Fall 1967

PRATYLENCHUS PENETRANS--ITS INTERACTION WITH VERTICILLIUS ALBO-ATRUM IN THE VERTICILLIUM WILT OF POTATOES AND ITS ATTRACTION BY VARIOUS CHEMICALS

FREDERICK MORSINK

MORSINK, Frederick, 1931-

PRATYLENCHUS PENETRANS -- ITS INTERACTION WITH VERTICILLIUM ALBO-ATRUM IN THE VERTICILLIUM WILT OF POTATOES AND ITS ATTRACTION BY VARIOUS CHEMICALS.

University of New Hampshire, Ph.D., 1967
Agriculture, plant pathology

University Microfilms, Inc., Ann Arbor, Michigan

PRATYLENCHUS PENETRANS --
ITS INTERACTION WITH VERTICILLIUM ALBO-ATRUM
IN THE VERTICILLIUM WILT OF POTATOES
AND
ITS ATTRACTION BY VARIOUS CHEMICALS

BY

FREDERICK MORSINK

A THESIS

Submitted to the University of New Hampshire

In Partial Fulfillment of

The Requirements for the Degree of

Doctor of Philosophy

Graduate School

Department of Botany

September, 1966

This thesis has been examined and approved.

Wilbur L. Bullock

M. C. Richards

Richard W. Schreibel

Lorne A. McFadden

Leroy E. Rick

Nov. 16, 1966
Date

ACKNOWLEDGEMENTS

The author of this thesis is greatly indebted to Dr. A. E. Rich who so generously provided advice for these researches and so patiently enabled the author to carry out the work herein reported. Also many thanks are here extended for critically reading the manuscript to Dr. W. L. Bullock, Dr. L. A. McFadden, Dr. M. C. Richards, to Dr. R. W. Schreiber, and, last but not least, to Dr. W. E. Urban who was of great assistance in working out the statistical analyses of the data.

TABLE OF CONTENTS

LIST OF TABLES

SECTION I

INTERACTION OF THE PLANT PARASITIC NEMOTODE PRATYLENCHUS PENETRANS AND VERTICILLIUM ALBO-ATRUM IN THE VERTICILLIUM WILT OF POTATOES

Introduction and Literature Review

Verticillium wilt of potatoes is a disease caused by a fungus commonly referred to as Verticillium albo-atrum Reinke & Berthold, 1879 (61). In the U. S. the disease was reported as early as 1914 (56), and it occurs in such widely separated areas as Maine (18), Oregon (40), Idaho (25), Long Island (10), Connecticut (46), and Wisconsin (63). The disease often results in considerable losses in tuber yields (45). It is further characterized by slowly progressive wilting and yellowing of the leaves. Symptoms are increased by conditions which hasten maturity such as earliness of variety, early planting, early irrigation, and high temperatures, which in turn result in early death of the plant (45). Thus the disease has also been referred to as the early maturity disease (10), and as the early dying of potatoes (25).

Rudolph (65) proposed the term Verticillium hadromycosis in preference to wilt since the wilting phenomenon is not always the most obvious symptom of the disease under field conditions. Instead, the leaves may turn yellow and wither from the base of the plant upward (51). Nielsen (51) describes the symptoms as follows: "Chlorosis and wilting usually start in

the basal leaves on one side of the plant and progress upward. In time most lower leaves wilt and die."

McLean (45) argues that the name wilt is confusing in that it suggests wilting of the entire plant which is not the case in many varieties. According to him some varieties show rapid wilting of the stem whereas others remain erect even after most of the leaves are dead and dry. The onset of wilt is often quite sudden, usually occurring about flowering time (42). In many cases only 1 stem of a plant is affected, or a single stem may escape infection while the rest of the plant dies (63). The fungus enters the roots, progresses through the vascular system of root and stem, turning it a reddish-brown color (22, 42). Hyphae are often observed in the lumen of the xylem vessels (63), and the fungus can be isolated from the xylem (65).

The symptoms of Verticillium wilt are often similar to and confused with those of Fusarium wilt (45). In Maine (18) and in Idaho (25) Fusarium spp. have never been found associated with potato wilt although they occur in potato soils in these regions. Edson and Shapovalov (18) found that the optimum temperature for growth was 30°C for F. oxysporum and 25°C for V. albo-atrum. Robinson et al. (63) found that the 2 types of V. albo-atrum have different temperature requirements in vitro. The micro- or pseudo-sclerotial type, referred to as MS type, and the dark mycelial type, referred to as DM type, both grow well over a temperature range of 16°

to 28°C with best growth at 20° and 24°C; but only the MS type is able to grow at 32°C. This may help to explain the apparent predominance of the DM type in cooler areas such as Maine (21) and Eastern Canada (63), and of the MS type in warmer areas such as Long Island (10) and Southern Idaho (51).

There still exists some confusion regarding the taxonomic status of the 2 types of the fungus. The original drawings and description of V. albo-atrum isolated from potatoes by Reinke and Berthold in 1879 (61), are interpreted by some authors to mean the microsclerotial type (43, 59, 65, 75), whereas others take the view that no microsclerotia are formed by this potato-attacking fungus (5, 27, 31). Reinke and Berthold (61) referred to the dark sclerotia-like masses of hyphae in their drawings as resting mycelium, but called them sclerotia in the description of the fungus.

In 1913 Klebahn (31) described a new species of Verticillium isolated from dahlias as V. dahliae, with distinct, black microsclerotia consisting of thick-walled dark formations resulting from septation and budding in all planes of one or more hyphae (5, 27). Isaac (27) reported that V. dahliae, the MS type, was also pathogenic on potatoes. Van Beyma Thoe Kingma (70) found in his studies of many isolates, that V. albo-atrum was a rare pathogen confined to potato and tomato, and that V. dahliae, the MS type, had a wide host range including potato and tomato.

Presley (59), working with Verticillium spp. from cotton, obtained many forms including all the MS types described

by Van Beyma, as variants of monospore cultures. He argued that the microsclerotial character should not be used in the differentiation of Verticillium spp., and that the MS types should be called V. albo-atrum. Apparently, Presley's monospore cultures were all derived from MS cultures only, and he does not report that any of these cultures ever changed to the DM form. His proposal that the MS character ought not to be used for species differentiation, apparently pertained only to his variants which were all derived from MS ancestors.

Isaac (28, 29) supports the view that V. albo-atrum does not include MS types, and that V. dahliae is a valid species. According to his findings black carbonized hyphae (the dark mycelium of other authors) normally occur in the life cycle of V. albo-atrum, and microsclerotia in that of V. dahliae.

Robinson et al. (63), in their extensive work with variants obtained from monospore colonies, found no instance of dark mycelium being present in variants of microsclerotial isolates, nor microsclerotia in variants of the dark mycelial isolates. They concluded that species differentiation between the MS type and the DM type is warranted, and endorsed the use of V. dahliae for the MS type, and V. albo-atrum for the DM type.

In preliminary work for this thesis several isolations were made from potatoes in New Hampshire. Some of the parent fungus cultures remained white for as long as 6 months. Others

turned a dark grey after 3 to 4 weeks and were identified as DM types. Subcultures made from several of the white parent cultures yielded a majority of DM types and more white types, but at least 3 cultures originating from white cultures were identified as MS types. Subcultures from DM types yielded either DM or white types; subcultures from MS types yielded either MS type or white type. Thus it is conceivable that Reinke and Berthold in 1879 isolated and described a mixture of DM and MS types.

Fordyce and Green (23), however, showed that anastomosis and parasexual recombination can be effected between cultures representative of V. dahliae and V. albo-atrum. They consider this additional evidence for the hypothesis that these two types are strains of a single species and should be recombined as V. albo-atrum. The author of this thesis will follow an old custom and refer to the fungus causing Verticillium wilt of potatoes as V. albo-atrum and specify as to type.

The plant parasitic nematode Pratylenchus penetrans (Cobb) Chitwood & Oteifa, 1952 (13, 14) is a common parasite of potato roots in Canada (26), Holland (53, 55), on Long Island (10, 38), and Wisconsin (16).

Dickerson et al. (16) reported that symptoms associated with high populations of P. penetrans in potato fields were generally characterized by circular areas of 30 to 150 feet in diameter. Plants in these areas were reduced in

vigor, turned yellow, ceased to grow in the latter part of the season, had reduced root systems, and lower yields.

Hastings and Bosher (26), in controlled pathogenicity tests, showed that P. penetrans reduced growth of potato seedlings by 59.6%.

Oostenbrink (52) reported that build-up of P. penetrans in the field was negligible in many trials with potatoes planted in soil artificially infested with the nematodes in the first year. In a pathogenicity test on potatoes grown in steam-disinfested sandy soil reinfested with clean nematodes no growth retardation of the plants was observed in the first year. Potatoes grown in the same infested soil a second year showed a marked growth retardation, and large numbers of nematodes were recovered from roots and soil (54). Infesting soil with nematodes (P. penetrans) for controlled experiments involves a lengthy process of extraction from roots of a previous host, or from soil, and adding them, usually in a suspension, to soil in which the new host is planted. Apparently, a high rate of mortality occurs during this process (52).

Dickerson et al. (16) avoided this problem by using naturally infested soil from a potato field, but they used autoclaved soil for the controls in their greenhouse experiments. They report similar symptoms of nematode-infected potato plants in greenhouse pathogenicity tests as in field tests, at constant temperatures of 24° and 28°C. But at

constant temperatures of 16° and 20°C only a reduction of secondary roots was observed. The nematode build-up on potatoes was highest at 16°, and on potatoes following corn at 20°C.

In 1959 Dr. M. B. Harrison (personal communication) found plant parasitic nematodes of the genera Pratylenchus and Tylenchorhyncus in rather large numbers in a potato field on Long Island in which Verticillium wilt occurred. In a survey of 6 potato fields on Long Island high numbers of Pratylenchus spp., recovered from roots and soil, appeared to be associated with a high degree of wilt incidence, whereas low numbers were found in potato fields without wilt symptoms or only mild ones. The nematodes of the genus Pratylenchus were identified as P. penetrans (Cobb) Chitwood & Oteifa (37).

Several workers have reported on soil fumigation in connection with the control of Verticillium wilt. Fall injections of 50 gal/A of Vapam (32.7% sodium methyldithiocarbamate, anhydrous) substantially reduced losses the next growing season, as was observed in decreased nematode populations, delayed expression of foliar symptoms, higher tuber yields, lower incidence of pink-eye and stem end browning (10). Fall injections of Vorlex (20% methyl isothiocyanate, 80% 1,3-dichloropropene and related C_3 hydrocarbons), and of Trapex (20% methyl isothiocyanate) resulted in similar responses, whereas DD (mixture of 1,3-dichloropropene and

1,2-dichloropropane) reduced the nematode population but failed to delay symptom expression and to improve yields (10).

In Connecticut (46), spring injections (13 days prior to planting) of 20 gal/A of DD or 10 gal/A of Vorlex gave excellent control of nematodes (mainly P. penetrans) at planting time and throughout most of the summer. In both treatments repopulation of the treated soil occurred 100 days after fumigation. Five gal/A of Vorlex gave only partial control at planting time and the population level in the treated plots was found to be twice that in untreated plots late in the season. All 3 treatments reduced by half the vascular discoloration of the stem ends of tubers, a symptom presumably caused by V. albo-atrum (46).

Guthrie's (25) soil fumigation experiments with Vapam, apparently applied as spring injections, did not reduce losses. According to him this may have been due to low soil temperature at the time of fumigation and consequently to lack of volatilization and dispersion of the chemical within the soil. Chloropicrim gave better control of Verticillium wilt, but was not considered economically feasible.

In Oregon (76), however, spring injections (10 days prior to planting) of 190 lbs/A of Vapam did result in effective control of the early maturity disease of potatoes which is in that region primarily attributed to V. albo-atrum. Young (76) emphasized the importance of the method of application of Vapam. In his experiments the Vapam was injected into the soil by means of a blade applicator which sprayed the

chemical in a continuous layer approximately 6 inches deep into the soil, whereas the usual method depended on downward percolation of the chemical with the water into the soil.

Faulkner and Skotland (19), working with the Verticillium wilt of mint (Mentha spp.) in Washington, found that Trizone (chloropicrin 31%, methyl bromide 61%, and propargyl bromide mixture) at 370 lbs/A, Picfume (chloropicrin) at 45 gal/A, Vorlex at 45 gal/A, and DD at 38 gal/A all gave significant nematode control and yield increases. Trizone, Picfume, and Vorlex were also effective in controlling Verticillium wilt; DD only reduced the initial incidence of the disease and by harvest time no significant differences were noted between the DD-treated and the control plots.

Rich and Miller (62) found that various antifungal treatments, particularly Terraclor (pentachloronitrobenzene, 75% wettable powder) at 170 lbs/A applied to the soil increased the incidence of Verticillium wilt of strawberries in the field. These increases in Verticillium wilt were correlated with increases in the number of meadow nematodes, P. penetrans. It has been shown that Terraclor increases the number of P. penetrans in field plots without strawberries or Verticillium wheat cultures (48). Rich and Miller (62) believe that treatments favoring an increase in nematode population in turn increase the incidence of Verticillium wilt in strawberry plants.

McKeen and Mountain (44), in their work with Verticillium wilt of eggplant (Solanum melongena L.), proved the

existence of a definite synergism of the fungus and the nematode (P. penetrans). In their growth room experiments steam-disinfested soil was inoculated with microsclerotia of V. albo-atrum alone, combined with nematodes, with nematodes alone and no inoculum. Both the fungus and nematode levels were varied. At low and medium levels of fungus inoculum, the nematodes increased wilt and the increase might have been a function of the number of nematodes present. Except at the high inoculum levels of the fungus, significantly larger numbers of nematodes occurred in eggplant roots in the presence than in the absence of the fungus. The nematodes alone had no adverse effect on root condition or on growth of eggplant.

In growth room experiments with Verticillium wilt of tomatoes, the same authors (50) found that plants in soil infested with both the fungus and the nematode, showed a higher incidence of wilt than those in soil with the fungus alone. The plants in the combined treatments also yielded significantly higher numbers of nematodes (P. penetrans) than plants in soil with nematodes only.

Miller and Edgington (47) added white pine sawdust and chopped paper to soil infested with P. penetrans. Ten days later 50,000 microsclerotia of V. albo-atrum were added to each pot, and again 10 days later var. Bonny Best tomatoes were planted in each pot. Twenty days after adding the sawdust and paper amendments the nematode population had already

been reduced to one-third and one-fourth, respectively, of that in unamended soil. Wilt symptoms rated 60 days after planting the tomatoes showed that sawdust and paper had reduced the Verticillium wilt by 90% and 50%, respectively. Apparently, the addition of cellulosic materials controlled nematode numbers and consequently wilt severity.

Abu-Gharbieh et al. (1) found an apparent interaction of the fungus and the nematode on varieties of strawberries susceptible to Verticillium wilt, but none on resistant varieties even though the nematodes reproduced readily on them.

Bergeson (3) found that simultaneous inoculation of peppermint (Mentha piperita L.) with P. penetrans and V. albo-atrum resulted in either nonsignificant or additive differences in growth reduction. When the inoculation with nematodes preceded the fungal inoculation by 2 months, diagnostic symptoms of Verticillium wilt appeared approximately 2 weeks earlier than in the treatments with the fungus alone.

Faulkner and Skotland (20) inoculated peppermint plants with microsclerotia alone, in combination with varying levels of nematodes, and with nematodes alone. Presence of the nematodes increased both the severity and incidence of Verticillium wilt symptoms, and reduced the incubation period. The rate of reproduction of the nematode (P. minyus Sher & Allen) was greater in plants infected with both pathogens than with the nematode alone. Dry weights of infected plants

were reduced up to 22% when the inoculum contained either pathogen alone. However, with combinations of the fungus and the nematode, the dry weights of infected plants were reduced by as much as 68% in comparison with the controls.

Mountain and McKeen (49) found that the relative rate of reproduction of P. penetrans in roots of tomato and eggplant increased in soil infested with microsclerotia of V. albo-atrum. It is not established that the increased rate of reproduction is the only factor contributing to the increase in the nematode population. They postulated that the fungus may affect the epidermal and cortical tissues of the host roots in such a way as to facilitate entry of the nematode into the root, or that metabolic substances evolved by the fungus-infected root may be attractive to the nematode.

In summary, it has been shown that treatments which reduce nematode populations (soil fumigation and soil amendments) also reduce the incidence and severity of wilt symptoms and yield losses. Application of a soil fungicide that is not toxic to V. albo-atrum increases the nematode population, and this increase is associated with an increase in wilt. Presumably the fungicide controls some of the soil antagonists of P. penetrans.

In the work on eggplant, tomato, strawberry, and peppermint results have revealed the existence of a definite interaction between the fungus and the nematode in the Verticillium wilt of these plants.

The purpose of this research was to determine whether or not a similar fungus-nematode interaction exists in the Verticillium wilt of potatoes.

Materials and Methods

Conidial Experiment

Nematode inoculum. Vetch (Vicia sativa L.), an excellent host for P. penetrans (55), was grown in 6 inch pots in the greenhouse. Before seeding the soil was mixed with chopped roots of previously grown vetch plants parasitized with nematodes (P. penetrans). After about 8 weeks of growth the tops of the vetch plants were cut off; the root masses were cut in small pieces, which were placed in layers alternating with soil in large 10 inch pots, and left to incubate for 7 days in the greenhouse. In previous tests it was found that under these conditions after 7 days most nematodes had migrated out of the roots into the surrounding soil. At longer periods of incubation the number of nematodes found in the soil declined sharply. This may be due to increased mortality of the nematodes as they fail to locate a suitable food source, or to the possibility that the proportion of nematodes emerging from the roots after 7 days is very small, or to a combination of these factors.

After 7 days' incubation the roots were separated from the soil by means of a 1/2-inch-mesh screen; the soil was thoroughly mixed and assayed for nematodes. Eight samples of 20 g soil were placed on Baermann funnels (2) and left overnight

at room temperature. The filters used were 6-1/2 inch milk filters, fluff type, distributed by Agway, Inc. The following morning 50 ml were drawn from each funnel and the nematodes counted in each of these 8 suspensions. During this assay the main body of soil later to be used as nematode inoculum, was stored in a cold room at 45-55°F. Storage at this temperature range did not affect the numbers of nematodes appreciably (less than 5% as was determined in previous tests).

Conidial inoculum. A culture of V. albo-atrum, a microsclerotial form, was kindly supplied by Dr. W. J. Tolmsoff. Subcultures were grown on 1.5% PDA with 100 ppm Streptomycin sulfate in Petri plates in the dark at room temperature. When verticillate conidiophores were observed growing on the surface of the agar, sterile water was poured on the fungus cultures; the dish was then gently roiled for about 30 sec and the liquid, containing the conidia of V. albo-atrum, was poured into a sterile beaker. Spore concentration was determined by means of counts on a hemacytometer.

Soil. The soil used for these experiments originated from a field near Madbury, N. H. This field had been planted to potatoes and tomatoes in most of the last 10 years. When no potatoes or tomatoes were grown it was left fallow. The last fallow period had occurred the same summer of the year (1965) in which the soil was taken. In this field no Verticillium- or Fusarium-wilt had been observed during the last 10 years, according to Dr. A. E. Rich who supervises the

cultivation of this field closely. In the fall top soil was taken and stored in the greenhouse at a temperature range of 80-90°F. Four months later and again 6 months later the soil was checked for V. albo-atrum and Fusarium spp. by means of the dilution-plate method. Four dilutions of 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were prepared of each of 4 soil samples of 1 g; 1 ml was poured on 1.5% PDA with 100 ppm Streptomycin sulfate in each of 4 Petri dishes for each dilution. No colonies of V. albo-atrum or of Fusarium spp. were observed at any of the dilutions either time. Hence the soil was considered to be free of both fungi, and disinfestation was unnecessary.

The soil was also sampled for nematodes (P. penetrans) after 2 and again after 4 months of storage in the greenhouse. At both times 8 samples of 20 g each were assayed on Baermann funnels. After 2 months only 2 specimens of P. penetrans were found per 160 g soil, which represents a concentration of only 0.0125 nematode/g of soil. This is sufficiently small compared to the inoculum level of 2.5 nematodes/g of soil used in the conidial experiment. After 4 months of storage only 1 nematode/g of soil was found, which represents a concentration of only 0.00625/g of soil. This is negligible compared to the concentrations of nematodes used in the microsclerotial experiment. Thus it was also considered unnecessary to disinfest the soil for nematodes. In the following parts this soil is referred to as "clean" soil.

Inoculation. Potato seed pieces of the variety Katahdin were surface-disinfested in a suspension of 50% Captan, and planted in "clean" soil in peat pots. When the plants were about 4 inches high they were transplanted to 6 inch pots and inoculated.

Ten plants received nematodes only (referred to as N treatment): 40 g of nematode soil, containing about 125 nematodes/g, was thoroughly mixed with about 2000 g "clean" soil (hand friable). Thus the inoculum level was approximately 5000 nematodes per pot. The bottom of the pot was covered with about 1 inch of "clean" soil; then the peat pot was removed and the potato plant placed in the pot. The previously nematode-infested soil was added around the root system and another layer of about 1/2 inch "clean" soil placed on top.

Ten plants received conidia only (referred to as F treatment): a suspension of about 400 ml containing about 2,500,000 conidia/ml was prepared. The root systems of these plants were first dipped in the suspension; then all 20 plants (10 for the F, and 10 for the FN treatment) were placed on some "clean" soil and about 20 ml of the conidial suspension was poured over each of the root systems and "clean" soil added for 10 plants of the F treatment. Thus each of the fungus-inoculated plants received an estimated 50,000,000 conidia.

The 10 remaining fungus-inoculated plants (referred to as FN treatment) also received nematodes: 2000 g of soil

containing about 5000 nematodes, was added around the root system of each plant, and 1/2 inch of "clean" soil was placed on top.

Growth conditions. All 30 plants were grown for 51 days from the time of planting to harvest. They were given a 20-20-20 mixture of N, P_2O_5, and K_2O in tap water every 2 weeks. Temperatures in the greenhouse during this experiment ranged from a minimum of 65°F at night and on cloudy days to an occasional 90°F at mid-day on sunny days in the fall. A photoperiod of about 16 hours was provided by means of incandescent light bulbs suspended about 1 foot over the plants.

Wilt observations. At harvest time the number of wilted stems per pot was recorded, and expressed as a percentage of the total number of stems per pot; the stems in each pot were checked for vascular discoloration. Stem sections about 3/4 inch long were saved and stored at 5°C for isolation of V. albo-atrum. To isolate the fungus, stem sections were surface-disinfested in 10% Clorox for 2 minutes followed by a 1-minute dip in sterile water. The stem sections were then dissected and xylem columns removed aseptically. The xylem strands were dipped in 10% Clorox for only 1 minute, followed by a 30-sec dip in sterile water. They were plated out on 1.5% PDA with 100 ppm Streptomycin sulfate in Petri dishes. Cross sections of the lower part of the stems were examined for fungus hyphae in the lumina of the

xylem vessels. Percentages of stems wilted per pot, of stems showing vascular discoloration, and hyphae in the xylem vessels were converted to angles (68), analyzed, and then again represented as average percentages.

Harvest procedures. Fresh and dry weights of tops, tubers and stolons, and roots were determined. For the dry weight determination, the tops were cut in pieces and predried at 65°C for about 12 hours in a ventilated oven; the tubers and stolons were sliced and predried at 65°C for about 18 hours. Both groups of plant parts were subsequently dried at 105°C for about 12 hours in a ventilated oven; the dried material was then transferred to desiccators, allowed to cool off to room temperature for a few hours, and weighed. Nematodes were extracted from the fresh roots by incubation for about 24 hours at room temperature in shallow layers of tap water in Erlenmeyer-flasks on a rotary-action shaker (11, 77). The nematode suspensions obtained were decreased in volume to 200 ml, and the nematodes in 4 aliquots of 5 ml for each plant were counted in Syracuse watch glasses with the help of a binocular dissecting microscope; the total number of nematodes for each plant was estimated by means of the average number found in the 5 ml aliquots.

Microsclerotial Experiment

Nematode inoculum. The production and preparation of nematode inoculum proceeded as described for the conidial experiment.

Microsclerotial inoculum. Production, collection, and preparation of microsclerotial inoculum proceeded following Tolmsoff's modification of a method developed by Luck (69). A microsclerotial culture of V. albo-atrum was kindly supplied by Dr. Tolmsoff. The surface of the agar in this tube was covered with a thick crust of microsclerotia. Small amounts of this microsclerotial mass were transferred aseptically to sterile test tubes containing about 5 ml of sterile, distilled water. The test tubes were shaken vigorously, and the suspensions poured on Petri plates under aseptic conditions. The plates contained 1.5% PDA and 100 ppm Streptomycin sulfate. The agar surface was covered with an autoclaved cellophane disc prior to pouring on the fungus suspension. After inoculation the Petri plates were stored in the dark at room temperature. When inoculum was needed for an experiment the cellophane discs were removed from the plates, and the surface growth of microsclerotia and hyphae was transferred to sterile water in a sterile beaker. The suspension of microsclerotia was stirred vigorously and poured over a known amount of air-dry "clean" soil; the material was thoroughly mixed and allowed to dry for several days at room temperature. The concentration of microsclerotia in this soil was estimated by means of the dilution-plate method. Dilutions of 10^{-3}, 10^{-4}, and 10^{-5} were prepared for each of 4 samples of 1 g each of the mixture. Four plates were prepared for each dilution, and each plate received 1 ml of the particular dilution.

Inoculation. The 5 levels of microsclerotial inoculum added were approximately 0; 70,000; 140,000; 210,000; and 280,000 per pot. These levels represented concentrations of 0, 35, 70, 105, and 140 microsclerotia/g of soil, respectively. They were chosen in the range of concentrations at which Tolmsoff (69) observed his first wilt symptoms after 82 days. Thus chosen they were thought to be sufficiently low to render possible interactions with the nematode discernible, and yet high enough to produce an observable wilt syndrome.

The levels of nematode inoculum were approximately 0, 2000, 4000, 6000, and 8000 nematodes per pot.

In the inoculation procedure the appropriate amounts of soil, containing the necessary inocula, were mixed with 500 g clean sand and enough "clean" soil to yield a total of 2000 g. One inch of "clean" soil was placed on the bottom of a 6 inch container, the plant added with the 2000 g mixture of sand, soil, and appropriate amounts of inocula, and a 1 inch layer of "clean" soil placed on top of this. Plants grown in the first period were planted in 6 inch clay pots. Those grown in the second and third period were planted in 6 inch cans.

The 5 fungus inoculum levels and the 5 nematode inoculum levels were combined in all possible combinations to make a total of 25 treatments in a 5^2 factorial design. Six replicates were grown of each treatment, 2 during each of

3 growing periods. The 50 plants grown in each period were numbered 1 to 50, and randomly assigned places on a greenhouse bench with the help of a table of random numbers (68).

Growing conditions. A photoperiod of 16 hours was provided with incandescent lights as described for the conidial experiment. Every 2 weeks the plants were given a 20-20-20 mixture of N, P_2O_5, and K_2O in tap water.

The first set of 2 replicates was grown in the winter for 50 days from inoculation to harvest. Greenhouse temperatures during this period ranged from frequent minima of 65°F to rare maximum of 85°F at mid-day on sunny days. The second set of 2 replicates was grown in the spring for 60 days from inoculation to harvest. Greenhouse temperatures ranged from nocturnal minima of 65°F to rare maxima of 95°F on sunny days.

The plants in the third period were grown in the early summer for 60 days. Greenhouse temperatures ranged from infrequent nightly minima of 50°F to very frequent daytime temperatures of 90-100°F, and occasional maxima of about 110°F.

The number of sprouting eyes was kept to 1 per pot; thus there was only 1 main stem with its side branches in each pot.

Lodging of vines grown in the first period was found to render cultivation and observation of wilt symptoms difficult. Therefore plants grown in the second and third periods were supported with 4-foot long bamboo stakes.

Wilt observations. Wilt symptoms in the microsclerotial experiment were rated on a scale from 0 to 6. The symptom classes were as follows:

0: no wilt, no yellowing of leaves, no leafdrop.

1: no wilt, slight yellowing of leaves, no leafdrop.

2: no wilt, moderate yellowing of leaves progressing upward along the vines, some leafdrop.

3: slight wilt, moderate yellowing of leaves progressing upward along the vines, moderate leafdrop.

4: moderate wilt, widespread yellowing and necrosis of leaves, moderate leafdrop.

5: severe wilt, very widespread yellowing of leaves and severe necrosis, some yellowing of stems progressing from the tops downward, extensive defoliation.

6: very severe wilt, some of the tops of plants dead, and widespread necrosis of leaves, extensive defoliation and yellowing of stems.

The frequency of each rating was computed; then for each rating, a transformed value was obtained by computing the mean of the corresponding area of the normal distribution. The values for the means were substituted for the wilt ratings and analyzed.

Harvest procedures. Fresh and dry weights of tops, tubers and stolons, and roots were determined; vascular discoloration of the stems recorded; sections of the stems

were saved and fungus isolations made; and nematodes were extracted from fresh roots, all as described for the conidial experiment.

In addition, soils were assayed for nematodes: the soil from each pot was thoroughly mixed; 4 samples of 10 g soil were assayed per pot. After 12 hours incubation on Baermann funnels at room temperature the nematodes in 4 samples of 50 ml each were counted. The average number of nematodes per 10 g of soil, obtained from 4 samples of 10 g each, was used to estimate the total number of nematodes in the soil of each pot.

Results

Conidial Experiment

The fresh weight (Table 1) of the tops of the FN treatment was not significantly smaller than that of the F treatment, but fresh tops of both FN and F treatments weighed significantly less than fresh tops of the N treatment. The dry weights (Table 2) of the tops of either FN and F treatments, or F and N treatments were not significantly different, but the FN value was significantly smaller than the N value.

The fresh weight (Table 1) of the tubers of treatment F was significantly larger than of both N and FN treatments, but fresh tubers of the N treatment did not weight significantly more than those of the FN treatment. Similar results were obtained for the dry weights of tubers (Table 2).

Table 1

Fresh weights[a/] of potatoes from fungus (F), nematode (N), and fungus-nematode (FN) treatments arranged in descending order.

	Tops		
Treatments	N	F	FN
Grams	75.70*	52.30	43.65

	Tubers		
Treatments	F	N	FN
Grams	22.20*	13.15	10.40

	Roots		
Treatments	N	FN	F
Grams	18.15	13.35	12.25

	Totals		
Treatments	N	F	FN
Grams	107.00	86.75	67.40

a/ Averages of 10 plants.

* Significant at 5%.

Table 2

Dry weights[a/] of potatoes from fungus (F), nematode (N), and fungus-nematode (FN) treatments arranged in descending order.

	Tops		
Treatments	N	F	FN
Grams	6.32	5.21	4.28

	Tubers		
Treatments	F	N	FN
Grams	2.77*	1.64	1.30

	Roots		
Treatments	N	FN	F
Grams	1.08	0.76	0.59

	Totals		
Treatments	N	F	FN
Grams	9.04	8.57	6.34

a/ Averages of 10 plants.

* Significant at 5%.

The fresh weight (Table 1) of the roots of the N treatment was significantly larger than that of the F treatment, but not larger than that of the FN treatment; the FN value also was not significantly larger than the F value. The results of the dry weights (Table 2) of roots were similar.

The total fresh weight (Table 1) of potato plants of the N treatment was significantly larger than that of the FN treatment, but not larger than that of the F treatment; the F value also was not significantly larger than the FN value. Similar results were obtained for the total dry weights (Table 2).

The numbers of nematodes (Table 3) obtained from potato roots of the N treatment was not significantly larger than that of the FN treatment. The number of nematodes per gram of roots (dry weight) of the FN treatment was not significantly larger than that of the N treatment.

The percentage of stems wilted (Table 4) of the FN treatment was not significantly larger than that of the F treatment. However, the percentage of stems showing vascular discoloration and hyphae in the xylem vessels (Table 5) was significantly larger in the FN treatment than in the F treatment, and zero in the N treatment.

Table 3

Numbers[a/] of nematodes (*P. penetrans*) recovered from potato roots in fungus (F), nematode (N), and fungus-nematode (FN) treatments arranged in descending order.

Total from roots			
Treatments	N	FN	F
Nematodes	12,330	10,840	160**

Per gram of roots (dry weight)			
Treatments	FN	N	F
Nematodes	16,440	12,200	310**

a/ Averages of 10 plants

** Significant at 1%.

Table 4

Percentages[a/] of stems of potatoes wilted from fungus (F), nematode (N), fungus-nematode (FN) treatments arranged in descending order.

Treatments	FN	F	N
	52%	35%	0%**

a/ Averages of 10 plants.

** Significant at 1%.

Table 5

Percentages[a/] of stems showing vascular discoloration and hyphae in the xylem vessels of potatoes from fungus (F), nematode (N), and fungus-nematode (FN) treatments arranged in descending order.

Treatments	FN	F	N
	98%*	63%*	0%*

a/ Averages of 10 plants.

* Significant at 5%.

Microsclerotial Experiment

As stated before, 2 replicates of each treatment were grown at the same time, each set of 2 during a different period; there were 3 periods which will be referred to in the following as P-I for the winter, P-II for the spring, and P-III for the early summer.

The analyses of variance of data of the 6 replicates together showed that the period effect had been large enough to mask treatment effects to a great extent. Hence, all combinations of 2 periods were analyzed and the results are given of those combinations in which the period effect was small enough to reveal effects of fungus and nematode treatments, and interaction of the 2, if any. Significance levels are at 5% unless otherwise stated.

For the dry weights of the tops (Table 6) in P-I and P-II both the fungus effect and the fungus-nematode interaction

Table 6

Dry weights[a/] in grams of tops of potatoes grown at 5 levels each of fungus and nematode inoculum.

Microsclerotia per pot	Nematodes per pot				
	0	2000	4000	6000	8000
0	13.03	11.96	14.31	13.59	13.16
70,000	9.85	11.94	16.22	14.74	12.88
140,000	10.53	12.68	10.63	10.41	12.09
210,000	12.16	12.75	18.38	10.40	13.71
280,000	11.94	12.79	9.74	12.04	12.45

a/ Averages of 4 replicates grown in P-I and P-II.

were found to be significant, but not the nematode effect. For the fresh weights of tops (Table 7) all 3 effects were non-significant. In the fungus-nematode treatments of the dry weights of tops (Table 6), yields at all 4 nematode levels were higher than those of the fungus only treatments. Yields at the lower inoculum levels for the combination treatments tended to be somewhat higher than for the corresponding single treatments. Yields at combinations of highest fungus and nematode levels were lower than those of corresponding nematode levels, but higher than at corresponding fungus levels.

For the dry weights and fresh weights of tubers (Tables 8 and 9) in P-II and P-III, a significant fungus-nematode interaction was found, but fungus and nematode effects

Table 7

Fresh weights[a/] in grams of tops of potatoes grown at 5 levels each of fungus and nematode inoculum.

Microsclerotia per pot	Nematodes per pot				
	0	2000	4000	6000	8000
0	135	137	155	138	134
70,000	95	127	185	154	129
140,000	105	137	114	95	132
210,000	137	140	187	119	141
280,000	133	142	97	126	115

a/ Averages of 4 replicates grown in P-I and P-II.

Table 8

Dry weights[a/] in grams of tubers of potatoes grown at 5 levels each of fungus and nematode inoculum.

Microsclerotia per pot	Nematodes per pot				
	0	2000	4000	6000	8000
0	11.76	19.54	24.30	21.83	8.11
70,000	22.96	20.34	12.60	10.55	16.21
140,000	31.11	22.08	19.79	19.92	15.82
210,000	10.28	24.11	21.76	26.28	19.80
280,000	21.31	25.51	22.65	17.36	20.28

a/ Averages of 4 replicates grown in P-II and P-III.

Table 9

Fresh weights[a/] in grams of tubers of potatoes grown at 5 levels each of fungus and nematode inoculum.

Microsclerotia per pot	Nematodes per pot				
	0	2000	4000	6000	8000
0	82	131	152	139	55
70,000	138	132	96	75	108
140,000	190	140	137	125	103
210,000	81	163	141	167	119
280,000	138	142	143	120	132

a/ Averages of 4 replicates grown in P-II and P-III.

by themselves were non-significant. At the lowest level of nematode inoculum, yields (Table 8) of the combined treatments were higher than of the fungus only treatment; then at increasing nematode levels yields decreased to values below that of the fungus only treatment. Yields at the 2 highest fungus levels in combination treatments tended to be somewhat larger than those at the 2 lowest fungus levels. In the nematode only treatments, yields increased first with increasing nematode inoculation level to a maximum in the second level, then decreased sharply in the third and fourth levels.

For the dry and fresh weights of roots (Tables 10 and 11) in P-II and P-III a highly significant (at 1%) nematode effect was found, but fungus and interaction effects were non-significant. Yields (Table 10) increased with increasing nematode inoculum levels with a maximum at the next highest

Table 10

Dry weights[a/] in grams of roots of potatoes grown at 5 levels each of fungus and nematode inoculum.

Microsclerotia per pot	Nematodes per pot				
	0	2000	4000	6000	8000
0	0.97	0.87	1.12	1.48	0.83
70,000	0.80	1.05	0.91	0.81	0.89
140,000	1.26	1.30	1.02	1.25	0.68
210,000	0.58	0.99	1.07	1.32	0.96
280,000	0.83	1.02	1.29	1.35	0.97

a/ Averages of 4 replicates grown in P-II and P-III.

Table 11

Fresh weights[a/] in grams of roots of potatoes grown at 5 levels each of fungus and nematode inoculum.

Microsclerotia per pot	Nematodes per pot				
	0	2000	4000	6000	8000
0	20	17	23	29	17
70,000	16	21	19	15	17
140,000	24	25	21	23	14
210,000	12	21	19	24	16
280,000	17	19	25	28	19

a/ Averages of 4 replicates grown in P-II and P-III.

nematode level and a decrease at the highest level. This effect was also apparent in the nematode only treatments.

For the number of nematodes recovered per gram of roots (dry weight) in P-I and P-III (Table 12) a strong nematode effect was found, but fungus and interaction effects were non-significant. Numbers of nematodes increased from low to high nematode inoculum levels, but for the fungus levels there was an initial increase in numbers with a maximum in the second fungus inoculum level and a decrease in numbers at the third and fourth fungus levels.

Table 12

Numbers[a/] of nematodes (*P. penetrans*) per gram of roots of potatoes grown at 5 levels each of fungus and nematode inoculum.

Micro-sclerotia per pot	Nematodes per pot				
	0	2000	4000	6000	8000
0	35	645	1240	1100	2040
70,000	36	1302	2055	2922	2180
140,000	30	682	2070	2600	3767
210,000	40	745	2095	2765	2160
280,000	37	1227	1462	1770	1917

a/ Averages of 4 replicates grown in P-I and P-III.

For the numbers of nematodes recovered from roots (Table 13) in P-I and P-III, both fungus and nematode effects were found to be significant, but not the interaction effect. Nematodes again increased in numbers from low to high nematode

inoculum levels, and at each of the fungus inoculum levels more nematodes were recovered than at the nematode only treatments.

Table 13

Numbers[a/] of nematodes (P. penetrans) recovered from roots of potatoes grown at 5 levels each of fungus and nematode inoculum.

Micro-sclerotia per pot	Nematodes per pot				
	0	2000	4000	6000	8000
0	37	785	2032	2142	1912
70,000	38	1747	2460	2907	3577
140,000	36	1252	2410	3377	3497
210,000	42	965	3670	3335	3042
280,000	50	2045	1760	2670	3127

a/ Averages of 4 replicates grown in P-I and P-III.

For the numbers of nematodes recovered from soil (Table 14) in P-I and P-III the nematode effect was again found to be highly significant, while the fungus and interaction effects were not. Numbers of nematodes increased with the first three nematode inoculum levels, and decreased in the fourth. The numbers recovered at all fungus inoculum levels were higher than without fungus, the highest number being obtained at the highest fungus level.

For the total number of nematodes recovered from soil and roots (Table 15) in P-I and P-III the nematode effect was significant, but fungus and interaction effects were not.

Numbers again increased in the first three nematode inoculum levels and decreased in the fourth level. Numbers recovered at all fungus inoculum levels were higher than without fungus, and highest at the fourth fungus level.

Table 14

Numbers[a/] of nematodes (P. penetrans) recovered from potato soil at 5 levels each of fungus and nematode inoculum.

Microsclerotia per pot	Nematodes per pot				
	0	2000	4000	6000	8000
0	100	983	2807	2462	1900
70,000	137	1640	3818	1785	5540
140,000	147	1093	3780	3943	3053
210,000	200	2462	2908	5512	2275
280,000	105	2405	4240	5122	4427

a/ Averages of 4 replicates grown in P-I and P-III.

Table 15

Total numbers[a/] of nematodes (P. penetrans) recovered from soil and roots of potatoes grown at 5 levels each of fungus and nematode inoculum.

Microsclerotia per pot	Nematodes per pot				
	0	2000	4000	6000	8000
0	137	1768	4839	4604	3812
70,000	175	3387	6278	4692	9117
140,000	183	2345	6190	7320	6550
210,000	242	3427	6578	8847	5317
280,000	155	4450	6000	7792	7554

a/ Averages of 4 replicates grown in P-I and P-III.

All plants inoculated with the fungus showed vascular discoloration and yielded the microsclerotial form of V. albo-atrum, while plants not inoculated with this fungus did not yield any V. albo-atrum nor was any vascular discoloration detected.

For the wilt ratings (Table 16) the fungus effect was significant, while the nematode and interaction effects were not. Higher ratings were obtained for the combined treatments than for the corresponding fungus only treatments; the highest rating was observed in the combined treatments with highest inoculum levels of both fungus and nematode.

Table 16

Wilt ratings[a/] of potatoes grown at 5 levels each of fungus and nematode inoculum.

Microsclerotia per pot	Nematodes per pot				
	0	2000	4000	6000	8000
0	0.00	1.00	0.75	1.00	0.50
70,000	2.75	3.00	3.25	2.75	3.75
140,000	3.00	3.00	4.00	3.25	3.75
210,000	3.50	3.75	3.50	4.00	3.75
280,000	4.00	5.00	4.75	4.50	5.50

a/ Averages of 4 replicates grown in P-II and P-III.

0 = no wilt
1 = no wilt, slight yellowing
2 = no wilt, moderate yellowing
3 = slight wilt
4 = moderate wilt
5 = severe wilt
6 = very severe wilt

(see also page 22)

Discussion and Conclusions

In the conidial experiment the results showed that tuber yields from the fungus-nematode treatment were lower than from the fungus only treatment. Subsequently in the microsclerotial experiment a fungus-nematode interaction was found, but the nature of this interaction was not entirely clear. It appeared that in the combined treatments at the higher inoculum levels of both fungus and nematodes the interaction resulted in lower yields than from the fungus only treatments.

At harvest time the plants were apparently at different stages of maturity. One of the more striking phenomena usually observed in Verticillium wilt of potatoes is that infected plants mature earlier with increasing inoculum levels of the fungus. Hence, harvesting plants inoculated with different amounts of fungus inoculum all at the same time is expected to yield results for plants at different stages of maturity. In the work here reported this became apparent in the higher tuber yields at higher fungus inoculum levels than at the lower levels.

The nematodes alone also appeared to hasten maturity as expressed in tuber yields increasing with increasing nematode inoculum in the treatments with nematodes only, while at the highest level the nematodes were apparently so harmful that the yield decreased sharply to below that of the controls. If this is true then the fungus-nematode interaction can be explained as follows: since both organisms tend

to speed up maturity of infected plants they can be expected to interact at the lower, less harmful inoculum levels to hasten maturity resulting in more and earlier yields than from the single treatments. At higher levels of both fungus and nematode the interaction becomes harmful resulting in lower yields. The interaction found for the tops can be explained similarly.

As regards the wilt characteristics in the conidial experiment only the percentages of stems showing vascular discoloration and hyphae in the xylem were found to be higher in the fungus-nematode treatment than in the fungus only treatment.

In the microsclerotial experiment, wilt ratings for combined treatments were higher than for fungus only treatments. Since no significant interaction effect was found for the wilt ratings additivity of fungus and nematode effects may be expected.

Increasing nematode inocula were strongly associated with increased root growth except at the highest nematode inoculum level which limited root production severely. This was also true for the fungus-nematode treatments but with more root growth than at each of the corresponding nematode only treatments. Although no significant fungus effect was detected for root production it is conceivable that fungus and nematode stimulate root growth in an additive manner.

Increasing nematode inocula yielded higher numbers of nematodes per gram of roots; this was also true for the total

number of nematodes recovered from roots except for a slight decrease at the highest nematode inoculum level, probably associated with decreased root production at that level.

The significance of the fungus effect found for the total number of nematodes from roots also seems to lie in higher numbers recovered at increasing fungus levels with a moderate decrease at the highest fungus level, probably due to less root growth. However, there were also fewer nematodes recovered per gram of roots at the higher fungus levels. Apparently, the nematode population in the roots not only decreases with decreasing root production but also with increasing colonization of the roots by the fungus. This point is supported by the fact that in soil the highest number of nematodes was found at the highest fungus inoculum level. At this level the roots apparently become less inhabitable for the nematodes, causing many to leave the roots. The results of Mountain and McKeen (49) may have differed due to lower inoculum levels in their experiments.

Summary

An interaction between Verticillium albo-atrum and Pratylenchus penetrans was demonstrated in the Verticillium wilt of potatoes.

In a greenhouse experiment on the combined effects of V. albo-atrum and P. penetrans on potatoes, significantly lower tuber yields were obtained in the fungus-nematode treatment than in treatments with either fungus or nematode alone,

when conidia of the fungus were used as inoculum. When fungus (microsclerotia) and nematodes were combined each at 5 inoculum levels in a 5^2 factorial design, significant interactions were found for the dry weights of both tops and tubers. Yields at the lower inoculum levels in fungus-nematode treatments tended to be higher than at corresponding levels of either fungus or nematode alone. Yields of tops at combinations of high inoculum levels tended to be lower than at corresponding nematode levels, but higher than at corresponding fungus levels. Yields of tubers at combinations of high inoculum levels tended to be somewhat larger at high fungus levels than at low fungus levels, but tuber yields were smaller at high nematode levels than at either low nematode levels or at fungus only treatments.

Root yields significantly increased with increasing nematode inoculum levels to a maximum in the next highest nematode level with a decrease at the highest nematode level in both combination treatments and nematode only treatments. Numbers of nematodes recovered per gram of roots (dry weight) significantly increased with increasing nematode inoculum levels in both combination and nematode only treatments. With increasing fungus inoculum in the combined treatments the nematodes increased with the lower levels, then decreased with the 2 higher levels.

Both fungus and nematodes were found to influence the total number of nematodes recovered from roots, the nematodes

increasing with increasing nematode inoculum level. At each of the fungus inoculum levels significantly more nematodes were recovered from roots in the combined treatments than at the nematode only treatments.

Numbers of nematodes recovered from soil were found to increase significantly with the nematode inoculum levels, with a decrease at the highest level. At all fungus levels more nematodes were found in the soil of combination treatments than in nematode only treatments, and the highest number found at the highest fungus level.

Wilt ratings in combined treatments were significantly higher than at corresponding fungus only treatments, and were highest at the combination of highest fungus and nematode inoculum.

SECTION II

ATTRACTION OF THE NEMATODE PRATYLENCHUS PENETRANS BY AMINO ACIDS, DICARBOXYLIC AND HYDROXY ACIDS, PURINES AND PYRIMIDINES, VITAMINS AND OTHER COMPOUNDS

Introduction

The manner in which plant parasitic nematodes locate the roots of their hosts in the soil has recently been the subject of many investigations. Work in this area has chiefly centered around the question of whether nematodes are able to find their host roots by means of directed, oriented migration or by random wandering. Orientation may be defined as the establishment and maintenance of body attitude in relation to the external environment (34).

Primary orientation serves to control the body attitude in relation to such factors as contact with surfaces, dorsal light, and to gravity, whereas secondary orientation is concerned with orientation to gradients of chemical concentrations, humidity, potentials and so on. Kineses are defined as undirected reactions to these stimuli, and taxes as directed ones (24).

Attraction is probably best defined as the process by which a given stimulus elicits a positive, directed response by the animal, and repulsion as an avoiding reaction (15, 34). Thus attraction in its narrow sense refers to directed orientation, klinotaxis or orthotaxis, and in a wider sense to

undirected orientation, orthokinesis or klinokinesis (34). Aggregation of animals may result from directed or undirected migration to a source of stimulation or from random wandering. Hence, any aggregation does not necessarily result from attraction as defined in its narrow sense.

Literature Review

It is a frequently observed fact that nematodes will aggregate around roots. Discussions as to how the nematodes reach the roots have led to the birth of 2 opposing hypotheses (34).

The first hypothesis postulates that there is no attraction, i.e. the nematodes migrate at random and find the roots by chance (35), after which they are simply detained to the root surface, e.g. by an orthokinetic reaction to root exudates or by trapping in water about roots which grew between agar and the container (66).

The second hypothesis supports the view that nematodes are attracted from some distance. In experiments with a membrane placed between the nematodes and plant roots (8, 39, 58, 71, 72) the nematodes were found to aggregate near the membrane close to the roots. Apparently the nematodes responded to a diffusible factor some distance away. Klingler (34) feels that the aggregations of nematodes near the membrane could have been the result of attraction in the wide sense, an orthokinetic reaction, e.g. a decrease in speed of the nematodes near the membrane. Whether directed or undirected attraction enticed the nematodes to travel to the

membrane, it is still clear that some factor induced more nematodes to remain there than to move to other areas.

Evidence for actual occurrence of attraction in the strict sense was provided by direct observation of nematodes during their migrations. When the free water has evaporated from agar surfaces nematode tracts can be made visible under vertically incident light (64, 67). From these nematode tracks it can be determined whether oriented or random movement has taken place. At first only random movement was observed to roots lying uncovered on agar surfaces (67), but after a glass plate had been placed about 1 mm above the agar surface a directed movement (klinotaxis) of Ditylenchus dipsaci to germinating seeds of various plant species was observed (33). Klingler (33) states that the placing of this glass plate facilitates the formation of any possible gaseous gradient and if this gradient is essential no orientation can take place without such a glass plate. Chen and Rich (12) also found a positive directed orientation of P. penetrans towards roots of clover plants growing in closed containers, and Lavallee and Rohde (36) obtained similar evidence for attraction in its narrow sense of this nematode to plant roots. Klingler (34) feels that it seems clear from the available evidence that nematodes are able to move towards roots of a host plant by means of orientation, whether directed or undirected, but that attraction in a strict sense is only to be expected in the immediate vicinity of the roots, probably within a range of 1-2 cm. Beyond this range random movements

are probable. Chen and Rich (12), however, proved the existence of attraction of nematodes to roots from a distance of 15 to 20 cm in U-tubes.

The stimuli to which nematodes have been reported to react can be divided into two groups, physical and chemical.

Among the physical stimuli studies on electric ones have yielded the following results. Galvanotaxis, reaction to an electrical current, has been reported to exist among nematodes (9). Jones (30), however, found that the tension of the current, not the strength, is decisive as to whether or not the nematodes will move to either cathode or anode. When platinum electrodes were used, D. dipsaci moved mostly to the cathode, but with copper electrodes the nematodes moved to the anode (32).

Bird (6) hypothesized that nematodes are primarily attracted to roots along a potential gradient caused by lower redox potentials. Later he (7) observed that only Heterodera schachtii and Meloidogyne javanica, out of several nematode species tested, were attracted to the reducing agent sodium dithionite, and that not all reducing agents attracted these 2 species. Klingler (32) found that D. dipsaci was attracted to reducing sodium dithionite as well as to oxidizing potassium permanganate which raises the redox potential. Also, Jones (30) found that H. schachtii was attracted to the positive pole.

Orientation of nematodes in an electric field near the plant root has the limitation that, although a sufficient

potential difference for nematodes to orient themselves (30) is present in the near vicinity of roots (34), the potential drop occurs only within perhaps 1 mm from the root surface. Temperature gradients have also been reported to facilitate orientation of nematodes (64, 73), over a range of 15 cm (74), but their importance is mainly thought to be concerned with vertical movement in the soil (74).

Work on chemical stimuli yielded the following insights. In pH gradients nematodes have been found to aggregate at any pH value (4, 6, 32) and extreme values may even have a repelling effect (6).

Moisture gradients have been found to counteract attraction to roots in the sense that nematodes aggregate at the wet end of a moisture gradient (72). Since we must assume that moisture in the soil decreases towards the roots and there is still migration to the roots, attraction by other factors must be stronger.

Considerable work has been done on attraction of nematodes by CO_2 and O_2. Since roots and microorganisms in the rhizosphere give off CO_2 and consume O_2, the research has centered around the use of increasing CO_2 and decreasing O_2 gradients presumably encountered by nematodes traveling towards the roots (34). Results have shown that nematodes show a directed orientation towards ascending CO_2 gradients, klinotaxis, but no orientation at all to a descending O_2 gradient (34).

The effect of root excretions on nematode behavior has only recently been investigated. Bird (6) found that of several amino acids tested only glutamic acid attracted the larvae of 2 species of Meloidogyne. Jones (30) found that D. dipsaci appeared to be repelled by glutamic and aspartic acid each at concentrations of 1:1000, but seemed to be attracted at concentrations of 1:100,000. Oteifa and Elgindi (57) found that of 14 amino acids, tyrosine was the most attractive to larvae of M. javanica.

Root excretions have been found to contain many amino acids, sugars, other organic acids, scopoletin (6-methoxy-7-oxycoumarin), nucleotides, flavanones, and acetaldehyde; many vitamins are found in the rhizosphere, and pea cotyledons excrete purines and pyrimidines during germination (60).

The work reported here deals with attempts to test the comparative attractiveness of a wide variety of these compounds.

Materials and Methods

The nematodes for these experiments were obtained from vetch (Vicia sativa L.) roots incubated in tap water for 24 hours on a rotary shaker.

Petri dishes were filled with 20 ml of a 1% Bacto agar solution and autoclaved for 15 minutes at 15 lbs. After the agar in the dishes had solidified a well was punched in the agar at one side of each dish with a 1 cm cork borer, and the agar disc removed under aseptic conditions. The test chemical

was placed in this well and the nematodes were deposited on the surface of the agar in a small drop of water. After the chemical and the drop with nematodes had been introduced into each dish under aseptic conditions the lid was replaced on the dish, and all dishes were stored in the dark at room temperature.

In the first tests the nematodes were placed at a distance of about 6 cm from the well containing the chemical. The chemicals tested in the first series of tests and their concentrations were sterile, distilled water (abbreviated as AD for aqua distillata), the ammonium salt of 2,4-dichlorophenoxyacetic acid (abbreviated as 2,4-D) at 2.0 mg/liter, calcium pantothenate at 2.5 mg/liter, alpha-naphthalene acetic acid (abbreviated as NAA) at 0.1 mg/liter, glycine at 3.0 mg/liter, thiamine.HCl at 0.1 mg/liter, asparagine at 6.0 mg/liter, bacto-asparagine at 6.0 mg/liter, succinic acid at 4.7 mg/liter, kinetin at 20 mg/liter dissolved with 2 ml of 1 N HCl (abbreviated as K-1), and kinetin at 2000 mg/liter dissolved with 20 ml 1 N HCl (abbreviated as K-2). All chemicals were dissolved in sterile, distilled water and stored at 1°C until needed.

The nematodes found in the well were counted at various time intervals. There were from 3 to 8 replicates and 3 to 6 subsamples in each replicate.

In the second test 23 amino acids were tested simultaneously with 7 sugars, 5 other organic acids, 2 purines,

3 pyrimidines, 4 vitamins, 2 growth inhibitors, and 4 other compounds reportedly associated with plants. Their names and concentrations are listed in Table 17.

Table 17

Concentrations of amino acids and other compounds tested for attractiveness to nematodes (Pratylenchus penetrans).

	Concentration mg/liter	Concentration gmol/liter
Aliphatic amino acids		
glycine	300	4×10^{-3}
alanine	356	"
valine	468	"
leucine	524	"
isoleucine	524	"
gamma-aminobutyric acid	413	"
Aromatic amino acids		
phenylalanine	661	"
tyrosine	725	"
tryptophan	817	"
Hydroxy-amino acids		
serine	420	"
threonine	477	"
Acidic amino acids (2 carboxyl groups)		
aspartic acid	533	"
glutamic acid	588	"
Amides		
asparagine	600	"
glutamine	585	"
Basic amino acids (2 amino groups)		
lysine.HCl	731	"
arginine.HCl	842	"
histidine.HCl.H_2O	838	"

Table 17. Continued.

	Concentration mg/liter	Concentration gmol/liter
Sulphur amino acids		
cystine	961	4×10^{-3}
cysteine (free base)	485	"
methionine	597	"
Imino acids		
proline	461	"
hydroxy-proline	525	"
Organic acids		
tartaric acid	600	"
oxalic acid	361	"
malic acid	536	"
citric.H_2O	840	"
succinic acid	472	"
Sugars		
glucose	721	"
fructose	721	"
mannose	721	"
galactose	721	"
maltose.H_2O	1441	"
xylose	601	"
ribose	601	"
Purines		
adenine	541	"
guanine	605	"
Pyrimidines		
thymine	505	"
cytosine	445	"
uracil	449	"
Vitamins		
thiamin.HCl	1349	"
biotin	976	"
riboflavin	1506	"
calcium pantothenate	1906	"

Table 17. Continued.

	Concentration mg/liter	Concentration gmol/liter
Others		
2,4-D (ammonium salt)	948	4×10^{-3}
NAA	744	"
coumarin	585	"
chlorogenic acid	1288	"
kinetin	86.1	4×10^{-4}
acetaldehyde	176	4×10^{-3}

In the second experiment the drop with nematodes was placed in the center of the Petri dish about 3 cm from the well containing the test chemical. Only those nematodes were counted that were found outside the drop limits. Separate counts were made of nematodes found in each of the four quadrants of the dish. The first of these quadrants included the area on both sides of the well, the second and third were adjacent to the first, while the fourth quadrant was opposite the first one.

There were 6 replicates of the second experiment, each treatment in each replicate consisting of one dish.

Results

In the first tests nematodes were counted after 12, 24, 36, 48, 60, 72, and 96 hours. It was found that the time after which the maximum number of nematodes was found in the well was longer when more free water was present in the dish at the start of the experiment. The nematodes were retained in the water drop until the drop had almost completely

evaporated leaving each of the migrating nematodes in its own film of water. On the other hand when the liquid in the well had dried nematodes were found to leave the well; when distilled water was added to the well some of them returned to the well, thus indicating their desire for free water.

It was estimated that only from 1-5% of the nematodes in the drop actively migrated away from this drop. Hence it was not thought relevant to express the number of nematodes found in the well as a proportion of the total number added since the majority of these would not migrate. Thus only the number found in the well is recorded for the first tests. Since the time needed by the nematode to escape from the water drop differed from one replicate to another, only the maximum number of nematodes is given for each replicate. In the following 2 tables (Tables 18 and 19) results are recorded of the numbers of nematodes found in wells after time lapses varying from 24 to 72 hours depending on the moisture condition in the particular replicate.

The data were analyzed, and the new multiple range test (17) was applied when treatment means were found to be significantly different at 5%.

Table 18

Numbers of nematodes[a/] (*P. penetrans*) found in wells with different test chemicals, arranged in ascending order and grouped in ranges of not significantly different* numbers of nematodes.

2,4-D	glycine	panthothenate	NAA	AD	thiamine
54	56	58	60	67	83

a/ Average of 8 replicates.

* Significant at 5%.

It follows that thiamine is significantly more attractive than 2,4-D, glycine, pantothenate, and NAA, but not more than AD. There was a very large replicate effect probably due to differences between the replicates in numbers of actively migrating nematodes.

Table 19

Numbers of nematodes[a/] found in wells with different test chemicals, arranged in ascending order and grouped in ranges of not significantly different* numbers of nematodes.

AD	asparagine	bacto-asparagine	succinic	K-1	K-2
13	15	18	31	32	102

a/ Averages of 3 replicates.

* Significant at 5%.

In the second experiment with 51 chemicals the numbers of nematodes were counted after 24 hours since it was found that in keeping the dishes more uniformly dry throughout all 6 replicates, this was the approximate time at which maximum numbers of nematodes would be found migrating. Nematodes found in the well were counted as well as those found in each of the 4 quadrants of the Petri dish outside the drop in which they had been deposited initially.

The numbers in the well were added to those of the first quadrant surrounding the well. The difference between this number and that found in the fourth quadrant was divided by the total number of migrating nematodes in all 4 quadrants including the well. The ratios thus obtained were multiplied by 100 for more convenient calculations, and termed the behavior index. Negative values were termed repellent, positive ones attractive, while a small numerical value was considered as a measure of mostly random movement by the nematodes and a large value as directed orientation. The 306 behavior index values were found to constitute a population with a normal distribution, and hence subjected to an analysis of variance (68). Subsequently the values for amino acids were analyzed separately, as well as those for the sugars, organic acids, purines and pyrimidines, and for the remaining 10 compounds, each group with the values for AD serving as a control. When significant differences for the means were found, the new multiple range test (17) was applied.

In Tables 20, 21, 22, 23, 24 and 25 the chemicals have been arranged in order of behavior index values, and grouped in ranges of chemicals with means not significantly different. Any chemical of the ones tested, not found in one particular range has a behavior index value significantly different from those found in that range.

Discussion and Conclusions

Thiamine was found to be significantly more attractive to the nematodes than any of the other chemicals tested.

Of the amino acids with positive behavior index values only lysine was significantly more attractive than alanine and distilled water (AD), but lysine was not significantly more attractive than arginine and histidine. AD was apparently almost neutral as far as nematode response was concerned. Arginine, histidine, and alanine were all slightly more attractive than AD but not significantly. All other amino acids tested had negative behavior index values, but none of them was significantly more negative than the value of AD. Hence they cannot truly be called repellent. Glutamic acid had a negative value, but was not significantly repellent to P. penetrans. While Bird (6) found that it was attractive to larvae of Meloidogyne spp., Jones (30) found that D. dipsaci appeared to be repelled by glutamic and aspartic acids at one concentration, but attracted at a smaller concentration. In our tests P. penetrans was neither repelled nor attracted to glutamic and aspartic acid.

Table 20

Chemicals arranged in descending order of behavior index, a/ and grouped in ranges of not significantly different* values.

POSITIVE VALUES	NEGATIVE VALUES
thiamine	AD
	uracil
lysine	methionine
arginine	succinic
citric	tartaric
histidine	NAA
fructose	oxalic
kinetin	aspartic
coumarin	tryptophan
riboflavin	leucine
2,4-D	cystine
galactose	threonine
alanine	cytosine
ribose	biotin
maltose	glutamic
	chlorogenic
	proline
to oxalic	glucose
	valine
	gamma-aminobutyric
	acetaldehyde
to phenylalanine	phenylalanine
to cysteine	cysteine
	hydroxyproline
	adenine
	glycine
	mannose
	malic
to guanine	guanine
	thymine
to asparagine	asparagine
	pantothenate
	serine
	isoleucine
to tyrosine	tyrosine
to xylose	xylose
to glutamine	glutamine

a/ Values are given in Tables 21, 22, 23, 24, and 25.

* Significant at 5%.

Table 21

Amino acids arranged in descending order of behavior index value, and grouped in ranges of not significantly different* values.

lysine	+25.97				
arginine	arginine	+18.55			
histidine	histidine	histidine	+10.15		
	alanine	alanine	alanine	+0.99	
	AD	AD	AD	AD	-0.30
	methionine	methionine	methionine	methionine	-0.74
		aspartic	aspartic	aspartic	-3.66
		tryptophan	tryptophan	tryptophan	-3.92
		leucine	leucine	leucine	-4.03
		cystine	cystine	cystine	-4.39
		threonine	threonine	threonine	-4.40
		glutamic	glutamic	glutamic	-5.83
		proline	proline	proline	-6.71
		valine	valine	valine	-6.99
		gamma-NH_2-butyric	gamma-NH_2-butyric	gamma-NH_2-butyric	-8.41
			phenylalanine	phenylalanine	-10.66
			cysteine	cysteine	-11.07
			OH-proline	OH-proline	-11.29
			glycine	glycine	-12.45
			asparagine	asparagine	-15.38
			serine	serine	-16.31
			isoleucine	isoleucine	-16.57
			tyrosine	tyrosine	-16.71
				glutamine	-20.16

* Significant at 5%.

Table 22

Sugars arranged in order of behavior index values, and grouped in ranges of not significantly different* values.

xylose	mannose	glucose	AD	maltose	ribose	galactose	fructose
-17.66	-12.95	-6.99	-0.30	+0.56	+0.62	+1.83	+7.48

* Significant at 5%.

Table 23

Acids arranged in order of behavior index values, and grouped in ranges of not significantly different* values.

malic	oxalic	tartaric	succinic	AD	citric
-13.22	-2.62	-1.47	-1.14	-0.30	+10.41

* Significant at 5%.

Table 24

Purines and pyrimidines arranged in order of behavior index values.*

thymine	guanine	adenine	cytosine	uracil	AD
-15.08	-13.71	-11.31	-5.18	-0.51	-0.30

* Means not significantly different at 5%.

Table 25

Vitamins, growth inhibitors, and some other compounds arranged in order of behavior index values, and grouped in ranges of not significantly different* values.

pantothenic	acetaldehyde	chlorogenic	biotin	NAA
-12.54	-9.36	-6.55	-5.82	-2.43

AD	2,4-D	riboflavin	coumarin	kinetin	thiamine
-0.30	+3.64	+4.24	+4.41	+5.70	+60.92

* Significant at 5%.

Oteifa and Elgindi (57) found that larvae of M. javanica were most attracted to tyrosine, which in our tests had almost the largest negative value, though not significantly different from AD. Testing the behavior of different nematode species simultaneously towards amino acids should reveal the existence of possible differences in response to chemicals by different species.

Of the sugars tested, only xylose was significantly more repellent than distilled water, but not more than mannose or glucose. The nematode was apparently indifferent to mannose and glucose with negative values, and to maltose, ribose, galactose, and fructose with positive values.

Of the acids tested, citric was more attractive than malic, but the nematodes did not seem to have any particular preference for malic, oxalic, tartaric, and succinic with negative values, or for citric with a positive value. The nematodes also behaved indifferently towards the purines and pyrimidines tested, all of which had negative behavior index values.

Thiamine was definitely attractive to the nematodes, but they showed no particular attraction or repulsion to kinetin, coumarin, riboflavin, and 2,4-D with positive values, and NAA, biotin, chlorogenic acid, acetaldehyde, and pantothenate with negative values.

In retrospect, the author feels that in view of the many other factors implicated in nematode attraction, some of these might have played important roles in the tests here reported; in future studies attempts should be made to control variables such as temperature, humidity, and CO_2 gradients.

Summary

In tests of the response of the nematode P. penetrans to 50 chemicals and distilled water, it was found that thiamine

was the most attractive, while distilled water was neutral to the nematodes.

Of the 23 amino acids tested, only lysine was more attractive than distilled water, while none of the others was particularly attractive or repellent. Of the 7 sugars tested, only xylose was repellent as compared to distilled water.

In addition the nematodes were indifferent to 5 organic acids, 2 purines, 3 pyrimidines, 3 vitamins, 1 growth hormone, 1 herbicide, acetaldehyde, kinetin, chlorogenic acid, and coumarin.

LITERATURE CITED

1. Abu-Gharbieh, W., E. H. Varney, and W. R. Jenkins. 1962. Relationship of meadow nematodes to Verticillium wilt of strawberries. Phytopathology 52: 921. (Abstr.)

2. Baermann, G. 1917. Eine einfache Methode zur Auffindung von Anchylostomum--(Nematoden)--Larven in Erdproben. Geneesk. Tijdschr. Ned.-Indie 57: 131-137.

3. Bergeson, G. B. 1963. Influence of Pratylenchus penetrans alone and in combination with Verticillium albo-atrum on growth of peppermint. Phytopathology 53: 1164-1166.

4. Bergman, B. H. H., and A. J. van Duuren. 1959. Het bietencystenaaltje en zijn bestrijding. VI. De invloed van wortels van waardplanten en excreten hiervan op de bewegingsrichting van larven van Heterodera schachtii in vitro. Inst. Suikerbietenteelt, Bergen op Zoom, Meded. 29: 1-24.

5. Berkeley, G. H., G. O. Madden, and R. S. Willison. 1931. Verticillium wilts in Ontario. Sci. Agr. 11: 739-759.

6. Bird, A. F. 1959. The attractiveness of roots to the plant parasitic nematodes Meloidogyne javanica and M. hapla. Nematologica 4: 322-335.

7. Bird, A. F. 1960. Additional notes on the attractiveness of roots to plant parasitic nematodes. Nematologica 5: 217.

8. Blake, C. D. 1962. Some observations on the orientation of Ditylenchus dipsaci and invasion of oat seedlings. Nematologica 8: 177-192.

9. Caveness, F. E., and J. D. Panzer. 1960. Nemic galvanotaxis. Proc. Helm. Soc. Wash. 27: 73-74.

10. Cetas, R. C., and M. B. Harrison. 1963. Evaluation of soil fumigants for the control of early maturity wilt of potatoes on Long Island. Phytopathology 53: 347-348. (Abstr.)

11. Chapman, R. A. 1957. The effects of aeration and temperature on the emergence of species of Pratylenchus from roots. Plant Dis. Reptr. 41: 836-841.

12. Chen, T., and A. E. Rich. 1963. Attraction of Pratylenchus penetrans to plant roots. Plant Dis. Reptr. 47: 504-507.

13. Chitwood, B. G., and B. A. Oteifa. 1952. Nematodes parasitic on plants. Annu. Rev. Microbiol. 6: 151-184.

14. Cobb, N. A. 1917. A new parasitic nema found infesting cotton and potatoes. J. Agr. Res. 11: 27-33.

15. Dethier, V. G. 1947. Definition of terms, p. 4. In V. G. Dethier, Chemical insect attractants and repellents, Blakiston, Philadelphia.

16. Dickerson, O. J., H. M. Darling, and G. D. Griffin. 1964. Pathogenicity and population trends of Pratylenchus penetrans on potatoes and corn. Phytopathology 54: 317-322.

17. Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11: 1-42.

18. Edson, H. A., and M. Shapovalov. 1920. Temperature relations of certain potato rot and wilt producing fungi. J. Agr. Res. 18: 511-524.

19. Faulkner, L. R., and C. B. Skotland. 1963. Control of Verticillium wilt of mint and plant parasitic nematodes assocciated with mint by soil fumigation. Plant Dis. Reptr. 47: 662-665.

20. Faulkner, L. R., and C. B. Skotland. 1965. Interactions of Verticillium dahliae and Pratylenchus minyus in Verticillium wilt of peppermint. Phytopathology 55: 583-586.

21. Folsom, D. 1957. Verticillium wilt of potatoes in relation to fungicides added to the fertilizer. Amer. Potato J. 34: 1-5.

22. Folsom, D., O. L. Wyman, and E. Westin. 1951. Potato Verticillium wilt. Maine Agr. Exp. Sta. Ext. Circ. 265.

23. Fordyce, C., and R. J. Green. 1964. Mechanisms of variation in Verticillium albo-atrum. Phytopathology 54: 795-798.

24. Fraenkel, G. S., and D. L. Gunn. 1961. Introduction, p. 1-10. In G. S. Fraenkel and D. L. Gunn, The orientation of animals, Dover Publications, New York.

25. Guthrie, J. W. 1960. Early dying (Verticillium wilt) of potatoes in Idaho. Idaho Agr. Exp. Sta. Res. Bull. 45: 24 p.

26. Hastings, R. J., and J. E. Bosher. 1938. A study of the pathogenicity of the meadow nematode and associated fungus Cylindrocarpon radicicola Wr. Can. J. Botany C, 16: 225-229.

27. Isaac, I. 1949. A comparative study of pathogenic isolates of Verticillium. Brit. Mycol. Soc., Trans. 32: 137-157.

28. Isaac, I. 1953. A further comparative study of pathogenic isolates of Verticillium. Brit. Mycol. Soc., Trans. 36: 180-195.

29. Isaac, I. 1954. Studies on the interactions between species of Verticillium. Ann. Appl. Biol. 40: 623-629.

30. Jones, F. G. W. 1960. Some observations and reflections on host finding by plant nematodes. Meded. Landb. Hoogesch., Gent 25: 1009-1024.

31. Klebahn, H. 1913. Beitrage zur Kenntnis der Fungi Imperfecti. I. Eine Verticillium-Krankheit auf Dahlien. Mycol. Centralbl. 3: 49-66.

32. Klingler, J. 1961. Anziehungsversuche mit Ditylenchus dipsaci unter Berucksichtigung der Wirkung des Kohlendioxyds, des Redoxpotentials und anderer Faktoren. Nematologica 6: 69-84.

33. Klingler, J. 1963. Die Orientierung von Ditylenchus dipsaci in gemessenen kunstlichen und biologischen CO_2-Gradienten. Nematologica 9: 185-199.

34. Klingler, J. 1965. On the orientation of plant nematodes and of some other soil animals. Nematologica 11: 4-18.

35. Kuhn, H. 1959. Zum Problem der Wirtsfindung phytopathogener Nematoden. Nematologica 4: 165-171.

36. Lavallee, W. H., and R. A. Rohde. 1962. Attractiveness of plant roots to Pratylenchus penetrans (Cobb). Nematologica 8: 252-260.

37. Loof, P. A. A. 1960. Taxonomic studies in the genus Pratylenchus (Nematoda). Tijdschr. Pl. ziekten 66: 29-90.

38. Lownsbery, B. F., J. W. Lownsbery, and W. F. Mai. 1951. Nematodes found in New York State fields with several different cropping histories. Amer. Potato J. 28: 681-686.

39. Lownsbery, B. F., and D. R. Viglierchio. 1960. Mechanism of accumulation of Meloidogyne incognita acrita around tomato seedlings. Phytopathology 50: 178-179.

40. McKay, M. B., 1921. Transmission of some wilt diseases in seed potatoes. J. Agr. Res. 21: 821-848.

41. McKay, M. B. 1926. Further studies of potato wilt caused by Verticillium albo-atrum. J. Agr. Res. 32: 437-470.

42. McKay, M. B. 1926. Potato wilt and its control. Oregon Agr. Exp. Sta. Bull. 221: 1-23.

43. McKeen, C. D. 1943. A study of some factors affecting the pathogenicity of Verticillium albo-atrum R. & B. Can. J. Res. C, 21: 95-117.

44. McKeen, C. D., and W. B. Mountain. 1960. Synergism between Pratylenchus penetrans (Cobb) and Verticillium albo-atrum R. & B. in eggplant wilt. Can. J. Botany 38: 789-794.

45. McLean, J. G. 1955. Selecting and breeding potatoes for field resistance to Verticillium wilt in Idaho. Idaho Agr. Exp. Sta. Res. Bull. 30: 19 p.

46. Miller, P. M., and L. V. Edgington. 1962. Controlling parasitic nematodes and soil-borne diseases of potatoes with soil fumigation. Amer. Potato J. 39: 235-240.

47. Miller, P. M., and L. V. Edgington. 1962. Effects of soil amendments on nematodes and subsequent Verticillium wilt. Phytopathology 52: 742. (Abstr.)

48. Miller, P. M., and P. E. Waggoner. 1963. Interaction of plastic mulch, pesticides and fungi in the control of soil-borne nematodes. Plant and Soil 18: 45-52.

49. Mountain, W. B., and C. D. McKeen. 1962. Effect of Verticillium dahliae on the population of Pratylenchus penetrans. Nematologica 7: 261-266.

50. Mountain, W. B., and C. D. McKeen. 1962. Interaction of Verticillium dahliae and Pratylenchus penetrans in tomato wilt. Phytopathology 52: 744. (Abstr.)

51. Nielsen, L. W. 1948. Verticillium wilt of potatoes in Idaho. Idaho Agr. Exp. Sta. Res. Bull. 13: 23 p.

52. Oostenbrink, M. 1954. Over de betekenis van vrijlevende wortelaaltjes in land- en tuinbouw. Versl. Pl. ziektek. Dienst, Wageningen 124: 196-233.

53. Oostenbrink, M. 1956. Over de invloed van verschillende gewassen op de vermeerdering van en de schade door Pratylenchus pratensis en Pratylenchus penetrans (Vermes, Nematoda), met vermelding van een afwijkend moeheidsverschijnsel bij houtige gewassen. Tijdschr. Pl.ziekten 62: 189-203.

54. Oostenbrink, M. 1958. An inoculation trial with Pratylenchus penetrans or potatoes. Nematologica 3: 30-33.

55. Oostenbrink, M., J. J. 's Jacob, and K. Kuiper. 1957. Over de waardplanten van Pratylenchus penetrans. Tijdschr. Pl.ziekten 63: 345-360.

56. Orton, W. A. 1914. The fungus genus Verticillium in relation to plant diseases. Phytopathology 4: 40-41.

57. Oteifa, B. A., and D. M. Elgindi. 1961. Physiological studies on host-parasite relationship of the root-knot nematode Meloidogyne javanica. Plant Dis. Reptr. 45: 928-929.

58. Peacock, F. C. 1959. The development of a technique for studying the host-parasite relationship of the root-knot nematode Meloidogyne incognita under controlled conditions. Nematologica 4: 43-55.

59. Presley, J. T. 1950. Verticillium wilt of cotton with particular emphasis on variation of the causal organism. Phytopathology 40: 497-511.

60. Quastel, J. H. 1963. Microbial activities of soil as they affect plant nutrition, p. 671-756. In F. C. Steward, Plant Physiology, Vol. III. Inorganic nutrition of plants. Academic Press, New York.

61. Reinke, J., and G. Berthold. 1879. Die Zersetzung der Kartoffel durch pilze. Untersuch. Bot. Lab. Univ. Gottingen 1: 1-100.

62. Rich, S., and P. M. Miller. 1964. Verticillium wilt of strawberries made worse by soil fungicides that stimulate meadow nematode populations. Plant Dis. Reptr. 48: 246-248.

63. Robinson, D. B., R. H. Larson, and J. C. Walker. 1957. Verticillium wilt of potato in relation to symptoms, epidemiology and variability of the pathogen. Univ. Wisconsin, Res. Bull. 202: 49 p.

64. Rode, H., and G. Staar. 1961. Die photographische Darstellung der Kriechspuren (Ichnogramme) von Nematoden und ihre Bedeutung. Nematologica 6: 266-271.

65. Rudolph, B. A. 1931. Verticillium hadromycosis. Hilgardia 5: 197-360.

66. Sandstedt, R., and M. L. Schuster. 1962. Liquid trapping of Meloidogyne incognita incognita about roots in agar medium. Phytopathology 52: 174-175.

67. Sandstedt, R., Thelma Sullivan, and M. L. Schuster. 1961. Nematode tracks in the study of movement of Meloidogyne incognita incognita. Nematologica 6: 261:265.

68. Snedecor, G. W. 1956. Statistical methods. The Iowa State College Press, Ames, Iowa. 534 p.

69. Tolmsoff, W. J. 1959. The influence of quantity of inoculum on the severity of Verticillium wilt. M. S. Thesis. Oregon State College, 137 p.

70. Van Beyma Thoe Kingma, F. H. 1940. Ueber einige Formen von Verticillium dahliae Klebahn. Antonie van Leeuwenhoek 6: 37-47. (Abstr. in Rev. Appl. Mycol. 19: 367-368.)

71. Viglierchio, D. R. 1961. Attraction of parasitic nematodes by plant root emanations. Phytopathology 51: 136-142.

72. Wallace, H. R. 1960. Movement of eelworms. VI. Influence of soil type, moisture gradients and host plant roots on the migration of the potato-root eelworm Heterodera rostochiensis Wollenweber. Ann. Appl. Biol. 48: 107-120.

73. Wallace, H. R. 1961. The orientation of Ditylenchus dipsaci to physical stimuli. Nematologica 6: 222-236.

74. Wallace, H. R. 1962. Observations on the behaviour of Ditylenchus dipsaci in soil. Nematologica 7: 91-101.

75. Wollenweber, H. W. 1929. Die Wirtelpilz-Welkekrankheit (Verticilliose) von Ulme, Ahorn, und Linde usw. Arb. biol. Reichsanstalt fuer Land- und Forstwirtsch., Berlin-Dahlem 17: 273-299. (Abstr. in Rev. Appl. Mycol. 9: 6.)

76. Young, R. A. 1956. Control of the early maturity disease of potatoes by soil treatment with Vapam. Plant Dis. Reptr. 40: 781-784.

77. Young, T. W. 1954. An incubation method for collecting migratory, endoparasitic nematodes. Plant Dis. Reptr. 38: 794-795.