AN ABSTRACT OF THE THESIS OF

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Title: The Roles of Verticillium dahliae, Colletotrichum

atramentarium, Erwinia carotovora subsp. carotovora

and E. carotovora subsp. atroseptica in "Early Dying"

Disease of Potatoes

Abstract approved: Redacted for Privacy
                          Mary L. Powelson

The primary cause of "early dying" disease of potatoes in

Oregon is Verticillium dahliae; however, Colletotrichum atramen-

tarium, Erwinia carotovora subsp. carotovora (Ecc) and E. caro-

tovora subsp. atroseptica (Eca) are also commonly isolated from

diseased plants. Therefore, field and greenhouse studies were

initiated to determine the relative involvement of each pathogen,

directly and/or interactively with V. dahliae in the disease.

Pathogenicity studies with 'Russet Burbank' potatoes were carried

out in greenhouse pot culture to determine if C. atramentarium,

Ecc and Eca can cause "early dying" disease similar to that caused

by V. dahliae. Fifty-three days after root-dip inoculation, plants

inoculated with Ecc, Eca or V. dahliae exhibited typical "early

dying" disease symptoms, whereas those inoculated with C. atramen-

tarium did not. Symptoms caused by V. dahliae were more severe

than those caused by Ecc or Eca. In field studies with 'Norgold

Russet' potatoes in 1980 (Columbia Basin and Klamath Basin) and

in 1981 (Columbia Basin and Wallowa Valley), plant infection by V. dahliae was most strongly associated with symptom expression in plots previously cropped several times to potatoes; whereas plant infection by Ecc or Eca was most strongly associated with symptom expression in plots previously cropped one year or less to potatoes. In basal stem isolations made late in "early dying" disease epidemics, reductions were observed in the recovery of Eca and of V. dahliae. Decreases in Eca recovery were linked to plant infection by V. dahliae, whereas decreases in V. dahliae recovery were caused by basal plant infection by C. atramentarium. In co-inoculation studies the severity of symptom expression in V. dahliae inoculated 'Russet Burbank' was increased by Eca or Ecc, but was not influenced by C. atramentarium. The effect of V. dahliae and Eca, or V. dahliae and Ecc, on symptom expression was additive, rather than synergistic, and was due to an enhancement of stem colonization by V. dahliae.

The Roles of <u>Verticillium</u> <u>dahliae</u>, <u>Colletotrichum</u> <u>atramentarium</u>,

<u>Erwinia</u> <u>carotovora</u> subsp. <u>carotovora</u> and <u>E</u>. <u>carotovora</u> subsp.

<u>atroseptica</u> in "Early Dying" Disease of Potatoes

by

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Typed by Dianne Lee Webster for Marjorie Lynn Kirkland.

Dedicated to my friend and mentor,

Dennis White

whose enthusiasm led me to a greater appreciation

of science and to a love of research

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

THE ROLES OF VERTICILLIUM DAHLIAE, COLLETOTRICHUM ATRAMENTARIUM,

ERWINIA CAROTOVORA SUBSP. CAROTOVORA AND E. CAROTOVORA

SUBSP. ATROSEPTICA IN "EARLY DYING" DISEASE OF POTATOES

INTRODUCTION

Oregon ranks fourth in potato (Solanum tuberosum L.) production

in the United States and, of the field crops in Oregon, potato is

ranked second only to wheat in terms of crop value. According to

Oregon State University Extension Service figures, a total of ca.

19,000 ha of potatoes, valued at ca. $90,000,000, was harvested

in Oregon in 1980 (Anonymous, 1981). Approximately 48% of this

acreage was in commercial production in Umatilla and Morrow

counties, which are located in the Columbia Basin, a warm growing

region with a mean annual temperature of ca. 12 C. Due to the

highly sandy soils of this region, crops are irrigated with center-

pivot systems and production costs are high, necessitating high

yields. Out of the remaining 9900 ha in potato production in

Oregon, ca. 3300 ha and 200 ha, respectively, were harvested in

1980 in the Klamath Basin and Wallowa Valley, both of which are

cool growing regions with mean annual temperatures of ca. 8 C

and with crops primarily under wheel-line irrigation.

"Early dying" disease of potatoes, which is widespread in Oregon

(McKay, 1926; Powelson, 1978; Young 1956; Young, 1958), may cause

yield reductions of ca. 50% in the Columbia Basin in both 'Russet

Burbank' and 'Norgold Russet', which are the two leading cultivars

grown in the state (Powelson, 1980a). Significant yield reductions

also have been reported for the Klamath Basin of Oregon (Young, 1958) and for Idaho (Guthrie, 1960).

"Early dying" disease, which, as its name suggests, causes the plant to senesce prematurely, is characterized by a progressive foliar chlorosis followed by necrosis of the leaves, beginning at the base of the plant (Guthrie, 1960; Isaac and Harrison, 1968; McKay, 1926; Nielsen, 1948; Robinson et al, 1957; Rudolph, 1931). Vascular necrosis, unilateral symptom expression, stunting and wilt may also be associated with the disease (Guthrie, 1960; Isaac and Harrison, 1968; McKay, 1926; Nielsen, 1948; Robinson et al, 1957; Rudolph, 1931); however, in the field these symptoms have been either variable or only occasionally observed in diseased plants in Oregon (McKay, 1926; Powelson, personal communication). A major area of confusion in diagnosis of this disease transpires because foliar chlorosis and necrosis, the primary symptom of "early dying" disease, may be a secondary symptom in a number of other potato diseases such as blackleg, stem soft rot and Sclero-tinia stalk rot (Bonde, 1960; Harrison and Nielsen, 1981; Morse, 1909; Powelson, 1981; Raeder, 1944). In addition, early stages of the disease can be difficult to diagnose because in normal healthy plants the lower leaves, or those on or near the soil surface, become chlorotic, then necrotic and eventually are "sloughed-off" (Harrison and Isaac, 1968; Rudolph, 1931). This is due to shading of the leaves lowest in the canopy (Milthorpe, 1963) and/or to an inability of older leaves to successfully compete for nutrients with more actively growing regions (Watson, 1963).

In 'Norgold Russet', which has a determinant growth habit, this "sloughing-off" generally occurs only in the lower leaves; whereas in 'Russet Burbank', which has an indeterminant growth habit, "sloughing-off" of leaves may occur anywhere along the stem where leaves are on or near the soil surface. In healthy appearing plants, however, progressive foliar chlorosis and necrosis is delayed until the onset of natural senescence, whereas in diseased plants symptom expression increases rapidly with little or no delay (Isaac and Harrison, 1968; Rudolph, 1931).

In disease surveys conducted in Idaho (Nielsen, 1948) and in North Dakota (Gudmestad et al, 1977) Verticillium spp. and Fusarium spps. were isolated from potato plants. In the above early Idaho survey the predominant pathogen isolated from diseased plants was identified as V. albo-atrum, however, relative frequencies of dauermycelial and microscloerotial forms were not quantified. Representative isolates of both forms were tested and found to be pathogenic on potato. In the North Dakota survey, in which both diseased and healthy appearing plants were sampled, the predominant pathogen isolated was Fusarium spp., with V. dahliae Kleb. second. However, representative isolates of Fusarium spp. were not tested for pathogenicity. As it is not uncommon to isolate non-pathogenic Fusarium spp. from the vascular tissue of potato stems and tubers (McLean and Walker, 1941; Nielsen, 1948; Raeder and Kraus, 1943), the relative importance of these two genera in association with "early dying" disease in North Dakota is still unclear. Nevertheless, in Oregon the predominant pathogen

associated with the disease is V. dahliae (McKay, 1926; Powelson, 1978). In 1979, 1980 and 1981, Fusarium spp. were rarely isolated from diseased potato plants in Oregon.

Numerous pathogens interact with V. dahliae and/or V. albo-atrum in eliciting disease responses in potato and other hosts. Martin et al (1982) reported a positive interaction, ranging from synergistic to not quite additive, between V. dahliae and P. pene-trans in inducing "early dying" disease symptoms in potato. Muller (1977) noted both positive (P. penetrans and P. vulnus) and negative (P. crenatus, P. fallax and P. thornei) interactions with V. dahliae in wilt induction in Impatiens balsamina. Tylen-chorus capitatus and Meloidogyne incognita increased incidence and severity of Verticillium wilt in tomato (Overman and Jones, 1970). Khoury (1970) reported that both M. incognita acrita and Rhizoctonia solani increased the susceptibility of cotton to V. albo-atrum. In maple and eggplant, Catani and Peterson (1967) noted an antagonism between V. dahliae and each of three other fungi, Aspergillus fumigatus, Trichoderma lignorum and Gliocladium roseum. Co-inoculation of cotton with Fusarium oxysporum f. sp. vasinfectum and V. dahliae increased the severity of wilt symptoms (Al-Shukri, 1968). Reports of an interaction between V. dahliae and Colletotrichum atramentarium (Berk. & Br.) Taub. in potatoes suggest that the situation may not be a simple one: Davis and Howard (1976) reported an increase in vine death in co-infected plants, and Otazu et al (1978) noted a possible positive association between the two; however, Goodell et al (1982) noted a suppression

in co-incidence of the two pathogens in basal stem assays. The
interaction between Thielaviopsis basicola and V. dahliae in
cotton also appears to be a complex situation: Schnathorst
(1964) reported an increase in sudden wilt and defoliation where
the two pathogens coincided; and Mathre et al (1967), while not
noting any increase in symptom severity in individual plants,
reported earlier onset of wilt symptoms with lower T. basicola
populations, but with higher T. basicola populations onset was
delayed and symptom incidence was reduced. Erwinia carotovora
subsp. carotovora (Ecc) (Jones) Dye reportedly increased potato
stem colonization by V. dahliae (Rahimian and Mitchell, 1981),
as did potato virus X (Goodell et al, 1982).

Pathogens other than Verticillium spp. and Fusarium spp. have
been implicated in "early dying" disease of potatoes. These
include Pratylenchus penetrans (Martin et al, 1982; Morsink, 1967;
Rowe and Riedel, 1975), non-Pratylenchus nematode species (Hoyman
and Dingman, 1967; Weingartner et al, 1974), C. atramentarium
(Davis and Howard, 1967; Gudmestad et al, 1977; Mitchell et al,
1981; Otazu et al, 1978; Thirumalachier, 1967) and Ecc (Mitchel
et al, 1981; Powelson, 1979). Many of these pathogens have been
implicated as playing an indirect, or interactive role with
V. dahliae and/or V. albo-atrum, rather than a direct role, in
disease development. As C. atramentarium, Ecc and E. carotovora
subsp. atroseptica (Eca) (van Hall) Dye are frequently isolated
from potato plants in Oregon (Powelson, 1979), it was considered
desirable to study the involvement of these pathogens in "early

dying" disease of potatoes in Oregon.

C. atramentarium is known to cause "black dot" disease of potatoes (Dickson, 1926; Harrison, 1963) and has been implicated in a skin necrosis of potato tubers (Mooi, 1959); however, to date, it has not been reported to be capable of causing the progressive foliar chlorosis and/or necrosis, in the absence of any other primary symptoms, that is typical of "early dying" disease. In tomatoes this pathogen is weakly parasitic on stems, leaves and green fruit, only becoming aggressive on ripe fruit (Kendrick and Walker, 1948). Nevertheless, wounding of the ripe fruit is required for formation of typical anthracnose type lesions. In potatoes C. atramentarium is considered by some to be a weak parasite (Gudmestad et al, 1977; Otazu et al, 1978), whereas others have intimated that it may be more aggressive (Davis and Howard, 1976; Stevenson et al, 1976).

Ecc and Eca are soft-rotting, gram negative bacteria which cause a variety of field symptoms in potatoes. These include seedpiece decay (Bonde, 1960; Harrison and Nielsen, 1981), blackleg (Bonde, 1960; Harrison and Nielsen, 1981; Leach, 1930; Molina and Harrison, 1977; Morse, 1909; Powelson, 1980b; Stanghellini and Meneley, 1975), below ground stem rot (Harrison and Nielsen, 1981) and stem soft rot (Powelson, 1980b).

The major objectives of this research were (1) to determine whether C. atramentarium, Ecc and/or Eca can cause typical "early dying" disease symptoms in potatoes and, if so, to compare their relative importance to V. dahliae, and (2) to describe interactions,

if existent, between $\underline{V}$. dahliae and $\underline{C}$. atramentarium, $\underline{Ecc}$ or $\underline{Eca}$. In an attempt to realistically define the involvement of each pathogen in "early dying" disease epidemics as they may actually occur in growers' fields, studies were conducted in the field, using standard farming practices, as well as in the controlled environment of the greenhouse.

MATERIALS AND METHODS

Field Experiments

<u>1980 Columbia Basin and Klamath Basin plots</u>. A single seed

lot of 'Norgold Russet' and a single seed lot of 'Russet Burbank'

were planted in separate blocks at each of three locations in

Oregon in 1980. The two plots in the Columbia Basin (RF and EF)

were planted on April 4 and the plot in the Klamath Basin (KF)

was planted on June 6. Each plot consisted of 17 sampling units;

each unit was a single row, 3.3 m in length. Spacing of seed-

pieces within a row was 23 cm and between rows was 86 cm. Plot

KF, located in a solid-set irrigated field, had previously been

cropped ca. eight times to potatoes. Plot RF was located in a

field which had previously been cropped three times to potatoes,

and plot EF was located in a field which had never been cropped

to potatoes. The latter two fields were center-pivot irrigated

circles. Potatoes at the KF and RF plots had been grown in rotation

with winter wheat. Prior to 1980 at the EF plot, alfalfa was grown

for six years on previously undeveloped desertland. Plots were

managed according to the standard farming practices of each

locality.

Disease readings were made in the 'Norgold Russet' blocks on

a tri-weekly basis beginning in early June at the RF plot, in late

June at the EF plot, and in late July at the KF plot. Readings

were continued until the crop was dead or ready to be harvested.

Incidence of "early dying" disease was visually estimated from

the percent diseased hills; a hill was determined to be diseased if at least one plant in that hill exhibited typical "early dying" disease symptoms. Whenever disease readings were made five or ten 'Norgold Russet' plants were randomly pulled from within each sampling unit. No more than one plant was pulled from a single hill at a single sampling date. Although disease readings were not made in the 'Russet Burbank' blocks, plants were pulled on all of the above sampling dates, with the exception of the first dates for the RF and KF plots. Except during transport at ambient temperatures, sampled plants were stored at 5 C until they could be assayed for V. dahliae, C. atramentarium, Ecc and Eca, and for internal stem populations of V. dahliae, taken to represent the relative amount of vascular colonization.

Immediately prior to planting, 12 soil samples were taken from the 5 to 20-cm depth at the RF and EF plots. Following the Anderson Sampler technique of Butterfield and DeVay (1977), 67 mg subsamples were plated onto a selective pectate medium (NPX) to assay for soil populations of V. dahliae. Also prior to planting, tubers were sampled from each seed lot and assayed for Ecc and Eca (Powelson, 1981). Using Ouchterlony double diffusion (Ouchterlony, 1958) isolates were tested for identity to known Erwinia serogroups (DeBoer et al, 1979; DeBoer, personal communication; Powelson, personal communication). Seed lots were assayed to determine if subsequent stem isolates of Ecc and Eca were serologically similar to isolates recovered from the seed tubers.

1981 Columbia Basin plot. A center-pivot irrigated circle in Oregon's Columbia Basin, which was undeveloped desertland in 1980, was planted to 'Norgold Russet' on March 15, 1981, and was managed according to the standard farming practices of the region. Beginning in late June, plants that exhibited typical "early dying" disease symptoms were sampled every 7 to 10 days until harvest. Except during transport at ambient temperatures, sampled plants were stored at 5 C until they could be assayed for V. dahliae, C. atramentarium, Ecc and Eca. Adjacent 6-m row lengths were sampled on consecutive sampling dates.

1981 Wallowa Valley plot. A wheel-line irrigated field in the Wallowa Valley of Oregon was planted to 'Norgold Russet' on June 20, 1981 and managed according to the standard farming practices of the region. Prior to 1981 at this plot, wheat had been grown for a total of three years, interrupted by croppings to potatoes in 1979 and to barley in 1978, on land that had previously been in blue grass pasture for ca. 60 years. In early September all plants were pulled from ten hills where at least one stem in each was exhibiting typical "early dying" disease symptoms. However, if any plant within a hill exhibited blackleg, stem soft rot or below ground stem rot, that hill was not considered to be exhibiting typical "early dying" disease symptoms and, therefore, was not sampled. Except during transport at ambient tempertures, sampled plants were stored at 5 C until they could be assayed for V. dahliae, C. atramentarium, Ecc and Eca.

Assay for Erwinia carotovora subsp. carotovora and E. carotovora subsp. atroseptica. The below ground portion of the main stem was

thoroughly rinsed in running tap water and a 1.5-cm segment from

just below the soil line was macerated with a sterile razor blade

and placed into 2 ml of distilled water. The suspension was vor-

texed and allowed to stand for 2 to 4 hr. An aliquot was then

streaked onto crystal violet-pectate medium (CVP) and incubated

in the dark at 24 C for 40 to 48 hr (Cuppels and Kelman, 1974).

If present, at least one typical E. carotovora-like colony was

subcultured onto fresh CVP medium, and from this a single colony

was transferred for maintenance onto a sugarless-nutrient medium

(SNA) consisting of 1.5 g beef extract, 5.0 g peptone and 10.0 g

Difco agar flake in 500 ml distilled water and autoclaved for 20

min. Each isolate was tested for ability to rot a potato tuber

slice. Two additional tests, acid production from α-methyl

glucoside (Graham, 1972) and growth at 36 C, were used to differ-

entiate between Ecc and Eca. Using Ouchterlony double diffusion,

isolates were tested for identity to serogroups developed from

known tuber and soil-borne E. carotovora isolates.

Assay for Verticillium dahliae and Colletotrichum atramentarium.

A 2.5-cm segment of the main stem removed from just above the soil

line was surface sterilized by a 3-min immersion in 0.5% sodium

hypochlorite followed by removal of the epidermis. Three 4 to 5-mm

thick cross-sections of the segment were placed onto streptomycin-

ethanol water agar (Nadakavukaren and Horner, 1959), incubated in

the dark at 22 C for seven days and then, using a dissecting micro-

scope, were scanned for structures characteristic of each fungus.

Incidence of V. dahliae and C. atramentarium was indicated by the

presence of at least one microsclerotium or acervulus, respectively, in or on at least one cross-section.

Assay for internal stem populations of Verticillium dahliae (Davis et al, 1977). The above ground portion of the main stem was air-dried at ca. 24 C for at least 3 mo and a 5-cm segment from within 10 to 15-cm of the stem apex was ground. Two 10-mg subsamples were each plated onto NPX medium and incubated in the dark at 22 C. After 2 wks, the surface of the medium was washed with tap water to facilitate observation and identification of microsclerotia embedded in the medium. With the aid of a dissecting microscope, colonies of V. dahliae were counted; each colony was presumed to have arisen from a single propagule, or colony forming unit (cfu).

Greenhouse Experiment

Preparation of inoculum. The isolates of V. dahliae, C. atramentarium, Ecc and Eca used in this experiment were isolated from potato plants exhibiting typical "early dying" disease symptoms and purified. The Ecc and Eca isolates were serologically typed to serogroups XXIX and I, respectively (DeBoer et al, 1979; DeBoer, personal communication). V. dahliae conidia were harvested from 7-day-old cultures grown in the dark on yeast extract-lactose medium (Bernearts and DeLeg, 1963). C. atramentarium conidia were harvested from 3-day-old cultures grown under cool white fluorescent lamps on V-8 medium (Barksdale, 1967) clarified by first autoclaving for 5 min and then centrifuging at 5000 rpm for 15 min a mixture of 1.5 g calcium carbonate and 100 ml V-8

juice. <u>Ecc</u> and <u>Eca</u> cells were harvested from 2-day-old cultures grown in the dark on casamino acid-peptone-glucose medium (Cuppels and Kelman, 1974). Conidia and bacterial cells were suspended in sterile 0.1 M phosphate buffer-saline solution (Lazar, 1972) (PBS) and suspension concentrations were adjusted to give two different inoculum densities for each pathogen, 3.7 X 10^5 cfu/ml and 3.7 x 10^7 cfu/ml. Approximately 24 hr later, just prior to inoculation, three pathogen combinations (<u>V</u>. <u>dahliae</u> and <u>C</u>. <u>atramentarium</u>, <u>V</u>. <u>dahliae</u> and <u>Ecc</u>, and <u>V</u>. <u>dahliae</u> and <u>Eca</u>) were each prepared at two inoculum densities. Low and high inoculum densities consisted of equal parts of the two pathogens both at 3.7 x 10^5 cfu/ml and 3.7 x 10^7 cfu/ml, respectively.

<u>Rooting of stem cuttings and inoculation procedure</u>. Cuttings, 8 to 10-cm in length, were made from stem apices of healthy appearing 'Russet Burbank' plants. Cut ends were dipped in Rootone F (Amchem Products, Inc., Fremont, CA 94536) to stimulate rooting and then placed through holes cut in the bottom of inverted 12-oz styrofoam cups. The cups were placed 60 cm beneath an equal mixture of cool white and gro-lux fluorescent lamps and subjected to 30 sec misting at 5 min intervals with 16-hr days and a day/night temperature regime of ca. 22/18 C. After 2 wks, healthy appearing cuttings with ample root systems were harvested and, immediately prior to inoculation, the root tips were excised. Cut roots of ca. 25 cuttings were immersed for 1.5 hr in one of the 15 treatments. The treatments consisted of a sterile PBS control, low and high inoculum densities for each pathogen, and low and high inoculum

densities for each pathogen combination. During inoculation

the cuttings were placed 50-cm beneath an equal mixture of cool

white and gro-lux fluorescent lamps to stimulate photosynthesis

and respiration and, thus, uptake of inoculum in the xylem

vessels. Immediately following inoculation the roots were

rinsed and, to allow the cuttings to recover from the wilting

incurred during inoculation, the roots were placed in fresh

tap water, using separate containers for each treatment, and

cuttings were again subjected to the intermittant misting for 45

min prior to planting. To determine whether the inoculum had

reached the stem apex, four cuttings per treatment were randomly

sampled prior to planting. These were stored in sealed plastic

bags containing damp paper towels and placed in the dark at 22 C

until isolations could be made. Two days later, 2-cm segments

from both the base and the apex of each cutting were assayed for

V. dahliae, C. atramentarium, Ecc and Eca.

Planting, maintenance and harvest. Inoculated plants were

grown three plants per 15-cm-diameter pot containing silica sand,

and pots were completely randomized on greenhouse benches. Three

days after planting, daylight was supplemented with an equal

mixture of cool white and gro-lux fluorescent lamps placed 120-cm

above the benches, and 16-hr days were instituted. The day/night

temperature regime was ca. 22/18 C. Pots were watered twice

daily with a modified Hoagland's solution (Hoagland and Arnon, 1938)

consisting of 2.36 g/l $Ca(NO_3)_2 \cdot 4H_2O$, 1.01 g/l KNO_3, 0.986 g/l

$MgSO_4 \cdot 7H_2O$, 0.272 g/l KH_2PO_4, 3.33 X 10^{-2} g/l 6% iron chelate

(Ciba Geigy, Greensborough, NC 27409), 5.72 X 10^{-3} g/l H_3BO_3,
3.62 X 10^{-3} g/l $MnCl_2 \cdot 4H_2O$, 4.44 X 10^{-4} g/l $ZnSO_4 \cdot 7H_2O$, 1.58 X
10^{-4} g/l $CuSO_4 \cdot 5H_2O$ and 3.08 X 10^{-5} g/l MoO_3 (99.5%). Periodi-
cally, as needed, plants were sprayed with diazinon (O,O-diethyl
O-[6-methyl-2-(1-methyletheyl)-40-pyrimidinyl] phosphoro =
thioate) to control insect pests. To reduce variability due
to shading and internal competition from secondary shoot growth,
each main stem was staked and all side stems removed ca. 30 days
from planting. At this time pots were re-randomized. New shoots
were removed weekly during subsequent plant growth.

Fifty-three days after inoculation, at bloom initiation, each
plant was visually assessed, on a scale of 0, 1, 5, 10, 25 and 50,
for the percent of total leaf area that exhibited chlorosis and/or
necrosis. (No plants exhibited more than 50% chlorotic and/or
necrotic leaf area.) Percent diseased leaf area was estimated
by subtracting the average visual assessment of percent foliar
chlorosis and/or necrosis of the control treatment from the
average visual assessment of another treatment. Three days
following the visual assessment, the basal 25-cm of each plant's
main stem was harvested, immersed for 30-sec in 0.25% sodium
hypochlorite to reduce surface contamination, and thoroughly
rinsed in running tap water. From each stem two 5-cm segments,
one from 0 to 5-cm above the root ball and the other from 15 to
20-cm above the root ball, were sampled and assayed for V. dahliae,
C. atramentarium, Ecc and Eca.

Assay for <u>Verticillium</u> <u>dahliae</u>, <u>Colletotrichum</u> <u>atramentarium</u>,

<u>Erwinia</u> <u>carotovora</u> subsp. <u>carotovora</u> and <u>E</u>. <u>carotovora</u> subsp.

<u>atroseptica</u>. Three random 4 to 5-cm thick cross-sections from

each stem segment were placed on streptomycin ethanol water

medium and incubated in the dark at 22 C for 7 days. With the

aid of a dissecting microscope, cross-sections were indexed for

microsclerotial and acervuli production to estimate the extent

of stem colonization by <u>V</u>. <u>dahliae</u> and <u>C</u>. <u>atramentarium</u>,

respectively. Indexing consisted of a visual rating of the

relative amount of total cross-section tissue within which

microsclerotia or upon which acervuli could be found. Rating

was on scales of 0 to 12 for <u>V</u>. <u>dahliae</u> and 0 to 9 for <u>C</u>. <u>atra-

mentarium</u>. Zero indicated complete absence of the respective

structures, 12 indicated the presence of microsclerotia in all

tissues of all three segments, and nine indicated the presence

of acervuli on all surfaces. The remaining cross-sections from

each stem segment were placed in sterile PBS (1 ml per cm of

stem segment). Suspensions were vortexed for 20 sec and then

allowed to stand at 22 C. Approximately 25 hr later the suspensions

were again vortexed and aliquots were streaked onto CVP medium and

incubated in the dark at 22 C for 40 to 48 hr. Streaked aliquots

were then indexed for <u>E</u>. <u>carotovora</u>-like colony production as an

estimate of relative stem colonization. Indexing consisted of a

visual rating on a scale of 0 to 3 with zero indicating no

<u>E</u>. <u>carotovora</u>-like colonies, one indicating a few well-defined

<u>E</u>. <u>carotovora</u>-like colonies (seven or less), two indicating several

E. carotovora-like colonies still relatively well-defined (eight
to ca. 20) and three indicating a mass(es) of confluent E. caroto-
vora-like colonies. If present, a typical E. carotovora-like
colony from each aliquot was subcultured on fresh CVP medium and
from this a single colony was transferred to SNA for maintenance.
Using Ouchterlony double diffusion, isolates were tested for
identity to serogroups XXIX or I.

Statistical Analysis

Probability of equality. Probability of equality (p), as
used herein, was obtained, interpolating when necessary, from the
"probability of a larger value, sign ignored" in the distribution
of Student's t, or from the "probability of a greater value" in
the cumulative distribution of chi-square (Snedecor and Cochran,
1980). Means or frequency distributions were considered to be
equal if $p > 0.25$. Significance of differences, where $p \leq 0.25$,
was considered as a continuum that varied inversely with the
probability of equality.

Field experiments. Frequencies of co-occuring events, such
as co-incidence of plant infection by two pathogens or incidence
of C. atramentarium in V. dahliae infected stems from which
V. dahliae was recovered from near the apex but not from the base
of the stem, were analyzed by preparing 2 X 2 contingency tables.
These tables were prepared with data pooled over all plots and
sampling dates, to obtain expected frequencies and chi-square

values. Chi-square values were used to test for equality between observed and expected frequencies.

Average $\log_e$ internal stem populations of V. dahliae were compared by testing the frequency distributions of individual samples, each a single plot for a single sampling date, for equality using chi-square values. These chi-square values were determined from R X 2 contingency tables, where $R \leq 7$, constructed on intervals of $\log_e$ original number of colonies counted per 20-mg stem tissue with $\log_e$ 0 defined as zero. Interval midpoints consisted of the whole numbers 0 through 6. Only those stems infected with V. dahliae were used in this analysis. For a sample to qualify for analysis of internal stem populations of V. dahliae, the sample size of each distribution being compared had to exceed 11. In addition to this criterion, for cases concerning E. carotovora involvement with internal stem populations of V. dahliae, analysis was only carried out on those samples where the corresponding incidence of "early dying" disease symptoms in the plot at the time was less than 95%.

Greenhouse experiment. For all analyses high and low inoculum densities were pooled for each treatment. Means of percent chlorotic and/or necrotic leaf in the single pathogen tests were tested for equality to the mean of the control treatment with one-tailed Student's t-tests. Equalities between means of stem colonization indices and between means of percent diseased leaf area were tested for equality with two-tailed Student's t-tests.

RESULTS

Field Experiments

Disease, plant infection and pathogen interactions in 'Norgold Russet' in the 1980 plots. Typical "early dying" disease symptoms were exhibited by 'Norgold Russet' grown in the Columbia Basin (RF and EF) and the Klamath Basin (KF) plots. Disease progress was similar for the KF and RF plots (Fig. 1). Symptom expression at the KF and RF plots were characterized by (1) early onset with 10% of the hills exhibiting "early dying" disease symptoms at ca. 61 and 70 days, respectively, from planting, (2) rapid build-up to 90% diseased hills within ca. 25 days and (3) premature death of the vines ca. 5 and 8 wks, respectively, prior to expected regional harvest times. However, at the EF plot symptom expression was characterized by (1) delayed onset with 10% of the hills exhibiting "early dying" disease symptoms ca. 94 days from planting, (2) slow build-up to a maximum of 64% diseased hills 152 days from planting and (3) only occasional vine death by the expected regional harvest time.

In the KF plot and in the RF plot, where preplant soil populations of V. dahliae were 14.38 cfu/g, development over time of plant infection by V. dahliae almost paralleled "early dying" disease progress (Fig. 1). Conversely, at the EF plot where preplant soil populations were 1.13 cfu/g, "early dying" disease progress did not parallel development over time of plant infection by V. dahliae.
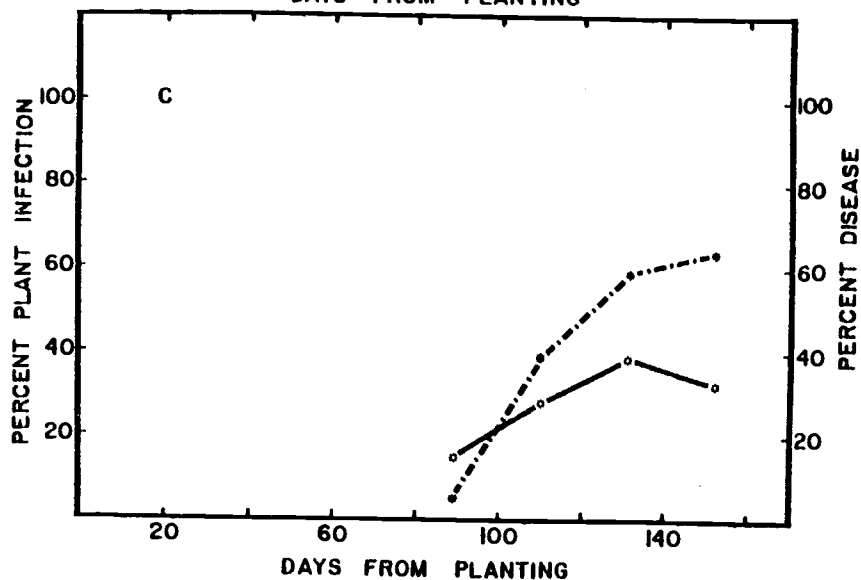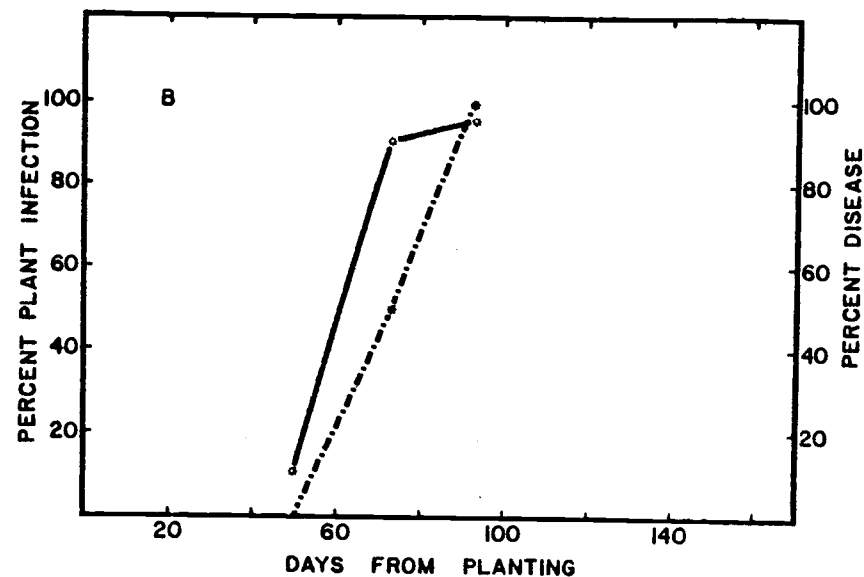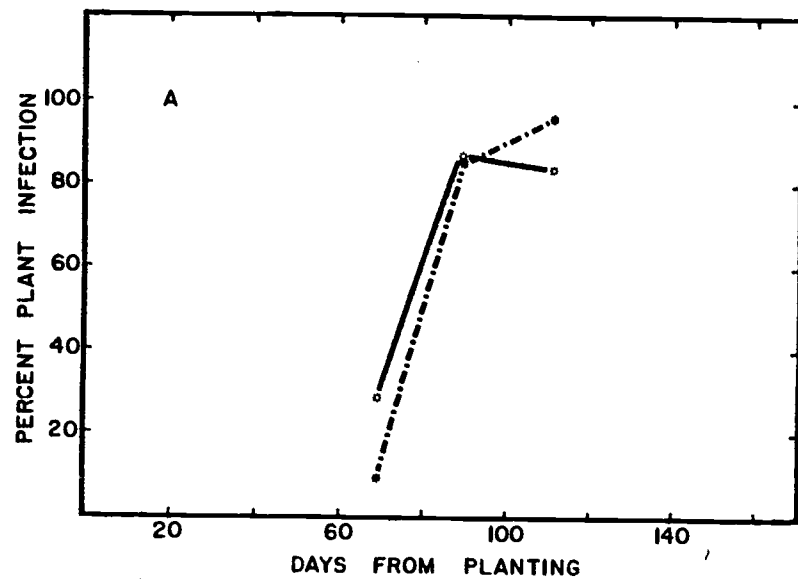
Fig. 1. "Early dying" disease symptoms (•—•—••) and plant infection by Verticillium dahliae (•———•) in field grown 'Norgold Russet' at three plots in Oregon in 1980. A. The RF plot, located in the Columbia Basin and previously cropped to potatoes. B. The KF plot, located in the Klamath Basin and previously cropped to potatoes. C. The EF plot, located in the Columbia Basin and not previously cropped to potatoes.

Isolations for V. dahliae were made from the base and from
within 10 to 15-cm of the apex of each plant. Out of the 781
plants infected with V. dahliae, 14% of the time the pathogen
could not be recovered from the basal portion of the stem; this
was most pronounced at the third sampling dates in the KF and
RF plots (Table 1). Possible involvement of C. atramentarium in
this basal suppression of V. dahliae was suggested by the suppressed
co-incidence of the two pathogens noted by Goodell et al (1982) in
basal stem isolations, and by the fact that C. atramentarium is
most often isolated from the base rather than the apex, of field
grown potatoes (Dickson, 1926; Otazu et al, 1978). Therefore,
frequency of basal infection by C. atramentarium in V. dahliae
infected plants was analyzed and found to be significantly greater
in plants with basal suppression of V. dahliae (p = 0.005) than
would be expected if this were a random event.

Development over time of plant infection by C. atramentarium
almost paralleled that of V. dahliae at the KF and EF plots, but
not at the RF plot (Fig. 2). Nevertheless, increases in plant
infection by V. dahliae always preceeded increases in plant
infection by C. atramentarium, and at any given time more plants
were infected with V. dahliae than with C. atramentarium. Develop-
ment over time of plant infection by Ecc was similar at all plots,
although onset was slightly delayed at the EF plot, when compared
to the KF and RF plots (Fig. 3). Plant infection by Eca at both
the KF and RF plots increased between the first and second sampling
dates and then decreased between the second and third sampling dates;

Table 1. <u>Verticillium dahliae</u> infected field grown 'Norgold
Russet', in Oregon in 1980, from which <u>V. dahliae</u>
was recovered from 10 to 15 cm of the apex but not
from the base of the plant.

Plot[x]	Date[y]	Lack of basal recovery (%)
KF	50	0.0
	73	1.9
	93	31.2
RF	69	--[z]
	89	--
	111	31.9
EF	89	--
	110	10.6
	131	1.6
	152	15.4

[x]Field plots in the Columbia Basin (RF and EF) and Klamath
Basin (KF).

[y]Number of days from planting.

[z]Missing data.
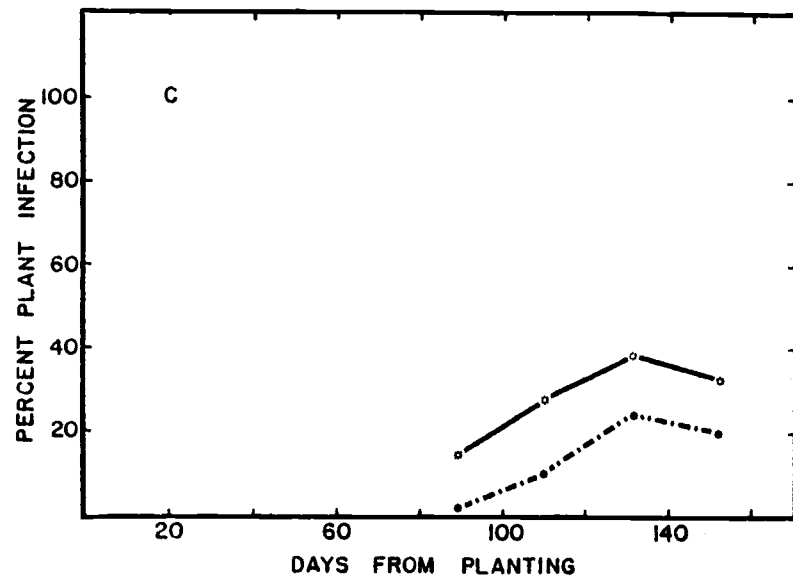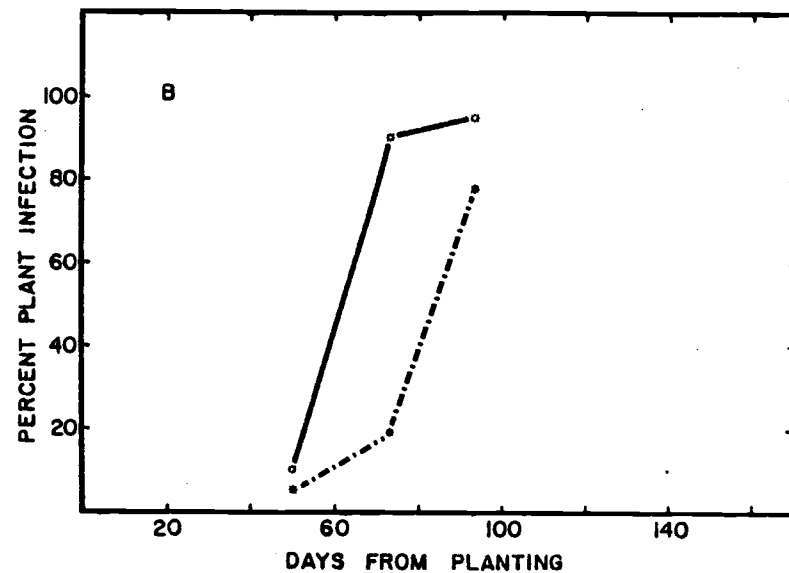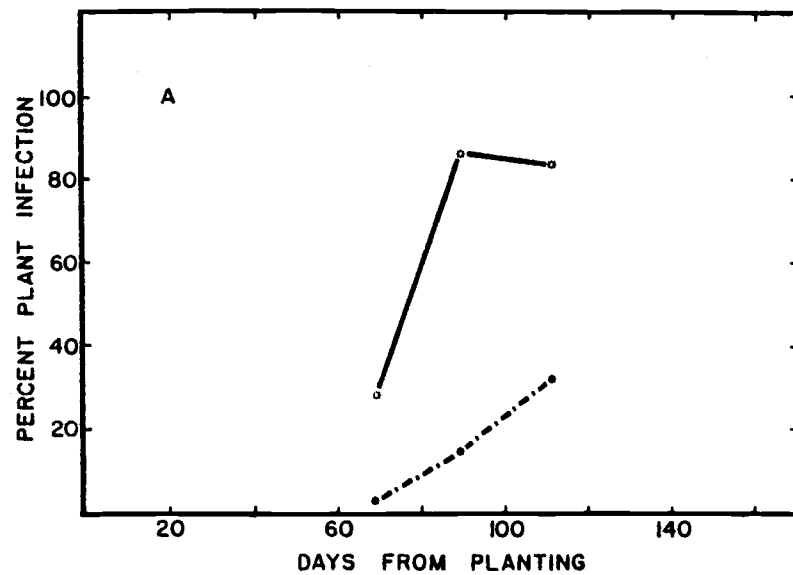
Fig. 2. Plant infection by <u>Verticillium</u> <u>dah-liae</u> (•———•) and by <u>Colletotrichum</u> <u>atramentarium</u> (•—·—·•) in field grown 'Norgold Russet' at three plots in Oregon in 1980. A. The RF plot, lo-cated in the Columbia Basin and pre-viously cropped to potatoes. B. The KF plot, located in the Klamath Basin and previously cropped to potatoes. C. The EF plot, located in the Colum-bia Basin and not previously cropped to potatoes.

this decrease was much more pronounced at the RF plot (Fig. 4).

However, at the EF plot plant infection by Eca remained fairly

constant during the second half of the growing season.

Detection of pathogen interactions with V. dahliae was

approached in two ways, (1) by investigating the frequency of

plant co-infection and (2) by investigating the effects of

co-infection on the severity of V. dahliae colonization, as

represented by internal stem populations. Co-infection frequencies

involving C. atramentarium, a soil-borne pathogen (Blakeman and

Hornby, 1966; Farley, 1972; Stevenson et al, 1976) or Eca, a

tuber-borne pathogen (Graham and Harper, 1967; Perombelong, 1971;

Perombelon, 1973), were pooled from all data; however, frequencies

involving Ecc, which may be soil-borne (Burr and Schroth, 1977;

Meneley and Stanghellini, 1976) or tuber-borne (Perombelon, 1971),

were partitioned into two corresponding groups. Frequency of

plants co-infected with V. dahliae and C. atramentarium, V. dahliae

and Eca, or V. dahliae and those Ecc isolates serologically similar

to isolates recovered from the seed tubers was significantly greater

than would be expected if co-infection were a random event (Table

2). However, analysis of plant co-infection by V. dahliae and

those Ecc isolates serologically similar to known Oregon soil-borne

isolates was not possible as these serogroups were only recovered

from seven stems. Approximately 60% of the Ecc stem isolates

could not be serologically identified and were excluded from the

above analysis. The positive effects observed in co-infection

were also observed in severity of V. dahliae colonization. Internal
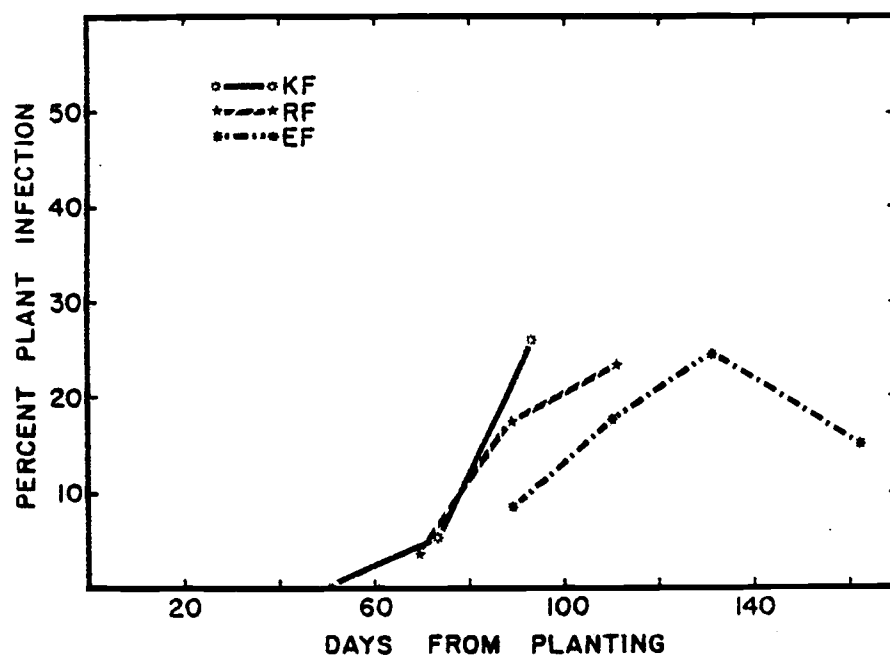
Fig. 3. Plant infection by <u>Erwinia</u> <u>carotovora</u> subsp. <u>caro-</u>
<u>tovora</u> in field grown 'Norgold Russet' in the Colum-
bia Basin (RF and EF) and in the Klamath Basin (KF)
of Oregon in 1980. The RF and KF plots had previous-
ly been cropped to potatoes; the EF plot had not.
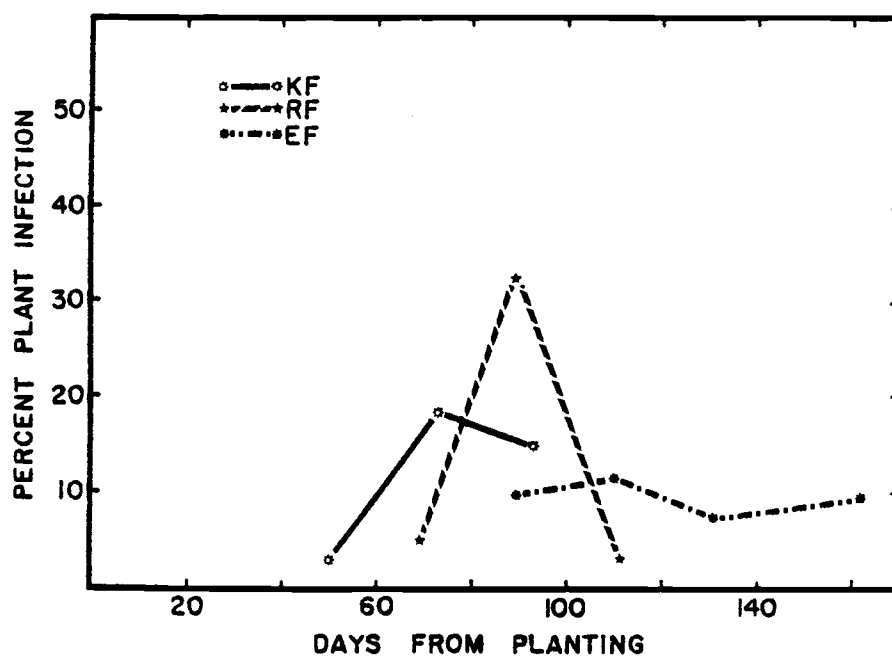
Fig. 4. Plant infection by <u>Erwinia</u> <u>carotovora</u> subsp. <u>atro-</u>
<u>septica</u> in field grown 'Norgold Russet' in the Colum-
bia Basin (RF and EF) and in the Klamath Basin (KF)
of Oregon in 1980. The RF and KF plots had previous-
ly been cropped to potatoes; the EF plot had not.

Table 2.   Comparison of observed and expected frequencies of
plant co-infection by Verticillium dahliae and
Colletotrichum atramentarium, V. dahliae and
Erwinia carotovora subsp. atroseptica (Eca), and
V. dahliae and those E. carotovora subsp. carotovora
(Ecc) isolates serologically similar to isolates
recovered from the seed tubers in field grown
'Norgold Russet' in the Columbia Basin and Klamath
Basin of Oregon in 1980.

| Pathogens | Frequency of plant infection[x] | | p^z |
	Observed	Expected[y]	
V. dahliae + C. atramentarium	239	200	0.005
V. dahliae + Eca	118	98	0.005
V. dahliae + Ecc	67	48	0.005

[x]Data pooled over all plots and sampling dates.

[y]Expected frequencies determined from 2 X 2 contingency tables.

[z]Probability of equality of observed and expected frequencies
using a chi-square value to test for equality of distributions.

stem populations of V. dahliae were generally higher in plants

infected with C. atramentarium, although this difference was

not statistically significant (Table 3). However, for both Eca

and Ecc, internal stem populations of V. dahliae were signifi-

cantly higher in plants infected with either Ecc or Eca (Tables

4 and 5).

Pathogen interactions in 'Russet Burbank' in the 1980 plots.

Statistical analysis of the relationship between V. dahliae and Eca

in 'Russet Burbank' was not possible as frequency of plant infection

by Eca was very low; nevertheless, Eca stem isolates were always

recovered from V. dahliae infected plants. Analysis of C. atramen-

tarium and Ecc interactions with V. dahliae involved the same two

parameter approach, used for the above 1980 'Norgold Russet' data,

involving (1) co-infection frequencies and (2) internal stem popu-

lations of V. dahliae; and, as Ecc may be both soil-borne and

tuber-borne, Ecc co-infection analysis was again partitioned into

the two corresponding groups. Frequency of plants co-infected

with V. dahliae and C. atramentarium, or V. dahliae and those Ecc

isolates serologically similar to isolates recovered from the seed

tubers, was significantly greater than would be expected if co-

infection were a random event; however, frequency of plants co-infected

with V. dahliae and those Ecc isolates serologically similar to known

Oregon soil-borne isolates was significantly lower than would be

expected if this were a random event (Table 6). Approximately

45% of the Ecc stem isolates could not be serologically identified

and were excluded from the above analysis. As per the 'Norgold

Table 3. Internal stem populations of <u>Verticillium</u> <u>dahliae</u> as
influenced by <u>Colletotrichum</u> <u>atramentarium</u> in field
grown 'Norgold Russet'.

Sample[v] (plot[w] – date[x])	Average log$_e$ <u>V. dahliae</u> populations (cfu/g)[u]		p[z]
	<u>C</u>. <u>atramentarium</u> infected[y]	<u>C</u>. <u>atramentarium</u> non–infected	
EF – 131	5.30	5.16	0.48
RF – 89	4.48	3.40	0.71
RF – 111	6.21	6.31	0.79
KF – 73	2.64	2.08	0.38
KF – 93	6.50	6.43	0.54

[u]Assayed within 10 to 15 cm of the plant apex.

[v]Sample size for each frequency distribution exceeded 11.

[w]1980 field plots in the Columbia Basin (RF and EF) and Klamath
Basin (KF) of Oregon.

[x]Number of days from planting.

[y]Assayed at the base of the plant.

[z]Probability of equality of internal stem populations using a
chi-square value to test for equality of frequency distributions.

Table 4. Internal stem populations of <u>Verticillium</u> <u>dahliae</u> as
influenced by <u>Erwinia</u> <u>carotovora</u> subsp. <u>carotovora</u>
(<u>Ecc</u>) in field grown 'Norgold Russet'.

Sample[v] (plot[w] - date[x])	Average log$_e$ V. dahliae populations (cfu/g)[u]		p[z]
	Ecc infected[y]	Ecc non-infected	
EF - 131	4.70	4.19	0.37
RF - 89	3.99	3.50	0.22

[u]Assayed within 10 to 15 cm of the plant apex.

[v]Sample size for each frequency distribution exceeded 11.

[w]1980 field plots in the Columbia Basin of Oregon.

[x]Number of days from planting.

[y]Assayed at the base of the plant.

[z]Probability of equality of internal stem populations using a
chi-square value to test for equality of frequency distributions.

Table 5. Internal stem populations of <u>Verticillium</u> <u>dahliae</u> as
         influenced by <u>Erwinia</u> <u>carotovora</u> subsp. <u>atroseptica</u>
         (<u>Eca</u>) in field grown 'Norgold Russet'.

| Sample[v] (plot[w] – date[x]) | Average log$_e$ <u>V.</u> <u>dahliae</u> populations (cfu/g)[u] | | p[z] |
	Eca infected[y]	Eca non-infected	
RF – 89	3.14	3.81	0.31
KF – 73	3.40	1.95	0.05

[u]Assayed within 10 to 15 cm of the plant apex.

[v]Sample size for each frequency distribution exceeded 11.

[w]1980 field plots in the Columbia Basin (RF) and Klamath Basin
(KF) of Oregon.

[x]Number of days from planting.

[y]Assayed at the base of the plant.

[z]Probability of equality of internal stem populations using a
chi-square value to test for equality of frequency distributions.

Table 6. Comparison of observed and expected frequencies of plant co-infection by <u>Verticillium</u> <u>dahliae</u> and <u>Colletotrichum</u> <u>atramentarium</u>, <u>V</u>. <u>dahliae</u> and those <u>Erwinia</u> <u>carotovora</u> subsp. <u>carotovora</u> isolates serologically similar to isolates recovered from the seed tubers (tuber-borne <u>Ecc</u>), and <u>V</u>. <u>dahliae</u> and those <u>E</u>. <u>carotovora</u> subsp. <u>carotovora</u> isolates serologically similar to known Oregon soilborne isolates (soil-borne <u>Ecc</u>) in field grown 'Russet Burbank' in the Columbia Basin and Klamath Basin of Oregon in 1980.

| Pathogens | Frequency of plant infection[x] | | p[z] |
	Observed	Expected[y]	
<u>V</u>. <u>dahliae</u> + <u>C</u>. <u>atramentarium</u>	213	179	0.005
<u>V</u>. <u>dahliae</u> + tuber-borne <u>Ecc</u>	44	38	0.14
<u>V</u>. <u>dahliae</u> + soil-borne <u>Ecc</u>	10	19	0.005

[x]Data pooled over all plots and sampling dates.

[y]Expected frequencies determined from 2 X 2 contingency tables.

[z]Probability of equality of observed and expected frequencies using a chi-square value to test for equality of distributions.

Russet' data, the positive effects observed in co-infection were also observed in severity of V. dahliae colonization. Internal stem populations of V. dahliae were significantly higher in plants infected with C. atramentarium (Table 7) or with Ecc (Table 8). The negative effect observed in the co-incidence of V. dahliae and Ecc stem isolates serologically identical to seed tuber isolates, therefore, was not observed as a negative effect on the severity of V. dahliae colonization.

Plant infection in the 1981 plots. In the Wallowa Valley plot, which was previously cropped one year to potatoes, the pathogen isolated most often from 'Norgold Russet' hills exhibiting typical "early dying" disease symptoms was Eca (21%), followed by Ecc (16%), C. atramentarium (3%) and V. dahliae (0%). In the Columbia Basin plot, which was not previously cropped to potatoes, Ecc was the predominant pathogen isolated at all sampling dates from 'Norgold Russet' plants exhibiting typical "early dying" disease symptoms (Table 9). The only other pathogen here to be isolated from at least 10% of the plants was Eca, but only at the first sampling date.

Greenhouse Experiment

Single pathogen tests. Just after inoculation, V. dahliae, C. atramentarium, Ecc and Eca were isolated from the base and apex of inoculated 'Russet Burbank' plants. After 53 days, plants which had been inoculated with V. dahliae, Ecc or Eca exhibited significantly more foliar chlorosis and/or necrosis than did the

Table 7. Internal stem populations of <u>Verticillium</u> <u>dahliae</u> as influenced by <u>Colletotrichum</u> <u>atramentarium</u> in field grown 'Russet Burbank'.

Sample[v] (plot[w] - date[x])	Average log$_e$ <u>V</u>. <u>dahliae</u> populations (cfu/g)[u]		p[z]
	<u>C</u>. atramentarium infected	<u>C</u>. atramentarium non-infected	
EF - 131	1.61	0.69	0.33
EF - 152	1.95	1.37	0.24
RF - 111	1.10	1.10	--
KF - 73	0.00	0.00	--
KF - 93	4.04	3.76	0.36

[u]Assayed within 10 to 15 cm of the plant apex.

[v]Sample size for each frequency distribution exceeded 11.

[w]1980 field plots in the Columbia Basin (RF and EF) and Klamath Basin (KF) of Oregon.

[x]Number of days from planting.

[y]Assayed at the base of the plant.

[z]Probability of equality of internal stem populations using a chi-square value to test for equality of frequency distributions.

Table 8.   Internal stem populations of <u>Verticillium</u> <u>dahliae</u> as
           influenced by <u>Erwinia</u> <u>carotovora</u> subsp. <u>carotovora</u>
           (<u>Ecc</u>) in field grown 'Russet Burbank'.

Sample[v] (plot[w] – date[x])	Average log$_e$ V. dahliae populations (cfu/g)[u]		p[z]
	Ecc infected[y]	Ecc non-infected	
EF – 152	2.20	1.39	0.08
KF – 93	4.04	3.76	0.02

[u]Assayed within 10 to 15 cm of the stem apex.

[v]Sample size for each frequency distribution exceeded 11.

[w]1980 field plots in the Columbia Basin (EF) and Klamath Basin
(KF) of Oregon.

[x]Number of days from planting.

[y]Assayed at the base of the plant.

[z]Probability of equality of internal stem populations using a
chi-square value to test for equality of frequency distributions.

Table 9. Incidence of <u>Verticillium</u> <u>dahliae</u>, <u>Colletotrichum</u>
<u>atramentarium</u>, <u>Erwinia</u> <u>carotovora</u> subsp. <u>carotovora</u>
(<u>Ecc</u>) and <u>E</u>. <u>carotovora</u> subsp. <u>atroseptica</u> (<u>Eca</u>) in
field grown 'Norgold Russet' plants in the Columbia
Basin of Oregon that exhibited typical "early dying"
disease symptoms in 1981.

Pathogen	Infected plants (%)			
	72^z	82	91	97
<u>V</u>. <u>dahliae</u>	2	4	6	8
<u>C</u>. <u>atramentarium</u>	0	0	2	0
<u>Ecc</u>	41	58	50	64
<u>Eca</u>	17	9	0	0

^{z}Days from planting.

controls, whereas those inoculated with C. atramentarium did

not (Table 10). Leaf chlorosis and/or necrosis in the controls,

assumed to represent the normal "sloughing-off" of leaves that

occurs in healthy appearing plants, was never above 20-cm from

the soil line. Therefore, plants with foliar chlorosis and/or

necrosis at least 30-cm from the soil line were considered to be

exhibiting typical "early dying" disease symptoms. Plants inocu-

lated with V. dahliae, Ecc or Eca exhibited typical "early dying"

disease symptoms, whereas those inoculated with C. atramentarium

did not. In isolations made from diseased plants, at least 71%

of the time the inoculated pathogen was isolated from the base

of the stem (Table 11); however, only V. dahliae was consistently

isolated above this region (Table 12). Diseased plants were only

infected with the inoculated pathogen. Incidence and severity of

"early dying" disease symptoms, as indicated by the percent chlorotic

and/or necrotic leaf area, were greatest in V. dahliae inoculated

plants; followed by Eca and then Ecc.

Verticillium dahliae and Colletotrichum atramentarium inter-

action test. Just after inoculation, V. dahliae and C. atramentarium

were isolated from the base and apex of 'Russet Burbank' plants to

which they had been inoculated either alone or in combination.

After 53 days, severity of "early dying" disease symptoms in plants

co-inoculated with V. dahliae and C. atramentarium did not differ

significantly from those inoculated with V. dahliae alone. V. dahliae

colonization at the base of the stem was significantly lower in the

co-inoculated plants than in those inoculated with V. dahliae;

Table 10. Foliar chlorosis and/or necrosis in greenhouse grown
'Russet Burbank' inoculated with <u>Verticillium</u> <u>dahliae</u>,
<u>Erwinia</u> <u>carotovora</u> subsp. <u>atroseptica</u> (<u>Eca</u>), <u>E</u>. <u>caro-</u>
<u>tovora</u> subsp. <u>carotovora</u> (<u>Ecc</u>) or <u>Colletotrichum</u>
<u>atramentarium</u>, as compared to foliar chlorosis and/or
necrosis in the controls.

Treatment	Chlorotic and/or necrotic leaf area (%)^x		p^z
	Average	Maximum	
Control^y	3.73	10	--
<u>V</u>. <u>dahliae</u>	9.42	50	0.002
<u>Eca</u>	5.75	25	0.08
<u>Ecc</u>	5.00	25	0.16
<u>C</u>. <u>atramentarium</u>	4.19	10	0.36

^{x}Pooled over two inoculum densities

^{y}Inoculated with sterile phosphate buffer-saline solution.

^{z}Probability of equality to the control treatment using a one-
tailed Student's t-test.

Table 11. Incidence of "early dying" disease and recovery of
pathogens from diseased plants in greenhouse grown
'Russet Burbank' inoculated with <u>Verticillium</u>
<u>dahliae</u>, <u>Erwinia</u> <u>carotovora</u> subsp. <u>carotovora</u>
(<u>Ecc</u>) or <u>E</u>. <u>carotovora</u> subsp. <u>atroseptica</u> (<u>Eca</u>).[y]

Pathogen	Disease (%)[z]	Recovery (%)
<u>V</u>. <u>dahliae</u>	38	93
Ecc	7	100
Eca	13	71

[y]Data pooled over two inoculum densities.

[z]Foliar chlorosis and/or necrosis at least 30 cm from the soil
line (foliar chlorosis and/or necrosis in the controls, repre-
senting normal "sloughing-off", was never greater than 20-cm
from the soil line).

Table 12. Incidence of <u>Verticillium</u> <u>dahliae</u>, <u>Colletotrichum</u>
<u>atramentarium</u>, <u>Erwinia</u> <u>carotovora</u> subsp. <u>carotovora</u>
(<u>Ecc</u>) or <u>E</u>. <u>carotovora</u> subsp. <u>atroseptica</u> (<u>Eca</u>) 15
to 20 cm above the root ball in inoculated green-
house grown 'Russet Burbank' stems.

Pathogen	Infection (%)[z]
<u>V</u>. <u>dahliae</u>	75
<u>C</u>. <u>atramentarium</u>	29
<u>Ecc</u>	12
<u>Eca</u>	20

[z]Data pooled over two inoculum densities.

however, there was no significant difference in V. dahliae
colonization above the basal region (Table 13). Colonization
by C. atramentarium was also significantly lower in the co-
inoculated plants than in those inoculated with C. atramentarium
alone (Table 14).

Verticillium dahliae and Erwinia carotovora subsp. carotovora
interaction test. Just after inoculation, V. dahliae and Ecc were
isolated from the base and apex of 'Russet Burbank' plants to which
they had been inoculated either alone or in combination. After 53
days, severity of "early dying" disease symptoms, in plants co-
inoculated with V. dahliae and Ecc did not deviate significantly
from the additive effect of each pathogen alone (Table 15).
V. dahliae colonization was significantly greater in the co-
inoculated plants than in those inoculated with V. dahliae alone;
this was more pronounced above the basal region of the stem (Table
16). Conversely, Ecc colonization in the co-inoculated plants was
either equal to or significantly less than Ecc colonization in
plants inoculated with Ecc alone (Table 17).

Verticillium dahliae and Erwinia carotovora subsp. atroseptica
interaction test. Just after inoculation, V. dahliae was isolated
from the base and apex of all 'Russet Burbank' plants to which it
had been inoculated, whereas Eca was isolated from all but the
apex of those plants which had been inoculated with the two patho-
gens at the low inoculum density. After 53 days, severity of "early
dying" disease symptoms in plants co-inoculated with V. dahliae and
Eca did not deviate significantly from the additive effect of each

Table 13. Verticillium dahliae colonization as influenced by
Colletotrichum atramentarium in inoculated green-
house grown 'Russet Burbank' stems.

| Height (cm)[x] | Average V. dahliae colonization index[w] | | p[z] |
	V. dahliae[y]	V. dahliae + C. atramentarium	
0 to 5	2.08	1.11	<0.001
15 to 20	2.32	1.91	0.37

[w]On a scale of 0 to 12 (no microsclerotia to microsclerota in
all visible tissues of stem cross-sections), pooled over two
inoculum densities.

[x]Distance from the root ball.

[y]Inoculated pathogen(s).

[z]Probability of equality using a two-tailed Student's t-test.

Table 14. Colletotrichum atramentarium colonization as influenced
by Verticillium dahliae in inoculated greenhouse grown
'Russet Burbank' stems.

| | Average $\underline{C}$. atramentarium colonization index[w] | | |
Height (cm)[x]	$\underline{C}$. atramentarium[y]	$\underline{V}$. dahliae + $\underline{C}$. atramentarium	p[z]
0 to 5	5.76	3.74	<0.001
15 to 20	0.52	0.15	0.08

[w]On a scale of 0 to 9 (no acervuli to acervuli on all visible tissues of stem cross-sections), pooled over two inoculum densities.

[x]Distance from the root ball.

[y]Inoculated pathogen(s).

[z]Probability of equality using a two-tailed Student's t-test.

Table 15. Severity of "early dying" disease symptoms in green-
house grown 'Russet Burbank' co-inoculated with
Verticillium dahliae and Erwinia carotovora subsp.
carotovora (Ecc), compared with the additive effect
of each pathogen.

Diseased leaf area (%)^w

V. dahliae^x	Ecc	V. dahliae + Ecc	Additive effect^y	p^z
5.70	1.27	9.13	6.97	0.35

^{w}Average % chlorotic and/or necrotic leaf area minus the average
in the controls, pooled over two inoculum densities.

^{x}Inoculated pathogen(s).

^{y}Addition of average % diseased leaf area in plants inoculated
with each pathogen alone, pooled over two inoculum densities.

^{z}Probability of equality of % diseased leaf area in co-inoculated
plants and the additive effect using a two-tailed Student's t-test.

Table 16. <u>Verticillium</u> <u>dahliae</u> colonization as influenced by
<u>Erwinia</u> <u>carotovora</u> subsp. <u>carotovora</u> (Ecc) in inocu-
lated greenhouse grown 'Russet Burbank' stems.

| Height (cm)[x] | Average $\underline{V}$. $\underline{dahliae}$ colonization index[w] | | p[z] |
	$\underline{V}$. $\underline{dahliae}$[y]	$\underline{V}$. $\underline{dahliae}$ + Ecc	
0 to 5	2.08	2.69	0.03
15 to 20	2.32	4.58	<0.001

[w]On a scale of 0 to 12 (no microsclerotia to microsclerotia in
all visible tissues of stem cross-sections), pooled over two
inoculum densities.

[x]Distance from the root ball.

[y]Inoculated pathogen(s).

[z]Probability of equality using a two-tailed Student's t-test.

Table 17. Erwinia carotovora subsp. carotovora (Ecc) coloni-
zation as influenced by Verticillium dahliae in
inoculated greenhouse grown 'Russet Burbank' stems.

	Average Ecc colonization index[w]		
Height (cm)[x]	Ecc[y]	V. dahliae + Ecc	p[z]
0 to 5	2.53	2.59	>0.50
15 to 20	0.16	0.04	0.08

[w]On a scale of 0 to 3 (0 = no E. carotovora-like colonies,
1 = a few well-defined E. carotovora-like colonies, 2 =
several relatively well-defined E. carotovora-like colonies,
3 = mass(es) of confluent E. carotovora-like colonies produced
on CVP medium in stem assays), pooled over two inoculum
densities.

[x]Distance from the root ball.

[y]Inoculated pathogen(s).

[z]Probability of equality using a two-tailed Student's t-test.

pathogen alone (Table 18). V. dahliae colonization was significantly greater in the co-inoculated plants than in those inoculated with V. dahliae alone: this was more pronounced above the basal region of the stem (Table 19). Conversely, Eca colonization in the co-inoculated plants was either equal to or significantly less than Eca colonization in plants inoculated with Eca alone (Table 20).

Table 18. Severity of "early dying" disease symptoms in green-
house grown 'Russet Burbank' co-inoculated with
<u>Verticillium</u> <u>dahliae</u> and <u>Erwinia</u> <u>carotovora</u> subsp.
<u>atroseptica</u> (<u>Eca</u>), compared with the additive effect
of each pathogen.

Diseased leaf area (%)^w

<u>V</u>. <u>dahliae</u>^{x}	<u>Eca</u>	$\underline{V.\ dahliae}$ + <u>Eca</u>	Additive effect^y	p^z
5.70	2.02	8.09	7.72	>0.50

^{w}Average % chlorotic and/or necrotic leaf area minus the average
in the controls, pooled over two inoculum densities.

^{x}Inoculated pathogen(s).

^{y}Addition of average % diseased leaf area in plants inoculated
with each pathogen alone, pooled over two inoculum densities.

^{z}Probability of equality of average % diseased leaf area in
co-inoculated plants and the additive effect using a two-tailed
Student's t-test.

Table 19. Verticillium dahliae colonization as influenced by Erwinia carotovora subsp. atroseptica (Eca) in inoculated greenhouse grown 'Russet Burbank' stems.

	Average V. dahliae colonization index[w]		
Height (cm)[x]	V. dahliae[y]	V. dahliae + Eca	p[z]
0 to 5	2.08	3.31	<0.001
15 to 20	2.32	5.87	<0.001

[w]On a scale of 0 to 12 (no microsclerotia to microsclerotia in all visible tissues of stem cross-sections), pooled over two inoculum densities.

[x]Distance from the root ball.

[y]Inoculated pathogen(s).

[z]Probability of equality using a two-tailed Student's t-test.

Table 20. <u>Erwinia</u> <u>carotovora</u> subsp. <u>atroseptica</u> (<u>Eca</u>) coloni-
zation as influenced by <u>Verticillium</u> <u>dahliae</u> in
inoculated greenhouse grown 'Russet Burbank' stems.

	Average <u>Eca</u> colonization index[w]		
Height (cm)[x]	<u>Eca</u>[y]	<u>V. dahliae</u> <u>Eca</u>	p[z]
0 to 5	2.15	1.81	0.19
15 to 20	0.11	0.22	0.35

[w]On a scale of 0 to 30 (0 = no <u>E</u>. <u>carotovora</u>-like colonies,
1 = a few well-defined <u>E</u>. <u>carotovora</u>-like colonies, 2 =
several relatively well-defined <u>E</u>. <u>carotovora</u>-like colonies,
3 = mass(es) of confluent <u>E</u>. <u>carotovora</u>-like colonies produced
on CVP medium in the stem assays), pooled over two inoculum
densities.

[x]Distance from the root ball.

[y]Inoculated pathogen(s).

[z]Probability of equality using a two-tailed Student's t-test.

DISCUSSION

In root-dip pathogenicity tests $\underline{V}$. $\underline{dahliae}$, $\underline{Eca}$ and $\underline{Ecc}$ were capable of causing typical "early dying" disease symptoms in 'Russet Burbank'. As determined by the severity of foliar symptoms 53 days after inoculation, $\underline{V}$. $\underline{dahliae}$ was the most virulent pathogen, followed by $\underline{Eca}$ and then $\underline{Ecc}$. In field experiments $\underline{V}$. $\underline{dahliae}$, $\underline{Eca}$ and $\underline{Ecc}$ were each associated with "early dying" disease symptoms, but relative involvement varied with location. $\underline{V}$. $\underline{dahliae}$ was most strongly associated with disease symptoms in plots which had previously been cropped several years to potatoes. Thus, while $\underline{V}$. $\underline{dahliae}$ was the predominant pathogen isolated from 'Norgold Russet' plants at all three 1980 plots, it was only at the RF and KF plots, which had previously been cropped at least three times to potatoes, that development of disease symptoms paralleled development of plant infection by $\underline{V}$. $\underline{dahliae}$. Conversely, $\underline{Eca}$ and $\underline{Ecc}$ were most strongly associated with symptom expression in plots cropped one year or less to potatoes. At the 1981 Wallowa Valley plot, which had been previously cropped one year to potatoes and where the average temperature for the 3-mo period of Jun, Jul and Aug was 16.7 C, $\underline{Eca}$ was the predominant pathogen isolated from diseased 'Norgold Russet' hills sampled in early September. However, at the 1981 Columbia Basin plot, which had not been previously cropped to potatoes and where the average temperature for the same 3-mo period was 20.2 C, $\underline{Ecc}$ was the predominant pathogen isolated from diseased 'Norgold Russet' plants sampled from late June to harvest. The association of $\underline{Eca}$ with cooler temperatures

and Ecc was warmer temperatures was previously reported (McCarter-Zoner, 1981; Molina and Harrison, 1977; Powelson, 1980b).

C. atramentarium was not capable of causing "early dying" disease symptoms in root-dip inoculated 'Russet Burbank'. This agrees with the results of previous pathogenicity studies conducted in Indiana and India (Stevenson et al, 1976; Thirumalacher, 1967). Conversely, in pathogenicity tests in Idaho, C. atramentarium was reported to cause foliar chlorosis; however, this may have been a secondary symptom because cortical sloughing and root rot were also observed (Davis and Howard, 1976). C. atramentarium is reported to be a weak pathogen on potatoes (Gudmestad et al, 1977; Mooi, 1959) that requires some type of additional stress before extensive colonization of the above ground stem can occur (Otazu et al, 1978). The 1980 and 1981 field data indicated that plant infection by V. dahliae predisposes the above ground stem to colonization by C. atramentarium. In the two 1981 'Norgold Russet' plots, where plant infection by V. dahliae was rare or absent, plant infection by C. atramentarium was consistently low. In the three 1980 'Norgold Russet' plots, increases in plant infection by V. dahliae always preceeded increases in plant infection by C. atramentarium, and C. atramentarium was more frequently isolated from V. dahliae infected plants, especially those with high internal stem populations of V. dahliae, than could be explained by chance.

In monitoring "early dying" disease, plant isolations must be made to determine the causal agent(s), since more than one

pathogen can cause typical disease symptoms. However, the pathogen(s) involved in symptom expression may not always be recovered from infected plants. A reduction in recovery of V. dahliae and of Eca was observed late in the epidemics at the 1980 plots, when plant infection by each pathogen decreased between the last two sampling dates. The reduction in Eca recovery was linked to plant infection by V. dahliae, and was influenced by high temperatures. V. dahliae decreased stem colonization by Eca in the greenhouse study. Nevertheless, in the field, a decrease in Eca recovery was observed only where there was high incidence of vine death and, thus, host desiccation. At two of the 1980 'Norgold Russet' plots (KF and RF), where plant infection by V. dahliae was high and many of the plants were dead, Eca recovery was reduced. This reduction was much greater in the warmer growing region (Columbia Basin) than in the cooler growing region (Klamath Basin). However, in the third 1980 plot (EF), where plant infection by V. dahliae was lower and where vine death was negligible, there was no reduction in Eca recovery.

Decreases in V. dahliae recovery were caused by C. atramentarium. In the greenhouse C. atramentarium reduced V. dahliae populations, but only at the base of the stem where colonization by C. atramentarium was most extensive. In the field C. atramentarium caused a reduction in recovery of V. dahliae at the base of the stem, but had no influence on internal stem populations or recovery of V. dahliae near the plant apex. Therefore, the inhibition of V. dahliae may only involve a competition, requiring

the physical presence of C. atramentarium, which affects the sapro-
phytic phase of the V. dahliae disease cycle that occurs in
senescing plant tissues.

Pathogen interactions with V. dahliae were determined by
monitoring the severity of "early dying" disease symptoms in
root-dip co-inoculation tests with 'Russet Burbank'. Severity
of symptom expression in V. dahliae inoculated plants was
influenced by Eca and Ecc, but not by C. atramentarium. Symptom
expression was more severe in plants co-inoculated with V. dahliae
and Eca, or V. dahliae and Ecc, than in those inoculated with
either pathogen alone. Contrary to the relationship reported
V. dahliae and P. penetrans (Martin et al, 1982), the relationship
between V. dahliae and Eca, or V. dahliae and Ecc was additive,
rather than synergistic. The increase in severity of symptom
expression in both cases was paralleled by an increase in stem
colonization by V. dahliae, which was more pronounced above the
basal stem region. A similar relationship was observed in the
field where internal stem populations of V. dahliae near the apex
were higher in Eca or Ecc infected plants. Thus, Eca and Ecc
each enhanced vascular colonization by V. dahliae, the parasitic
phase of its disease cycle, as well as the saprophytic colonization
of senescing pith and cortical tissues. Therefore, in addition
to causing typical disease symptoms, Eca and Ecc play an important
role in "early dying" disease epidemics by enhancing V. dahliae
colonization and thereby intensifying symptom expression.

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