Iprodione

Composition: 3-(3,5-dichlorohenyl)-N-(1-methylethyl) 2,4-dioxo-1-imidazolinecarboxamide (CAS) (Meister, 2000).

Action: A broad-spectrum contact and locally systemic fungicide with action on both fungal spores and mycelium. It translocates through the cuticle and across leaves (Ware, 1991).

Use: It provides excellent activity against *Alternaria, Botrytis, Helminthosporium, Monilinia, Rhizoctonia, Sclerotinia, Penicillium, Aspergillus, Rhizopus* and *Mucor.* Iprodione is equally efficient on mycelial growth and conidial germination (Bendahmane et al., 1992).

Formulation used in this study: An Aliette® /Rovral® 15G premix containing fosetyl-aluminum, and iprodione, respectively.

Literature Review:

Iprodione is a member of the dicarboximide (imide) group of fungicides for which little is known regarding the precise mode of action. This group is known to inhibit spore germination, mycelial growth and DNA synthesis (Cremlyn, 1991). The imides are particularly effective against *Botrytis, Monilinia* and *Sclerotinia.* Iprodione is also active

against *Alternaria*, *Helminthosporium*, *Rhizoctonia*, *Corticium*, *Typhula*, *Fusarium* (Ware, 1991), *Phoma*, and *Polyscytalum* species (Edgington, 1981).

Resistance of the Sclerotineaceae to the dicarboximides developed in both laboratory and field research (Edgington, 1981). To extend the antifungal spectrum, iprodione may be mixed with maneb, thiram, or thiophanate-methyl (Cremlyn, 1991).

Rovral® 30 FL applied as a seed treatment at the rate of 3.75 g kg^{-1} of seed was ineffective in controlling bean root rot (Abawi et al., 1988). Neither iprodione, mancozeb, benomyl, nor *Streptomyces griseoviridis* prevent Fusarium wilt of sweet basil when applied as a drench 3 days prior to inoculation (Keinath, 1994).

Two applications of Rovral® 4F as a foliar spray (10 April and 2 May) at 2.24 kg h^{-1} significantly reduced scape blight caused by *Botrytis allii* in seed onions. There were no differences, however, for soil-line rot, seed set or weight per umbel (Butler and Crowe, 1997). Rovral® 4F applied alone (1.2 liters h^{-1}) and in combination with Triton® CS7 (2.4 liter h^{-1}) as foliar sprays significantly (P=0.05) reduced purple blotch (*Alternaria porri*) but had no effect on final yield (Miller and Amador, 1991). Efficacy trials using Rovral® 50WDG (1.7 kg h^{-1}) and Rovral® 4SC (1.8 liters h^{-1}) were effective (P=0.05) in reducing purple blotch on two evaluation dates but no significant difference was observed for downy mildew (*Peronospora destructor*). Plots were sprayed weekly for a total of eight applications. Yield differences compared to the untreated control were not significantly

different (Hausbeck and Cortwright, 1997).

Four week old sweet basil plants (*Ocimum basilicum*) grown in a greenhouse were drenched with 100 ml of iprodione (1.88 ml liter^{-1}) three days before being inoculated with *Fusarium oxysporum* f. sp. *basilicum*. A significant increase in plant height and dry weight of leaves harvested 3 weeks after treatment resulted. When a concentration of 0.019 and 0.19 ml liter^{-1} was tested, no significant differences in plant height, disease severity, leaf area, or fresh or dry leaf weight occurred (Keinath, 1994).

Biodegradation

Effect of pH

Iprodione degrades little below pH 5.0. At pH 5.7, 50% loss occurs in 60 days. At pH 6.5, 50% loss occurs within 30 days in soil previously untreated with iprodione. In soil with a pH of 6.5 which had three prior applications of iprodione, 50% loss occurred within 4 days (Walker, 1987).

It is generally agreed that the two most important degradation forces operating on chemicals in the environment are microorganisms and sunlight (Matsumura, 1982). The ability of microbes to rapidly degrade ("enhanced degradation") iprodione can be induced with a single pre-treatment at a very low initial concentration. In thirty-three different

soils, the mean time to 90% disappearance varied from 50 days in soil that had not been treated previously with iprodione, to about 8 days in soils treated previously on three or more occasions (Walker and Welch, 1990).

Metalaxyl

Composition: [N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester]

Action: Fungicide seed dressing, soil and foliar fungicide.

Uses: Foliar or soil with curative and systemic properties. Controls foliar diseases such as downy mildew and late blights; soilborne diseases cause by *Phytophthora* and *Pythium* in many crops (Meister, 2000).

Formulations used in this study:

Ridomil® (CGA-48988) - a systemic fungicide active against Phycomycetes (Raid, 1998).

Apron® - for use as a seed dressing. It is used to control seedling damping-off caused by *Pythium* spp., *Phytophthora* spp., and certain downy mildews (Raid, 1998). According to Edgington (1981), metalaxyl is only toxic to Oomycetes.

Literature Review:

Metalaxyl, [N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester], was originally developed as a chemical for control of foliar pathogens (Bruck et al., 1980), and is widely used for the control of soil borne pathogens (Anderson and Buzzell, 1982).

It was introduced by Ciba Geigy (Novartis) in 1977 as one of the first members of the phenylamide group of fungicides (Cremlyn, 1991) and registered as a pesticide in the United States in 1979. EPA issued 'Registration Standards' for metalaxyl in June 1981 (NTIS#PB82-172297) and September 1988 (NTIS #PB89-128979) (U.S. EPA, 1994).

Metalaxyl has a highly inhibitory effect on sporangium formation by various *Phytophthora* spp., as well as on the production of chlamydospores and oospores (Bruck et al., 1980). This compound has systemic and curative, as well as residual-protectant activities (Ware, 1991). Metalaxyl is translocated mainly in the transpiration stream (Bruin and Edgington, 1983). Its characteristics of translocation include local penetration as well as acropetal movement. Depending on crop species, basipetal translocation is reported (Schwinn and Urech, 1986). It has activity against fungal diseases caused by a few non-Oomycetes (Gutter, 1983) to include *Fusarium sambucinum, Fusarium culmorum* and *Alternaria solani* (Barak et al., 1984). Ware (1991), however, reports metalaxyl is not effective against Ascomycetes, Basidiomycetes, or Fungi Imperfecti.

While inhibiting one group of rots, there is the possibility that a fungicide may encourage another. During research with metalaxyl in the post harvest control of *Phytophthora* crown rot of citrus, there was an unexpected increase of green and blue molds, caused by *Penicillium digitatum* and *P. italicum*, respectively (Cohen, 1981).

All the phenylamides show cross-resistance to one another indicating a common

biochemical mode of action (Cremlyn, 1991). At the biochemical level, metalaxyl is effective by inhibiting RNA synthesis at a single site (Schwinn and Urech, 1986).

Seed and Soil Applications of Metalaxyl

Research by Bradford et al. (1988), found that metalaxyl applications to muskmelon seed or soil generally improved seedling emergence, but the effect varied with cultivar, location, and planting method. None of the treatments significantly influenced final fruit yield. Treatments of metalaxyl (Apron®) alone or in combination with quintozene[80] did not increase germination or yield of soybean. Apron in combination with captan, however, resulted in a significant (P=0.05) increase in seedborne *Cercospora kukuchii* (Fernandez et al., 1989). Apron® 25 applied as a seed treatment was ineffective in reducing the incident of beet root rot caused by *Pythium*, *Rhizoctonia*, or *Fusarium* (Abawi and Cobb, 1986).

In 1985, Abawi and Crosier conducted field evaluations of fungicides for control of bean root rot caused by *Pythium ultimum*, *Rhizoctonia solani*, *Thielaviopsis basicola*, and *Fusarium solani* f. sp. *phaseoli*. Seed treatments of metalaxyl (Apron® 25W) alone and in combination with Rizolex[81] 50W, SN84364 50W and NC28410 50W at varying rates

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[80]Quintozene = PCNB - pentachloronitrobenzene (Meister, 2000).

[81] Tolclofos-methyl - 0-(2,6-Dichloro-4-methylphenyl) O,O-dimethyl phosphorothioate) - Sumitomo Chemical Co, Ltd. (Meister, 2000).

increased the number of plants per plot; root disease also increased when compared with the nontreated check. Soil treatments of metalaxyl (Ridomil®) with PCNB and SN84364 had no significant effect on the final number of plants per plot, stem rot or root rot. None of these treatments significantly increased yield (Abawi and Crossier, 1986). Soil treatments of Ridomil® 2E were ineffective against root rot of 'Bush Blue Lake' beans caused by several soil-borne pathogens to include *F. s.* f. sp. *phaseoli*, even in combination with Rizolex®. Seed treatment of Apron® 28.35 FL at 1.25 g kg^{-1} was likewise ineffective (P=0.05). Root rot in the Ridomil® plus Benlate® plots was significantly less than the root rot in untreated check plots (Abawi and Crossier, 1988). In 1988, Abawi and Crossier applied Ridomil® 5G as a soil treatment at the rate of 4.5 kg h^{-1} without producing a significant difference (P=0.05) when compared with the untreated check (Abawi and Crossier, 1989). Ridomil® PC 11G applied as a soil treatment at the rates of 0.47, 0.65, 0.93 and 1.86 grams $meter^{-1}$ of row of Navy bean did not result in differences in stand count or yield (Venette and Gross, 1991).

Research on cotton *(Gossypium hirsutum)* for seedling diseases (*Rhizoctonia solani, Pythium* spp. and *Fusarium* spp.) resulted in the following:

1. Applications of Ridomil® 5G (2.8 kg h^{-1}) and Ridomil® PC 11G at 14 and 19.6 kg h^{-1} did not increase the plant population of cotton when plants were counted two weeks after germination. These treatments applied in the furrow at seeding, however, were responsible for a significant increase (P=0.05) in plant population six weeks after seed

germination (Colyer, 1988).

2. There was no significant difference in the plant populations of cotton two and six weeks after germination when Ridomil® PC 11G was applied at the rate of 19.6 kg h^{-1} at planting (Colyer and Vernon, 1990).

3. Population counts taken two weeks after germination were significantly higher (P=0.05) when the granular formulation of Terraclor® Super X was applied in-furrow at the rate of 11.2 kg h^{-1}. Seed applied Apron®-Terraclor® and Apron®-Terraclor®-Vitavax® combinations were not significantly different from the untreated control (Colyer and Vernon, 1991a).

4. Ridomil® PC 11G at 11.2 kg h^{-1} applied in the planting furrow resulted in a significant increase (P=0.05) in plant population 2 weeks after germination. This significant difference was not apparent, however, by the six week plant population count. No significant difference occurred for the liquid formulation of Ridomil® 2E at 2.6 ml kg^{-1} or the Apron®-Terraclor® combination (6.25-25D) at 5.2 ml kg^{-1} applied as a hopper-box treatment (Colyer and Vernon, 1991b).

5. No significant differences at either the 2- or 6-week plant population count occurred for Ridomil® PC 11G at 11.2 kg h^{-1} applied as an in-furrow treatment, the combination

treatment of Ridomil® PC 11G (7.84 kg h^{-1}; in-furrow treatment) plus Prevail® [82] (5.2 ml kg^{-1}; hopper box treatment), or the Apron® - Terraclor® combination (6.25-25D) at 5.2 ml kg^{-1} treatment when compared with the untreated control (Colyer and Vernon, 1992).

Apron® 25W was applied as a seed treatment at the rate of 1.25 g kg^{-1} to a seed lot of 'Tyler' soft red winter wheat contaminated with Fusarium (57% infection). The treatment did not increase stand count, shoot dry weight, or yield (Bergstrom et al., 1989). Kalb et al. (1988) applied Apron® 25W at 1.3 ml kg^{-1} of seed alone and in combination with Baytan® 30F (0.81 ml kg^{-1} of seed) to *Triticum aestivum* 'Houser' and found no significant difference (P=0.05) in stand count, yield, or foot rot incidence caused by *Fusarium* spp. Seed treatments of Apron® FL (0.48 ml kg^{-1}) used for the control of seed rot and seedling blight of grain sorghum (*Sorghum bicolor;* 'Fredonia', 'Powhattan', and 'Hesston' cultivars), caused by *Fusarium* and *Pythium* spp., respectively, alone and in combination with *Trichoderma* did not increase stand count or yield (Jardine et al., 1991).

Foliar Applications of Metalaxyl

Ridomil® is used as a foliar treatment. However, such treatments are not always effective. Foliar applications of Ridomil® and Bravo®, alternately applied every 7 days

[82] Carboxin + metalaxyl + PCNB - 3.12-15-15D - Trace Chemicals, Inc.

for a total of eight applications, significantly reduced onion purple blotch (*Alternaria porri*) when compared with the untreated check, but yield differences were not significant (Hausbeck and Cortright, 1997). Miller (1992b) treated onions for purple blotch control when the bulbs were 1.25 to 1.87 cm in diameter on a 7 to 10 day schedule alternating with Ridomil® and Bravo®. In this study there was no significant difference in yield when compared with the untreated control.

Biodegradation of Metalaxyl

Metalaxyl persists for several months in soils which have no previous history of metalaxyl treatments. Bailey and Coffey (1985) compared soils from metalaxyl-treated sites with similar texture soils that had not received prior treatments of metalaxyl (controls) to determine the rate of biodegradation of metalaxyl and other acylanilide herbicides. In three of five soils with a history of metalaxyl treatment, there was a more rapid degradation of metalaxyl when compared with soils without a metalaxyl history. The average half-life of metalaxyl in soils previously treated with metalaxyl was 28 days. In a previously treated soil with accelerated biodegradation, the half-life of metalaxyl was 14 days. One of the control soils also was capable of actively degrading metalaxyl. When the metalaxyl-active control soil was tested, the other three acylanilide pesticides were not degraded. The other materials used in this study were oxadixyl (2-methoxy-N-[2-oxo-1,3-oxazalidin-3-y]-acet-2',6'-xylidine), RE 26745 (2-methoxyl-N-[2,6-dimethylphenyl]-N-[tetrahydro-2-oxo-3-furanyl]-acetamide), and the chemically similar

herbicide metolachlor (2-chloro-N-[2-ethyl-6-methylphenyl]-N-[2-methoxy-1-methylethyl] acetamide).

A follow-up report by Bailey and Coffey in 1986, provided additional information on metalaxyl degradation and characterized the microbes involved. Using the same acylanilide pesticides as in their 1985 report, they found metalaxyl was completely degraded in 70 days in soil with a history of metalaxyl treatments. In chloroform-sterilized soil, even after 45 days, metalaxyl was still assayed at its original level. While metolachlor was degraded in the presence of metalaxyl, it was not degraded when it was the sole source of carbon. A wide range of bacteria and fungi were involved in the degradation process.

A clear relationship exists between the rate of degradation and frequency of prior applications of fungicide (Walker and Welch, 1990). This phenomenon is termed "enhanced degradation" or "accelerated degradation", because a more rapid rate of pesticide degradation occurs in previously treated than in previously untreated fields (Racke, 1990).

Resistance to Metalaxyl

Naturally occurring outbreaks of resistance to metalaxyl have occurred with *Phytophthora infestans* on potato (Coffey and Young, 1984), and downy mildew

(Georgopoulos and Grigoriu,1981) and *Pythium* blight on turf (Samoucha and Cohen, 1985). The phenylamide fungicides are generally formulated as wettable powders mixed with non-systemic fungicides to combat resistance problems (Cremlyn, 1991). Different oomycetous species and isolates from a single species, can vary significantly in their sensitivity (both *in vitro* and *in vivo*) (Coffey and Young, 1984).

Effects of Metalaxyl on Soil Microbes

Metalaxyl appears to have no adverse effects on either vesicular-arbuscular mycorrhizae (Groth and Martinson, 1983; Nemec, 1980) or ectomycorrhizae (Kennerlye et al., 1984.), and may actually promote the growth of these mycorrhizae (Groth and Martinson, 1983). However, Jabaji-Hare and Kendrick (1987) found treatment of leek (*Allium porrum* L.) with metalaxyl resulted in significantly decreased mycorrhizal infection and plant growth of plants drenched with metalaxyl. Growth rates of treated VAM plants (0.5 to 2.0 mg a.i. $plant^{-1}$) were not significantly different from each other ($P>0.05$), but were significantly different from the untreated controls. Metalaxyl increases the tolerance of crops to nematodes (Kaplan, 1983).

Additional Comments

In 1996 the United State Environmental Protection Agency announced the voluntary cancellation of registrations of the active ingredient metalaxyl and the end-use product

registrations held by Ciba (Novartis) Crop Protection that contain metalaxyl. The cancellation of metalaxyl registrations was said to allow for the full environmental benefit provided by the registration of mefenoxam and end-use products containing mefenoxam. Mefenoxam is the R-enantiomer[83] of metalaxyl and at half the application rate provides the same level of efficacy as metalaxyl[84]. All of the registered uses of metalaxyl-based products were replaced by the registration of parallel and equivalent mefenoxam-based products. Under an agreement with Ciba (Novartis) Crop Protection, metalaxyl products could be sold until December 31, 1998 (U.S. EPA, 1996).

Metam-sodium

Composition: Sodium N-methyldithiocarbamate.

Action: Fungicide, herbicide, insecticide, nematicide, soil fumigant.

Uses: Soil disinfectant for soil fungi, nematodes, soil insects. Controls germinating weeds (Draper and Wakeham, 1993; Meister, 2000).

Formulation used in this study: Busan ® 1020

Literature Review:

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[83] Enantiomer - the optical isomer of a compound containing at least one asymmetric atom; such isomers often have different biological activity (Cremlyn, 1991).

[84] "A survey of the literature pertaining to the formulation of fungicides quickly reveals that almost anything that is done to the active ingredient affects its biological activities" (Backman, 1978).

Methyl isothiocyanate (MIT) was introduced in 1959 as a nematicide and general soil fumigant for control of soil fungi, insects, nematodes, and weed seeds. Methyl isothiocyanate is phytotoxic and must be applied sufficiently early to allow the toxicant to decompose before the crop is planted. MIT is effective when applied as a soil drench in the form of metam-sodium (Cremlyn, 1991).

Formulated as a 32.7% solution, metam-sodium is diluted with water and applied directly to soil where it decomposes to MIT, a volatile, biologically active product believed to account for metam-sodium's fumigant action (Draper and Wakeham, 1993). This decomposition process is thought to occur within minutes to hours (Turner and Corden, 1963). MIT inactivates the -SH groups in amino acids, proteins and enzymes (Ware, 1991).

The half-life of MIT varies from 0.5 to 50 days depending on soil temperature and texture (Smelt et al., 1989). The reported photochemical half-lives for metam-sodium are very short, between 30 (Spurgeon, 1990) and 96 (Woltz and Jones, 1973) minutes. Draper and Wakeham (1993) reported photodegradation half-lives ranged from 2.9 to 8.4 minutes, depending on light intensity (3500 to 6400 micro watts/cm^2).

The review by Leistra and Smelt (1974) noted slow diffusion of methyl isothiocyanate and a higher decomposition rate as compared with other fumigants caused a poor overlap of the dose pattern. They further noted application with a shank injector requires a great

deal of horsepower power and subsequently growers are occasionally inclined to decrease the number of shanks and increase the distance between the shanks at the same time. Even with careful application by shank injector under favorable soil conditions, the authors note the overlap of the pattern of effective doses may be poor. With increasing distance between the shanks, the overlap of the effective-dose patterns becomes much worse. They recommend shanks be spaced 20 cm or less apart. With fumigation under cold and wet conditions late in autumn, there is a great risk of damage being done to the crop grown in spring. When injecting metam-sodium in a line, precultivation of the soil is a very important factor. In practice, cultivation is often shallow which will be an obstacle for the achievement of the desired effectivity. With injection in ploughed soil, the dose pattern is much more favorable.

Research by Macalady et al. (1998) suggests that fumigation with metam-sodium results in persistent changes (at least 18 weeks) in the microbial biomass. McGovern et al. (1995) reported metam-sodium could reduce Fusarium crown rot of tomato only when thoroughly incorporated in the planting bed prior to bed formation.

Miller (1992a) applied Busan® 1020 and Telone® C17 [85] to a field with a history of pink root. Busan® 1020 was injected into the soil in the center of a 40 cm bed using a winged-shank with orifices at 30, 22.5, and 15 cm depths. Telone® C17 was injected with two

[85] Telone® C17 - Chloropicrin; 1,3-Dichloropropene - Dow AgroSciences LLC. (Meister, 2000).

shanks, 30 cm deep. Disease ratings of roots in plots treated with Busan® 1020 at 186- and 467- liters per hectare and Telone® C17 at 186 liters per hectare were significantly lower (P=0.05) than the disease rating in control plots. Yields were significantly higher for Telone® C17 treatments than untreated controls. Yields of bulbs in plots treated with Busan® 1020, however, were not significantly different from the control (Miller, 1992a). In follow-up research by Miller (1993b), Busan® 1020, injected as in the previous research, resulted in no significant difference (P=0.05) in disease ratings with 'TG 502y' when compared with the untreated check. The total yield, however, was significantly greater for both the 58 and 117 liter h^{-1} rate of Busan® 1020 when compared with the untreated check. A similar analysis with 'TG 1015Y' resulted in a significant difference (P=0.05) in disease ratings for Busan® 1020 applied at 117 liters $hectare^{-1}$, but no difference when Busan® 1020 was applied at 58 liters h^{-1}. There was no significant difference in total yield when the Busan® treatments were compared with the untreated check (Miller, 1993a).

Miller et al. (1990) conducted research with Fusarium root rot of muskmelons (*Cucumis melo* 'Magnum 45') and showed no increase in yield or vine length when Busan® 1020 was injected with a winged-shank with orifices at a depth of 30, 22.5, and 15 cm at three different rates (70, 140 and 210-l h^{-1}). The *Fusarium* population in mid season was significantly lower (P=0.05) than the untreated control, but this significance had been lost by the end of the season.

Miller et al. (1991 and 1992) conducted research with metam-sodium on *Monosporascus cannonballus*, a root rot and vine decline pathogen of muskmelon. Busan® 1020 (186 liters h^{-1}) injected in an 8 to 10 inch band had no significant effect on vine length or yield when compared with the untreated check. When applied in various band widths (30, 60, and 90 cm) and application rates (140, 196 and 261 liters $hectare^{-1}$, respectively), no significant (P=0.05) differences were found when compared with the untreated check. Some combinations of Busan® 1020 and Telone® C17 were, however, effective in increasing vine length and yield (P=0.05).

Schwartz (1986) found similar results with Vapam® [86] (metam-sodium) even with Vapam® rates of 91.8 liters per hectare. In his study, the incidence of Fusarium basal rot was 56% in the untreated check plots and 55% in the Vapam® treated plots.

Nusan® 30 EC[87] applied at 0.81 ml kg^{-1} of seed, significantly increased percent germination of certified wheat seed ('Tam' W-101) in one of two fields contaminated by *Bipolaris*, *Fusarium*, and *Pythium* spp. In the second field, however, there was no significant difference between the Nusan® treatment and untreated control (Williams, 1988). Nusan® 30 EC applied at the rate of 0.65 ml kg^{-1} of scabby wheat seed resulted in a significant increase (p=0.05) in both yield per hectare and weight (Buechley et al.,

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[86] Vapam® - Amvac Chemical Corp. (Meister, 2000).

[87] Nusan® - metam-sodium - Wilbur-Ellis Co. (Meister, 2000).

1989). When applied to soybean seed, there were no significant differences in yield or germination (Fernandez et al., 1989). Recommended application rates range from 150 to 400 kg ha^{-1} based on a 10-cm soil depth (Leistra and Smelt, 1974).

Mycorrhizal fungi appear to be especially sensitive to metam-sodium (Trappe et al., 1984). The reduction in intensity of mycorrhizal infection of cotton by metam-sodium resulted in shorter plants, fewer nodes and less yield (Davis et al., 1996b).

Combining metam-sodium at reduced doses with short solarization is more effective in controlling fungal pathogens than either treatment alone for the control of *Fusarium oxysporum* f. spp. *basilici*, *melonis*, and *radicis-lycopersici*, the causal agents of Fusarium root rot of basil, melon, and tomato, respectively (Eshel et al., 2000). A reduction in the density of *Pseudomonas solanacearum* occurs when solarization is combined with fumigation (methyl bromide: chloropicrin) (Chellemi et al., 1994).

Thiophanate methyl (Topsin® M)

Composition: 1,2-bis(3-methoxy-carbonyl-2-thioureido)-benzene

Action: The thiophanates[88] are an important group of systemic fungicides with a high

[88] A subgroup within the benzimidazoles (Erwin, 1973).

level of persistent systemic activity by root uptake (Cremlyn, 1991).

Use: Thiophanate® M is applied as a seed treatment, soil drench, soil mixture and foliar spray with systemic activity demonstrated in all methods of treatment (Erwin, 1973).

Formulation used in this study: Topsin ® M

Literature Review:

The thiophanates are not themselves fungicidal but are converted to active benzimidazole derivatives. Thiophanate methyl, like benomyl, is retained in the roots and released gradually to other parts of the plant (Cremlyn, 1991). The systemic toxophore within plants treated with Topsin® M is carbendazim (Edgington, 1981). Erwin (1973) indicates the fungitoxic conversion product of thiophanate methyl is MBC (methyl-2-benzimidazolecarbamate). This conversion is hastened by fungal and plant metabolic activity. When applied to roots of cotton plants, thiophanate methyl is detected in relatively high quantities after 1 day of root uptake, but by 6 days, very little thiophanate methyl and a relatively high quantity of MBC are detected (Erwin, 1973). MBC interferes with DNA synthesis and with some post-DNA synthesis aspect of the cell replication process (Sisler, 1982). Transformation to MBC is four times as rapid in soil at pH 7.4 as in soil at 5.6 (Fleeker et al., 1974). The rate of conversion is reduced by steam treatment (Sisler, 1982).

The lifetime of thiophanatemethyl in moist soil is rather short. Eighty to 90% of applied

TM disappears from soil (Towner sand and Fargo clay soil) within 5 days of application. In air-dried soil, with no moisture added, 10-20% of applied TM is present 30 days after application (Fleeker et al., 1974).

Certain isolates of fungi are tolerant of the benzimidazole fungicides (Erwin, 1973). When the benzimidazole fungicides were first introduced they were effective against a wide spectrum of fungi. Due to the specific mode of action of these fungicides, resistance appeared quite rapidly (Büchel, 1986).

Two applications of Topsin® M 70 W at 1.12 kg h^{-1} as a foliar spray (10 April and 2 May) significantly reduced scape blight caused by *Botrytis allii* in seed onions. There were no differences, however, for soil-line rot, seed set or weight per umbel (Butler and Crowe, 1997). Significant differences were detected in disease incidence and severity of white mold (*Sclerotinia sclerotiorum*) of beans (*Phaseolus vulgaris* 'Viva' Pink) when Topsin® M 70W was applied at 1.7 kg h^{-1}; no significant differences in yield were detected (Forster and Strausbaugh, 1999).

An in-furrow application of Topsin® 5G (31.36 kg h^{-1}) had no effect on the severity of Fusarium root rot of beans (*Phaseolus vulgaris* 'Harris') caused by *Fusarium solani* f. sp. *phaseoli*. Yield and total biomass, likewise, was not significantly (P=0.05) changed (Kerr, 1991). Research with dry bean varieties 'Fargo' and 'Carrington', indicated Topsin® 5G as an in-furrow application at 31 kg h^{-1} had no effect on yield. Root rot was

significantly (P=0.05) increased, however, in the 'Carrington' trial when compared with the untreated check (Venette and Gross, 1991). In trials in 1991, no significant (P=0.05) differences were found with 'Fargo' or 'Carrington' for stand count, root rot or total yield when Topsin® M was tested as an in-furrow application at 15.7-, 23.5- and 31.4 kg h^{-1}, when compared with the untreated check (Venette and Gross, 1992).

Topsin® M 70 applied at the rate of 60 mg liter $^{-1}$ to chrysanthemum (*Chrysanthemum morifolium* 'Royal Trophy') infested with the fusarium wilt organism (*Fusarium oxysporum* f. sp. *chrysanthemi*) significantly (P=0.05) reduced disease (Engelhard and Woltz, 1988). Cyclamen (*Cyclamen persicum* 'Firmament') was drenched on a 7 day interval with 100 ml of Topsin M 70W per each 15 cm diameter pot to determine its efficacy against *Fusarium oxysporum* f. sp. *cyclaminis*. At the end of 12 weeks, the 1g 100 ml^{-1} rate had a significant (P=0.05) increase in diseased plants as compared to the infested control. There was no significant difference between the infested control and the 2g 100 ml^{-1} rate Topsin® M 70W treatment. The authors report that none of the treatments used were phytotoxic to the plants (Padmanabhan and Merriman, 1989).

Applications of Topsin® M F 4.5 at the rate of 1.5 liters h^{-1} and 2.9 liters h^{-1} four times during the season for control of black dot (*Colletotrichum coccodes*), Sclerotinia stem rot (*Sclerotinia sclerotiorum*) and Verticillium wilt (*Verticillium dahliae*) of potato (*Solanum tuberosum* 'Russett Burbank') did not increase tuber number or weight (Easton, 1989).

Thiram

Composition: Bis (dimethylthiocarbamoyl) disulfide; or tetramethylthiuram disulfide (IUPAC[89]) (Meister, 1999)

Action: Fungicide, seed protectant, animal repellent.

Use: Reduces loss from seed decay, damping off and blight caused by many seedborne and soilborne organisms (Meister, 2000).

The dithiocarbamates[90], such as thiram, probably owe their fungitoxicity to their ability to chelate with certain metals, especially copper (Cremlyn, 1991). Ware (1991) says the mode of action of thiram is due to its metabolizing to the isothiocyanate radical which inactivates the sulfhydryl (-SH) group in amino acids and enzymes within the pathogen cell.

Thiram is not systemic (Ware, 1991). Thiram (tetramethylthiuram disulphide) was the first compound to be applied as a fungicide and is still used as a seed dressing against soil fungi causing damping-off diseases (Ivens, 1988).

The rate of breakdown of thiram is dependent on the concentration applied. Thiram is not

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[89] IUPAC - International Union of Pure and Applied Chemistry (Meister, 2000).

[90] Sisler places thiram in the dialkydithiocarbamate group (Sisler, 1982).

detectable after 49 days at application rates of 250 ppm. In soils treated with 1000 ppm, much of the fungicide remains after 64 days (Sisler, 1982).

Greenhouse and Field Trials

Methods and Materials

Greenhouse and field trials were conducted between 1992 and 1994 to determine the efficacy of various soil and seed treatments and the fumigant metam-sodium. Each of these trials is discussed separately.

Analysis of variance (ANOVA2) was conducted to compare treatments. Means were separated with the LSD test. MSTAT-C, Michigan State University's microcomputer statistical program was used for all tests.

Greenhouse Trials; Tables 4a1 and 4a2

Methods and Materials

These trials were designed to determine the efficacy of various soil and seed treatments against *F. o. cepae*. Infested soil containing 10,000 propagules gm^{-1} of soil was used in

this efficacy study.

The soil used in these trials was a potting mix prepared by mixing 80% sand and 20% peat moss. This mix was steam sterilized, and stored in galvanized trash cans lined with black plastic bags.

The inoculum was prepared as follows:

Fifty grams of rolled oats was added to 500 grams of sand in a 2 liter flask and thoroughly mixed. The sand used for inoculum preparation was screened out of the potting mix used for these trials. Adequate distilled water was added to moisten the mixture. The flask was sealed with a plug of paper toweling which was in turn covered with a layer of aluminum foil. The mixture was autoclaved at 15 psi for 45 minutes. After the mixture cooled, the soil/rolled oats mixture was infested with a suspension of *F. o. cepae* in aseptic conditions in a sterile laminar flow hood.

The *F. o. cepae* suspension was prepared from a virulent isolate collected from the Clarence Wallace farm, Delta, Colorado. Petri plates of Komada medium were infested with the isolate and grown for 14 days under cool white fluorescent light at 25C. Sterile distilled water was applied to the surface of the agar and a sterile scalpel used to scrape the spores and mycelium from the agar. This mix of

water and propagules was then added to the sterile flasks of soil and oats. This procedure was conducted in aseptic conditions in a sterile laminar flow hood.

The infested flasks were maintained under fluorescent lights at room temperature until a heavy mycelial mat developed on the soil surface. The soil in the flasks was again mixed and allowed to develop mycelium. This mixing process was done to ensure a uniform level of inoculum throughout the soil mix. The mix was then allowed to dry. It was then carefully ground, screened through a 50 mesh screen and tested for the level of colony forming units using the serial dilution method discussed in Chapter 2. The soil was then diluted with the appropriate quantity of sterilized sand necessary to produce an inoculum level of 10,000 cfu g^{-1} of soil.

Four inches of steam pasturized soil was placed in new 15 centimeter pots. One and one-quarter centimeters of the infested sand was applied over the pasteurized soil. Ten Brown Beauty onion seeds were inserted 6 mm deep in each pot using a sterile dibble. The seed or soil was treated according to label recommendations as indicated in Table 4a1.

Ten pots were used per treatment for a total of 100 seeds. The pots were placed in a completely randomized pattern under cool white fluorescent lights in the greenhouse[91].

[91] Located at the Colorado State University Orchard Mesa Research Center, 3186 B ½ Road, Grand Junction, Colorado.

The temperature was maintained at 13 ± 2C. The lights were on chains permitting them to be raised or lowered as needed. The lights were maintained at a height of 15 centimeters above the soil/plant. The pots were watered by drip irrigation as needed. Readings on percent germination were collected one month after planting and statistically evaluated using MSTAT-C.

Table 4a1. Fungicides Used in Greenhouse Trials in 1993 to Determine their Efficacy Against *F. o. cepae*, the Causal Agent of Fusarium Basal Rot of Onion.

Product	Application Rate	Application Method
Untreated Check		
Ridomil® metalaxyl	11.2 kg h^{-1}	Soil Applied
Flo-Pro® imazalil	0.98 ml kg^{-1} of seed	Seed Applied
Pro-Gro® carboxin/thiram	25 g dust kg^{-1} of seed	Seed Applied
Aliette®/Rovral® fosetyl-Al/iprodione	0.34 g m^{-1} of row	Soil Applied
Apron® 25W metalaxyl	1.25 g kg^{-1} of seed	Seed Applied
Topsin® M thiophanate methyl	31.4 kg h^{-1}	Soil Applied

Non-healthy plants were not included in the analysis. Non-healthy plants were considered to be plants:

1. with roots above soil,
2. with stubby roots,
3. laying on the soil surface with no attempt to root into soil, or
4. with rotting roots.

Results and Discussion

The analysis of fungicide treatments in both trials were consistent in their order of significance. The untreated check in all cases had a greater number of living plants at the end of the trials as compared with the fungicide treatments.

In greenhouse trial number 1 (data collected 27 February, 1993), the Flo-Pro®, Ridomil®, Aliette®/Rovral® and Topsin® M treatments had significantly fewer healthy plants at the end of the study than the untreated check. There was no significant difference (P=<0.01) for the Pro-Gro® and Apron® treatments when compared with the untreated check.

In greenhouse trial number 2 (data collected 24 May, 1993), the Ridomil®, Aliette®/Rovral® and Topsin® M treatments resulted in significantly less (P=<0.01) healthy plants at the end of the study than the untreated check.

A comparison of the two trials (ANOVA-2) revealed the Pro-Gro® treatment (25 g dust kg^{-1} of seed) was statistically the same (P=<0.01) as the untreated check. All other treatments resulted in significantly fewer living plants at the end of the trials than occurred with the untreated check and Pro-Gro® seed treatment.

At the level of infestation tested and the results of these greenhouse trails, it is logical to assume that no treatments with these products would be effective in pasteurized soil. The

reduction in living plants due to Apron®, Flo-Pro®, Ridomil®, Aliette®/Rovral® and Topsin® M treatments can be explained by their phytotoxic effect. This effect could have been due to cell/seed death, cell damage increasing the pathogenicity of the inoculum (*F. oxysporum* f. sp. *cepae*) or plant stress leading to increased infection by the organism. The data indicates Pro-Gro® (at 25 g kg^{-1} of seed; label rate) is less phytotoxic than the other fungicides used in this study.

Treatments at label rates in non-pasteurized field soil is likely non-phytotoxic due to the abundance of pesticide degrading microbes. Previous *in vitro* studies report the effectiveness of fungicides at much lower concentrations than is required for field application. This study further supports the thesis that much lower concentrations of fungicides are needed for pathogen control in pasteurized soil and that fungicides are phytotoxic at high rates of application. Additional testing is needed at varying levels of *F. o. cepae* infestation and varying levels of product.

Table 4a2. Results of Two Greenhouse Trials Conducted in 1993 to Determine the Efficacy of Selected Fungicides against *F. o. cepae*, the Causal Agent of Fusarium Basal Rot of Onion.

Treatment	Trial #1 *	Trial #2 **	Combined Trials
Untreated Check	5.9 a	4.2 a	5.1 a
Pro-Gro® - carboxin/thiram - 25 g dust kg^{-1} of seed	5.7 ab	3.9 a	4.8 ab
Apron® - metalaxyl - 1.25 ml kg^{-1} of seed	4.9 ab	3.0 ab	3.9 ab
Flo-Pro® - imazalil - 0.98 ml kg^{-1} of seed	3.8 bc	2.8 ab	3.3 bc
Ridomil® - metalaxyl - 11.2 kg h^{-1}	2.2 cd	1.8 b	2.0 cd
Aliette®/Rovral® - fosetyl-Al/iprodione - 0.34 g m^{-1} of row	2.2 cd	1.5 b	1.9 cd
Topsin® M - 31.4 kg h^{-1}	1.5 d	1.5 b	1.5 d
Probability	0.000	0.000	0.000
C.V.%	45.43	50.90	39.28
LSD	2.030 $^{0.01}$	1.624 $^{0.01}$	1.504 $^{0.01}$

* Number of live plants per pot. Ten pots were planted with 10 seeds each for each treatment. Data collected on 02/27/1993, one month after planting.

** Number of live plants per pot. Ten pots were planted with 10 seeds each for each treatment. Data collected on 05/24/1993, one month after planting.

Field Trials 1992, 1993 and 1994

Materials and Methods

Research trials were conducted in 1992, 1993 and 1994 to determine the efficacy of various soil and seed treatments against *F. o. cepae*, the causal agent of Fusarium basal rot on onions. Studies were conducted in commercial onion fields of Jerry Hines (Delta, CO) and Bill English (Olathe, CO). Brown Beauty onion seeds were planted in early spring of each year.

1992 Field Study; Table 4b1 and 4b2

Field Plots

Experimental units consisted of four 15.25 meter beds planted with 2 rows of onions per bed at a spacing of 7.5 cm between plants. Plots were 170 cm wide. Plots were arranged in a completely randomized block design with four replications per treatments. This trial was conducted in a commercial field of Jerry Hines (Delta, CO). Seeding was done using a MiniNibex precision planter. Plots were maintained by the cooperator. Products applied in this study are as follows.

Table 4b1. Materials Used in the 1992 Field Study at the Hines Farm in Delta, CO., to Determine their Efficacy against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions.

Product	Application Rate
Topsin® M 5G thiophanate methyl	28 kg hectare^{-1}
Ridomil® PC 11G metalaxyl	22.4 kg hectare^{-1}
Aliette®/ Rovral® fosetyl-Al/iprodione	0.36 ml meter^{-1} of row
Pro-Gro® carboxin/ thiram	0.025 g kg^{-1} of seed
Apron® 25W metalaxyl	0.00125 g kg^{-1} of seed
Flo-Pro® IMZ imazalil	0.98 ml kg^{-1} of seed

Data Collection

All onions from the center 1.5 meters of the middle two beds of each plot were harvested. Diseased and small onions were discarded. Remaining marketable onions (colossal to prepack) were weighed and recorded.

Results and Discussion

Topsin® M 5G significantly (P=0.01) increased yield over the untreated control. The

Aliette®/Rovral®, Ridomil® PC 11G, Pro-Gro®, Apron® and Flo-Pro® treatments were equal to the control (Table 4b2).

Soil treatments in general produced better results than dusts or liquids applied directly to the seed. Ridomil® (metalaxyl), reported to be effective against Pythiaceae fungi, was not effective against *Fusarium* in this study. The marketable yield of the Apron® and Flo-Pro® treatments, while not significantly different from the control, were lower. This may have been due to direct application of these products to the seed and the resulting phytotoxic response.

Table 4b2. Results of the Field Study Conducted in 1992 at the Hines Farm in Delta, CO., to Determine the Efficacy of Various Fungicides against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions.

Treatment	Total Marketable Weight Onion Bulbs (kgs 1.275 m^2)
Untreated Check	3.4 bc
Pro-Gro® - 25 g dust kg^{-1} of seed carboxin/thiram	3.6 abc
Apron® - 1.25 ml kg^{-1} of seed metalaxyl	6.7 c
Flo-Pro® - 0.98 ml kg^{-1} of seed imazalil	1.8 c
Ridomil® 11.2 kg h^{-1} metalaxyl	5.0 abc
Aliette®/Rovral® - 0.34 g m^{-1} of row	6.5 ab
Topsin® M - 31.4 kg h^{-1} thiophanate methyl	6.7 a
Probability	0.0025
C.V.%	38.27
LSD	<0.01

1993 and 1994 Field Trials

Materials and Methods

Experimental units consisted of four 30.5 meter beds planted with 2 rows of onions per bed (30 cm between rows). Fields were planted by the cooperator with vacuum seeders. The Hines plots were 170 cm wide; English plots were 150 cm wide. Plots were arranged in a completely randomized block design with three replicates per treatment. These fields had been planted to onions over several decades. Fusarium basal rot was reported to be a problem in both fields.

Products selected for these trials were based on the 1992 field results with those being most effective in 1992 selected. Metam-sodium was band-applied in the fall of 1992, and broad-cast applied in the fall of 1993, for studies in 1993 and 1994, respectively. Products applied in the 1993 and 1994 trials are given below:

Product		**Application Rate**
Topsin® M 5G	thiophanate methyl	31.5 kg h^{-1}
Ridomil® PC 11G	metalaxyl	22.5 kg h^{-1}
Aliette®/Rovral® 15G	fosetyl-Al/Iprodione	34.5 g 100 m^{-1} of row

Treatments of thiophanate-methyl (Topsin® M 5G) at the rate of 31.5 kg h^{-1}, metalaxyl (Ridomil® PC 11G) at the rate of 22.5 kg h-1, and a combination of fosetyl-aluminum/iprodione (Aliette®/Rovral® 15G) at the rate of 34.5 g 100 m^{-1} row were uniformly applied to their respective plots at the Hines field and raked in to the top 1.25 centimeters of soil. Due to a delay in receiving the materials in 1993, they were applied approximately 1 month after the Hines field was planted instead of at seeding time as preferred. In 1994, these treatments were applied at planting.

Data Collection

Emergence data were collected from the Hines and English farms on 21 May, and 4 June, 1993, respectively. In 1994, data were collected on 20 May, 16 June, 15 July, and 12 August in the Hines field, and on 6 June, 1 July, and 29 July in the English field. All living seedlings from the center 15.2 meters of the two middle beds of each plot were counted. Plots were 11.4 m^2 and 12.9 m^2 in the English and Hines fields, respectively. Plot data were converted to a plot size of 12.5 m^2 for the purpose of analysis.

Harvest data were collected in August and September 1993, prior to the scheduled commercial harvest in the English and Hines farms, respectively. All onions from the center 15.25 meters of the two middle beds of each plot were harvested. Data for each line on each bed were kept separate resulting in four sets of data for each treatment/plot

being collected. Data on the number and weight (in kgs) on U.S. No. 1 colossal, jumbo, medium, and prepack onions were collected in accordance with the United States Standards for Grades (USDA, 1995). Data for doubles, culls, and Fusarium infected onions were also collected. These categories are described below:

Colossal - a diameter of 95.3 mm or larger
Jumbo - 76.2 to 95.2 mm in diameter
Medium - 50.8 to 76.1 mm in diameter
Prepack - 44.5 to 50.7 mm in diameter

Data for the following were also collected:

Doubles - onions which had developed more than one distinct bulb joined at the base
Fusarium infected - onions which had obvious symptoms of Fusarium basal rot
Culls - all other discards.

1993 Emergence Studies; Tables 4c1 and 4c2

Results and Discussion

Hines Farm, Delta, Colorado

There was a significant ($P=<0.01$) increase in the number of emerged seedlings as a result of the metam-sodium applications when compared with the untreated control and other treatments. There was no significant difference, however, between the two higher rates (327.4 and 280.7 l h^{-1}) of metam-sodium. The lower rate of metam-sodium (223.8 l h^{-1})

was significantly better than the untreated control but not as effective as the two higher rates.

The treatments of thiophanate-methyl (Topsin M®), metalaxyl (Ridomil®), and fosetyl-aluminum/iprodione (Aliette ®/Rovral ®) showed no increase in seedling emergence over the untreated control. This may have been due to the inability to apply the products at planting time. However, a previous greenhouse trial using these soil treatment materials applied at the time of seeding produced similar results (Table 4a2).

English Farm, Olathe, Colorado

There were no significant differences between treatments of metam-sodium and the untreated control.

Hines and English Farms Data Combined

A significant (P=0.05) difference occurred when metam-sodium treatments from both farms were compared with the untreated control. As occurred at the Hines farm, there was no significant difference between the 280.7 and 327.4 l hectare^{-1} application rates. The plots of the lower rate of Busan® 1020 (223.8 liter hectare^{-1}) had significantly more seedlings than the untreated control. This, however, was not as significant a difference as occurred with the two higher rates of metam-sodium. More basic studies are needed to

assess the relationship of seed vigor to yield and its relationship to seedling emergence and early growth.

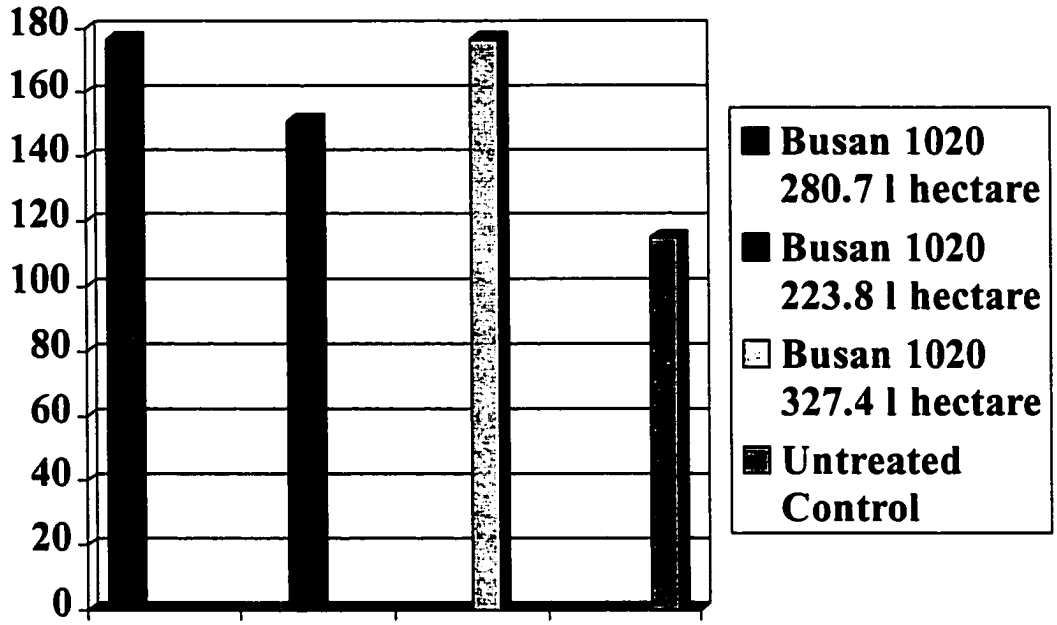
Table 4c1. Stand Count Data Collected in the Summer of 1993 in Fields of Jerry Hines, Delta, and Bill English, Olathe, CO., Treated with Various Fungicides to Determine Their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions .

Treatment	Hines Field *	English Field **	Combined Fields
Busan® 1020 - 327.4 l hectare^{-1}	156.6 a	188.0	176.6 a
Busan® 1020 - 280.7 l hectare^{-1}	163.4 a	185.2	177.3 a
Busan® 1020 - 223.8 l hectare^{-1}	107.6 b	192.3	150.6 b
Ridomil® - 22.5 kg h^{-1}	81.4 c	---	---
Aliette®/Rovral® - 34.5 g 100 m^{-1} of row	54.1 c	---	---
Topsin® M - 31.5 kg h^{-1}	55.8 c	---	---
Untreated	58.7 c	166.4	114.9 c
Probability	0.0023	0.1031	0.0167
C.V.%	30.85	6.01	11.71
LSD	<0.01	n.s.	0.05

* Data collected 5/21/1993; based on number of living plants 12.5 m^2.

** Data collected 6/4/1993; based on number of living plants 12.5 m^2. Treatments for Ridomil®, Aliette®/Rovral®, and Topsin® M were not applied in the English field due to receiving these materials late.

Table 4c2. Summary Table for the 1993 Band-applied Metam-sodium Trials Conducted in Onion Fields of Jerry Hines, Delta, and Bill English, Olathe CO., Showing the Average Number of Living Plants 12.5 meter^{-2} treatment^{-1} in *F. o. cepae* infested soil.



1994 Emergence Studies

Results and Discussion

Hines Farm, Delta, Colorado; Tables 4d1 and 4d2

Ridomil® had significantly less (P=0.05) seedlings when counts were taken on 20 May, 16 June, and 15 July than the Topsin® M, Ridomil®, Aliette®/Rovral® and Busan ® 1020 treatments.

A review of the monthly stand counts (Table 4d2) shows a drop in the final stand count for all treatments with the exception of the lowest (561 liter hectare^{-1}) metam-sodium treatment. All the treatments except for the Aliette®/Rovral® treatment experienced an increase in seedling population by the second counting date. This table demonstrates the great difference between Ridomil® and the other treatments.

English Farm, Olathe, Colorado; Tables 4d3 and 3d4

Ridomil® (metalaxyl) consistently had significantly less live seedling than the other treatments. There was no significant difference in the number of living seedlings in the untreated control or plots treated with Topsin® M (thiophanate methyl), Aliette®/Rovral® (fosetyl-Al/iprodione), or the 701, 608, or 561 l h-01 (broadcast-applied) rates of Busan®

1020 (metam-sodium).

The variation in seedling population in the English field was not as great as in the Hines field. Other than this field having been planted about 1 month ahead of the Hines field and the seedlings being more mature when the first seedling count was taken, there was no other significant difference between fields.

Hines and English Data Combined; Table 4d5

The average of all stand count data confirms plots treated by metalaxyl had significantly less seedlings than the other treatments.

Table 4d1. Stand Count Data Collected During the Summer of 1994 at the Hines Farm, Delta, CO., from Plots Treated with Various Fungicides to Determine Their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions .

Treatments	5/20/1994	6/16/1994	7/15/1994	8/12/1994
Topsin® M - thiophanate methyl - 31.5 kg h^{-1}	656.7 ab	674.0 a	667.9 a	660.9 a
Ridomil® - metalaxyl - 22.5 kg h^{-1}	489.2 d	513.3 c	503.4 c	491.8 c
Busan® 1020 - metam-sodium - 701 l $hectare^{-1}$	645.1 ab	648.0 a	648.7 a	628.7 ab
Busan® 1020 - metam-sodium - 561 l $hectare^{-1}$	617.5 b	652.2 a	646.1 a	647.7 a
Busan® 1020 - metam-sodium - 608 l $hectare^{-1}$	670.2 a	680.8 a	664.7 a	664.1 a
Aliette®/Rovral® - 34.5 g 100 m^{-1} of row	671.2 a	661.8 a	667.0 a	659.6 a
Untreated Control	563.5 c	579.5 b	576.0 b	575.0 b
Probability:	0.0208	0.0250	0.0200	0.0484
% C.V.:	9.56	8.72	8.67	10.28
LSD:	52.40 $^{0.05}$	48.87 $^{0.05}$	48.20 $^{0.05}$	56.52 $^{0.05}$

Plot size was 12.9625 m^2. Converted to a plot size of 12.5 m^2.
Metam-sodium treatments were broadcast applied in the fall of 1993.

Table 4d2. Comparison of Monthly Stand Count Data Collected during the Summer of 1994 at the Hines Farm, Delta, CO., from Plots Treated with Various Fungicides to Determine Their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions .

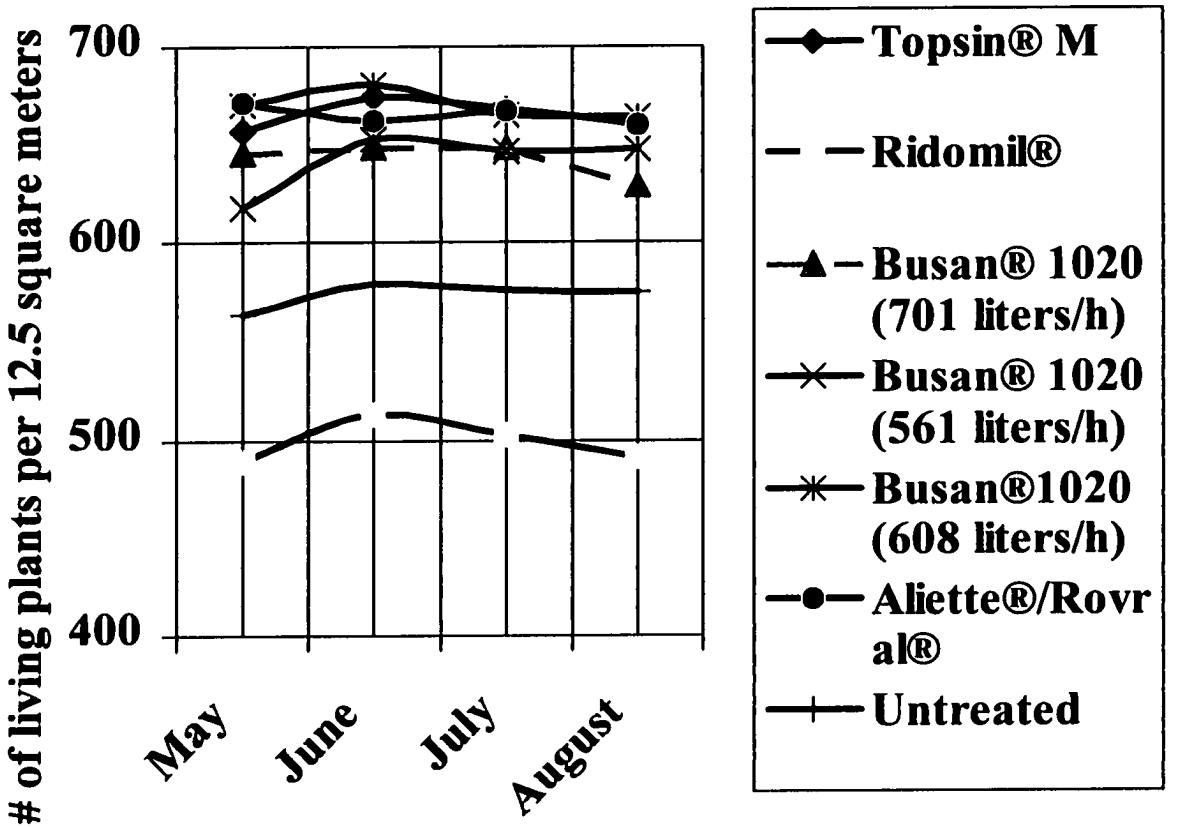


Table 4d3. Stand Count Data Collected During the Summer of 1994 at the English Farm, Olathe, CO., from Plots Treated with Various Fungicides to Determine Their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions .

Treatments	6/6/1994	7/1/1994	7/29/1994
Topsin® M - thiophanate methyl - 31.5 kg h^{-1}	413.5 b	431.7 b	417.8 b
Ridomil® - metalaxyl - 22.5 kg h^{-1}	115.8 c	115.1 c	115.5 c
Busan® 1020 - metam-sodium - 701 l $hectare^{-1}$	567.9 a	551.5 a	562.5 a
Busan® 1020 - metam-sodium - 561 l $hectare^{-1}$	493.6 ab	504.9 ab	499.1 ab
Busan® 1020 - metam-sodium - 608 l $hectare^{-1}$	491.1 ab	488.9 ab	478.3 ab
Aliette®/Rovral® - 34.5 g 100 m^{-1} of row	456.5 ab	439.3 a	439.3 b
Untreated Control	515.5 ab	486.3 ab	476.1 ab
Probability:	0.0104	0.0195	0.0168
% C.V.:	27.10	29.11	28.74
LSD:	147.4 $^{0.01}$	111.6 $^{0.05}$	109.1 $^{0.05}$

Plot size was 11.4375 m^2. Converted to a plot size of 12.5 m^2.
Metam-sodium treatments were broadcast applied in the fall of 1993.

Table 4d4. Comparison of Monthly Stand Count Data Collected During the Summer of 1994 at the English Farm, Olathe, CO., from Plots Treated with Various Fungicides to Determine Their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions .

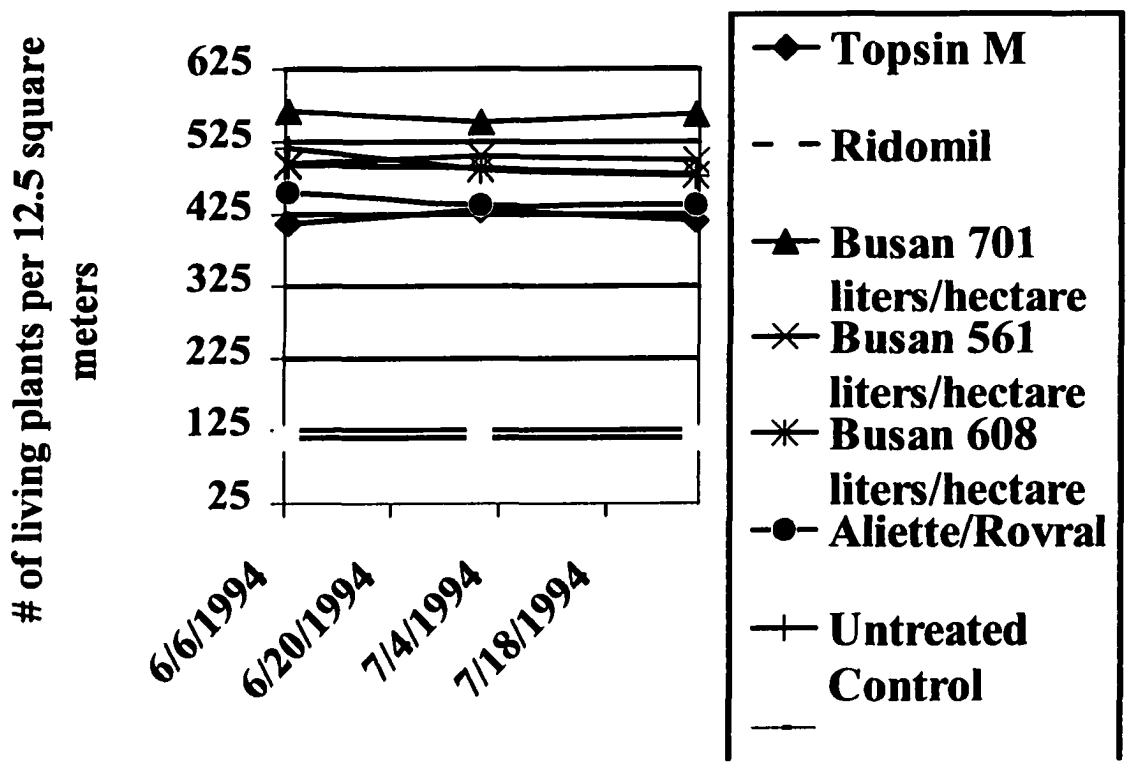


Table 4d5. Average of Stand Count Data Collected During the Summer of 1994 at the Hines Farm, Delta, and English Farm, Olathe, CO., from Plots Treated with Various Fungicides to Determine Their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions .

Treatments	# of Living Plants
Topsin® M - thiophanate methyl - 31.5 kg h^{-1}	560.4 bc
Ridomil® - metalaxyl - 22.5 kg h^{-1}	334.9 d
Busan® 1020 - metam-sodium - 701 l $hectare^{-1}$	607.5 a
Busan® 1020 - metam-sodium - 561 l $hectare^{-1}$	508.2 ab
Busan® 1020 - metam-sodium - 608 l $hectare^{-1}$	591.2 ab
Aliette®/Rovral® - fosetyl-aluminum/iprodione - 34.5 g 100 m^{-1} of row	570.7 abc
Untreated Control	538.9 c
Probability:	0.0042
% C.V.:	12.21
LSD:	$31.10^{0.01}$

Plot size was 11.4375 m^2. Converted to a plot size of 12.5 m^2.
Metam-sodium treatments were broadcast applied in the fall of 1993.

1993 and 1994 Harvest Studies; Hines and English farms

Materials and Methods

The purpose of these trials was in part to determine the rate of the soil fumigant sodium N-methyl dithiocarbamate (metam-sodium), and soil treatments of metalaxyl, thiophanate methyl, and the combination product fosetyl-aluminum/iprodione, that were most effective against Fusarium basal rot of onion, caused by *F. o. cepae*, as determined by stand count and yield.

Experimental units consisted of four 30.5 meter beds planted with 2 rows of onions per bed (30 cm between rows). Fields were planted by the cooperators with vacuum seeders. The Hines plots were 170 cm wide. The English plots were 150 cm wide. Plots were arranged in a completely randomized block design with three replicates per treatment. These fields had been planted to onions in a 3-4 year crop rotation over several decades. Fusarium basal rot was reported to be a problem in both fields.

Data were evaluated using MSTAT-C, Michigan State University's microcomputer statistical program.

Metam-sodium Fumigation Tests.

Two commercial onion fields in the Olathe, Colorado area were fumigated in October, 1992 with a band applicator provided by Buckman Laboratories. Fumigation was done under the guidance of a Buckman representative. The fields were plowed, beds shaped and the application rig used to inject 223.8, 280.7 and 327.4 liters of product (Busan® 1020) hectare^{-1} to a depth of 15.2 and 30.5 centimeters in a band under each seedling row. Beds were then packed by the grower to retain the fumigant in the soil.

In September 1993, two commercial onion fields in the Olathe, CO area, were fumigated with a broadcast applicator/rototiller unit used under the guidance of a Buckman representative. Five hundred and sixty one, 608 and 701 liters of product hectare^{-1} were applied and tilled into the top 8 inches of soil. The soil was bedded and packed to retain the fumigant.

Soil Treatments

Soil treatments consisted of thiophanate methyl (Topsin® M at 31.5 kg hectare^{-1}), metalaxyl (Ridomil® PC 11G at 22.5 kg h^{-1}), and the combination product fosetyl-aluminum/iprodione (Aliette®/Rovral® 15G at 34.5 g 100 m^{-1} of row). Due to a delay in

receiving the materials in 1993, materials were applied approximately 1 month after the Hines field was planted instead of at seeding time as preferred. The materials were applied at planting in 1994 as specified on the label.

Data Collection

Harvest data were collected on the number and weight of colossal, jumbo, medium and prepack size onions based on United States standards[92] Data were also collected on double, Fusarium-infected and cull onions.

Statistics were conducted on total onion data, as well as data on soil treatments (fosetyl-aluminum/iprodione, metalaxyl and thiophanate methyl) and the three metam-sodium rates.

Results and Discussion

1993 Harvest Study; Tables 4e1 - 4e5

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[92] United States Standards for Grades of Bermuda-granex-grano type onions. 1995. USDA, Agricultural Marketing Service, Fruit and Vegetable Division, Fresh Products Branch.

Hines Farm, Delta, Colorado; Tables 4e1 - 4e3

There were no significant differences at the Hines farm for total marketable yield, double, colossal, or jumbo onions when all treatments were compared. The metam-sodium applications produced the greatest weight of medium and prepack onions (P=0.05). There were no significant differences between the untreated control, fosetyl-aluminum/iprodione, metalaxyl, or thiophanate methyl treatments for medium or prepack onions. The greatest number of bulbs infected by *F. o. cepae* were at the two high metam-sodium rates (P=0.05); The untreated control had the lowest number of unmarketable and *Fusarium*-infected bulbs.

Comparisons of the soil treatments (thiophanate-methyl, metalaxyl, and fosetyl-aluminum/iprodione) and the untreated control showed no significant differences for any analysis conducted. Comparisons of the three metam-sodium rates with the untreated control gave no significant differences for total marketable, double, colossal, or jumbo onion bulbs. The two high rates of metam-sodium (327.4 and 280.7 liters hectare^{-1}) produced a significantly higher weight of medium and prepack (P=0.10) as well as number of *Fusarium*-infected and unmarketable (P=0.05) onion bulbs.

English Farm, Olathe, Colorado; Table 4e4

At the English farm, significant differences existed for total marketable, double, colossal, jumbo, and unmarketable onion bulbs. No significant differences were found for medium, prepack, or *Fusarium*-infected onion bulbs. The untreated control produced a greater volume of double onion bulbs than the three metam-sodium rates (P=0.10).

The untreated control produced a higher yield of total marketable, colossal, and jumbo onion bulbs than the metam-sodium treatments (P=0.10 and P=0.01, respectively). Fewer jumbo onions were produced at the highest rate of metam-sodium (P=0.01).

The number of total unmarketable onion bulbs was greatest (P=0.05) at the highest metam-sodium rate (327.4 l hectare^{-1}), followed by the medium and low rates of metam-sodium, and finally the untreated control. There was no difference in *Fusarium*-infected bulbs, therefore the difference in unmarketable onions was the result of distorted and other cull onions. The 327.4 liter hectare^{-1} rate of metam-sodium consequently produced more cull onions than the other rates of metam-sodium. This may have been a result of increased phytotoxicity due to more chemical residual remaining in the soil from the high metam-sodium rate.

The high rate of metam-sodium (327 l h^{-1}) had a significantly lower yield than the untreated control, low or medium rates of metam-sodium. This may have been due to a phytotoxic response to residual MIT.

Hines and English Data combined; Table 4e5

A comparison of data for metam-sodium from both farms provided the following::

1. No differences were found for weight of double, colossal, or medium onion bulbs.

2. The metam-sodium application of 280.7 liters $hectare^{-1}$ produced a greater overall weight of marketable onion bulbs (P=0.10) than the untreated control. The highest rate of metam-sodium (327.4 liters $hectare^{-1}$) produced the lowest weight of marketable onion bulbs. This can be explained by the increased number of unmarketable onion bulbs produced by the high metam-sodium rate. As mentioned previously, this may have been due to residual phytotoxicity of the high metam-sodium rate. This residual effect would be more likely in the English farm than at the Hines farm, as the English farm was planted earlier. No tests, however, were conducted to determine if toxic

residuals remained after treating. Future efficacy trials with metam-sodium should include a test for toxic residuals by either a bioassay or chemical analysis.

3. Weight of jumbo onion bulbs was significantly lower at the highest metam-sodium rate than the other treatments (P=0.05).

4. Weight of prepack onion bulbs was greatest for the metam-sodium treatments than the untreated control. The 280.7 liter hectare^{-1} application of metam-sodium produced the highest yield as compared to the untreated control (7.92 vs. 3.53 kg plot^{-1}, respectively). The 327.4 liter hectare^{-1} rate of metam-sodium produced a significantly lower yield of prepack onions than the 280.7 liter hectare rate.

5. Fewer *Fusarium*-infected bulbs were found in the untreated control than in the metam-sodium treatments. The high and mid-rates of metam-sodium had significantly more *Fusarium*-infected bulbs than the low metam-sodium rate or untreated control (P=0.05).

6. Fewer unmarketable onion bulbs (doubles, *Fusarium*-infected, and culls) occurred in the untreated control. The highest rate of metam-sodium had the highest number of unmarketable onion bulbs (P=0.01).

Table 4e1. 1993 Yield Data for the Hines farm, Delta, CO., from Plots Treated with Various Fungicides to Determine Their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Doubles - weight in kgs
Aliette®/Rovral® - fosetyl-aluminum/ iprodione - 34.5 g 100 m^{-1} of row	27.0 b	90.6 bc	3.4 c	8.5 c	31.1	4.1	47.1	1.8
Busan® 1020 - metam- sodium - 223.8 liters $hectare^{-1}$	25.4 b	115.7 a	5.2 b	14.1 b	32.6	1.9	53.8	1.3
Busan® 1020 - metam- sodium - 280.7 liters $hectare^{-1}$	48.9 a	198.3 a	7.6 a	18.4 a	40.3	2.5	68.7	2.3
Busan® 1020 - metam- sodium - 327.4 liter $hectare^{-1}$	49.2 a	214.4 a	7.3 a	17.2 ab	34.5	2.4	61.3	2.7

Ridomil® PC 11G - metalaxyl - 22.5 kg hectare	20.3 bc	59.7 c	2.3 c	6.2 c	21.2	3.1	32.8	1.4
Topsin® M - thiophanate methyl - 31.5 kg hectare	23.8 bc	65.9 bc	3.1 c	6.7 c	21.9	2.5	34.1	1.8
Untreated Control	14.5 c	47.8 c	2.6 c	5.1 c	22.8	4.5	35.0	0.6
Probability:	0.0132	0.0010	0.0173	0.0128	0.3422	0.7120	0.1243	0.3468
% C.V.:	37.63	35.65	42.22	41.43	38.89	70.88	35.75	63.44
LSD:	9.99 $^{0.05}$	50.34 $^{0.01}$	1.682 $^{0.05}$	4.010 $^{0.05}$	n.s.	n.s.	n.s.	n.s.

Table 4e2. A Comparison of the 1993 Yield Data for the Hines farm, Delta, CO., from Plots Treated with Metalaxyl, Fosetyl-aluminum/Iprodione and Thiophanate Methyl to Determine Their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Doubles - weight in kgs
Aliette®/Rovral® - fosetyl aluminum/iprodione - 34.5 g 100 m^{-1} of row	27.0	90.6	3.4	8.5	31.1	4.1	47.1	1.8
Ridomil® PC 11G - metalaxyl - 22.5 kg hectare^{-1}	20.3	59.8	2.3	6.2	21.2	3.1	32.8	1.3
Topsin® M - thiophanate methyl -31.5 kg hectare^{-1}	23.8	65.9	3.1	6.7	21.9	2.5	34.1	1.8
Untreated Control	14.5	47.6	2.6	5.1	22.8	4.5	35.0	0.6
Probability:	0.3521	0.2837	0.6425	0.4536	0.5051	0.6414	0.3559	0.3908

% C.V.:	37.71	37.45	41.22	36.45	35.38	57.17	26.93	66.48
LSD:	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 4e3. A Comparison of the 1993 Yield Data for the Hines farm, Delta, CO., from Plots Treated with Various Rates of Metam-sodium (Band-Applied) to Determine its Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Doubles - weight in kgs
Busan® 1020 - metam-sodium - 223.8 liters hectare^{-1}	25.4 b	115.7 b	5.2 b	14.1 a	32.6	1.9	53.8	1.3
Busan® 1020 - metam-sodium - 280.7 liters hectare^{-1}	48.9 a	198.3 a	7.6 a	18.4 a	40.3	2.5	68.7	2.3
Busan® 1020 - metam-sodium - 327.4 liter hectare^{-1}	49.2 a	214.4 a	7.3 a	17.4 a	34.5	2.4	61.3	2.7
Untreated Control	14.5 b	47.9 c	2.6 c	5.1 b	22.8	4.5	35.0	0.6
Probability:	0.0317	0.0274	0.593	0.0965	0.5143	0.6768	0.3262	0.2299
% C.V.:	35.90	36.91	34.01	41.45	41.92	97.69	38.48	70.43
LSD:	12.36 $^{0.05}$	53.15 $^{0.05}$	1.525 $^{0.10}$	4.50 $^{0.10}$	n.s.	n.s.	n.s.	n.s.

Table 4e4. A Comparison of the 1993 Yield Data for the English farm, Olathe, CO., from Plots Treated with Various Rates of Metam-sodium (Band-Applied) to Determine its Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Doubles - weight in kgs
Busan® 1020 - metam-sodium - 223.8 liters hectare	6.9	136.3 b	9.7	36.0	79.7 a	1.5 b	127.0 a	5.3 bc
Busan® 1020 - metam-sodium - 280.7 liters hectare	10.2	151.2 b	8.3	34.5	77.9 a	0.9 b	122.4 a	5.6 b
Busan® 1020 - metam-sodium - 327.4 liter hectare	3.3	216.8 a	6.5	20.0	40.88 b	1.2 b	68.9 b	3.1 c
Untreated Control	9.1	99.8 c	4.6	28.3	95.9 a	4.3 a	133.4 a	10.6 a
Probability:	0.37222	0.0154	0.1025	0.1078	0.0106	0.0644	0.0084	0.0907

% C.V.:	64.15	19.61	29.44	23.92	17.71	66.63	14.06	48.36
LSD:	n.s.	29.59 $^{0.05}$	n.s.	n.s.	19.73 $^{0.01}$	1.04 $^{0.10}$	24.03 $^{0.01}$	2.360 $^{0.10}$

Table 4e5. A Comparison of the Average of the 1993 Yield Data Collected from the Hines Farm, Delta, and English Farm, Olathe, CO., from Plots Treated with Various Rates of Metam-sodium (Band-Applied) to Determine its Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Doubles - weight in kgs
Busan® 1020 - metam-sodium - 223.8 liters hectare^{-1}	16.2 b	126.1 c	7.5 ab	25.3	56.17 a	1.7	90.6 ab	3.3
Busan® 1020 - metam-sodium - 280.7 liters hectare^{-1}	29.5 a	174.8 b	8.0 a	26.6	59.1 a	1.7	95.4 a	3.9
Busan® 1020 - metam sodium - 327.4 liter hectare^{-1}	26.2 a	215.6a	6.9 b	18.8	37.7 b	1.8	65.2 c	2.9
Untreated Control	11.8 c	73.9 d	3.6 c	16.9	59.4 a	4.4	84.2 b	5.6
Probability:	0.0116	0.0096	0.0733	0.1340	0.0187	0.1152	0.0187	0.2285
% C.V.	22.75	22.82	26.80	22.75	12.34	55.36	12.34	38.69
LSD:	13.07 $^{0.05}$	36.04 $^{0.01}$	0.9734 $^{0.10}$	n.s.	4.628 $^{0.05}$	n.s.	6.942 $^{0.10}$	n.s.

1994 Harvest Studies; Tables 4f1 - 4f9

Hines Farm, Delta, Colorado; Tables 4f1 - 4f3

There were no significant differences in weight of total marketable , colossal, jumbo, medium, or prepack onion bulbs when all treatments were analyzed.

The thiophanate methyl, fosetyl-aluminum/iprodione and two metam-sodium treatments (701 and 561 liters hectare^{-1}) had a significantly greater (P=0.10) numbers of bulbs infected by *F. o. cepae* than the untreated control, metalaxyl or 561 l h^{-1} rate of metam-sodium. When the thiophanate methyl, fosetyl-aluminum/iprodione and metalaxyl treatments were compared with the untreated control, the order of significance (P=0.05) was as follows: thiophanate methyl > fosetyl-aluminum/iprodione > untreated control /metalaxyl.

The weight of double onions harvested (in kgs) was greatest (P=0.10) for the two high treatment rates of metam-sodium (701 and 608 liters hectare^{-1}); the other treatments were statistically equal to the untreated control.

When all treatments were compared, the two high rates of metam-sodium (701 and 608 liters hectare^{-1}) produced the greatest number of unmarketable onions. The number of unmarketable onions was the same for the lowest rate of metam-sodium (561 liters

hectare^{-1}), fosetyl-Al/iprodione, thiophanate methyl, metalaxyl and the untreated control (P=0.10). No significant differences in any of the analyses were noted when the three rates of metam-sodium were compared with the untreated control.

When the soil treatments, fosetyl-aluminum/iprodione, thiophanate methyl, and metalaxyl, were compared with the untreated control, significant differences were found with the jumbo, prepack and number of *Fusarium*-infected measurements. The fosetyl-aluminum/iprodione and thiophanate methyl treatments produced a greater weight of jumbo onion bulbs than the untreated control (P=0.10). Fosetyl-aluminum/iprodione and thiophanate methyl had a significantly higher weight of prepack onion bulbs (kgs) than the untreated control and metalaxyl. The metalaxyl treatment had a significantly lower weight of jumbo onions than other treatments.

English Farm, Olathe, Colorado; Tables 4f4 - 4f6

There were no significant differences in weight of total marketable onions harvested (kgs) for any of the treatments with the exception of metalaxyl. When all treatments were compared, the weight of marketable onions for the metalaxyl treatment was significantly lower (P=0.01) than the other treatments; metalaxyl had a significantly lower total marketable weight (P=0.05) when compared with fosetyl-aluminum/iprodione, thiophanate and the untreated control. There were no significant differences in total marketable weight for the three metam-sodium rates and untreated

control.

The untreated control and medium rate of metam-sodium (608 liter hectare^{-1}) had a significantly higher yield of colossal onions than the other treatments (P=0.05). There was no significant difference, however, between the medium and high rate (701 liters hectare^{-1}) of metam-sodium . Of the three metam-sodium treatments, the two higher rates produced a significantly greater weight of colossal onion bulbs than the low rate (561 liters hectare^{-1}). The metalaxyl treatment had a significantly lower yield of total marketable, colossal, and jumbo onion bulbs than other treatments.

With jumbo onions, the highest level of metam-sodium (701 l hectare^{-1}) produced the highest weight. There was no significant difference in weight of jumbo onions for thiophanate methyl, fosetyl-aluminum/iprodione, or metalaxyl.

The weight of double onions (one bulb with a split growing point at the base) was significantly greater for the two high metam-sodium treatments (701 and 608 l hectare^{-1}). The weight of doubles in the lowest metam-sodium rate (561 l hectare^{-1}) was equal to fosetyl-aluminum/iprodione, thiophanate methyl, metalaxyl and the untreated control.

The number of *Fusarium*-infected bulbs was significantly greater in the untreated plots. The three metam-sodium treatments were statistically equal. The thiophanate-methyl and metalaxyl plots had the fewest *Fusarium*-infected bulbs.

The greatest number of unmarketable onions occurred in fosetyl-aluminum/iprodione, untreated control and the two higher rate metam-sodium plots. The metalaxyl treatment had the lowest weight (P=0.05) of unmarketable onions.

Hines and English Data Combined; Tables 4f7 - 4f9

To increase the accuracy of probability, data from both farms were combined. The following results were found:

1. Double onions (weight in kgs) were more prevalent at the mid and high metam-sodium rates. The other treatments produced the same weight of doubles as the untreated control. The high incidence of doubles in the high and medium metam-sodium treatments may have been due to the residual phytotoxicity of the material. It would also appear that fosetyl-aluminum/iprodione, metalaxyl, metam-sodium (561 liters hectare^{-1}), and thiophanate methyl treatments were not phytotoxic at the treatment rates used. These treatments were statistically equal to the untreated control in regard to double onions produced.

Analyses to determine if the increased total weight of double onions in the metam-sodium plots was the result of an elevated bulb weight in those treatments was carried out. Total weight was divided by the number of bulbs. No significant differences were found with any of the computations conducted.

2. The broad-cast application rate of 701 l h-1 of metam-sodium resulted in a significantly higher (P=0.01) yield than the untreated control, fosetyl-Al/iprodione, thiophanate methyl, or metalaxyl treatments. The three metam-sodium rates were statistically equal in total marketable yield. The total marketable weight of onions for the metalaxyl treatment was significantly less than the other treatments.

3. The untreated control produced the greatest weight of colossal onion bulbs, followed by the three rates of metam-sodium. The fosetyl-aluminum/iprodione and thiophanate methyl treatments produced significantly less weight in colossal onions. The weight of colossal onions in the metalaxyl plots was significantly lower than any of the other treatments (P=0.05).

An analysis of the weight of colossal onions for each treatment (total weight/number of bulbs) indicated a significantly greater weight per bulb for the metalaxyl plots at the English farm, but no significant difference when data from the Hines and English farms were combined.

4. The analysis of treatments for jumbo onions revealed the metam-sodium rate of 701 liters hectare^{-1} resulted in a significantly greater yield (P=0.05) than the fosetyl-aluminum/iprodione, thiophanate methyl, metalaxyl, metam-sodium at 608 liters hectare^{-1}, or untreated plots.

5. The greatest number of *Fusarium*-infected onions was found in the untreated control plots. The number in the untreated control plots and fosetyl-aluminum/iprodione plots were not statistically different (P=0.01). The other treatments, however, had significantly fewer infected onions. Metalaxyl had the lowest number of infected onion bulbs per plot.

6. The number of unmarketable onions was evaluated. The two high application rates of metam-sodium as well as the fosetyl-aluminum/iprodione treatment had significantly more unmarketable onions than the other treatments (P=0.01). The metalaxyl plots had significantly fewer unmarketable onions.

7. There were no significant differences in medium or prepack onions for any of the treatments.

Summary

Based on the data resulting from this set of trials, the following observations can be made:

1. The use of thiophanate methyl, fosetyl-aluminum/iprodione, and metalaxyl as soil treatments are ineffective in controlling *Fusarium* basal rot of onions.

2. The broadcast application of metam-sodium at 701 liters hectare^{-1} as a

broadcast application is effective in increasing total onion bulb yield in fields subjected to *F. o. cepae* pressure.

3. Application rates of metam-sodium of 608 and 701 liters hectare^{-1}, increase the number of double and unmarketable onions. The weight of individual bulbs of double onions was statistically the same for all treatments tested in this set of trials. A physiological change may have occurred due to phytotoxic effects of the fumigant stimulating the development of double onions.

4. Broadcast applications of metam-sodium at 701, 608 and 561 liters hectare^{-1}, as well as in-furrow applications of thiophanate methyl, metalaxyl, and fosetyl-aluminum/iprodione, do not increase the weight of colossal onions harvested.

5. The weight of individual double and colossal onion bulbs is fairly consistent regardless of the soil or fumigant treatment used.

6. A broadcast application of metam-sodium at 701 liters hectare^{-1} produces a significantly greater weight of jumbo onions than lower application rates of metam-sodium, thiophanate methyl, metalaxyl, fosetyl-aluminum/iprodione, or untreated control plots.

7. Broadcast applications of metam-sodium at 701, 608 and 561 liters hectare^{-1},

or soil applications of thiophanate methyl, metalaxyl, or fosetyl-aluminum/iprodione, are not effective in increasing medium or prepack onion bulbs in soil infested with *F. o. cepae*.

8. By the end of the growing season, fosetyl-aluminum/iprodione is ineffective in preventing *Fusarium* basal rot.

9. The use of metalaxyl in onion fields is responsible for a significant loss in total marketable onion yield and colossal size onions. Stand count data from the Hines and English fields confirm a low initial germination due to in-furrow applications of metalaxyl.

10. The metalaxyl plots had fewer *Fusarium*-infected onions at harvest than other treatment plots. This was likely due to the reduced number of bulbs available for infection and the infection/disintegration of injured and stressed bulbs earlier in the growing season.

Table 4f1. A Comparison of the 1994 Yield Data for the Hines Farm, Delta, CO., from Plots Treated with Various Fungicides to Determine their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Doubles - weight in kgs
Aliette®/Rovral® - fosetyl-aluminum/ iprodione - 34.5 g 100 m^{-1} row	10.9 a	26.0 bc	2.6	34.9	112.2	10.7	160.3	1.7 b
Busan® 1020 - metam-sodium - 561 liters hectare^{-1}	11.3 a	23.1 cd	2.2	28.0	116.7	14.8	161.7	1.3 bc
Busan® 1020 - metam-sodium - 608 liters hectare^{-1}	7.1 b	28.9 ab	2.5	35.8	104.2	10.2	152.7	2.9 a
Busan® 1020 - metam-sodium - 701 liter hectare^{-1}	12.2 a	31.8 a	2.5	32.1	107.8	15.9	158.4	2.4 a

Ridomil® PC 11G - metalaxyl - 22.5 kg hectare^{-1}	7.1 b	20.3 d	1.4	20.9	88.5	24.6	135.3	1.3 bc
Topsin® M - thiophanate-methyl - 31.5 kg hectare^{-1}	12.9 a	21.5 d	2.4	29.9	110.3	9.4	152.1	0.9 c
Untreated Control	8.0 b	19.6 d	2.1	29.0	99.5	15.5	146.0	1.3 bc
Probability:	0.0787	0.0790	0.8257	0.4354	0.1745	0.4478	0.1367	0.0906
% C.V.:	26.93	20.48	44.57	27.95	11.35	61.41	7.36	47.19
LSD:	1.944 $^{0.10}$	3.648 $^{0.10}$	n.s.	n.s.	n.s.	n.s.	n.s.	0.5780 $^{0.10}$

Table 4f2. A Comparison of the 1994 Yield Data for the Hines Farm, Delta, CO., from Plots Treated with the Fungicides Metalaxyl, Fosetyl-aluminum/Iprodione and Thiophanate Methyl to Determine their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Doubles - weight in kgs
Aliette®/Rovral® - fosetyl-aluminum/ iprodione - 34.5 g 100 m^{-1} row	10.9 b	25.6	2.6 a	34.8	112.2 a	10.7	160.3	1.7
Ridomil® PC 11G - metalaxyl - 22.5 kg $hectare^{-1}$	7.1 c	29.9	1.4 c	20.9	88.5 c	24.6	135.3	1.3
Topsin® M - thiophanate-methyl - 31.5 kg $hectare^{-1}$	12.9 a	21.1	2.4 a	29.9	110.3 a	9.4	152.1	0.9
Untreated Control	8.0 c	19.2	2.1 b	29.0	99.5 b	15.5	146.0	1.3

Probability:	0.0110	0.1934	0.0525	0.2925	0.0873	0.3391	0.1922	0.5124
% C.V.:	15.44	15.62	19.09	27.90	9.83	67.50	8.30	48.86
LSD:	1.499 $^{0.05}$	n.s.	0.3213 $^{0.10}$	n.s.	8.004 $^{0.10}$	n.s.	n.s.	n.s.

Table 4f3. A Comparison of the 1994 Yield Data for the Hines Farm, Delta, CO., from Plots Treated with various rates of Metam-sodium to Determine its Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Doubles - weight in kgs
Busan® 1020 - metam-sodium - 561 liters hectare^{-1}	11.3	23.1	2.2	28.0	116.7	14.8	161.7	1.3
Busan® 1020 - metam-sodium - 608 liters hectare^{-1}	7.1	28.9	2.5	35.8	104.2	10.2	150.4	2.9
Busan® 1020 - metam-sodium - 701 liter hectare^{-1}	12.2	31.8	2.5	32.1	107.8	15.9	152.8	2.4
Untreated Control	8.1	19.6	2.1	29.0	99.5	15.5	146.0	1.3
Probability:	0.1630	0.1987	0.9740	0.4297	0.5143	0.6115	0.4664	0.1750
% C.V.:	28.43	25.37	55.64	19.03	12.77	40.39	7.80	46.04
LSD:	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 4f4. A Comparison of the 1994 Yield Data for the English Farm, Olathe, CO., from Plots Treated with Various Fungicides to Determine their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number of Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Doubles - weight in kgs
Aliette®/Rovral® - fosetyl-aluminum/ iprodione - 34.5 g 100 m^{-1} row	12.7 b	22.7 a	0.46	3.9	97.6 b	98.4 c	218.9 b	1.8 b
Busan® 1020 - metam-sodium - 561 liters hectare^{-1}	9.1 cd	14.2 c	0.47	5.3	118.1 ab	109.7 c	2355.2 ab	0.6 b
Busan® 1020 - metam-sodium - 608 liters hectare^{-1}	11.3 bc	20.4 ab	0.37	4.6	97.03 b	128.7 ab	252.2 ab	4.3 a
Busan® 1020 - metam-sodium - 701 liter hectare^{-1}	8.7 cd	18.9 abc	0.43	5.4	144.9 a	111.4 bc	286.6 a	3.8 a

Ridomil® PC 11G - metalaxyl - 22.5 kg hectare^{-1}	3.2 e	4.7 d	0.41	1.8	12.8 d	57.0 d	78.7 c	0.8 b
Topsin® M - thiophanate-methyl - 31.5 kg hectare^{-1}	5.8 de	16.4 bc	0.80	4.8	89.4 bc	95.4 c	208.0 b	1.0 b
Untreated Control	17.5 a	22.2 a	0.45	3.8	66.0 c	130.5 a	252.1 ab	1.6 b
Probability:	0.0008	0.0296	0.9502	0.7435	0.0676	0.0147	0.0032	0.0375
% C.V.:	27.85	33.73	100.56	67.47	45.51	19.81	20.99	70.67
LSD:	3.339 $^{0.01}$	5.137 $^{0.05}$	n.s.	n.s.	31.02 $^{0.10}$	18.41 $^{0.05}$	58.03 $^{0.01}$	1.249 $^{0.05}$

Table 4f5. A Comparison of the 1994 Yield Data for the English Farm, Olathe, CO., from Plots Treated with the Fungicides Metalaxyl, Fosetyl-aluminum/Iprodione and Thiophanate Methyl to Determine their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Doubles - weight in kgs
Aliette®/Rovral® - fosetyl-aluminum/ iprodione - 34.5 g 100 m^{-1} row	12.8 b	23.0 a	0.46	3.9	89.4	98.4 b	218.9 a	1.8
Ridomil® PC 11G - metalaxyl - 22.5 kg $hectare^{-1}$	3.3 d	4.7 c	0.40	1.8	12.8	56.9 c	78.68 b	0.8
Topsin® M - thiophanate-methyl - 31.5 kg $hectare^{-1}$	5.8 c	16.4 b	0.79	4.8	88.4	95.4 b	208.0 a	1.0
Untreated Control	17.5 a	22.2 ab	0.45	3.8	95.9	130.5 a	252.1 a	1.6
Probability:	0.000	0.0458	0.8721	0.7679	0.2206	0.0042	0.0362	0.6722
% C.V.:	12.28	39.48	121.63	99.0	68.34	14.69	29.52	83.07
LSD:	$1.829^{0.01}$	$6.538^{0.05}$	n.s.	n.s.	n.s.	$21.19^{0.01}$	$55.86^{0.05}$	n.s.

Table 4f6. A Comparison of the 1994 Yield Data for the English Farm, Olathe, CO., from Plots Treated with Various Rates of Metam-sodium to Determine its Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Doubles - weight in kgs
Busan® 1020 - metam-sodium - 561 liters hectare^{-1}	9.1 b	14.2	0.47	5.3	118.1	109.7	255.6	0.6 b
Busan® 1020 - metam-sodium - 608 liters hectare^{-1}	11.3 b	20.4	0.36	4.6	97.0	128.7	252.2	4.3 a
Busan® 1020 - metam-sodium - 701 liter hectare^{-1}	8.7 b	18.9	0.43	5.4	144.9	111.4	286.6	3.8 a
Untreated Control	17.5 a	22.2	0.45	3.8	95.9	130.5	252.1	1.6 b
Probability:	0.0546	0.2972	0.9582	0.8020	0.3473	0.6864	0.5420	0.0971
% C.V.:	28.17	25.24	57.81	48.09	30.23	22.19	12.50	64.37
LSD:	2.605 $^{0.10}$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1.316 $^{0.10}$

Table 4f7. A Comparison of the Average 1994 Yield Data collected from the Hines Farm, Delta, and English Farm, Olathe, CO., from Plots Treated with Various Fungicides to Determine their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Number Double Onion Bulbs
Aliette®/Rovral® (34.5 g 100 m^{-1} row)	11.8 ab	24.5 a	1.5	19.4	104.9 bc	54.6 d	180.3 bc	1.7 b
Busan® 1020 (561 liters $hectare^{-1}$)	10.2 c	18.7 b	1.4	16.6	117.4 ab	62.2 c	197.6 ab	1.0 c
Busan® 1020 (608 liters $hectare^{-1}$)	9.2 c	24.8 a	1.9	20.2	100.6 c	69.5 ab	191.7 ab	3.6 a
Busan® 1020 (701 liter $hectare^{-1}$)	10.5 bc	25.4 a	1.4	18.8	126.4 a	63.7 bc	210.6 a	3.1 a
Ridomil® PC 11G (22.5 kg $hectare^{-1}$)	5.2 d	12.5 c	0.9	11.3	50.6 d	40.8 e	103.7 d	1.1 c

Topsin® M (31.5 kg hectare^{-1})	9.3 c	19.0 b	1.6	17.3	99.9 c	52.4 d	171.2 c	0.9 c
Untreated Control	12.8 a	20.9 b	1.3	16.4	97.7 c	73.0 a	188.3 bc	1.5 bc
Probability:	0.0083	0.0038	0.7973	0.4339	0.0460	0.0484	0.0018	0.0157
% C.V.:	19.02	15.41	40.22	28.74	23.80	18.49	12.54	49.31
LSD:	1.652 $^{0.01}$	2.827 $^{0.10}$	n.s.	n.s.	14.62 $^{0.05}$	6.813 $^{0.05}$	19.64 $^{0.01}$	0.5689 $_{0.05}$

Table 4f8. Summary Table for the 1994 Yield Averaged from Data Collected at the Hines Farm, Delta, and English Farm, Olathe, CO., from Plots Treated with the Fungicides Metalaxyl, Fosetyl-aluminum/Iprodione and Thiophanate Methyl to Determine their Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Doubles - weight in kgs
Aliette®/Rovral® - fosetyl-aluminum/ iprodione - 34.5 g 100 m^{-1} row	11.8 a	24.5 a	1.5	19.4	104.9	54.6 b	180.3 a	1.7
Ridomil® PC 11G - metalaxyl - 22.5 kg $hectare^{-1}$	5.2 c	12.5 c	0.9	11.3	50.6	40.8 c	103.7 b	1.1
Topsin® M - thiophanate-methyl - 31.5 kg $hectare^{-1}$	9.3 b	19.0 b	1.6	17.3	99.9	52.4 b	171.2 a	0.9
Untreated Control	12.8 a	20.9 b	1.3	16.4	97.7	73.0 a	188.3 a	1.4

Probability:	0.0004	0.0351	0.2468	0.3525	0.1612	0.0498	0.0361	0.4785
% C.V.:	10.53	19.11	29.94	31.96	31.67	19.17	17.69	49.17
LSD:	1.102 $^{0.01}$	2.592 $^{0.05}$	n.s.	n.s.	n.s.	7.475 $^{0.05}$	20.11 $^{0.10}$	n.s.

Table 4f9. A Comparison of the 1994 Yield Averaged from Data Collected at the Hines Farm, Delta, and English Farm, Olathe, CO., from Plots Treated with Various Rates of Metam-sodium to Determine its Efficacy Against *Fusarium oxysporum* f. sp. *cepae*, the Causal Agent of Fusarium Basal Rot of Onions. Based on a Plot Size of 12.5 Meter^2.

Treatment	Number *Fusarium* -infected bulbs	Total Number Unmarketable bulbs	Prepack - weight in kgs	Medium - weight in kgs	Jumbo - weight in kgs	Colossal - weight in kgs	Total Marketable - weight in kgs	Doubles - weight in kgs
Busan® 1020 - metam-sodium - 561 liters hectare^{-1}	10.2	18.7 b	1.4	16.6	117.4	62.2	197.6	1.0 b
Busan® 1020 - metam-sodium - 608 liters hectare^{-1}	9.2	24.7 a	1.4	20.2	100.6	69.5	191.7	3.6 a
Busan® 1020 - metam-sodium - 701 liter hectare^{-1}	10.5	25.40 a	1.4	18.8	126.4	63.7	210.3	3.1 a
Untreated Control	12.8	20.9	1.3	16.4	97.7	73.0	188.3	1.4 b
Probability:	0.3988	0.0901	0.9839	0.5614	0.3125	0.7590	0.2636	0.0698
% C.V.:	22.82	13.11	47.77	20.42	17.64	20.59	6.49	48.08
LSD:	n.s.	1.648 $^{0.10}$	n.s.	n.s.	n.s.	n.s.	n.s.	0.6140 $^{0.10}$

References

Abawi, G.S. 1965. Cultural variability and suscept (Onion)-pathogen relationship of *Fusarium oxysporum* f. sp. *cepae*. M.S. Thesis, Cornell Univ., Ithaca, N.Y. 80 pages.

Abawi, G.S. 1971. The Ecology of *Fusarium oxysporum* f. sp. *cepae* in organic soil and its pathological reaction on selected onion varieties. Ph.D. Thesis, Cornell Univ., Ithaca, N.Y. 165 pages.

Abawi, G.S., and Cobb, A.C. 1986. Evaluation of Ridomil as seed and soil treatments for the control of beet root rot. Fungicide and Nematicide Tests 41:126-127.

Abawi, G.S., and Crossier, D.C. 1986. Field evaluation of selected fungicides for control of bean root rot, 1985. Fungicide and Nematicide Tests 41:126.

Abawi, G.S., and Crosier, D.C. 1988. Field Testing of fungicides as seed and soil treatments for the control of bean root rots, 1987. Fungicide and Nematicide Tests 43:104.

Abawi, G.S., and Crosier, D.C. 1989. Effect of an in-furrow application of fungicides on bean root rot incidence and severity, 1988. Fungicide and Nematicide Tests 44:96.

Abawi, G.S., and Lorbeer, J.W. 1965. Cultural variability and pathogenicity of *Fusarium oxysporum* f. sp. *cepae*. Phytopathology 55:1051 (Abstr.)

Abawi, G.S., and Lorbeer, J.W.. 1971a. Pathological histology of four onion cultivars infected by *Fusarium oxysporum* f. sp. *cepae*. Phytopathology 61(10):1164-1169.

Abawi, G.S., and Lorbeer, J.W. 1971b. Populations of *Fusarium oxysporum* f. sp. *cepae* in organic soils in New York. Phytopathology 61(9):1042-1048.

Abawi, G.S., and Lorbeer, J.W. 1971c. Reaction of selected onion varieties to infection by *Fusarium oxysporum* f. sp. *cepae*. Plant Dis. Reptr. 55 (11):1000-1004.

Abawi, G.S., and Lorbeer, J.W. 1972. Several aspects of the ecology and pathology of *Fusarium oxysporum* f. sp. *cepae*. Phytopathology 62(8):870-876.

Adams, M.J., and Welham, S.J. 1995. Use of the most probable number technique to quantify soil-borne plant pathogens. Annals of Applied Biology 126(1):181-196.

Aganostopoulus, P.T. 1932. Some Diseases of Fruit Trees, Vegetables and Flowers Caused by *Fusarium* sp. in Greece. A.B. Pasco, Athens, Greece.

Agrios, G.N. 1997. Plant Pathology: Fourth Edition. Academic Press Inc., San Diego, CA. 656 pages.

Ahn, I.P., Chung, H.S., and Lee, Y.-H. 1998. Vegetative compatibility groups and pathogenicity among isolates of *Fusarium oxysporum* f. sp. *cucumerinum*. Plant Dis. 82:244-246.

Ainsworth, G.C. 1973. Introduction and keys to higher taxa. Pp. 1-7, in: The Fungi: An Advanced Treatise, Vol. IVB. Ainsworth, G.C., Sparrow, F.K., and Sussman A.S., eds. Academic Press. New York

Alexander, J.V., Bourret, J.A., Gold, A.H., and Snyder, W.C. 1966. Induction of chlamydospore formation by *Fusarium solani* in sterile soil extracts. Phytopathology 56: 353-354.

Alexander, M. 1982. Most probable number method for microbial populations. Pp. 815-820, in: Methods of soil analysis, Part 2. Chemical and microbiological properties - Agronomy Monograph no. 9 (2nd Edition). ASA-SSA. Madison, WI.

Alexopoulos, C.J., Mims, C.W., Blackwell, M. 1996. Introductory Mycology, 4th edition. John Wiley and Sons Inc., New York, NY. 868 pages.

Aloi, C., and Baayen, R.P. 1993. Examination of the relationships between vegetative compatibility groups and races in *Fusarium oxysporum* f. sp. *dianthi*. Plant Pathology 42:839-850.

Alves-Santos, F.M., Benito, E.P., Eslava, A.P., and Diaz-Minguez, J.M. 1999. Genetic diversity of *Fusarium oxysporum* strains from common bean fields in Spain. Appl. Environ. Micro. 65(8):3335-3340.

An, Z.Q., Hendrix, J.W., Hershman, D.E., and Henson, G.T. 1990. Evaluation of the "most probable number" (MPN) and wet-sieving methods for determining soil-borne populations of endogonaceous mycorrhizal fungi. Mycologia 82(5):576-581.

Anagnostakis, S.L. 1977. Vegetative incompatibility in *Endothia parasitica*. Exp. Mycology 1:306-316.

Anderson, J.P.E. 1993. Side-effects of pesticides on carbon and nitrogen transformations in soils. Pp. 61-67, in: Proc. Int. Symp. on Environmental Aspects of Pesticide Microbiol. Sigluna, Sweden. 17-21 Aug 1992. Dept. of Microbiology. Swedish Univ. of Agr. Sci.,

Uppsala, Sweden.

Anderson, T.R., and Buzzell, R.I. 1982. Efficacy of metalaxyl in controlling *Phytophthora* root rot and stalk rot of soybean cultivars differing in field tolerance. Plant Dis. 66:1144-1145.

Anonymous. 1958. Annual report of the Department of Agriculture, Tanganyika. 1957 (part II).

Appel, D.J., and Gordon, T.R. 1994. Local and regional variation in populations of *Fusarium oxysporum* from agricultural field soils. Phytopathology 84:486-491.

Appel, D.J., and Gordon, T.R. 1995. Intraspecific variation within populations of *Fusarium oxysporum* based on RFLP analysis of the intergenic spacer region of the rDNA. Exp. Mycol. 19:120-128.

Appel, O., and Wollenweber, H.W. 1910. Gruhdlagen einer Monographie der Gattung *Fusarium* (Link). Arb. Kais. Biol. Inst. Land. Forstwirtch. 8(1):1-207.

Arbelaez, G., and Calderon, O.L. 1992. Determination of the physiological races of *Fusarium oxysporum* f. sp. *dianthi* on carnation in Colombia. Acta Hort. 307:43-49.

Armstrong, G.M., and Armstrong, J.K. 1948. Nonsusceptible hosts as carriers of wilt fusaria. Phytopathology 38:808-826.

Armstrong, G.M., and Armstrong, J.K. 1966. *Fusarium oxysporum* f. *cassiae* form. nov., causal agent of wilt of *Cassia tora* and other plants. Phytopathology 56:699-701.

Armstrong, G.M., and Armstrong, J.K. 1975. Reflections on the wilt Fusaria. Ann. Rev. Phytopathol. 13:95-103.

Armstrong, G.M., and Armstrong, J.K. 1981. Formae speciales and races of *Fusarium oxysporum* causing wilt diseases. Pp. 391-399, in: Fusarium: Disease, Biology, and Taxonomy. Nelson, P.E., Toussoun, T.A., and Cook, R.J., eds. Pennsylvania State Univ. Press, University Park, PA.

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. Pp.107-108, in: California Celery Research Program 1981-1982 Annual Report.

Awuah, R.T., and Lorbeer, J.W. 1986. A sorbose-based selective medium for enumerating propagules of *Fusarium oxysporum* f. sp. *apii* race 2 in organic soil. Phytopathology 76:1202-1205.

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 Disease 70:1154-1158.

Awuah, R.T., and Lorbeer, J.W. 1988. Nature of cultural variability in *Fusarium oxysporum* f. sp. *apii* race 2. Phytopathology 78:385-389.

Awuah, R.T., and Lorbeer, J.W. 1989. Role of light, temperature, and method of propagation in cultural variability of *Fusarium oxysporum* f. sp. *apii* race 2. Mycologia 81(2):278-283.

Baayen, R.P., van Dreven, R., Krijger, M.C., and Waalwijk, C. 1997. Genetic diversity in *Fusarium oxysporum* f. sp. *dianthi* and *Fusarium redolens* f. sp. *dianthi*. Euro. J. Plant Path. 103:395-408.

Baayen, R.P., Förch, M.G., Waalwijk, C., Bonants, P.J.M., Löffler, H.J.M., and Roebroeck, E.J.A. 1998. Pathogenic, genetic and molecular characterisation of *Fusarium oxysporum* f. sp. *lilii*. Euro. J. Plant Path. 104:887-894.

Baayen, R.P., and Kleijn, J. 1989. The Elegans fusaria causing wilt disease of carnation. II: Distinction of vegetative compatibility groups. Neth. J. Plant Path. 95:185-194.

Bacher, J.W., Pan, S., and Ewart, L. 1989. Inheritance of resistance to *Fusarium oxysporum* f. sp. *cepae* in cultivated onions. Pp. 85-91, in:1998 National Onion (and other Allium) Research Conference. Voss, R.E., ed. Sacramento, California.

Backman, P.A. 1978. Fungicide formulation: relationship to biological activity. Ann. Rev. Phytopath. 16:211-237.

Bailey, A.M., and Coffey, M.D. 1985. Biodegradation of metalaxyl in avocado soils. Phytopathology 75:135-137.

Bailey, A.M., and Coffey, M.D. 1986. Characterization of microorganisms involved in accelerated biodegradation of metalaxyl and metolachlor in soils. Can. J. Micro. 32(7):562-569.

Baker, K.F. 1981. Biological control. Pp. 523-561, in: Fungal Wilt Diseases of Plants. Marshall, E.M., Bell, A.A., and Beckman, C.H., eds. Academic Press Inc., New York, NY.

Baker, R. 1969. Fungal populations and incidence of disease. Pp. 8-11, in: Nature of the Influence of Crop Residues on Fungus-induced Root Disease. Cook, R.J., and Watson, R.D., eds. Washington Agr. Exp. Sta. Bull. 716.

Baker, R. 1970. Use of population studies in research on plant pathogens in soil. Pp. 11-15, in: Root Diseases and Soil-borne Pathogens. Toussoun, T.A., Bega, R.V., and Nelson, P.E., eds. Univ. of California Press, Berkeley, CA.

Baker, R. 1981. Ecology of the fungus, *Fusarium*: competition. Pp. 245-249, in: Fusarium: Disease, Biology and Taxonomy. Nelson, P.E., Toussoun, T.A., and Cook, R.J., eds. Pennsylvania State Univ. Press, University Park, PA.

Baker, R., and Scher, F.M. 1987. Enhancing the activity of biological control agents. Pp. 1-17, in: Innovative Approaches to Plant Disease Control. Chet, I., ed. John Wiley and Sons, Inc., New York, NY.

Baker, R., Maurer, C.L., and Maurer, R.A. 1967. Ecology of plant pathogens in soil. VII. Mathematical models and inoculum density. Phytopathology 57:622-666.

Bald, J.G. 1970. Measurement of host reactions to soil-borne pathogens. Pp. 37-41, in: Root Diseases and Soil-borne Pathogens. Toussoun, T.A., Bega, R.V., and Nelson, P.E., eds. Univ. of California Press, Berkley, CA.

Banihashemi, Z., and de Zeeuw, D.J. 1973. The effect of soil temperature on survival of *Fusarium oxysporum* f. *melonis* (Leach and Currence) Snyder and Hansen. Plant Soil 38:465-468.

Barak, E., Edgington, L.V., and Ripley, B.D. 1984. Bioactivity of the fungicide metalaxyl in potato tubers against some species of *Phytophthora, Fusarium* and *Alternaria*, related to polyphenoloxidase activity. Can. J. Plant Path. 6:304-308.

Barnett, H.L., and Hunter, B.B. 1998. Illustrated Genera of Imperfect Fungi: Fourth Edition. APS Press, St. Paul, MN. 241 pages.

Beach, B.G.W., Chalandon, A., Gallinelli, G., and Horriere, D. 1979. The control of various Phytophthora diseases in tropical crops with aluminum tris ethyl phosphonate. Proc. 1979 Br. Crop Prot. Conf. - Pests Dis. 2:319-329. British Crop Prot. Council Pub., Croydon, England.

Bell, A.A., and Mace, M.E. 1981. Biochemistry and physiology of resistance. Pp. 431-486, in: Fungal Wilt Diseases of Plants. Marshall, E.M., Bell, A.A., and Beckman, C.H. eds. Academic Press Inc., New York, NY.

Bendahmane, B., Barrault, G., Albertini, L., and Toubia-Rahme, H. 1992. An in vitro study of the activity of several fungicides on the development of *Drechslera teres* f. *teres* and *F. maculata*. Phytopath. Medit. 31(2):77-84.

Bentley, S., Pegg, K.G., and Dale, J.L. 1995. Genetic variation among a world-wide collection of isolates of *Fusarium oxysporum* f. sp. *cubense* analysed by RAPD-PCR fingerprinting. Mycol. Res. 99(11):1378-1384.

Bentley, S., Pegg, K.G., Moore, N.Y., Davis, R.D., and Buddenhagen, I.W. 1998. Genetic variation among vegetative compatibility groups of *Fusarium oxysporum* f. sp. *cubense* analyzed by DNA fingerprinting. Phytopathology 88(12):1283-1293.

Ben-Yephet, Y., Reuven, M., Szmulewich, Y., and Mor, Y. 1994. Effect of methyl bromide on the control of *Fusarium oxysporum* f. sp. *dianthi* propagules in carnation greenhouse soil and on inoculum increase after one growth cycle of carnation. Crop Prot. 13(5):357-361.

Bergstrom, G.C., Kalb, D.W., Harman, G.E., Samimy, C., and Sorrells, M.E. 1989. Effect of seed treatments on agronomic performance of soft red winter wheat from 'scabby' seed, 1988. Fungicide and Nematicide Tests 44:218.

Bilai, V.I. 1978. Principles of the taxonomy and phytopathogenic structure of species of the genus *Fusarium* Lk. ex Fr. Mikrobiol. Zh. 40:148-156.

Blok, W.J., and Bollen, G.J. 1996. Inoculum sources of *Fusarium oxysporum* f. sp. *asparagi* in asparagus production. Ann. Appl. Biol. 128:219-231.

Blok, W.J., and Bollen, G. J. 1997. Host specificity and vegetative compatibility of Dutch isolates of *Fusarium oxysporum* f. sp. *asparagi*. Can. J. Bot. 75:383-393.

Bompeix, G., and Saindrenan, P. 1984. *In vitro* antifungal activity of fosetyl-Al and phosphorous acid on *Phytophthora* species. Fruits 39:777-786.

Booth, C. 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England. 237 pages.

Booth, C. 1981. Perfect States (Teleomorphs) of *Fusarium* species. Pp. 446-452, in: Fusarium: Diseases, Biology, and Taxonomy. Nelson, P.E., Toussoun, T.A., and Cook, R.J., eds. Pennsylvania State Univ. Press, University Park, PA.

Booth, C. 1984. The *Fusarium* problem: historical, economic, and taxonomic aspects. Pp. 1-13, in: The Applied Mycology of *Fusarium*. Moss, M.O., and Smith, J.E., eds. British Mycological Society. Cambridge Univ. Press, Cambridge, England.

Bos, C.J. 1996. Biology of Fungi. Pp. 1-12, in: Fungal Genetics, Principles and Practice. Bos, C.J., ed. Marcel Dekker Inc., New York, NY.

Bos, C.J. 1987. Induction and isolation of mutants in fungi at low mutagen doses. Curr.

Genet. 12:472-474.

Bos, C.J., and Stadler, D. 1996. Mutation. Pp. 13-42, in: Fungal Genetics, Principles and Practice. Bos, C.J., ed. Marcel Dekker Inc., New York, NY.

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.

Bradford, K.J., May, D.M., Hoyle, B.J., Skibinski, Z.S., Scott, S.J., and Tyler, K.B. 1988. Seed and soil treatments to improve emergence of muskmelon from cold or crusted soils. Crop Sci. 28:1001-1005.

Brake, V.M., Pegg, K.G., Irwin, J.A.G., and Langdon, P.W. 1990. Vegetative compatibility groups within Australian populations of *Fusarium oxysporum* f. sp. *cubense*, the cause of Fusarium wilt of bananas. Aust. J.Agr. Res. 41:863-870.

Brandes, E.W. 1919. Banana wilt. Phytopathology 9:339-390.

Brewster, J.L. 1994. Onions and Other Vegetable Alliums. Crop Production Science in Horticulture Series. Atherton, J., and Rees, A., eds. CAB International. Cambridge Univ. Press, Cambridge, UK. 236 pages.

Brown, W. 1922. On the germination and growth of fungi at various temperatures and in various concentrations of oxygen and of carbon dioxide. Annals of Botany 36:257-283.

Brown, W., and Horne, A.S. 1926. Studies in the genus Fusarium. III. An analysis of factors which determine certain microscopic features of *Fusarium* strains. Annals of Botany 40:203-221.

Bruck, R.I., Fry, W.E., and Apple, A.E. 1980. Effect of metalaxyl, an acylalaine fungicide, on developmental stages of *Phytophthora infestans*. Phytopathology 70:597-601.

Bruin, G.C.A., and Edgington, L.V. 1983. The chemical control of disease caused by zoosporic plant pathogens. Pp. 193-232, in: Zoosporic Plant Pathogens: A Modern Perspective. Buczacki, S.T., ed. Academic Press, New York, NY.

Büchel, K.H. 1986. The history of azole chemistry. Pp. 1-23, in: Fungicide Chemistry: Advances and Practical Applications. Green, MB., and Spilker, D.A., eds. American Chemical Society, Washington, DC.

Buechley, G., Lehman, J., and Shaner, G. 1989. Effect of seed treatment on stand establishment from scabby seed, 1989. Fungicide and Nematicide Tests 44: 220.

Burges, A. 1965. The soil microflora - its nature and biology. Pp. 21-32, in: Ecology of Soil-Borne Plant Pathogens; Prelude to Biological Control. Baker, K.F., Snyder, W.C., Baker, R.R., Menzies, J.D., Clark, F.E., Miller, L.I., Dimock, A.W., Patrick, Z.A., Kreutzer, W.A., and Rubo, M., eds. Univ. of California Press, Berkeley, Los Angeles, CA.

Burgess, L.S. 1981. General Ecology of the Fusaria. Pp. 225-235, in: Fusarium: Diseases, Biology, and Taxonomy. Nelson, P.E., Toussoun, T.A., and Cook, R.J., eds. Pennsylvania State Univ. Press, University Park.

Burgess, L.W., and Trimboli, D. 1986. Characterization and distribution of *Fusarium nygamai*, sp.nov. Mycologia 78(2):223-229.

Burgess, L.W., Wearing, A.H., and Toussoun, T.A. 1975. Surveys of Fusaria associated with crown rot of wheat in eastern Australia. Aust. J. Agr. Res. 26:791-799.

Burgess, L.W., Nelson, P.E., and Toussoun, T.A. 1982. Characterization, geographic distribution and ecology of *Fusarium crookwellense* sp.nov. Trans. Br. Mycol. Soc 79(3):497-505.

Burgess, L.W., Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1985. *Fusarium scirpi*: emended description and notes of geographic distribution. Mycologia 77(2):212-218.

Burke, D.W., Hagedorn, D.J., and Mitchell, J.E. 1970. Soil conditions and distribution of pathogens in relation to pea root rot in Wisconsin soils. Phytopathology 60:403-406.

Burnett, J.H. 1984. Aspects of *Fusarium* genetics. Pp. 39-69, in: The Applied Mycology of Fusarium. Moss, M.O., and Smith, J.E., eds. Cambridge Univ. Press, Cambridge, England.

Butler, M.D., and Crowe, F.J. 1997. Evaluation of fungicides for control of *Botrytis allii* in seed onions, 1996. Fungicide and Nematicide Tests 52:118.

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

Byther, R. 1965. Ecology of plant pathogens in soil. V. Inorganic nitrogen utilization as a factor of competitive saprophytic ability of *Fusarium roseum* and *F. solani*. Phytopathology 55:852-858.

Campbell, C., Farrokhi-Nejad, R., Leslie, J.F. 1992. Genetic diversity of *Fusarium moniliforme* in seed from two maize cultivars. Phytopathology 82:1082 (Abstr.).

Cappellini, R.A., and Peterson, J.L. 1971. pH, nutrients and macrospore germination in *Gibberella zeae*. Mycologia 63:641-643.

Carnegie, S.F., Cameron, A.M., Linday, D.A., Sharp, E., and Nevison, I.M. 1998. The effect of treating seed potato tubers with benzimidazole, imidazole and phenylpyrrole fungicides on the control of rot and skin blemish diseases. Ann. Appl. Biol. 133:343-363.

Chellemi, D.O., Olson, S.M., and Mitchell, D.J. 1994. Effects of soil solarization and fumigation on survival of soilborne pathogens of tomato in northern Florida. Plant Dis. 78:1167-1172.

Clark, C.A., Hoy, M.W., and Nelson, P.E. 1995. Variation among isolates of *Fusarium lateritium* from sweet potato for pathogenicity and vegetative compatibility. Phytopathology 85:624-629.

Clark, F.E. 1965. The concept of competition in microbial ecology. Pp. 339-345, in: Ecology of Soil-borne Plant Pathogens. Baker, K.F., and Snyder, W.C., eds. Univ. of California Press, Berkeley, CA.

Claudius, G.R., and Mehrotra, R.S. 1973. Root exudates from lentil *(Lens culinaris medic)* seedlings in relation to wilt disease. Plant and Soil 38:315-320.

Clinton, G.P. 1916. Notes on Plant Diseases of Connecticut. Conn. Agr. Expt. Sta. Ann. Rpt. 1915:421-451.

Cochran, J.C., Cochrane, V.W., Simon, F.G., and Spaeth, J. 1963. Spore germination and carbon metabolism in *Fusarium solani*. I. Requirements for spore germination. Phytopathology 53:1155-1160.

Coffey, M.D., and Bower, L.A. 1984. *In vitro* variability among isolates of eight *Phytophthora* species in response to phosphorous acid. Phytopathology 74:738-742.

Coffey, M.D., and Young, L.H. 1984. Responses to metalaxyl of sensitive and resistant isolates of *Phytophthora infestans*. Phytopathology 74:615-620.

Coffey, M.D., and Joseph, M.C. 1985. Effects of phosphorous acid and fosetyl-Al on the life cycle of *Phytophthora cinnamomi* and *P. citricola*. Phytopathology 75:1042-1046.

Cohen, E. 1981. Metalaxyl for postharvest control of brown rot of citrus fruit. Plant Dis. 65:672-675.

Cohen, Y., and Coffey, M.D. 1986. Systemic fungicides and the control of Oomycetes. Annu. Rev. Phytopath. 24:311-338.

Colorado Agricultural Statistics 1998. Annual Report, Colorado Dept. of Agr., Denver, CO.

Colorado Agricultural Statistics 1999. Annual Report, Colorado Dept of Agr., Denver, CO.

Colyer, P.D. 1988. Evaluation of soil fungicides for seedling disease control in cotton, 1987. Fungicide and Nematicide Tests 43:208.

Colyer, P.D., and Vernon., P.R. 1990. Evaluation of soil fungicides for seedling disease control in cotton, 1989. Fungicide and Nematicide Tests 45:234.

Colyer, P.D., and Vernon, P.R. 1991a. Evaluation of hopper-box fungicides for seedling disease control in cotton, 1990. Fungicide and Nematicide Tests 46:274.

Colyer, P.D., and Vernon, P.R. 1991b. Evaluation of soil fungicides for seedling disease control in cotton, 1990. Fungicide and Nematicide Tests 46:242.

Colyer, P.D., and Vernon, P.R. 1992. Evaluation of hopper-box fungicides for seedling disease control in cotton, 1991. Fungicide and Nematicide Tests 47:218.

Cook, R. J., and Schroth. M.N. 1965. Carbon and nitrogen compounds and germination of chlamydospores of *Fusarium solani* f. *phaseoli.* Phytopathology 55:254-256.

Cook, R.J., and Snyder, W.C. 1965. Influence of host exudates on growth and survival of germlings of *Fusarium solani* f. *phaseoli* in soil. Phytopathology 55:1021-1025.

Cormack, M.W. 1951. Variation in the cultural characteristics and pathogenicity of *Fusarium avenaceum* and *F. arthrosporioides.* Can. J. Bot. 29:32-45.

Correll, J.C. 1991. The relationship between *formae speciales*, races and vegetative compatibility groups in *Fusarium oxysporum.* Phytopathology 81:1061-1064.

Correll, J.C., Gordon, T.R., and McCain, A.H. 1992. Genetic diversity in California and Florida populations of the pitch canker fungus *Fusarium subglutinans* f. sp. *pini.* Phytopathology 82:415-420.

Correll, J.C., Puhalla, J.E., and Schneider, R.W. 1985a. Distinct vegetative compatibility groups (populations) of *Fusarium oxysporum* colonizing celery roots from California. Phytopathology 75(11):1347 (Abstr.).

Correll, J.C., Puhalla, J.E., Schneider, R.W., and Kraft, J.M. 1985b. Differentiating races of *Fusarium oxysporum* f. sp. *pisi* based on vegetative compatibility. Phytopathology 75(11): 1347 (Abstr.).

Correl, 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 76:396-400.

Correl, 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.

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., Klittich, C.J.R., and Leslie, J.F. 1989. Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). Mycol. Res. 93:21-27.

Cotten, T.K., and Munkvold, G.P. 1998. Survival of *Fusarium moniliforme*, *F. proliferatum*, and *F. subglutinans* in maize stalk residue. Phytopathology 88:550-555.

Couteaudier, Y., and Alabouvette, C. 1990 Survival and inoculum potential of conidia and chlamydospores of *Fusarium oxysporum* f.sp. *lini* in soil. Can. J. Micro.biol. 36:551-556.

Cove, D.J. 1976. Chlorate toxicity in *Aspergillus nidulans*: the selection and characterisation of chlorate resistant mutants. Heredity 36(2):191-203.

Cremlyn, R. J. 1991. Agrochemicals: Preparation and Mode of Action. John Wiley and Sons, New York, N.Y. 396 pages.

Crowe, F. 1998. An analysis of the growth and reproduction of *Sclerotium cepivorum* on *Allium* crops in relation to traditional fungicide use and white rot incidence. Pp. 201-208, in: 1998 Nat. Onion (and other Allium) Res. Conf. Voss, R.E. ed. Sacramento, CA.

Daboussi, M., Langin, T., and Brygoo, Y. 1992. Fot1, a new family of fungal transposable elements. Mol. Gen. Genet. 232:12-16.

Davis, G.N., and Henderson, W.J. 1937. The interrelation of the pathogenicity of a *Phoma* and a *Fusarium* on onions. Phytopathology 27: 763-772..

Davis, R.D., Moore, N.Y., and Kochman, J.K. 1996a. Characterisation of a population of *Fusarium oxysporum* f. sp. *vasinfectum* causing wilt of cotton in Australia. Aust. J. Agr. Res. 47:1143-1156.

Davis, R.M., Nunez, J.J., Vargas, R.N., Weir, B.L., Wright, S.D. and Munier, D.J. 1996b. Metam-sodium kills beneficial soil fungi as well as cotton pests. Calif. Agr. 50:42-44.

Davis, R.M., Laemmlen, F.F., and Voss, R.E. 1998. Basal Rot. Page B10, in: UC IPM Pest

Management Guidelines: Onion/Garlic. Publication 3339, Univ. CA., Cooperative Extension, Oakland, CA.

Dercks, W., and Creasy, L.L. 1989. Influence of fosetyl-Al on phytoalexin accumulation in the *Plasmopara viticola*-grapevine interaction. Physiol. and Mol. Plant Path. 34:203-213.

Dimond, A.E., and Horsfall, J.G. 1965. The theory of inoculum. Pp. 404-415, in: Ecology of soil-borne plant pathogens. Baker, K.F., and Snyder, W.C., eds. Univ. of California Press, Berkeley, CA.

Dimond, A.E., and Horsfall, J.G. 1970. The theory of inoculum. Pp. 404-419, in: Root Diseases and Soil-borne Pathogens. Toussoun, T.A., Bega, R.V., and Nelson, P.E., eds. Univ. of California Press, Berkeley, CA.

Domsch, K.H., Jagnow, G., and Anderson, T.H. 1983. An ecological concept for the assessment of side-effects of agrochemicals on soil microorganisms. Res. Rev. 86:65-105.

Draper, W.M., and Wakeham, D.E. 1993. Rate constants for metam-sodium cleavage and photodecomposition in water. J. Agric. Food Chem. 41:1129-1133.

Duffy, B.K., and Defago, G. 1999. Macro- and microelement fertilizers influence the severity of Fusarium crown and root rot of tomato in a soil-less production system. HortScience 34(2):287-291.

Duncan, J.M. 1985. Effect of fungicides on survival, infectivity and germination of *Phytophthora fragariae* oospores. Trans. Brit. Mycol. Soc. 85:585-593.

Easton, G.D. 1989. Chemical control of *Colletotrichum coccoides*, *Sclerotinia sclerotiorum*, and *Verticillium dahliae*, 1988. Fungicide and Nematicide Tests 44:116.

Edel, V., Steinberg, C., Gautheron, N., and Alabouvette, C. 1997. Differentiation of Fusarium species by restriction fragment length polymorphism analysis of PCR amplified ribosomal DNA. Pp. 421-424, in: Diagnosis and Identification of Plant Pathogens. Proc. of the 4th Int. Symposium of the European Foundation for Plant Pathology, September 9-12, 1996, Kluwer Academic Publishers, Bonn, Germany.

Edgington, L.V. 1981. Structural requirements of systemic fungicides. Ann. Rev. Phytopath. 19:107-124.

Elias, K.S., and Schneider, R.W. 1992. Genetic diversity within and among races and vegetative compatibility groups of *Fusarium oxysporum* f. sp. *lycopersici* as determined by isozyme analysis. Phytopathology 82:1421-1427.

Elias, K.S., Schneider, R.W., and Lear, M.M. 1991. Analysis of vegetative compatibility groups in nonpathogenic populations of *Fusarium oxysporum* isolated from symptomless tomato roots. Can. J. Bot. 69:2089-2094.

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

Elmer, W.H., Summerell, B.A., Burgess, L.W., and Nigh, E.L., Jr. 1999. Vegetative compatibility groups in *Fusarium proliferatum* from asparagus in Australia. Mycologia 91(4):650-654.

Engelhard, A.W., and Woltz, S.S. 1988. Evaluation of chemotherapeutants for control of Fusarium wilt of chrysanthemum, 1986. Fungicide and Nematicide Tests 43:291.

Entwistle, A.R. 1990. Root Diseases. Pp. 103-154, in: Onions and Allied Crops. Volume II. Agronomy, Biotic Interactions, Pathology, and Crop Protection. Rabinowitch, H.D., and Brewster, J.L., eds. CRC Press, Boca Raton, FL.

Erwin, D.C. 1973. Systemic fungicides: disease control, translocation and mode of action. Ann. Rev. Phytopath. 11:389-422.

Eshel, D., Gamliel, A., Grinstein, A., DiPrimo, P., and Katan, J. 2000. Combined soil treatments and sequence of application in improving the control of soilborne pathogens. Phytopathology 90:751-757.

Everts, K.L., Schwartz, H.F., Epsky, N.D., and Capinera, J.L. 1985. Effects of maggots and wounding on occurrence of Fusarium basal rot of onions in Colorado. Plant Dis. 69(10):878-882.

Farley, J.D., Hubbeling, N., and Jaberg, C. 1974. Vertical distribution of *Fusarium oxysporum* f. sp. *lycopersici* Race 2 in a greenhouse soil. Plant Dis. Reptr. 58(4):320-321.

Federation of British Plant Pathologists. 1973. A guide to the use of terms in Plant Pathology. Phytopathology Paper number 17, Commonwealth Mycological Institute, Kew, Surrey, UK. 55 pages.

Fenn, M.E., and M.D. Coffey. 1984. Studies on the *in vitro* and *in vivo* antifungal activity of fosetyl-Al and phosphorous acid. Phytopathology 74:606-611.

Fenn, M.E., and Coffey, M.D. 1985. Further evidence for the direct mode of action of fosetyl-Al and phosphorous acid. Phytopathology 75:1064-1068.

Fenn, M.E., and Coffey, M.D. 1989. Quantification of phosphonate and ethyl phosphonate

in tobacco and tomato tissues and significance of the mode of action of two phosphonate fungicides. Phytopathology 79:76-82.

Fernandez, F.A., McClary, R.D., and Sinclair, J.B. 1989. Soybean seed treatments, 1988. Fungicide and Nematicide Tests 44:210.

Fernandez, D., Assigbetse, K., Dubois, M.P., and Geiger, J.P. 1994. Molecular characterization of races and vegetative compatibility groups in *Fusarium oxysporum* f. sp. *vasinfectum*. Appl. Environ. Micro. 60:4039-4046.

Fisher, N.L., Burgess, L.W., Toussoun, T.A., and Nelson, P.E. 1982. Carnation leaves as a substrate for preserving cultures of *Fusarium* species. Phytopathology 72:151-153.

Fisher, N.L., and Toussoun, T.A. 1983. Symptom response and colonization as measures of resistance in chrysanthemum cultivars inoculated with *Fusarium oxysporum* f. sp. *chrysanthemi*. Plant Dis. 67:376-378.

Fleeker, J.C., Lacy, H.M., Schultz, I.M., and Hokum, E.C. 1974. Persistence and metabolism of thiophanate-methyl in soil. J. Agr. Food Chem. 22(4):592-595.

Forster, R.D., and Strausbaugh, C.A. 1999. Control of white mold of dry beans with foliar sprays, 1998. Fungicide and Nematicide Tests 54:345.

Gabor, B.K. ed. 1996. Onion diseases: a practical guide for seedsmen, growers and agricultural advisors. Seminis Vegetable Seeds, Cetacea, CA.

Gams, W., and Nirenberg, H.I. 1989. A contribution to the generic definition of *Fusarium*. Mycotaxon 35:407-416.

Garrett, S.D. 1951. Ecological groups of soil fungi: a survey of substrate relationships. New Phytology 50:149-166.

Georgopoulos, S.G., and Grigoriu, A.C. 1981. Metalaxyl-resistant strains of *Pseudoperonospora cubensis* in cucumber greenhouses in southern Greece. Plant Dis. 65:729-731.

Gerlach, W. 1981. The present concept of *Fusarium* classification. Pp. 413-426, in: Fusarium: Diseases, Biology and Taxonomy. Nelson, P.E., Toussoun, T.A., and Cook, R.J., eds. Pennsylvania State Univ. Press, University Park, PA.

Glass, N.L., and Kuldau, G.A. 1992. Mating type and vegetative incompatibility in filamentous ascomycetes. Ann. Rev. Phytopath. 30:201-224.

Glynne, M.D. 1970. Crop sequence in relation to soil-borne pathogens. Pp. 423-435, in: Root Diseases and Soil-borne Pathogens. Toussoun, T.A., Bega, R.V., and Nelson, P.E., eds. Univ. of California Press. Berkeley, CA.

Gordon, W.L. 1956. The occurrence of *Fusarium* species in Canada. V. Taxonomy and geographic distribution of *Fusarium* species in soil. Can. J. Bot. 34:833-846.

Gordon, T.R., and Martyn, R.D. 1997. The evolutionary biology of *Fusarium oxysporum*. Annu. Rev. Phytopath. 35:111-128.

Gordon, T.R., and Okamoto, D. 1991. Vegetative compatibility groupings in a local population of *Fusarium oxysporum*. Can. J. Bot. 69:168-172.

Gordon, T.R., and Okamoto, D. 1992a. Variation in mitochondrial DNA among vegetatively compatible isolates of *Fusarium oxysporum*. Exp. Mycol. 16:245-250.

Gordon, T.R., and Okamoto, D. 1992b. Variation within and between populations of *Fusarium oxysporum* based on vegetative compatibility and mitochondrial DNA. Can. J. Bot. 70:1211-1217.

Gordon, T.R., Storer, A.J., and Okamoto, D. 1996. Population structure of the pitch canker pathogen, *Fusarium subglutinans* f. sp. *pini*, in California. Mycol Res. 100(7):850-854.

Goss, R.J., Johnson, B.E., and Stack, R.W. 1989. Effect of potassium chloride, imazalil, and method of imazalil application on barley infected with common root rot. Can J. Plant Sci. 69:437-444.

Goyal, J.P., Maraite, H., and Meyer, J.A. 1973. Abundant production of chlamydospores by *Fusarium oxysporum* f. sp. *melonis* in soil extract with glucose. Neth. J. Plant Path. 79:162-164.

Green, R.J., Jr. 1981. An Overview. Pp. 1-24 in: in: Fungal Wilt Diseases of Plants. Marshall, E.M., Bell, A.A., and Beckman, C.H. eds. Academic Press Inc., New York, NY.

Gregory, P.J., Guthrie, E.J., and Bunce, M.E. 1959. Experiments in splash dispersal of fungus spores. J. Gen. Micro. 20:328-354.

Griffin, G.J. 1964. Influence of carbon and nitrogen nutrition on chlamydospore formation by *Fusarium solani* f. *radicola*. Phytopathology 54:894 (Abstr.)

Griffin, G.J. 1969. *Fusarium oxysporum* and *Aspergillus flavus* spore germination in the rhizosphere of peanut. Phytopathology 59:1214-1218.

Griffin, G.J. 1970a. Carbon and nitrogen requirements for macroconidial germination of *Fusarium solani:* dependence on conidial density. Can. J. Micro.16:733-740.

Griffin, G.J. 1970b. Exogenous carbon and nitrogen requirements for chlamydospore germination by *Fusarium solani*: dependence on spore density. Can. J. Micro. 16:1366-1368.

Griffin, G.J. 1973. Modification of the exogenous carbon and nitrogen requirements for chlamydospore germination of *Fusarium solani* by contact with soil. Can. J. Micro. 19:999-1005.

Griffin, G.J. 1976. Roles of low pH, carbon, and inorganic nitrogen source use in chlamydospore formation by *Fusarium solani.* Can. J. Micro. 22:1381-1389.

Griffin, G.J. 1981. Physiology of conidium and chlamydospore germination. Pp. 331-339, in: Fusarium: Diseases, Biology, and Taxonomy. Nelson, P.E., Toussoun, T.A., and Cook, R.J., eds. Pennsylvania State Univ. Press, University Park, PA.

Griffin, G.J., and Pass, T. 1969. Behavior of *Fusarium roseum* 'Sambucinum' under carbon starvation conditions in relation to survival in soil. Can. J. Micro. 15:117-126.

Grinstein, A., Fishler, G., Katan, J., and Hakohen, D. 1983. Dispersal of the Fusarium wilt pathogen in furrow-irrigated cotton in Israel. Plant Dis. 67:742-743.

Gross, D. 1991. Plant growth regulatory substances both of microbial and plant origin. Pp. 1-49, in: Herbicide Resistance - Brassinosteroids, Gibberellins, Plant Growth Regulators. Broner, H., Martin, D., Sjut, V., Stan, H.J., and Stetter, J., eds. Springer-Verlag, Berlin, Germany.

Groth, D.E., and Martinson, C.A. 1983. Increased endomycorrhizal infection of maize and soybeans after soil treatment and metalaxyl. Plant Dis. 67:1377-1378.

Guest, D.I. 1984a. Modification of defense responses in tobacco and capsicum following treatment with fosetyl-Al [aluminum tris(o-ethyl phosphonate)]. Physiol. Plant Path. 25:125-134.

Guest, D.I. 1984b. The influence of cultural factors on the direct antifungal activities of fosetyl-Al, Propamocarb, Metalaxyl, SN75196 and Dowco 444. Phytpath. Z. 111:15-164.

Guest, D.I., Upton, J.C.R., and Rowan, K.S. 1989. Fosetyl-Al alters the respiratory response in *Phytophthora nicotianae* var. *nicotianae*-infected tobacco. Physiol. Mol. Plant Path. 34: 357-365.

Gutter, Y. 1983. Supplementary antimold activity of phosethyl Al, a new brown rot fungicide for citrus fruits. Phytopath. Z. 107:301-308.

Gutter, Y., Shachnai, A., Schiffmann-Nadel, M., and Dinoor, A. 1981. Chemical control in citrus of green and blue molds resistant to benzimidazoles. Phytopath. Z. 102:127-138.

Hall, R., and MacHardy, W.E. 1981. Water relations. Pp. 255 - 298, in: Fungal Wilt Diseases of Plants. Marshall, E.M., Bell, A.A., and Beckman, C.H., eds. Academic Press Inc., New York, NY.

Hanelt, P. 1990. Taxonomy, evolution, and history. Pp. 1-26, in: Onions and Allied Crops. Volume I. Botany, Physiology, and Genetics. Rabinowitch, H.D., and Brewster, J.L., eds. CRC Press, Boca Raton, FL.

Hansford, C.G. 1926. The Fusaria of Jamaica. Bulletin of miscellaneous information No.7:257-288. Royal Botanic Gardens, Kew, England.

Hargreaves, J.A., and Fox, R.A. 1977. Survival of *Fusarium avenaceum* in soil. Trans. Brit. Mycol. Soc. 69:425-428.

Hartl, D.L. 1980. Principles of Population Genetics. Sinauer Associates, Inc. Publishers, Sunderland, MA. 488 pages.

Harveson, R.M., and Rush, C.M. 1997. Genetic variation among *Fusarium oxysporum* isolates from sugar beet as determined by vegetative compatibility. Plant Dis. 81:85-88.

Hausbeck, M.K., and Cortright, B.D. 1997. Chemical control of onion purple blotch and downy mildew, 1996. Fungicide and Nematicide Tests 52:119.

Hawthorne, B.T., and Rees-George, J. 1996. Use of nitrate non-utilizing mutants to study vegetative incompatibility in *Fusarium solani* (*Nectria haematococca*), especially members of mating populations I, V and VI. Mycol. Res. 100(9):1075-1081.

Helbig, J.B., and Carroll, R.B. 1984. Dicotyledonous weeds as a source of *Fusarium oxysporum* pathogenic on soybean. Plant Dis. 68:694-696.

Hillis, D.M., and Dixon, M.T. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Quart. Rev. Biol. 66:411-453.

Hirst, J.M. 1965. Dispersal of soil microorganisms. Pp. 69-81, in: Ecology of Soil-Borne Plant Pathogens; Prelude to Biological Control. Baker, K.F., Snyder, W.C., Baker, R.R., Menzies, J.D., Clark, F.E., Miller, L.I., Dimock, A.W., Patrick, Z.A., Kreutzer, W.A., and Rubo, M., eds. Univ. of California Press, Berkely, Los Angeles, CA.

Hoffman, M.P., Petzoldt, C.H., and Frodsham, A.C. 1996. Integrated Pest Management for Onions. New York State IPM Program Publication No. 119. Cornell Cooperative Extension, Ithaca, NY. 78 pages.

Holmes, G.J., Lancaster, M.E., and Pollard, D.W. 1999. Evaluation of materials for control of Phytophthora crown rot of summer squash, 1998. Fungicide and Nematicide Tests 54:240.

Holz, G., and Knox-Davies, P.S. 1974. Resistance of onion selections to *Fusarium oxysporum* f. sp. *cepae*. Phytophylactica 6:153-156.

Hopkins, D.L., Lobinske, R.J., and Larkin, R.P. 1992. Selection for *Fusarium oxysporum* f. sp. *niveum* Race 2 in monocultures of watermelon cultivars resistant to Fusarium wilt. Phytopathology 82:290-293.

Horst, R.K., P.E. Nelson, and T.A. Toussoun. 1970. Aerobiology of *Fusarium* spp. associated with stem rot of *Dianthus caryophyllus*. Phytopathology 60:1296 (Abstr.)

Houston, B.R., and Knowles, P.F. 1949. Fifty-years survival of flax *Fusarium* wilt in the absence of flax culture. Plant Dis. Rept. 33:38-39.

Hsu, S.C., and Lockwood, J.L. 1973. Soil fungistasis: behavior of nutrient-independent spores and sclerotia in a model system. Phytopathology 63:334-337.

Irving, H.R., and Kuc, J.A. 1990. Local and systemic induction of peroxidase, chitinase and resistance in cucumber plants by K_2HPO_4. Physiol. Mol. Plant Path. 37:355-366.

Ivens, G.W., ed. 1988. The UK Pesticide Guide. British Crop Protection Council, Wallingford, Oxfordshire, UK. 565 pages.

Jabaji-Hare, S.H., and Kendrick, W.B. 1985. Effects of fosetyl-Al on root exudation and on composition of extracts of mycorrhizal and nonmycorrhizal leek roots. Can. J. Plant Path. 7:118-126.

Jabaji-Hare, S.H., and Kendrick, W.B. 1987. Response of an endomycorrhizal fungus in *Allium porrum* L. to different concentrations of the systemic fungicides, metalaxyl (Ridomil) and Fosetyl-Al (Aliette). Soil Biol. Biochem. 19(1):95-99.

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

Jacobson, D.J., and Gordon, T.R. 1990a. Further investigations of vegetative compatibility within *Fusarium oxysporum* f. sp. *melonis*. Can. J. Bot. 68:1245-1248.

Jacobson, D.J., and Gordon, T.R. 1990b. Variability of mitochondrial DNA as an indicator of relationships between populations of *Fusarium oxysporum* f. sp. *melonis*. Mycol. Res. 94(6):734-744.

Jacobson, D.J., and Gordon, T.R. 1991. *Fusarium oxysporum* f. sp. *melonis*: A case study of diversity within a forma specialis. Phytopathology 81:1064-1067.

Jalali, B.L., and Domsch, K.H. 1975. Effect of systemic fungitoxicants on the development of endotrophic mycorrhiza. Pp. 619-626, in: Endomycorrhizas: Proc. Symposium held at the Univ. Leeds, 22-25 July 1974. Sanders, F.W., Mosse, B., and Tinker, P.B., eds. Academic Press, London, England.

Jardine, D.J., Kilgore, G.L., Long, J.H., and Claassen, M. 1991. Effect of seed treatment fungicides on stand and yield of grain sorghum. Fungicide and Nematicide Tests 46:277.

Jarvis, W.R., and Shoemaker, R.A. 1978. Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. Phytopathology 68:1679-1680.

Jaworski, C.A., McCarter, S.M., Johnson, A.W., and Williamson, R.E. 1978. Response of onions grown for transplants to soil fumigation. J. Amer. Soc. Hort. Sci. 103:385-388.

Jenkinson, P., and Parry, D.W. 1994. Isolation of *Fusarium* species from common broad-leaved weeds and their pathogenicity to winter wheat. Mycol. Res. 98(7):776-780.

Jhamaria, S.L. 1972. Nutritional requirement of *Fusarium oxysporum* f. *niveum*. Indian Phytopath. 25:29-32.

Joffe, A.Z. 1986. Fusarium Species: their biology and toxicology. John Wiley & Sons, New York, N.Y. 588 pages.

Joffe, A.Z., and Palti, J. 1977. Species of *Fusarium* found in uncultivated desert type soils in Israel. Phytoparasitica 5:119-121.

Johanson, A., and Blazquez, B. 1992. Fungi associated with banana crown rot on field-packed fruit from the Windward Islands and assessment of their sensitivity to the fungicides thiabendazole, prochloraz and imazalil. Crop Prot. 11:79-83.

Jones, J.P., and Woltz, S.S. 1969. Fusarium wilt (race 2) of tomato: Calcium, pH and micronutrient effects on disease development. Plant Dis. Rep. 53:276-279.

Jones, J.P., Engelhard, A.W., and Woltz, S.S. 1989. Management of Fusarium wilt of vegetables and ornamentals by macro- and microelement nutrition. Pp. 18-32, in: Soilborne Plant Pathogens: Management of Disease with Macro- and Microelements. Engelhard,

A.W., ed. American Phytopathological Society, St. Paul, MN.

Jones, R.N. 1990. Cytogenics. Pp. 199-214, in: Onions and Allied Crops. Volume I. Botany, Physiology, and Genetics. Rabinowitch, H.D., and Brewster, J.L., eds. CRC Press, Boca Raton, FL.

Kalb, D.W., Bergstrom, G.C., and Sorrells, M.E. 1988. Effect of seed treatments on yield and disease development in winter wheat in a continuous wheat culture site with a history of foot rot diseases, 1987. Fungicide and Nematicide Tests 43:243.

Kalb, D.W., Samimy, C., and Bergstrom, G.C. 1989. Effects of Baytan seed treatments on emergence and growth of soft white winter wheat, 1988. Fungicide and Nematicide Tests 44:221.

Kannaiyan, S., and Prasad, N.N. 1974. Influence of foliar nutrient sprays on the population of *Fusarium oxysporum* f. *melonis* and other soil microflora in the rhizosphere of muskmelon. Indian Phytopath. 27:527-531.

Kaplan, D.T. 1983. Influence of metalaxyl on three nematodes of citrus. J. Nematol. 15:454-460.

Katan T. 1999. Current status of vegetative compatibility groups in *Fusarium oxysporum*. Phytoparasitica 27:51-64.

Katan, T., and Di Primo, P. 1999. Current status of vegetative compatibility groups in *Fusarium oxysporum*: Supplement (1999). Phytoparasitica 27(4):273-277.

Katan, T., Katan, J., Gordon, T.R., and Pozniak, D. 1994. Physiologic races and vegetative compatibility groups of *Fusarium oxysporum* f. sp. *melonis* in Israel. Phytopathology 84: 153-157.

Katan, T., Shlevin, E., and Katan, J. 1997. Sporulation of *Fusarium oxysporum* f. sp. *lycopersici* on stem surfaces of tomato plants and aerial dissemination of inoculum. Phytopathology 87:712-719.

Kawamoto, S.O., and Lorbeer, J.W. 1976. Protection of onion seedlings from *Fusarium oxysporum* f. sp. *cepae* by seed and soil infestation with *Pseudomonas cepacia*. Plant Dis. Rept. 60(3):189-191.

Kedera, C.J., Leslie, J.F., and Claflin, L.E. 1994. Genetic diversity of *Fusarium* section *Liseola* (*Gibberella fujikuroi*) in individual maize stalks. Phytopathology 84:603-607.

Kehr, A.E., O'Brien , M.J. , and Davis, E.W. 1962. Pathogenicity of *Fusarium oxysporum*

f. sp. *cepae* and its interaction with *Pyrenochaeta terrestris* on onion. Euphytica 11:197-20.

Keinath, A.P. 1994. Pathogenicity and host range of *Fusarium oxysporum* from sweet basil and evaluation of disease control methods. Plant Dis. 78:1211-1215.

Keinath, A.P. 1998. Resistance to benomyl and thiophanate-methyl in *Didymella bryoniae* from South Carolina and New York. Plant Dis. 82:479-484.

Kennerlye, C.M., Brouck, R.I., and Grand, L.F. 1984. Effects of metalaxyl on growth and ectomycorrhizae of Fraser fir seedlings. Plant Dis. 68:32-35.112

Kenneth, J.H. 1963. A dictionary of biological terms. D. Van Nostrand Company, Inc., Princeton, New Jersey. 640 pages.

Kerr, E.D. 1991. Evaluation of fungicides for control of Fusarium and Rhizoctonia root rots of dry bean, 1990. Fungicide and Nematicide Tests 46:236.

Kistler, H.C., and Miao, V. P.W. 1992. New modes of genetic change in filamentous fungi. Ann. Rev. Phytopath. 30:131-152.

Kistler, H.C., and Momol, E.A. 1990. Molecular genetics of plant pathogenic *Fusarium oxysporum*. Pp. 49-54, in: Fusarium Wilt of Banana. Ploetz, R.C., ed. APS Press, St. Paul, MN.

Kistler, H.C., Benny, U., Boehm, E.W.A., and Katan, T. 1995. Genetic duplication in *Fusarium oxysporum*. Current Genetics 28(2):173-176.

Kistler, H.C., Alabouvetter, C., Baayen, R.P., Bentley, S., Brayford, D., Coddington, A., Correll, J., Daboussi, M.-J., Elias, K., Fernandez, D., Gordon, T.R., Katan, T., Kim, H.G., Leslie, J.F., Martyn, R.D., Migheli, Q., Moore, N.Y., O'Donnell, K., Ploetz, R.C., Rutherford, M.A., Summerell, B., Waalwijk, C., and Woo, S. 1998. Systematic numbering of vegetative compatibility groups in the plant pathogenic fungus *Fusarium oxysporum*. Phytopathology 88 (1):30-32.

Klittich, C.J.R., and Leslie J.F. 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics 118:417-423.

Klittich, C.J.R., and Leslie, J.F. 1989. Chlorate-resistant, nitrate-utilizing (*crn*) mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). J. Gen. Micro. 135:721-727.

Klotz, L.J., Wong, P.P., and DeWolfe, T.A. 1959. Survey of irrigation water for the presence of *Phytophthora* spp. pathogenic to citrus. Plant Dis. Rept. 43:830-832.

Klotz, L.V., Nelson, P.E., and Toussoun, T.A. 1988. A medium for enhancement of chlamydospore formation in *Fusarium* species. Mycologia 80(1):108-109.

Knaflewski, M. 1990. Influence of irrigation, fertilization and spacing on the healthiness of asparagus seedlings. Acta Hort. 271:269-271.

Ko, W., and Lockwood, J.L. 1970. Mechanism of lysis of fungal mycelia in soil. Phytopathology 60:148-154.

Koenig, R.D., Ploetz, R.C., and Kistler, H.C. 1997. *Fusarium oxysporum* f. sp. *cubense* consists of a small number of divergent and globally distributed clonal lineages. Phytopathology 87:915-923.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soils. Rev. Plant Prot. Res. 8:114-125.

Kommedahl, T., Windels, C.E., and Lang, D.S. 1975. Comparison of Fusarium populations in grasslands of Minnesota and Iceland. Mycologia 67:38-44.

Kondo, N., Kodama, F., and Ogoshi, A. 1997. Vegetative compatibility groups of *Fusarium oxysporum* f. sp. *adzukicola* and nonpathogenic *Fusarium oxysporum* on adzuki bean isolated from adzuki bean fields in Hokkaido. Ann. Phytopath. Soc. Jpn. 63:8-12.

Kope, H.H., and Fortin, J.A. 1989. Inhibition of phytopathogenic fungi *in vitro* by cell free culture media of ectomycorrhizal fungi. New Phytol. 113:57-63.

Krelle, W., Adams, D., and Williamson, M. 1993. Cultural alternative for control of *Fusarium oxysporum* on non-fumigated soil at Magalia Nursery. Pp. 136-138, in: Proc. Western Forest Nursery Association, 1992. Landis, T.D., ed. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, General Technical Report RM-221.

Kreutzer, W.A. 1963. Selective toxicity of chemicals to soil microorganisms. Ann. Rev. Phytopath. 1:101-125.

Langton, F.A. 1969. Interactions of the tomato with two formae speciales of *Fusarium oxysporum*. Ann. Appl. Biol. 62:413-427.

Larkin, R.P., Hopkins, D.L., and Martin, F.N. 1990. Vegetative compatibility within *Fusarium oxysporum* f. sp. *niveum* and its relationship to virulence, aggressiveness, and race. Can. J. Micro. 36:352-358.

Larkin, R.P., Hopkins. D.L., and Martin, F.M. 1996. Suppression of Fusarium wilt of watermelon by nonpathogenic *Fusarium oxysporum* and other microorganisms recovered

from a disease-suppressive soil. Phytopathology 86:812-819.

Lawrence, E.B., Nelson, P.E., and Ayers, J.E. 1981. Histopathology of sweet corn seed and plants infected with *Fusarium moniliforme* and *F. oxysporum*. Phytopathology 71:379-386.

Lawrence, E.B., Nelson, P.E., and Toussoun, T.A. 1986. Spontaneous mutability and colony morphology of *Gibberella baccata*. Can. J. Genet. Cytol. 28:932-941.

Leistra, M., and Smelt, J.H. 1974. Concentration-time relationships for methyl isothiocyanate in soil after injection of metham-sodium. Pest. Sci. 5:409-417.

Leong, J. 1986. Siderophores: their biochemistry and possible role in biocontrol of plant pathogens. Ann. Rev. Phytopath. 24:187-209.

Leslie, J.F. 1990. Genetic exchange within sexual and asexual populations of the genus *Fusarium*. Pp 37-48, in: Fusarium Wilt of Banana. Ploetz, R.C., ed. APS Press, St. Paul, MN.

Leslie, J.F. 1993. Vegetative compatibility in fungi. Annual Rev. Phytopath. 31:127-151.

Linfield, C.A. 1993. A rapid serological test for detecting *Fusarium oxysporum* f. sp. *narcissi* in *Narcissus*. Ann. Appl. Biol. 123:685-693.

Link, G.K.K., Bailey, A.M., and Coffey, A.A. 1926. *Fusaria* causing bulb rot of onions. J. Agr. Res.. 33:929-952.

Lloyd, AB., and Lockwood, J.L. 1966. Lysis of fungal hyphae in soil and its possible relation to autolysis. Phytopathology 56:595-602.

Lodwig, E.M., Bridge, P.D., Rutherford, M.A., Kung'u, J., and Jeffries, P. 1999. Molecular differences distinguish clonal lineages within East African populations of *Fusarium oxysporum* f. sp. *cubense*. J. Applied Micro. 86:71-77.

Löffler, H.J.M., and Rumine, P. 1991. Virulence and vegetative compatibility of Dutch and Italian isolates of *Fusarium oxysporum* f. sp. *lilii*. J. Phytopath. 132:12-20.

Lopez-Serrano, M., Ferre, M.A., Calderon, A.A., Munoz, R., Ros Barcelo, A., and Pedreno, M.A. 1994. Aluminum-mediated fosetyl-Al effects on peroxidase secreted from grapevine cells. Envir. Exp. Bot. 34(3):329-336.

Lopez-Serrano, M., Ferrer M.A, Pedreno, M.A., and Ros Barcelo, A. 1997. Ca2+ and Mg2+ ions counteract the reduction by fosetyl-Al (aluminum tris[ethyl phosphonate]) of peroxidase activity from suspension-cultured grapevine cells. Plant Cell, Tissue and Organ

Culture 47:207-212.

Lorbeer, J.W. 1982. The New York Celery Industry. P. 87, in: California Celery Research Program 1981-1982 Annual Report.

Lorbeer, J.W., and Stone, K.W. 1965. Reaction of onion to Fusarium basal rot. Plant Dis. Rept. 49(6):522-526.

Lynch, J.M. 1990. Fungi as antagonists. Pp. 243-253, in: New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases. Baker, R.R., and Dunn, P.E., eds. Alan R. Liss, Inc., New York, N.Y.

Macalady, J.L., Fuller, M.E., and Scow, K.M. 1998. Effects of metam-sodium fumigation on soil microbial activity and community structure. J. Environ. Qual. 27:54-63.

Maloy, O.C., and Alexander, M. 1958. The 'most probable number' method for estimating populations of plant pathogenic organisms in the soil. Phytopathology 48:126-128.

Marchant, R., and White, M.F. 1966. Spore swelling and germination in *Fusarium culmorum*. J. Gen. Micro. 42:237-244.

Marasas, W.F.O., Nelson, P.E., and Toussoun, T.A. 1985. *Fusarium dlamini*, a new species from Southern Africa. Mycologia 77(6):971-975.

Marasas, W.F.O., Rabie, C.J., Lubben, A., Nelson, P.E., and Toussoun, T.A. 1987. *Fusarium napiforme*, a new species from millet and sorghum in Southern Africa. Mycologia 79(6):910-914.

Marlatt, R.B. 1958. Onion Fusarium basal rot in Arizona. Plant Dis. Rep. 42(5):667-668.

Martinetti, G., and Loper, J.E. 1992. Mutational analysis of genes determining antagonism of *Alcaligenes* sp. strain MFA1 against the phytopathogenic fungus *Fusarium oxysporum*. Can. J. Micro. 38:241-247.

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

Matheron, M.E., and Porchas, M. 2000. Impact of azoxystrobin, dimethomorph, fluazinam, fosetyl-Al, and metalaxyl on growth, sporulation, and zoospore cyst germination of three *Phytophthora* spp. Plant Dis. 84:454-458.

Matsumura, F. 1982. Degradation of pesticides in the environment by microorganisms and sunlight. Pp. 67-87, in: Biodegradation of Pesticides. Matsumura, F., and Krishna Murti,

C.R., eds. Plenum Press, New York, N.Y.

Matuo, T. 1972. Taxonomic studies of phytopathogenic fusaria in Japan. Rev. Plant Prot. Res. 5:32-45.

Maude, R.B.1990. Storage Disease of Onions. Pp. 273-196, in: Onions and Allied Crops. Volume II. Agronomy, Biotic Interactions, Pathology, and Crop Protection. Rabinowitch, H.D., and Brewster, J.L., eds. CRC Press, Boca Raton, FL.

Maurer, C.L., and Baker, R. 1965. Ecology of plant pathogens in soil. II. Influence of glucose, cellulose, and inorganic nitrogen amendments on development of bean root rot. Phytopathology 55:69-72.

McCrady, M.H. 1915. The numerical interpretation of fermentation-tube results. J. Infectious Diseases 17:183-212.

McDonald, M.R., Janse, S., and Vander Kooi, K. 1998. Evaluation of furrow fungicide treatments and drenches for control of onion smut, 1997. Fungicide and Nematicide Tests 53:161.

McDonald, M.R., Janse, S., and Vander Kooi, K. 1999. Evaluation of furrow fungicide and drench treatments for control of onion smut, 1998. Fungicide and Nematicide Tests 54:151.

McGovern, R.J., Vavrina, C.S., Obreza, T.A., and Capece, J.C. 1995. Reduction of fusarium crown and root rot of tomato by combining soil solarization and metam-sodium. Annual Int. Res. Conf. on Methyl Bromide Alternatives and Emissions Reductions. November 6-8, 1995, Red Lion Inn, San Diego, CA.

McKay, R. 1953. Some unusual parasitic and non-parasitic diseases of plants observed in Ireland in 1953. J. Dept. Agr. Eire 50:151-155.

McKeen, C.D., and Wensley, R.N. 1961. Longevity of *Fusarium oxysporum* in soil tube culture. Science 134:1528-1529.

Meister, R.T., ed. 1999 Farm Chemical Handbook, Vol 85. Meister Publishing Co. Willoughby, OH.

Meister, R. T. ed. 2000 Farm Chemical Handbook, Vol. 86. Meister Publishing Co. Willoughby, OH.

Menzies, J.D. 1963. The direct assay of plant pathogen populations in soil. Ann. Rev. Phytopath. 1:127-141.

Menzies, J.D. 1970. Factors affecting plant pathogen population in soil. Pp. 16-21, in: Root Diseases and Soil-borne Pathogens. Toussoun, T.A., Bega, R.V., and Nelson, P.E., eds. Univ. of California Press, Berkeley, CA.

Menzies, J.D., and Gilbert, R.G. 1967. Responses of the soil microflora to volatile compounds in plant residues. Soil Sci. Soc. Amer. Proc. 31:495-496.

Messiaen, C.M., and Cassini, R. 1968. Recherches sur les fusarioses. IV. La systematique des Fusarium. Ann. Epiphyt. 19:387-454.

Meyer, J.A., and Maraite, H. 1971. Multiple infection and symptom mitigation in vascular wilt diseases. [Muskmelons, Fusarium oxysporum melonis]. Trans. Br. Mycol. Soc. 57:371-377.

Miller, M.E. 1992a. Evaluation of fumigants for pink root control of onions, 1991. Fungicide and Nematicide Tests 47:103.

Miller, M.E. 1992b. Evaluation of fungicides for purple blotch control on onions, 1991. Fungicide and Nematicide Tests 47:103.

Miller, M.E. 1993a. Evaluation of fumigants for pink root control on 'TG 1015Y' onions, 1992. Fungicide and Nematicide Tests 48:140.

Miller, M.E. 1993b. Evaluation of fumigants for pink root control on 'TG 502y' onions, 1992. Fungicide and Nematicide Tests 48:139.

Miller, M.E. 1998. Evaluation of fungicides for purple blotch control in onions, 1997. Fungicide and Nematicide Tests 53:163-164.

Miller, M.E., and Amador, J.M. 1991. Evaluation of fungicides for purple blotch control on onions, 1990. Fungicide and Nematicide Tests 46:140.

Miller, M.E., Amador, J.M., Martyn, R.D., and Lander, C. 1990. Evaluation of fumigants for root rot control on muskmelons, 1989. Fungicide and Nematicide Tests 45:115.

Miller, M.E., Amador, J.M., Martyn, R.D., and Lander, C. 1991. Evaluation of fumigants for root rot control on muskmelons, 1990. Fungicide and Nematicide Tests 46:138.

Miller, M.E., and Martyn, R.D. 1992. Evaluation of fumigants for root rot/vine decline control on muskmelon, 1991. Fungicide and Nematicide Tests 47:101.

Moore, N.Y., Hargreaves, P.A., Pegg, K.G., and Irwin, J.A.G. 1991. Characterization of strains of *Fusarium oxysporum* f. sp. *cubense* by production of volatiles. Aust. J. Bot.

39:161-166.

Moore, N.Y., Pegg, K.G., Allen, R.A., and Irwin, J.A.G. 1993. Vegetative compatibility and distribution of *Fusarium oxysporum* f. sp. *cubense* in Australia. Aust. J. Exp. Agr. 33:797-802.

Morris, C., ed. 1996. Academic Press Dictionary of Science and Technology CD-ROM, 1996. San Diego, CA

Morton, V. 1999. Current status of seed treatment. Phytopathology News 33(10): 188.

Mount, M.S., and Lacy, G.H. 1982. Phytopathogenic Prokaryotes: Vol 2. Academic Press, New York, NY. 506 pages.

Munkvold, G.P., McGee, D.C., and Carlton, W.M. 1997. Importance of different pathways for maize kernel infection by *Fusarium moniliforme*. Phytopathology 87:209-217.

Nash, S.M., and Alexander, J.V. 1965. Comparative survival of *Fusarium solani* f. *cucurbito* and *F. solani* f. *phaseoli* in soil. Phytopathology 55: 963-966.

Nash, S.M., Chistous, T., and Snyder, W.C. 1961. Existence of *Fusarium solani* f. *phaseoli* as chlamydospores in soil. Phytopathology 51:164-168.

Nash, S.M., and Snyder, W.C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot Fusarium in field soils. Phytopathology 52:567-572.

Nash, S.M., and W.C. Snyder. 1965. Quantitative and qualitative comparisons of Fusarium populations in cultivated fields and non-cultivated parent soils. Can. J. Bot. 43:939-945.

Nelson, P.E. 1964. Carnation as a symptomless carrier of *Fusarium oxysporum* f.sp. *dianthi*. Phytopathology 54:323-329.

Nelson, P.E. 1981. Life cycle and epidemiology of *Fusarium oxysporum*. Pp. 51-80, in: Fungal Wilt Diseases of Plants. Marshall, E.M., Bell, A.A., and Beckman, C.H., eds. Academic Press, Inc., New York, NY.

Nelson, P.E. 1990. Taxonomy of Fungi in the genus *Fusarium* with emphasis on *Fusarium oxysporum*. Pp. 27-35, in: Fusarium Wilt of Banana. Ploetz, R.C., ed. APS Press, St. Paul, MN.

Nelson, P.E. 1991. History of Fusarium systematics. Phytopathology 81(9): 1045-1048.

Nelson, P.E., Toussoun, T.A., and Burgess, L.W. 1987. Characterization of *Fusarium*

beomiforme Sp. Nov. Mycologia 79(6):884-889.

Nelson, P.E. Toussoun, T.A., and Cook, R.J., eds. 1981. Fusarium: Diseases, Biology and Taxonomy. Pennsylvania State Univ. Press, University Park, PA.

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

Nemec, S. 1980. Effect of 11 fungicides on endomycorrhizal development in sour orange. Can. J. Bot. 58:522-526.

Nemestothy, G.S., and Guest, D.I. 1990. Phytoalexin accumulation, phenylaline ammonia lyase activity and ethylene biosynthesis in fosetyl-Al treated resistant and susceptible tobacco cultivars infected with *Phytophthora nicotianae* var. *nicotianae*. Physiol. Mol. Plant Path. 37:207-219.

Newcomb, M. 1960. Some effects of water and anaerobic conditions on *Fusarium oxysporum* f. *cubense* in soil. Trans. Brit. Mycol. Soc. 43:51-59.

Ooka, J.J., and Kommedahl, T. 1977. Wind and rain dispersal of *Fusarium moniliforme* in corn fields. Phytopathology 67:1023-1026.

Owens, L.D., Gilbert, R.G., Griebel, G.E., and Menzies, J.D. 1969. Identification of plant volatiles that stimulate microbial respiration and growth in soil. Phytopathology 59 (10): 1468-1472.

Padmanabhan, K., and Merriman, P.R. 1989. Evaluation of fungicides for control of wilt in cyclamen, 1987. Fungicide and Nematicide Tests 44:269.

Palo, M.A.1928-1929. A Fusarium causing bulb rot of onion in the Philippines. Philippine Agriculturist 17:301-316.

Parmeter, J.C. 1970. Mechanisms of variation in culture and soil. Pp. 63-68, in: Root Diseases and Soil-borne Pathogens. Toussoun, T.A., Bega, R.V., and Nelson, P.E., eds. Univ. of California Press, Berkeley, CA.

Parmeter, J.C., Snyder, W.C., and Reichle, R.E. 1963. Heterokaryosis and variability in plant-pathogenic fungi. Ann. Rev. Phytopath. 1:51-76.

Paulitz, T.C. 1990. Biochemical and ecological aspects of competition in biological control. Pp. 713-724, in: New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases. Baker, R.R., and Dunn, P.E., eds. Alan R. Liss, Inc., New York, N.Y. 837 pages.

Paulitz, T.C., Park, C.S., and Baker, R. 1987. Biological control of Fusarium wilt of cucumber with nonpathogenic isolates of *Fusarium oxysporum*. Can. J. Micro. 33:349-353.

Pegg, G.F., Whiley, A.W., Saranah, J.B., and Glass, R.J. 1985. Control of Phytophthora root rot of avocado with phosphorous acid. Aust. Plant Path. 14:25-29.

Pfleger, F.L., Pokorny, J.D., and Gould, S.L. 1990. Evaluation of aliette on control of *Pythium ultimum* root rot on geraniums. Minn. State Florist Bull. 39(5):5-8.

Ploetz, R.C. 1990. Population biology of *Fusarium oxysporum* f. sp. *cubense*. Pp. 63-76, in: Fusarium wilt of banana. Ploetz, R.C., ed. APS Press, St. Paul, MN.

Ploetz, R.C., and Shepard, E.S. 1989. Fusarial wilt of banana in Florida. Mycol. Res. 93(2):242-245.

Postma, J., and Rattink, H. 1991. Biological control of Fusarium wilt of carnation with nonpathogenic *Fusarium* isolates. Meded. Fac. Landbouwwet. Rijksuniv. Gent. 56:179-184.

Postma, J., and Rattink, H. 1992. Biological control of Fusarium wilt of carnation with a nonpathogenic isolate of *Fusarium oxysporum*. Can. J. Bot. 70:1199-1205.

Price, D. 1984. Fusarium and plant pathology: the reservoir of infection. Pp. 71-93, in: The Applied Mycology of *Fusarium*: Symposium of the British Mycological Society, Queen Mary College, London, September 1982. Moss, M.O., and Smith, J.E., eds. Cambridge Univ. Press. Cambridge, England.

Pugsley, A.T. 1938. Root rot of onions. J. Dept. Agr. Vict. 36:320.

Puhalla, J.E. 1981. Genetic considerations of the genus *Fusarium*. Pp. 291-305, in: Fusarium: Diseases, Biology, and Taxonomy. Nelson, P.E., Toussoun, T.A., and Cook, R.J., eds. Pennsylvania State Univ. Press, University Park, PA.

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 Spieth, P.T. 1983. Heterokaryosis in *Fusarium moniliforme*. Exp. Mycology 7:328-335.

Puhalla, J.E., and Spieth, P.T. 1985. A comparison of heterokaryosis and vegetative

incompatibility among varieties of *Gibberella fujikuroi (Fusarium moniliforme)*. Exp. Mycology 9:39-47.

Qureshi, A.A., and Page, O.T. 1970. Observations on chlamydospore production by *Fusarium* in a two-salt solution. Can. J. Micro. 16:29-32.

Rabinowitch, H.E., and Brewster, J.L., eds. 1990. Onions and Allied Crops. Volume I. Botany, Physiology, and Genetics. CRC Press, Boca Raton, FL.

Racke, K.D. 1990. Pesticides in the soil microbial ecosystem. Pp. 1-12, in: Enhanced biodegradation of pesticides in the environment. Racke, K.D., and Coats, J.C., eds. American Chemical Society, Washington, DC.

Raillo, A.I. 1936. Systematics and methods for determination of the species of the genus *Fusarium*. Acta Bot. Inst. Acad. Sci. USSR Ser. 2, 3, 803-857.

Raillo, A.I. 1950. Fungi of the genus *Fusarium*. Government Publishers of Agriculture Literature, Moscow. 415 pages.

Ramsey, G.R., and Lorbeer, J.W. 1980. Seedborne fungi of onion in New York. (Abstr.) Phytopathology 70:468.

Ratnayake, M., Leonard, R.T., and Menge, J.A. 1978. Root exudation in relation to supply of phosphorus and its relevance to mycorrhiza formation. New Phytologist 81:543-552.

Reuven, M., Nitzani, Y., Szmulewich, Y., Ben-Yephet, Y., and Mor, Y. 1992. Distribution of *Fusarium oxysporum* and *Fusarium oxysporum* f. sp. *dianthi* inoculum in carnation greenhouses. Acta Hort. 307: 51-56.

Richard, C., Fortin, J.A., and Fortin, A. 1971. Protective effect of an ectomycorrhizal fungus against the root pathogen *Mycelium radicis atrovirens*. Can. J. Forest Res. 1:246-251.

Richardson, L.T., and Munnecke, D.E. 1964. Effective fungicide dosage in relation to inoculum concentration in soil. Can. J. Bot. 42:301-306.

Robertson, K.J., Gladder, P., and Wafford, J. 1987a. The evaluation of prochloraz, fenpropimorph and imazalil as drench treatments against pea foot rot. Tests of Agrochemicals and Cultivars 8:70-71.

Robertson, K.J., Gladders, P, and Wafford, J. 1987b. The evaluation of prochlorza, fenpropimorpe and imazalil as seed treatments against pea root rot. Tests of Agrochemicals and Cultivars 8:68-69.

Rosewich, U.L., Pettway, R.E., Katan, T., and Kistler, H.C. 1999. Population genetic analysis corroborates dispersal of *Fusarium oxysporum* f. sp. *radicis-lycopersici* from Florida to Europe. Phytopathology 89:623-630.

Rowe, R.C., Farley, J.D., and Coplin, D.L. 1977. Airborne spore dispersal and recolonization of steamed soil by *Fusarium oxysporum* in tomato greenhouses. Phytopathology 67:1513-1517.

Saindrenan, P. G., Darakis, G.A., and Bompeix, P. 1985. Determination of ethyl phosphite, phosphite and phosphate in plant tissues by anion-exchange high performance liquid chromatography and gas chromatography. J. Chromatog. 347:267-273.

Salgado, M., Schwartz, H.F., and Brick, M.A.. 1991. Dry bean genotypes resistant to Fusarium Wilt. Ann. Rept. Bean Improv. Coop. 34:54-55.

Samoucha, Y., and Cohen, L. 1985. Occurrence of metalaxyl-resistant isolates of *Pseudoperonospora cubensis* in Israel: a 5-year survey. EPPO Bull. 15:419-422.

Scher, F.M., and Baker, R. 1982. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to Fusarium wilt pathogens. Phytopathology 72:1567-1573.

Schippers, B., and Old, K.M. 1974. Factors affecting chlamydospore formation by *Fusarium solani* F. *cucurbitae* in pure culture. Soil Biol. Biochem. 6:153-160.

Schippers, B., and van Eck, W.H. 1981. Formation and survival of chlamydospores in *Fusarium*. Pp. 250-260, in: Fusarium: Diseases, Biology, and Taxonomy. Nelson, P.E., Toussoun, T.A., and Cook, R.J., eds. Pennsylvania State Univ. Press. University Park, PA.

Schippers, B., and Voetberg, J. S. 1969. Germination of chlamydospores of *Fusarium oxysporum* f. sp. *pisi* race 1 in the rhizosphere, and penetration of the pathogen into roots of a susceptible and a resistant pea cultivar. Neth. J. Plant Path. 75:241-258.

Schippers, B., Bakker, A.W., and Bakker, P.A.H.M. 1987. Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Ann. Rev. Phytopath. 25:339-358.

Schneider, R.W., Katan, Y., and Katan, T. 1992. *Fusarium oxysporum*: Genetic relations of pathogenic and nonpathogenic populations. Louisiana Agric. Exp. Sta. BARD Project no. US-1408-87. 24 pages w/ illustrations.

Schroth, M.N., and Hendrix, F.F., Jr. 1962. Influence of non-susceptible plants on the survival of *Fusarium solani* f. *phaseoli* in soil. Phytopathology 52:906-909.

Schwartz, H.F. 1986. Soil fumigation effects upon onion diseases and onion yield in Colorado, 1985. Fungicide and Nematicide Tests 41:57.

Schwartz, H.F., Westra, P., and Cranshaw, W. 1990. Colorado Integrated Pest Management. Colorado State University Cooperative Extension Bulletin 547A, Fort Collins, CO.

Schwartz, H.F., Bartolo, M.E., and Schweissing, F.C. 1991. Onion disease management - environmental damage study. Pp. 32-37, in: Nat. Onion Res. Conf. Proc., December 5 & 6, 1991, DeSoto Hilton, Savannah, Georgia.

Schwartz, H.F., and Bartolo, M.E., eds. 1995. Colorado Onion Production and Integrated Pest Management. Revised. Bulletin 547a. Colorado State University, Cooperative Extension, Fort Collins, CO. 40 pages.

Schwartz, H.F., and Mohan, S.K. , eds. 1995. Compendium of Onion and Garlic Diseases. APS Press, St. Paul, MN. 54 pages.

Schwinn, F.J., and Urech, P.A. 1986. Progress in the chemical control of diseases caused by Oomycetes. Pp. 89-106, in: Fungicide Chemistry: Advances and Practical Applications. Green, M.B., and Spilker, D.A., eds. American Chemical Society, Washington, DC.

Scovel, G. 1987. Improved agrotechnical and sanitation methods versus resistant cultivars as a means of avoiding *Fusarium* wilt. Acta Hort. 216:55-61.

Selby, A.D. 1910. A Brief Handbook of the Diseases of Cultivated Plant in Ohio. Ohio Agr. Expt. Sta. Bul. 214:304-456.

Shaner, G., Stromberg, E.L., Lacy, G.H., Barker, K.R., and Pirone, T.P. 1992. Nomenclature and concepts of pathogenicity and virulence. Annu. Rev. Phytopath. 30:47-66.

Sherbakoff, C.D. 1915. *Fusaria* of Potatoes. Cornell Agr. Exp. Sta. Mem. 6. 271 pages.

Sherf, A.F., and McNab, A.A. 1986. Chapter 13: Onion, garlic, leeks and shallots. Pp. 432-470, in: Vegetable Diseases and their Control. John Wiley & Sons, New York, N.Y. 728 pages.

Shimabukuro, R.H., Lamoureux, G.L., and Frear, D.S. 1982. Pesticide metabolism in plants: reactions and mechanisms. Pp. 21-66, in: Biodegradation of Pesticides. Matsuma, F., and Murti Krishna, C.R., eds. Plenum Press, New York, NY.

Shokes, F.M., and McCarter, S.M. 1979. Occurrence, dissemination , and survival of plant pathogens in surface irrigation ponds in southern Georgia. Phytopathology 69:510-516.

Short, G.E., and Lacy, M.L. 1974. Germination of *Fusarium solani* f. sp. *pisi* chlamydospores in the spermosphere of pea. Phytopathology 64:558-562.

Siddiqi, M.A., and Alexander, M. 1991. Use of Aliette, a basipetally translocated antimicrobial compound, to enhance rhizosphere colonization and nodulation by root-nodule bacteria. Biol. Fert. Soils 12:141-146.

Simeoni, LA. 1987. Critical iron level associated with biological control of Fusarium wilt. PhD dissertation, CSU, Fort Collins, CO. 63 pages.

Sinclair, W.A., Sylvia, D.M., and Larsen, A.O. 1982. Disease suppression and growth promotion in Douglas-fir seedlings by the ectomycorrhizal fungus *Laccaria laccata*. Forest Science 28:191-201.

Sisler, H.D. 1982. Biodegradation of agricultural fungicides. Pp. 133-155, in: Biodegradation of Pesticides. Matsumura, F., and Murti Krishna, C.R., eds. Plenum Press, New York, N.Y.

Sisler, H.D., and Cox, C.E. 1954. Effects of tetramethylthiuram disulfide on metabolism of *Fusarium roseum.* Amer. J. Bot. 41:338-385.

Smelt, J.H., Crum, S.J.H., and Teunissen, W. 1989. Accelerated transformation of the fumigant methyl isothiocyanate in soil after repeated application of metam-sodium. Environ. Sci. Health Bull. 24:437-455.

Snyder, W.C. 1981. Introduction. Pp. 3-8, in: Fusarium: Diseases, Biology, and Taxonomy. Nelson, P.E., Toussoun, T.A., and Cook, R.J., eds. Pennsylvania State Univ. Press, University Park, PA.

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

Snyder, W.C., and Hansen, H.N. 1941. The species concept in *Fusarium* with reference to section Martiella. Amer. J. Bot. 28:738-742.

Snyder, W.C., and Hansen, H.N. 1945. The species concept in Fusarium with reference to Discolor and other sections. Amer. J. Bot. 32:657-666.

Snyder, W.C., Hansen, H.N., and Oswald, J.W. 1957. Cultivars of the fungus, *Fusarium*. J. Madras Univ. B,2:185-192.

Snyder, W.C., and Smith, S.N. 1981. Current Status. Pp. 25 - 50, in: Fungal Wilt Diseases of Plants. Marshall, E.M., Bell, A.A., and Beckman, C.H., eds. Academic Press, Inc., New

York, NY.

Spurgeon, C. 1990. Metam-sodium -- aqueous photolysis at 25° C; Registration Document BASF 90/5038, ICI Americas, Inc., April 30, 1990.

Stein, J., and Urdang, L., eds. 1967. The Random House Dictionary of the English Language: The Unabridged Edition. Random House, New York, N.Y. 2059 pages.

Stoddard, D.L. 1947. Nitrogen, potassium and calcium in relation to *Fusarium* wilt of musk melon. Phytopathology. 37:875-884.

Stover, R.H. 1959. Studies on Fusarium wilt of banana. IV. Clonal differentiation among wild type isolates of *Fusarium oxysporum* f. sp. *cubense*. Can. J. bot. 37:245-255.

Straathof, Th.P., and Löffler, H.J.M. 1994. Resistance to *Fusarium oxysporum* at different developmental stages of Asiatic hybrid lilies. J. Amer. Soc. Hort. Sci. 119(5):1068-1072.

Sumner, D.R., Baird, R.E., Gitaitis, R.D., and Smittle, D.A. 1991. Fungi isolated from onions before and after controlled atmosphere storage in Georgia. Pp. 35-37, in: Nat. Onion Res. Conf. Proc., December 5 & 6, 1991, DeSoto Hilton, Savannah, Georgia.

Sussman, A.S. 1965. Dormancy of soil microorganisms in relation to survival. Pp. 99-109, in: Ecology of Soil-borne Plant Pathogens. Baker, K.F., and Snyder, W.C., eds. Univ. of California Press, Berkeley, CA.

Tammen, J. 1958. Pathogenicity of *Fusarium roseum* to carnation and to wheat. Phytopathology 48:423-426.

Tantaoui, A., Ouinten, M., Geiger, J-P. and Fernandez, D. 1996. Characterization of a single clonal lineage of *Fusarium oxysporum* f. sp. *albedinis* causing Bayoud disease of date palm in Morocco. Phytopathology 86:787-7902.

Taylor, A.G., and Harman, G.E. 1990. Concepts and technologies of selected seed treatments. Annu. Rev. Phytopath. 28:321-339.

Thornton, M.K., Mohan, S.K., and Torell, J. 1992a . Response of onion cultivars to basal plate rot and pink root, 1990. Biological and Cultural Tests 7:16.

Thornton, M.K., Mohan, S.K., and Torell, J. 1992b. Response of onion cultivars to basal plate rot and pink root, 1991. Biological and Cultural Tests 7:17.

Tio, M., Burgess, L.W., Nelson, P.E., and Toussoun, T.A. 1977. Techniques for the isolation, culture and preservation of the Fusaria. Aust. Plant Path. Soc. Newsl. 6:11-13.

Toth, K.R., and Lacy, M.L. 1991. Comparing vegetative compatibility and protein banding patterns for identification of *Fusarium oxysporum* f. sp. *apii* race 2. Can. J. Micro. 37:669-674.

Toussoun, T.A., and Nelson, P.E. 1976. A pictorial guide to the identification of Fusarium species, 2nd ed. Pennsylvania State Univ. Press, Univ. Park, PA. 43 pages.

Toyota, K., and Kimura, M. 1993. Colonization of chlamydospores of *Fusarium oxysporum* f. sp. *raphani* by soil bacteria and their effects on germination. Soil Biol. Biochem 25(2):193-197.

Toyota, K., and Kimura, M. 1994. Earthworms disseminate a soil-borne plant pathogen, *Fusarium oxysporum* f. sp. *raphani*. Biol. Fert. Soils 18:32-36.

Trappe, J.M., Molina, R., and Castellano, M. 1984. Reactions of mycorrhizal fungi and mycorrhiza formation to pesticides. Ann. Rev. Phytopath. 22:331-359.

Trujillo, E.E., and Snyder, W.C. 1963. Uneven distribution of *Fusarium oxysporum* f. *cubense* in Honduras soils. Phytopathology 53:167-170.

Turner, N.J., and Corden, M.E. 1963. Decomposition of sodium n-methyldithiocarbamate in soil. Phytopathology 53:1388-1394.

Ulloa, M., and Hanlin, R.T. 2000. Illustrated dictionary of mycology. APS Press, St. Paul, MN. 448 pages.

United States Environmental Protection Agency. Fosetyl-Al (Aliette) R.E.D. Facts. EPA-738-F090-100, January 1991.

United States Environmental Protection Agency. Metalaxyl R.E.D. Facts. EPA-736-F-94-013, September 1994.

United States Environmental Protection Agency. 1996. Notice of voluntary cancellation of Ciba Crop Protection registrations of metalaxyl technical and end-use products that contain metalaxyl. [OPP-66225; FRL 5364-7].

United States Standards for Grades of Bermuda-granex-grano type onions, 1995. USDA, Agricultural Marketing Service, Fruit and Vegetable Division, Fresh Products Branch.

VanDemark, P.J., and Batzing, B.L. 1987. The Microbes: An Introduction to Their Nature and Importance. Benjamin/Cummings Publishing Company, Inc., Menlo Park, CA. 991 pages.

Van Eck, W.H. 1976. Ultrastructure of forming and dormant chlamydospores of *Fusarium solani* in soil. Can. J. Micro. 22:1634-1642.

Vazquez, C., Reyes, F., and Martinez, M.J. 1993. Comparative study of pectic activities from different formae speciales of *Fusarium oxysporum*. Letters in Applied Microbiology 16: 210-213.

Venette, J.C., and Gross, P.L. 1991. Evaluation of seed treatments for control of seedling disease on dry edible beans, 1990. Fungicide and Nematicide Tests 46:271.

Venette, J.C., and Gross, P.L. 1992. Evaluation of in-furrow seed treatments for control of seedling disease and root rot on dry bean, 1991. Fungicide and Nematicide Tests 47:245.

Walker, A. 1987. Further observations on the enhanced degradation of iprodione and vinclozolin in soil. Pest. Sci. 21 (3):219-231.

Walker, A., and Welch, S.J. 1990. Enhanced biodegradation of dicarboxim fungicides in soil. Pp. 53-67, in: Enhanced Biodegradation of Pesticides in the Environment. Racke, K.D., and Coats, J.C., eds. American Chemical Society, Washington, DC.

Walker, J.C. 1919. Onion diseases and their control. Farmers Bull. No. 1060. U.S.D.A. 26 pages.

Walker, J.C., and Tims, E.C. 1924. A *Fusarium* bulb rot of onion and the relation of environment to its development. J. Agr. Res. 28:683-694.

Warcup, J.H. 1955. On the origin of colonies of fungi developing on soil dilution plates. Brit. Mycol. Soc. Trans. 38:298-301.

Warcup, J.H. 1965. Growth and reproduction of soil microorganisms in relation to substrate. Pp. 52-688, in: Ecology of Soil-Borne Plant Pathogens; Prelude to Biological Control. Baker, K.F., Snyder, W.C., Baker, R.R., Menzies, J.D., Clark, F.E., Miller, L.I., Dimock, A.W., Patrick, Z.A., Kreutzer, W.A., and Rubo, M., eds. Univ. of California Press, Berkely, CA.

Ware, G.W. 1991. Fundamentals of Pesticides: A Self-instruction Guide. Third Edition, Thomson Publications, Fresno, CA. 307 pages.

Watanabe, T. 1994. Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species. Lewis Publishers, Boca Raton, FL. 411 pages.

Wensley, R.N., and McKeen, C.D. 1962. A soil suspension-plating method of estimating populations of *Fusarium oxysporum* f. *melonis* in muskmelon wilt soils. Can. J. Micro.

8:57-68.

Wensley, R.N., and McKeen, C.D. 1964. Some relationships between plant nutrition, fungal populations, and incidence of *Fusarium* wilt of muskmelon. Can. J. Micro. 11:581-594.

Wickramasinghe, R.H. 1990. The Cytochrome P-450 Proteins: Environmental and General Aspects. 295 pages.

Williams, D.J., Beach, B.G.W., Horriere, D, and Marechal, G. 1977. LS 74-783, a new systemic fungicide with activity against phycomycete diseases. 1977 Brit. Crop Prot. Conf. Proc. 2:565-573.

Williams, E. 1988. Effect of fungicide seed treatments on wheat seedling emergence, 1987. Fungicide and Nematicide Tests 43:249.

Windels, C.E. 1991. Current status of Fusarium taxonomy. Phytopathology 81(9):1048-1051.

Wolfe, M.S., and Barrett, J.A., 1986. Response of plant pathogens to fungicides. Pp. 245-346, in: Pesticide Resistance: strategies and tactics for management. National Academy Press, Washington, DC.

Wollenweber, H.W. 1913. Studies on the Fusarium problem. Phytopathology 3:24-51.

Wollenweber, H..W., and Reinking, O.A. 1935 Die Fusarien, ihre Beschreibung, Schadwirkiung und Kekampfung. Berlin: Paul Parey. 355 pages.

Wollenweber, H.W., Sherbakoff, C.D., Reinking, O.A., Johann, H., and Bailey, A.A. 1925. Fundamentals for taxonomic studies of *Fusarium*. J. Agr. Res. 30:833 -843.

Woltz, S.S. 1974. Gladiolus Fusarium disease: assay of soilborne inoculum potential and cultivar susceptibility. Plant Dis. Rept. 58(2):184-187.

Woltz, S.S., and Jones, J.P. 1973. Interactions in source of nitrogen fertilizer and liming procedure in the control of Fusarium wilt of tomato. HortScience, 8(2):137-138.

Woo, S.L., Zoina, A., Del Sorbo, G., Lorito, M., Nanni, B., Scala, F., and Noviello, C. 1996. Characterization of *Fusarium oxysporum* f. sp. *phaseoli* by pathogenic races, VCGs, RFLPs and RAPD. Phytopathology 86:966-973.

Woolliams, B.G.E. 1928. Fusarium bulb-rot of onions at Summerland, B.C. Pp. 189-192, in: Report of Dominican Botanist for the year 1927. Div. of Botany, Canada Dept Agr.

Worthing, C.R., ed. 1991. The Pesticide Manual: A World Compendium, 9th ed. British Crop Protection Council, Surrey, U.K. 1141 pages.

Woudt, L.P., Neuvel, A., Sikkema, A., van Grinsven, M.Q.J.M., de Millian, W.A.J., Campbell, C.L., and Leslie, J.F. 1995. Genetic variation in *Fusarium oxysporum* from cyclamen. Phytopathology 85:1348-1355.

Wright, G.F.K., Guest, D.I., Wimalajeewa, D.L.S., and van Heeswijck, R. 1996. Characterisation of *Fusarium oxysporum* isolated from carnation in Australia based on pathogenicity, vegetative compatibility and random amplified polymorphic DNA (RAPD) assay. Eur. J. Plant Path. 102:451-457.

Yoo, S.J., Watanabe, H. Kobayashi, K., Ogoshi, A., and Kodama, F. 1993. [Vegetative compatibility grouping of *formae speciales* of *Fusarium oxysporum* pathogenic to the Liliaceae.] Ann. Phytopath. Soc. Jpn. 59:3-9. (In Japanese).

Zak, B. 1964. Role of mycorrhizae in root disease. Ann. Rev. Phytopath. 2:377-392.

Appendix: Media Recipes

Ammonium Tartrate (NH_4) - Phenotyping Medium

ammonium tartrate	1.0 g
sucrose	30.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
KCl	0.5 g
Agar (Bacto)	20.0 g
trace element solution	0.2 ml
distilled water	1 liter

Carnation-leaf Agar [93]

This medium is prepared by placing several sterile carnation leaf pieces in a petri dish on a 2% water agar cooled to 45 C. Radiated leaf pieces were purchased from Dr. Paul Nelson, Pennsylvania State University, University Park, PA.

L-asparagine Chlorate Medium (minimal medium supplemented with L-asparagine)

L-asparagine	1.6 g
$KClO_3$	15 g for 1.5% chlorate; 20 g for 2.0% chlorate; 30 g for 3.0%, etc.
$NaNO_3$	2.0 g
sucrose	30.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
KCl	0.5 g
Agar (Bacto)	20.0 g
trace element solution	0.2 ml
distilled water	1 liter

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[93] Nelson et al., 1983. *Fusarium* species: An Illustrated Manual for Identification. Pennsylvania State Univ. Press, University Park, PA.

Complete Medium

NaNO2	2.0 g
N-Z caseine	2.5 g
yeast extract	1.0 g
sucrose	30.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
KCl	0.5 g
Agar (Bacto)	20.0 g
trace element solution	0.2 ml
vitamin stock solution	10.0 ml
distilled water	1 liter

Hypoxanthine (HX) - Phenotyping Medium

hypoxanthine	0.2 g
sucrose	30.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
KCl	0.5 g
Agar (Bacto)	20.0 g
trace element solution	0.2 ml
distilled water	1 liter

Komada Medium[94]

K_2HPO_4	1.0 g
KCl	0.5 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Fe-Na-EDTA	0.01 g
L - Asparagine	2.0 g
D-Galactose	20.0 g
distilled water	1 liter

the following is added when the basal medium is autoclaved and cooled below 50 ° C

PCNB (75% WP) 1.0 g

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[94] Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soils. Rev. Plant Protec. Res. 8:114-125.

Oxgall [95]	0.5 g
$Na_2B_4O_4 \cdot 10\ H_2O$	1.0 g
Streptomycin sulfate	0.3 g

the medium is then adjusted to pH 3.8 ±0.2 with a 10% solution of phosphoric acid

L-threonine chlorate medium (minimal medium supplemented with L-threonine[96])

L-threonine	2.3 g
sucrose	30.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
KCl	0.5 g
Agar (Bacto)	20.0 g
trace element solution	0.2 ml
distilled water	1 liter

Potato Dextrose Chlorate Medium

Potato Dextrose Agar	39 g
Potassium chlorate ($KClO_3$)	15 or 30 g (for 1.5% or 3% chlorate, respectively)
Distilled water	1 liter

Sodium Nitrate ($NaNO_3$) - Minimal Medium & Phenotyping Medium

$NaNO_3$	2.0 g
sucrose	30.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
KCl	0.5 g
Agar (Bacto)	20.0 g
trace element solution	0.2 ml
distilled water	1 liter

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[95] Oxgall is found to suppress rapidly growing saprophytic fungi (Littman, 1947)

[96] Hawthorne and Rees-George, 1996.

Sodium Nitrite - Phenotyping Medium

$NaNO_2$	2.0 g
sucrose	30.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
KCl	0.5 g
Agar (Bacto)	20.0 g
trace element solution	0.2 ml
distilled water	1 liter

Trace Element Solution

citric acid	5 g
$ZnSO_4 \cdot 6H_2O$)	5 g
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	1 g
$CuSO_4 . 5H_2O$	250 mg
$MnSO_4$	50 mg
H_3BO_3 (boric acid)	50 mg
$Na_2MoO_4 . 2H_2O$	50 mg
distilled water	95 ml

To reduce contamination store at 2-5 ° C

Vitamin Stock Solution

B1	thiamine	100 mg
B2	riboflavin	30 mg
B6	pyridoxine	75 mg
B5	Ca pantothenate	200 mg
B3	nicotinamide	75 mg
C	ascorbic acid	50 mg
	p-aminobenzoic acid	5 mg
	choline-cl	200 mg
	folic acid	5 mg
	biotin	5 mg
	inositol	4 g
	50% ethanol	1 liter

To reduce contamination store at 2-5 ° Cs