INCREASED SUSCEPTIBILITY TO ROOT-ROTS IN VIRUS-INFECTED PEAS

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

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1963

THESIS

ABSTRACT

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by James D. Farley

Miragreen peas infected with a virus [pea mosaic virus (PMV) strains PMV-1 or PMV-2, bean yellow mosaic virus (BYMV) strains BYMV 61-35 or BYMV 61-36, alfalfa mosaic virus (AMV), or pea enation mosaic virus (PEMV)] and a fungus, [Aphanomyces euteiches or Fusarium solani f. pisi], developed more severe root disease symptoms than peas infected with a root-rot fungus only. The length of virus establishment (2 or 5 days) prior to fungus inoculation did not significantly affect disease severity in virus-fungus-infected plants. The age of the plant at virus-fungus inoculations (13, 18 or 27 days) did not significantly affect the disease severity of virus-fungus or fungus-only-infected plants.

Pea varieties Perfected Wales and P.I. 169604 infected with PMV-1 or BYMV 61-35 and F. solani or A. euteiches developed more severe root-rot symptoms than plants infected with either root-rot fungus alone.

Virus infection did not appear to increase root-rot development in pea fields at Jackson, Michigan; however, field observations were too limited to make an accurate estimation of the importance of virus infection in increasing root-rot development in the field.

Young pea seedlings inoculated 3-5 days previously with A. euteiches or F. solani were transplanted into A. euteiches or F. solani-infected soil or into non-infested

soil. Before transplanting, the roots were rinsed several times with distilled water to remove spores or mycelium adhering to the root surfaces; some of the plants were inoculated with PMV-1 at the time of transplanting. Virus-fungus-infected plants had no more root-rot than fungus-only-infected plants when both were transplanted into non-infested soil. However, when plants were transplanted into infested soil, virus-fungus-infected peas had more severe root-rot than transplants infected with a fungus only. The results of the transplanting experiments suggest that PMV-1 infection enhances the infectivity of the fungal inoculum at the root surface.

Attempts to compare sterile root exudates from virus-infected peas with extracts from virus-free plants were not successful.

There was no increase in the number of actinomycetes, bacteria or fungi in the rhizospheres of PMV-1 infected Mira-green peas as compared to rhizospheres of virus-free peas.

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By

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A THESIS

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

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1963

ACKNOWLEDGEMENTS

Thanks to Dr. E. S. Beneke, Dr. E. H. Barnes and Dr. R. P. Scheffer for reviewing this manuscript. Special thanks to Dr. J. L. Lockwood who suggested the problem and gave unselfishly of his time and ideas.

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INTRODUCTION

A survey of Michigan pea diseases in 1955 and 1956 revealed that root-rots caused by *Aphanomyces euteiches* Drechs. and *Fusarium solani* f. *pisi* (F. R. Jones) Snyder and Hansen were by far the most important group of parasitic diseases of peas (17). Virus diseases were second in importance to root-rots in Michigan pea fields in these two years.

Recent studies on the association of virus and fungus infections have indicated that such multiple infections may play an important role in disease development in the field (2,7,9,11,20,22). A preliminary experiment in June, 1961 suggested that bean yellow mosaic virus (BYMV) infected peas may be more susceptible to *A. euteiches* or *F. solani* root-rots than BYMV-free peas.

To obtain information on combined virus-fungus infections, three pea varieties were inoculated with each of four viruses and *A. euteiches* or *F. solani*. The effect of the age of the plant, the time interval between virus-fungus inoculations and different strains of pea mosaic virus (PMV) and BYMV on disease severity of virus-fungus-infected peas was studied.

Several experiments were designed to study the mechanism(s) involved in the increased fungus root-rot susceptibility in virus-infected peas.

LITERATURE REVIEW

Nature provides numerous instances of associative and antagonistic relationships between living organisms, some of these being the effects of the microscopic forms of life upon higher plants and animals and upon one another. In 1931, Fawcett emphasized the need for studying multiple infections of plants (8). He suggested that many plant diseases were caused by an association of microorganisms and could not be produced by infection by one microorganism alone. An extensive literature has accumulated concerning the specific effects, either favorable or unfavorable, of microbes upon one another. The subject of microbial association has been reviewed by Fawcett (8), Waksman (27), Weindling (28), and Porter and Carter (21).

Due to the more recent recognition of the existence of viruses, studies of associative effects of fungi and viruses on disease development are relatively few. These interactions can be outlined as follows: 1) fungus infection is increased, decreased or not affected in virus-infected plants. 2) virus infection is increased, decreased or not affected in fungus-infected plants.

Fungus infection is increased: Hooker and Fronek observed that early blight [Alternaria solani (Ell. and G. Martin) L. R. Jones and Groat] symptoms on leaves of several potato varieties in the field was usually greater on mosaic-infected plants than on mosaic-free plants (11). A non-necrotic

strain of virus Y was believed to be associated with the increased severity of early blight. This association was not consistent in all mosaic-infected fields. Bateman reported an increase (10-15% to 60-87%) in post-emergence damping-off of cucumber caused by Rhizoctonia sp. as a result of cucumber mosaic virus infection (CMV) (2). It was suggested that the increased respiration of the virus-infected cotyledons caused movement of materials from the roots to the leaves, thereby increasing the susceptibility of the cucumber to fungus attack. Field observations with potato Ulster Supreme indicated that the late blight fungus [Phytophthora infestans (Mont.) DBy.] was present on about twice as many tubers on plants infected with the leaf-roll virus as on virus-free plants (22). In the laboratory leaf-roll-infected leaves were less susceptible to fungus infection than virus-free leaves. Since leaves of leaf-roll-infected plants in the field remained damp for longer periods than virus-free leaves, it was suggested that the leaf-roll leaves provided a more favorable microclimate for the blight organism.

Fungus infection is decreased: Goheen and Schnathorst (9) found that powdery mildew [Uncinula necator (Schw.) Burr.] infection was abundant on the canes of leaf-roll-free vines growing in the field, but absent or nearly so, on the virus-diseased ones. A greater accumulation of carbohydrates was shown in leaves of grape vines infected with the leaf-roll virus than in healthy leaves. Since accumulation of carbohydrates in leaves and canes tends to increase osmotic concentration, it was suggested that this may account for the decreased susceptibility of the leaves to powdery mildew. Cucumber plants infected with cucumber mosaic virus were less susceptible to scab infection caused by (Cladosporium cucumerinum Ell. & Arth. than were virus-free plants (7). The protection afforded by the virus was greater for scab-susceptible varieties than for scab-resistant varieties. The mechanism responsible for reducing scab severity was not ascertained. Muller and Munro (20) observed retarded growth of Phytophthora infestans on leaves of potato plants that had been inoculated with virus X or virus Y. The more severe the systemic virus symptoms, the greater was the reduction in blight development. It was suggested that the constituents of the host plants essential for optimal development of P. infestans were eliminated or decreased by virus infection. Tobacco, first inoculated with tobacco mosaic virus (TMV), and later challenge-inoculated with Thielaviopsis basicola (Berk.) Ferr. developed fewer and smaller fungus lesions than were developed on virus-free plants (4). Inoculations of one half-leaf with TMV induced resistance to T. basicola in the opposite half-leaf.

Virus infection is increased: Yarwood (29) found that the concentrations of tobacco mosaic virus, tobacco ringspot virus, tobacco necrosis virus, alfalfa mosaic virus or cucumber mosaic virus in rusted /Uromyces phaseoli (Pers.)

Wint.] bean leaves were increased compared with virus concentration in non-rusted tissue. An assay of sunflower leaves infected with TMV or tobacco ringspot virus and *Puccinia helianthi* Schw. showed more virus in rusted than in the non-rusted tissue. When small amounts, (0.003%-0.01%) of rusted bean tissue were mixed with TMV sap, the number of local lesions on half-leaves of tobacco was 177% greater than in plants inoculated with the virus alone. Increased susceptibility of rusted tissues to virus infection was not considered due to the mechanical punctures of cell walls by the rust haustoria since infection of bean with *Erysiphe polygoni* DC., which punctures the cell walls, did not favor virus infection.

Virus infection is decreased: Bozarth et al (4) reported a nonspecific resistance to viruses induced by *Thielaviopsis basicola* in tobacco. The effect appears to be similar to the localized resistance induced by viruses causing local lesions (23). Virus lesions were smaller in size and fewer in number when the lower leaves of Samsun tobacco were first inoculated with *T. basicola*, then followed 7 days later by a challenge inoculation of the upper leaves with TMV.

No change in disease severity: The virulence of four species of root-infecting fungi, [*Fusarium oxysporum* Schlect., *F. roseum* (Lk.) emend. Snyder and Hansen (*F. avenaceum*), *Rhizoctonia solani* Kühn and *Sclerotium bataticola* Taub.], was neither increased or decreased on red clover infected with bean yellow mosaic virus (18). Goth (10) found that in the greenhouse, Fusarium sp. and Rhizoctonia sp. were no more pathogenic on white clover infected with bean yellow mosaic virus, red clover mosaic virus or pea mosaic virus than on virus-free clover. Both fungi were isolated with equal frequency from virus-infected and virus-free plants from the field.

Little is known about the metabolic interactions in a host-virus-fungus complex. Several of the papers discussed have suggested that constituents essential for pathogenic development may have been decreased or increased by virus or fungus infection (7,20,29). Stimulatory or inhibitory effects of one pathogen on another are not surprising, for the physiology of an infected plant is not that of a healthy one, e.g., it is known that an infection by a virus (3) or a fungus (1) may result in abnormal accumulation of certain metabolities, or in the formation of materials not normally present.

A few workers suggested the development of physical resistance mechanisms as a result of virus infection, e.g., increase in osmotic pressure affording host resistance (9) and development of a favorable microclimate thus rendering the host more susceptible to subsequent fungus infection (22).

MATERIALS AND METHODS

Source and maintenance of fungus and virus cultures: Isolates of *Fusarium solani* f. *pisi* and *Aphanomyces euteiches* from Michigan pea fields were maintained on potato-dextrose agar (PDA) slants at 24°C. Both fungi are known to be pathogenic on peas (16). Pea mosaic virus (PMV), bean yellow mosaic virus (BYMV), pea enation mosaic virus (PEMV), and alfalfa mosaic virus (AMV) were maintained in a greenhouse (70-90°F) by periodic sap inoculation on pea variety Miragreen. Viruses were also stored in finely-chopped leaves over $CaCl_2$ in a freezer. PMV-1 and AMV were supplied by Dr. D. J. Hagedorn, Dept. of Plant Pathology, University of Wisconsin. BYMV strains, 61-35 and 61-36, and PEMV were supplied, respectively, by Dr. A. L. Andersen and Mr. Antonio Bustrillos, Dept. of Botany and Plant Pathology, Michigan State University. PMV-2 was isolated from a Michigan pea field.

Preparation of Inoculum: Fungus inoculum was prepared by growing *F. solani* or *A. euteiches* for 5-6 days at 24°C in 500 ml Erlenmeyer flasks containing 100 ml of potato-dextrose broth (PDB) or in petri dishes containing 15 ml of PDA. The mycelial mats grown on PDB were washed once with distilled water. The agar cultures or the mycelial mats were ground in a Waring blendor for approximately 1 minute, diluted with water (50 ml/mat of *F. solani* and 40 ml/mat of *A. euteiches*) and the suspensions used to infest autoclaved soil. Tests showed that the inoculum grown on PDA or PDB gave similar disease indices (Table 1). The use of agar cultures resulted in fewer disease escapes and in greater ease of handling, and was selected for further use.

Virus inoculum was prepared by growing virus-infected Miragreen pea plants in the greenhouse. Plants inoculated 10-20 days previously were ground in a mortar together with a small amount of distilled water and carborundum.

Inoculation procedures: Seeds of pea varieties Miragreen, Perfected Wales or P.I. 169604, dusted lightly with Captan to prevent decay, were planted in an autoclaved loam-muck (3:1) soil in 4 in. clay pots. Twelve seeds were planted per pot. When the plants were about 3 in. high, two expanded leaves on each plant were dusted with carborundum and rubbed twice with a cotton swab containing the infected sap. The soil was infested 1-5 days (usually 3 days) later with a homogenate of either *F. solani* or *A. euteiches*. Twenty ml of inoculum (equivalent of ½ of a 5-day culture of *A. euteiches* or 1/3 of a 5-day culture of *F. solani*) was added to each pot by applying 4 ml portions in 5 positions with a hypodermic syringe inserted to a depth of 1 in. below the soil surface. Disease was evaluated approximately 2-3 weeks after fungus inoculation.

Evaluation of disease: Disease index was estimated using the method of Lockwood and Ballard (16). Tops of the plants with only the lower one or two leaves wilted were rated 1, completely wilted tops were rated 3 and intermediate stages were rated 2. Water-soaked *A. euteiches*-infected collars (area immediately above and below seed attachment) were rated 1 and completely collapsed collars, 2. Slight, moderate and severe *F. solani* collar infections were rated 1, 2 and 3, respectively. Slight, moderate and severe root decay were rated 1, 2 and 3, respectively, for both diseases. The ratings were made for the group of plants in each pot and not for individual plants. Fresh weight of the plants was recorded.

Table 1. Comparison of potato-dextrose agar and potato-dextrose broth cultures of *A. euteiches* and *F. solani* as root-rot inocula for Miragreen peas

		Disease indices for plants infected with indicated pathogens[a]		
Culture	Fungus	Fungus only	Fungus + PMV	LSD 1%
Agar	*A. euteiches*	4.0	7.0	1.1
Broth	"	4.0	7.5	
Agar	*F. solani*	2.0	5.0	0.5
Broth	"	1.8	4.0	

[a]Disease indices were based on a scale of increasing severity from 0-8 (*A. euteiches*) or from 0-9 (*F. solani*). Each figure is a mean index of 4 pots, each with 12 plants. PMV is pea mosaic virus.

RESULTS

Increased root-rot severity of Miragreen peas infected with a virus and a root-rot fungus: In several greenhouse experiments, done throughout the year under varying temperature and light conditions, Miragreen peas were infected with a virus (PMV, BYMV, AMV, or PEMV) and a fungus (*A. euteiches* or *F. solani*). In all cases, irrespective or pathogens used, a combined virus-fungus infection resulted in more severe symptoms of root disease than a root-rot fungus infection produced alone (Tables 2 and 3; Figs. 1, 2, 3, 4, and 5). The disease indices of virus-fungus-infected plants were usually twice those of plants infected with a fungus only. Moreover, the sum of the disease indices of virus-only-infected plants and fungus-only-infected plants was always less than the disease index of a combined infection with the same virus and the same fungus. Separate disease ratings of foliage, collars and roots of plants illustrates that disease in all portions of the plant was increased by virus infection (Tables 4 and 5). These data and the fact that foliage symptoms in virus-fungus-infected plants were typical of severe root decay suggests that the increased disease in all parts of the plant was due to increased fungus infection.

Relationship between disease indices and plant weights: Pea plants in selected experiments were weighed to determine the relation of fresh plant weight to disease indices (Figs. 6 and 7).

Table 2. Results of several greenhouse experiments done at various times of the year which show the effects of combined virus and *Fusarium solani* infection on disease severity in Miragreen peas

Virus	Disease indices for plants infected with indicated pathogens[a] *F. solani* + virus	*F. solani* only	LSD 1%
Pea mosaic	4.0	1.9	1.3
"	2.8	1.1	0.5
"	6.2	3.5	1.6
"	4.8	2.0	0.4
Bean yellow mosaic	4.0	1.9	1.3
"	4.6	1.1	0.5
Alfalfa mosaic	5.2	2.6	2.5
Pea enation mosaic	4.0	1.8	1.2
"	2.2	0.6	0.9

[a]Disease indices were based on a scale of increasing severity from 0-9. Each figure is a mean index of 4 pots, each with 12 plants.

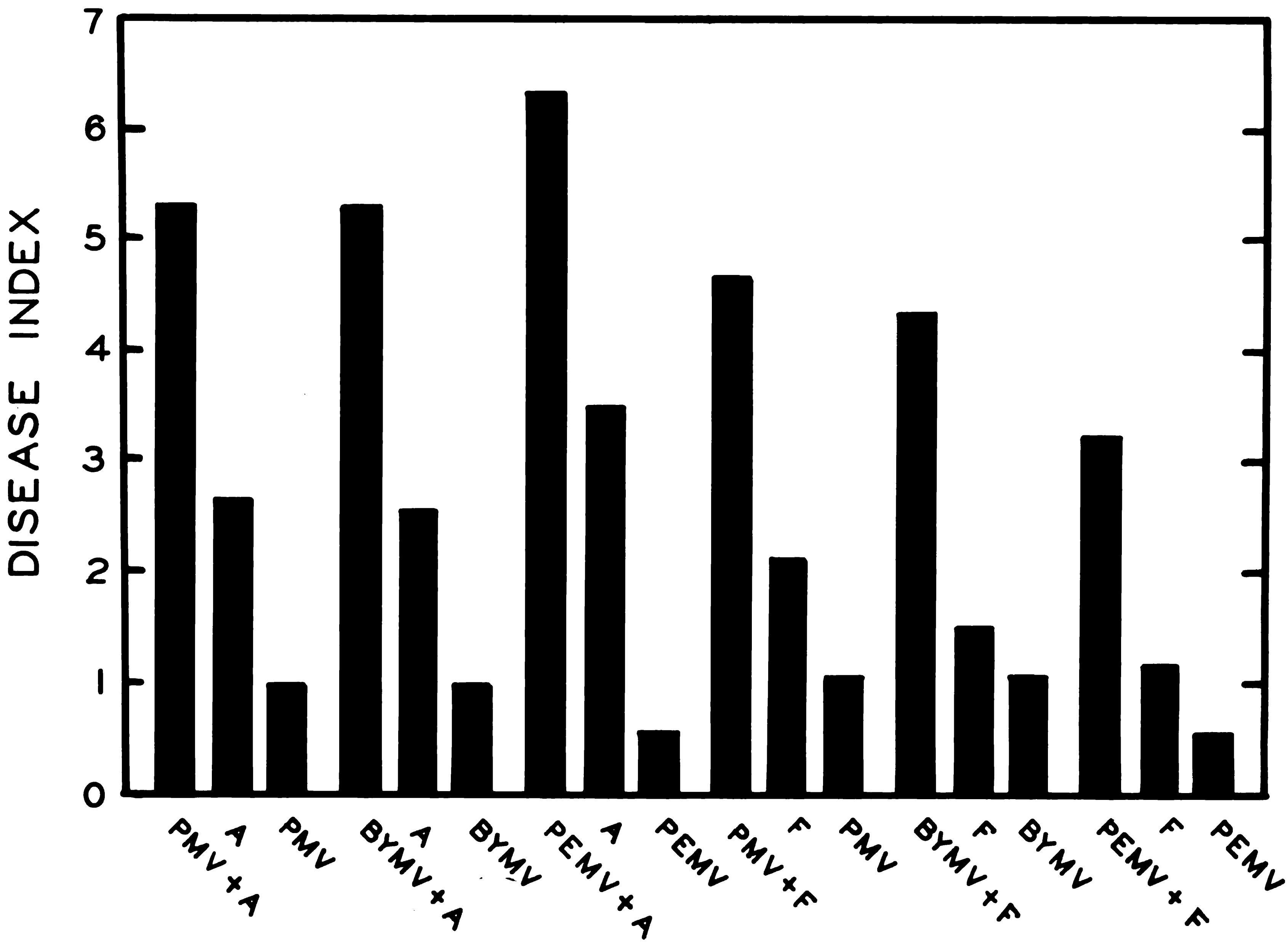
Table 3. Results of several greenhouse experiments done at various times of the year which show the effects of combined virus and *Aphanomyces euteiches* infection on disease severity in Miragreen peas

Virus	Diseased indices for plants infected with indicated pathogens[a] *A. euteiches* + virus	*A. euteiches* only	LSD 1%
Pea mosaic	5.5	2.9	1.6
"	4.4	2.2	1.4
"	3.8	2.0	1.4
"	5.5	2.0	1.7
"	7.5	4.0	1.1
Bean yellow mosaic	5.5	2.9	1.6
"	5.0	2.2	1.4
"	5.0	2.0	1.4
Alfalfa mosaic	6.5	3.6	1.5
Pea enation mosaic	7.0	3.5	1.8
"	5.6	3.4	1.3

[a]Disease indices were based on a scale of increasing severity from 0-8. Each figure is a mean index of 4 pots, each with 12 plants.

Fig. 1. Effect of combined virus and root-rot fungus infection on disease severity in Miragreen peas. Disease indices were based on a scale of increasing severity from 0-8 (_A. euteiches_) and 0-9 (_F. solani_). Each bar represents the mean of disease indices from 2-5 experiments. PMV is pea mosaic virus, strain 1, BYMV is bean yellow mosaic virus, strain 61-35, PEMV is pea enation mosaic virus, A. is _A. euteiches_, and F. is _F. solani_.

FIGURE I



DISEASE INDEX
0
1
2
3
4
5
6
7
PMV+A
A
PMV
BYMV+A
A
BYMV
PEMV+A
A
PEMV
PMV+F
F
PMV
BYMV+F
F
BYMV
PEMV+F
F
PEMV

A B C

Fig. 2. Miragreen peas infected with: A) pea mosaic virus (PMV-1), B) *F. solani*, C) *F. solani* and PMV-1.

Fig. 3. Miragreen peas infected with: A) pea mosaic virus (PMV-1), B) A. euteiches, C) A. euteiches and PMV-1.

Fig. 4. Miragreen peas infected with: A) bean yellow mosaic virus (BYMV 61-35), B) F. solani, C) F. solani and BYMV.

Fig. 5. Miragreen peas infected with: A) bean yellow mosaic virus (BYMV 61-35), B) A. euteiches, C) BYMV and A. euteiches.

Table 4. Collar, root and foliage ratings of Mira-green peas infected with *A. euteiches* and a virus or *A. euteiches* only

	Disease ratings for collar, root, foliage and whole plants infected with *A. euteiches*[a]			
Virus	Foliage	Collar	Root	Total
Pea mosaic	1.7	1.8	2.0	5.5
None	0.7	1.1	1.1	2.9
Bean yellow mosaic	2.1	1.5	1.9	5.5
None	0.7	1.1	1.1	2.9
Alfalfa mosaic	2.4	1.8	2.3	6.5
None	1.2	0.8	1.6	3.6
Pea enation mosaic	2.5	2.0	2.5	7.0
None	0.4	1.8	1.3	3.5

[a]Disease ratings were based on the following scales: collar 0-2, root 0-3, foliage 0-3, total 0-8. Each figure is a mean index of 4 pots, each with 12 plants.

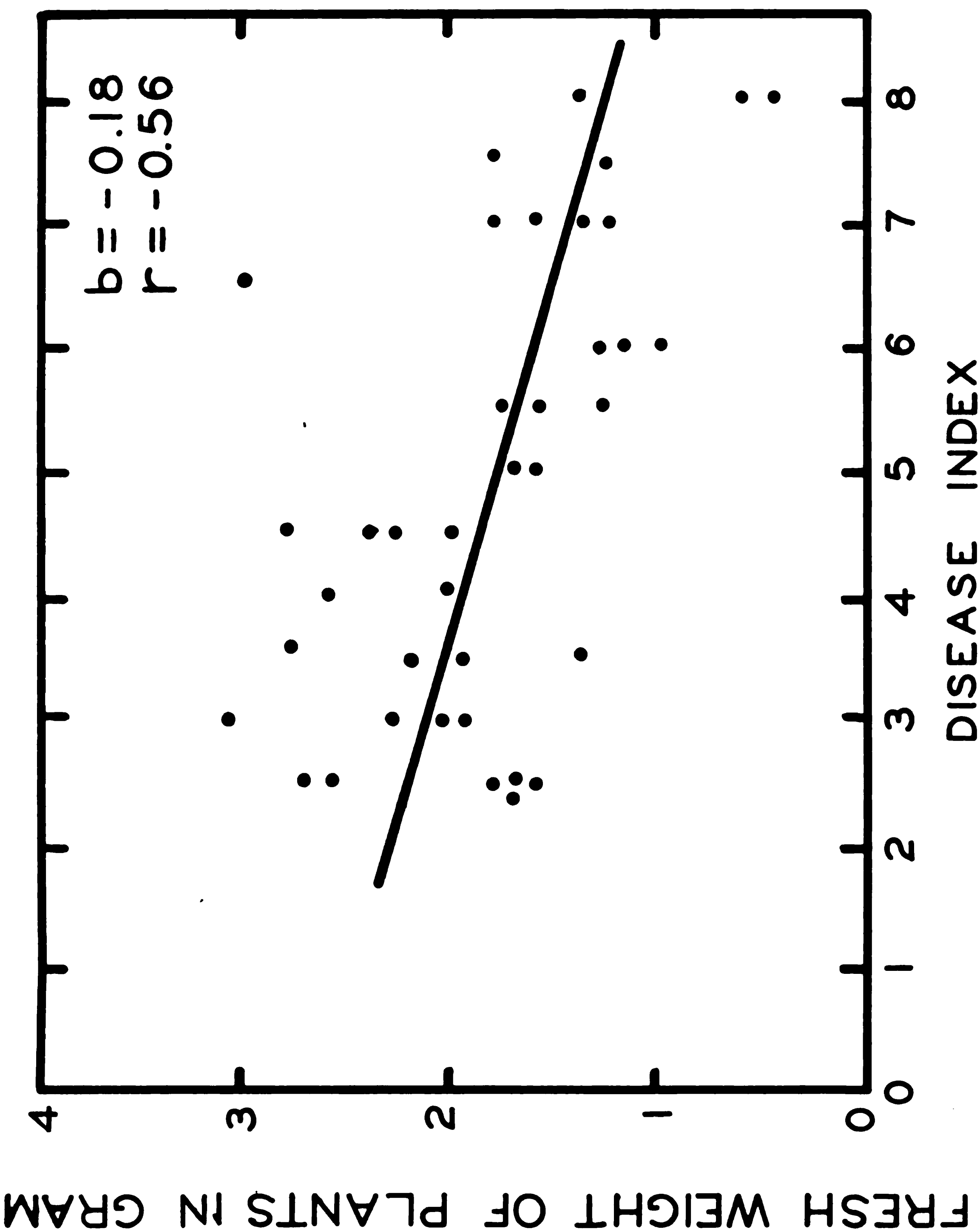
Table 5. Collar, root and foliage ratings of Mira-green peas infected with F. solani and a virus of F. solani only

Virus	Disease ratings for collar, root, foliage and whole plants infected with F. solani[a]			
	Foliage	Collar	Root	Total
Pea mosaic	1.0	2.4	0.6	4.0
None	0.4	1.3	0.2	1.9
Bean yellow mosaic	2.8	2.9	1.4	7.1
None	0.4	1.3	0.2	1.9
Alfalfa mosaic	1.9	1.6	1.7	5.2
None	0.8	0.8	1.0	2.6
Pea enation mosaic	1.3	1.4	1.3	4.0
None	0.2	1.1	0.5	1.8

[a]Disease ratings were based on the following scales: collar 0-3, root 0-3, foliage 0-3, total 0-9. Each figure is a mean index of 4 pots, each with 12 plants.

Fig. 6. Relation of disease indices and fresh weights of *A. euteiches*-infected Miragreen peas. Each point represents the mean index and fresh weight of 12 plants.

FIGURE 6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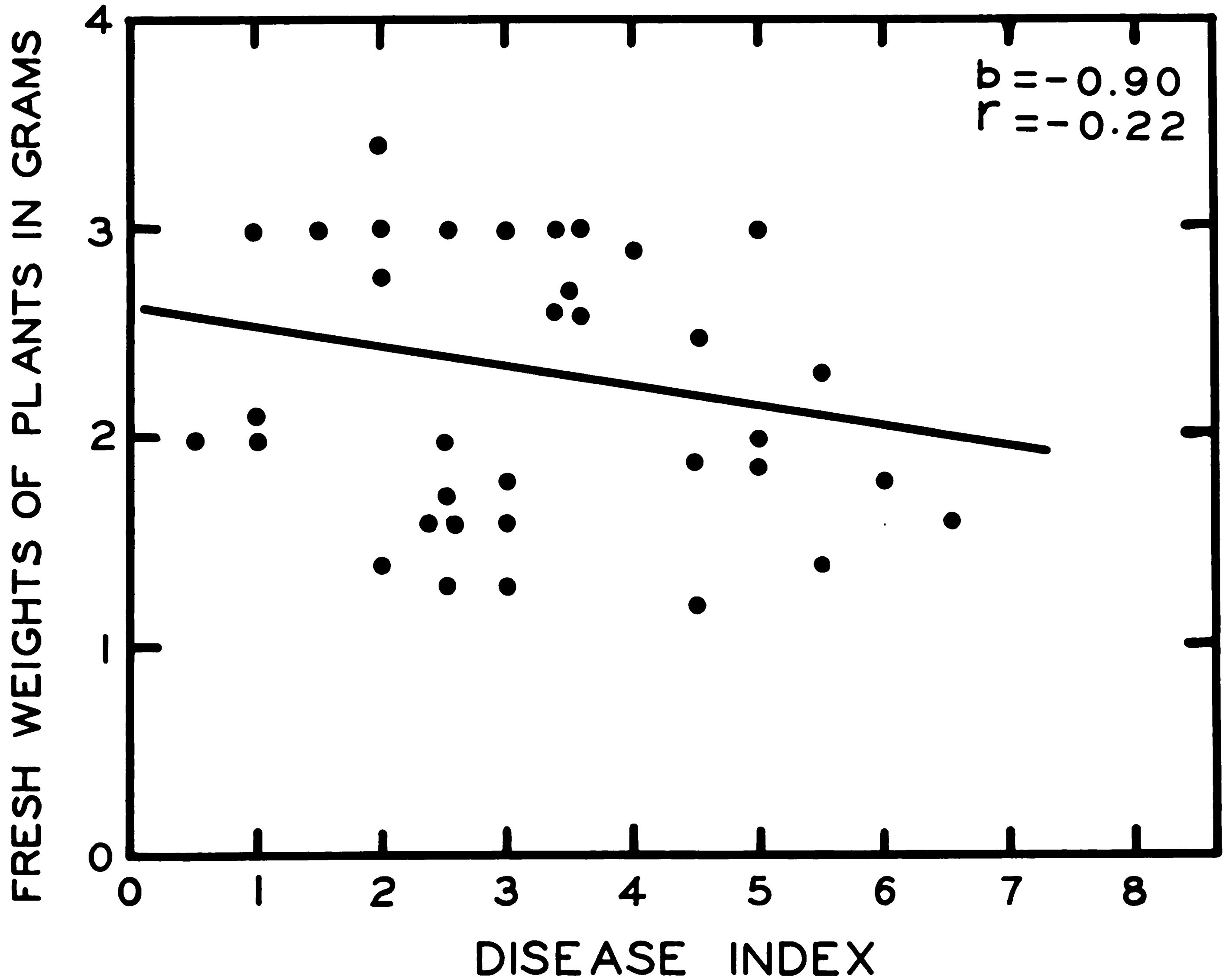
r = -0.56
DISEASE INDEX
FRESH WEIGHT OF PLANTS IN GRAMS
0
1
2
3
4
5
6
7
8
0
1
2
3
4

Fig. 7. Relation of disease indices and fresh weights of *F. solani*-infected Miragreen peas. Each point represents the mean index and fresh weight of 12 plants.

FIGURE 7


b = -0.90
r = -0.22
FRESH WEIGHTS OF PLANTS IN GRAMS
DISEASE INDEX
0
1
2
3
4
5
6
7
8

Disease index was a more sensitive measurement of disease severity than plant weight, i.e., disease indices of 2.0 and 3.0 of _A. euteiches_-infected plants corresponded to respective fresh plant weight of 2.3 g and 1.2 g. Fresh plant weights changed even less with _Fusarium_-infected plants. A statistically significant correlation existed between disease indices and fresh weights of _A. euteiches_-infected peas. The estimated correlation coefficient, r, was -0.560 (to be significant at the 1% level r must be 0.393 or more). Correlations were not significant for disease indices and fresh weights of _F. solani_-infected peas. _A. euteiches_ invades both vascular and cortical root tissues, interfering with translocation and causing severe wilting and stunting within a few days after infection (6). This may account for the significant correlation between _A. euteiches_ disease indices and fresh plant weights. _F. solani_, primarily a cortical invader (5) does not cause wilting or stunting of the foliage until the root-rot is well-established. This probably explains the lack of correlation between _Fusarium_ disease indices and plant weights.

Effect of combined virus-fungus infection on different pea varieties: Pea variety Perfected Wales and P.I. 169604 were inoculated with virus-fungus combinations to determine whether the increase in root-rot severity associated with combined virus-fungus infections would occur in pea varieties other than Miragreen. Perfected Wales and

P.I. 169604, infected with PMV-1 or BYMV 61-35 and *F. solani* or *A. euteiches*, developed more severe root-rot symptoms than plants infected with either root-rot fungus alone (Table 6). The relatively lower disease indices recorded for P.I. 169604 are probably due to its slight resistance to both root-rot pathogens (16).

Effect of different strains of PMV and BYMV: Miragreen pea seedlings were inoculated with either of two strains of PMV (PMV-1 or PMV-2) or either of two BYMV strains (BYMV 61-35 or BYMV 61-36). Three days later the soil was infested with *F. solani* or *A. euteiches*. Peas infected with either strain of PMV or BYMV and *F. solani* or *A. euteiches* developed more severe root-rot symptoms than plants infected with either root-rot fungus only (Table 7).

The effect of the length of virus establishment on root-rot severity: The effect of the time interval between virus and fungus inoculation was studied using Miragreen pea plants. Inoculations with PMV-1 and BYMV 61-36 were made when the first leaves were fully expanded and fungus inoculations were made 2 or 5 days later. The length of virus establishment (2 or 5 days) prior to fungus inoculation did not differentially affect root-rot development in virus-fungus-infected plants (Table 8).

The effect of the age of plants on root-rot severity in virus-fungus-infected peas: An experiment was designed to determine the effect of the age of the pea plant on combined

Table 6. Effect of combined virus and root-rot fungus infection on disease severity in 3 pea varieties

Pea variety	Fungus	Disease indices for plants infected with indicated pathogens[a] BYMV + Fungus	PMV + Fungus	Fungus only	LSD 1%
Miragreen	_F. solani_	7.1	4.0	1.9	1.3
	A. euteiches	5.5	5.5	2.9	1.6
Perfected Wales	_F. solani_	5.6	2.5	1.6	1.0
	A. euteiches	5.4	5.7	3.1	1.3
P.I. 169604	_F. solani_	-	2.3	0.5	1.2
	A. euteiches	-	3.6	1.9	1.1

[a]Disease indices were based on a scale of increasing severity from 0-8 (_A. euteiches_) or from 0-9 (_F. solani_). Each figure is a mean index of 4 pots, each with 12 plants. PMV is pea mosaic virus. BYMV is bean yellow mosaic virus.

Table 7. Effect of combined virus and root-rot fungus infection on disease severity in Miragreen peas using two strains of pea mosaic virus and 2 strains of bean yellow mosaic virus

Virus	Disease indices for plants infected with indicated pathogens[a]		
	A. euteiches + virus	*A. euteiches* only	LSD 1%
Pea mosaic (PMV-1)	5.5	2.9	1.6
Pea mosaic (PMV-2)	4.2	2.2	1.2
Bean yellow mosaic (61-35)	5.5	2.9	1.6
Bean yellow mosaic (61-36)	4.9	3.0	1.5

[a]Disease indices were based on a scale of increasing severity from 0-8. Each figure is a mean index of 4 pots, each with 12 plants.

Table 8. The effect of the time interval between virus and fungus inoculation on combined virus-fungus infection in Miragreen peas

Interval between virus and fungus inoculations	Fungus	Disease indices for plants infected with indicated pathogens[a]: Fungus Only	Fungus + PMV	Fungus + BYMV	LSD 1%
2 days	*A. euteiches*	2.2	4.4	4.3	1.4
5 days	"	2.0	3.8	5.0	
2 days	*F. solani*	0.7	2.2	5.0	0.5
5 days	"	1.1	2.8	4.6	

[a]Disease indices were based on a scale of increasing severity from 0-8 (*A. euteiches*) or from 0-9 (*F. solani*). Each figure is a mean index of 4 pots, each with 12 plants. PMV is pea mosaic virus. BYMV is bean yellow mosaic virus.

Table 9. The effect of the age of Miragreen peas on combined virus-root-rot fungus infection

Age of plants at virus inoculation	Fungus	Disease indices for plants infected with indicated pathogens[a] Fungus only	Fungus + PMV	Virus only	Control	LSD 1%
13 days	*A. euteiches*	4.5	6.5	3.0	0.5	
18 "	"	4.5	8.0	3.0	1.8	1.7
27 "	"	5.5	7.8	2.0	0.5	
13 "	*F. solani*	3.5	5.1	3.0	0.5	
18 "	"	3.5	6.2	3.0	1.8	1.6
27 "	"	4.4	6.1	2.0	0.5	

[a]Disease indices were based on a scale of increasing severity from 0-8 (*A. euteiches*) or from 0-9 (*F. solani*). Each figure is a mean index of 4 pots, each with 12 plants. PMV is pea mosaic virus.

virus-fungus infection. Miragreen peas were sown at varying times such that they would be 13, 18 and 27 days old when inoculated with PMV-1. Three days after inoculation with the virus, soil in the pots was infested with _F. solani_ or _A. euteiches_. Disease was evaluated 3 weeks later (Table 9). Disease indices of virus-fungus-infected peas were significantly higher than those of peas infected with a fungus only in 13-, 18-, and 27-day old plants. Results were similar for both fungi. The greatest difference in disease indices between fungus-only and virus-fungus-infected plants was in plants inoculated with the virus at 18 days. In this experiment plants infected with PMV-1 only had higher disease indices than uninoculated plants. Collars of PMV-1-infected plants were infected by _F. solani_ from an unknown source. This effect was observed in several experiments and provides additional evidence that virus-infected plants succumb more easily to root-rot than virus-free plants.

<u>Comparisons of roots of PMV-1-infected and virus-free' Miragreen pea plants:</u> No apparent difference was observed in tap or secondary root development in virus-infected pea plants as compared with roots of virus-free plants. The average fresh weights of 10 roots of PMV-1-infected and virus-free Miragreen peas were, respectively, 0.29 g and 0.30 g.

<u>Observations of peas in the field:</u> In June 1962, pea plants in 3 root-rot infested fields in Jackson, Michigan were examined to see if root-rot severity was greater in virus-fungus-infected plants than in plants infected with a

fungus only. Twenty virus-infected plants and 20 apparently virus-free plants surrounding them were dug up from each field. No attempt was made to identify virus or root-rot organisms. Plants were rated individually (Table 10). Virus infection did not increase root-rot development in these plants. The plants from the field were near maturity and were older and larger than plants used in the greenhouse tests.

Attempts to determine the mechanism(s) responsible for the increased root-rot virus-infected peas: Virus-infected peas are more susceptible to fungus root-rot than non-virus-infected peas for one or both of two reasons: 1) virus infection enhances root-rot inoculum potential at the root surface, 2) virus infection enhances development of root-rot following establishment of infection. To clarify whether the effect is one of pre-penetration or post-penetration the following experiments were conducted: Miragreen pea seeds were soaked in 0.5% sodium hypochlorite (10% Clorox) for 10 minutes and planted in rows in white silica sand in galvanized metal pans. When the plants were 1-2 in. high, the peas in each row were inoculated with 10 ml of a zoospore suspension of *A. euteiches* (5 x 10^4/ml) or a conidial suspension of *F. solani* (10 x 10^5/ml). *A. euteiches* zoospores were prepared by the method of Llanos and Lockwood (14). Two-5 days after fungus inoculation, 2 fully expanded leaves on each plant were inoculated with PMV-1. Immediately after

Table 10. Collar and root ratings of field peas infected with a virus and a root-rot fungus or with a root-rot fungus only

	Disease ratings[a]					
	Field 1		Field 2		Field 3	
	Collar	Root	Collar	Root	Collar	Root
Virus + fungus	2.2	1.6	1.8	1.4	1.3	1.3
Fungus only	2.0	1.8	1.8	1.6	1.2	1.3

[a]Disease ratings were based on a scale of increasing severity from 0-3. Each figure is a mean index of 15 or more plants.

virus inoculation, inoculated and uninoculated plants were transplanted to soil infested with A. euteiches or F. solani or to non-infested soil. The soil was infested using a hypodermic syringe just before transplanting. Before placing in soil, the roots were rinsed several times in distilled water to remove any surface mycelium and spores. Disease ratings were made 2 weeks after transplanting.

Virus-fungus-infected plants had no more root-rot than fungus-only-infected plants when both were transplanted into non-infested soil (Table 11). However, when plants were transplanted to infested soil, virus-fungus-infected peas had more severe root-rot than transplants infected with a fungus only. Disease indices of virus-fungus-infected plants transplanted into non-infested soil and fungus-only-infected plants transplanted into infested or non-infested soil were essentially the same (Fig. 8). Results were similar with both root-rot fungi and in all 3 experiments. The results from these tests suggest that PMV-1 infection enhances the infectivity of the fungal inoculum at the root surface.

The pathogenicity (inoculum potential) of several root-infecting fungi has been shown to be enhanced by nutrients exuded from plant roots (12,24,25,26,30). Therefore, attempts were made to compare total carbohydrates and amino compounds exuded from sterile roots of virus-infected plants and virus-free plants. Due to bacterial contamination,

Table 11. The effect on disease severity when PMV-fungus or fungus-only-infected Miragreen peas were transplanted into non-infested soil or soil infested with *A. euteiches* or *F. solani*

Test	Seedling transplanted into:	Fungus	Disease indices for plants infected with indicated pathogens[a]: Fungus only	Fungus + PMV	LSD 1%
1	Non-infested soil	*A. euteiches*	3.8	4.1	1.4
	"	*F. solani*	2.9	3.0	1.3
2	Non-infested soil	*A. euteiches*	3.1	3.7	1.2
	Infested soil	"	3.6	5.0	
	Non-infested soil	*F. solani*	3.1	3.2	1.1
	Infested soil	"	3.2	6.2	
3	Non-infested soil	*A. euteiches*	3.8	4.0	1.5
	Infested soil	"	3.4	5.9	
	Non-infested soil	*F. solani*	3.1	3.4	1.0
	Infested soil	"	3.1	5.0	

[a]Disease indices were based on a scale of increasing severity from 0-8 (*A. euteiches*) or from 0-9 (*F. solani*). Each figure is a mean index of 4 pots, each with 6 plants. PMV is pea mosaic virus.

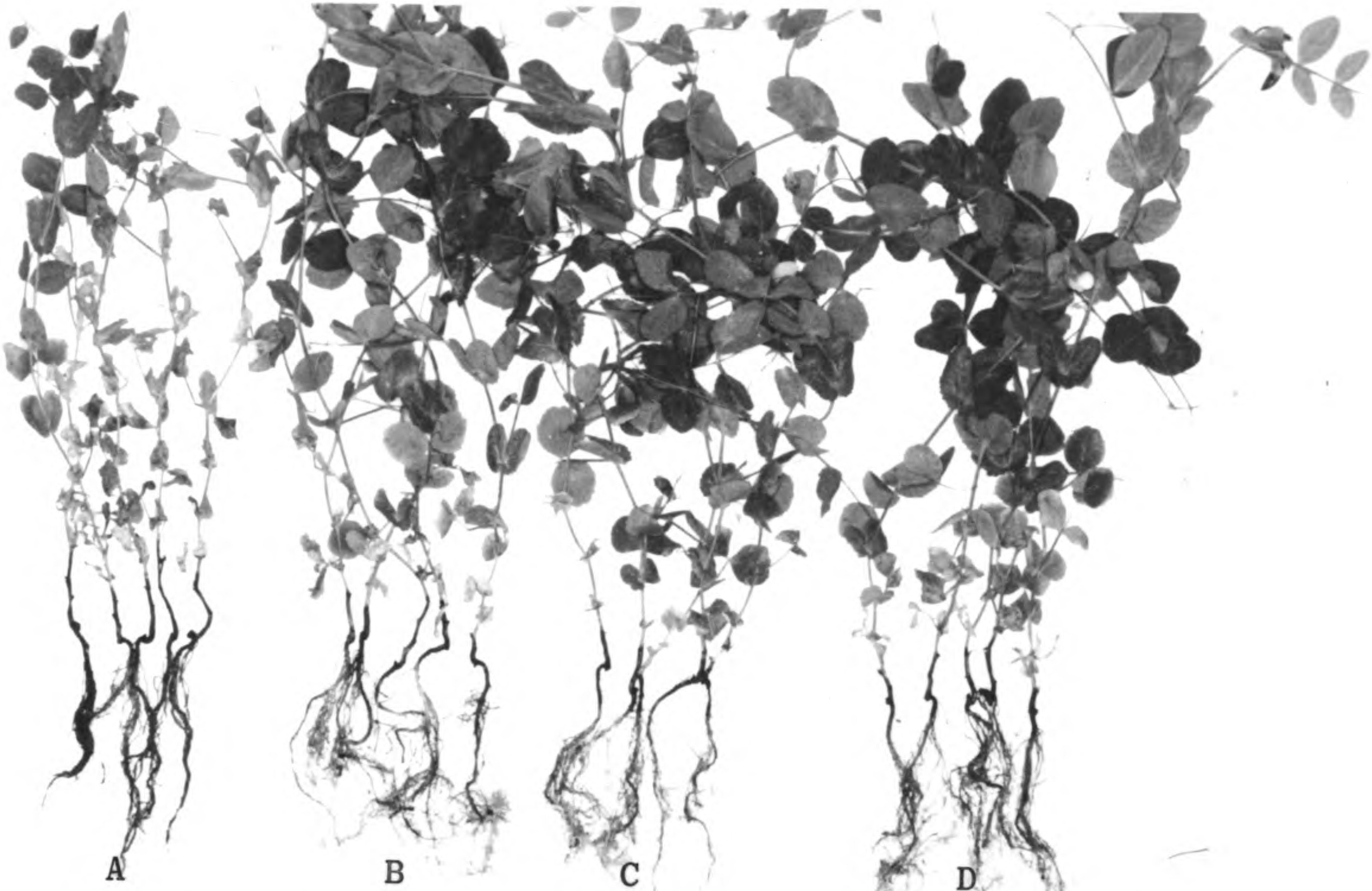


Fig. 8. The effect of transplanting virus-fungus and fungus-only-infected Miragreen peas into non-infested or F. solani infested soil. A) PMV + F. solani-infected peas transplanted into Fusarium infested soil. B) F. solani-only-infected peas transplanted into Fusarium-infested soil. C) PMV + F. solani-infected peas transplanted into non-infested soil. D) F. solani-only-infected peas transplanted into non-infested soil.

varying plant sizes or root breakage in the process of collecting the exudates, results were extremely variable. In some tests roots of PMV-1-infected peas exuded more carbohydrates and amino acids than roots of uninoculated peas; in other tests this result was reversed. No conclusions could be drawn.

Actinomycete, bacteria and fungus counts were made of the rhizosphere soil from PMV-1-infected peas and from uninoculated peas. Eight or 16 days after virus inoculation, 8 virus-infected plants and 8 uninoculated plants were carefully removed from clay pots containing loam muck (3:1) soil. The roots were gently shaken to remove non-rhizosphere soil, then placed along with adhering soil particles in flasks containing 100 ml of sterile water. After thorough shaking, serial dilutions from 10^{-4} to 10^{-10} were made in sterile water. One ml of a suitable dilution (10^{-5} for fungi, 10^{-7} for actinomycetes, or 10^{-10} for bacteria) was pipetted into 6 sterile petri dishes and 15 ml of melted (45°C) agar medium [fungi, OAES agar (19); actinomycetes, 2% chitin agar (15); bacteria, sodium albimate agar (13)] was added. Plates were incubated at 28°C and colonies were counted after 3-9 days. The dry weight of the rhizosphere soil was determined by transferring the contents of the flasks containing the original dilution into weighing bottles, and evaporating to dryness in an oven at 100°C.

There was no increase in the numbers of actinomycetes, bacteria or fungi in the rhizosphere of PMV-1 infected Miragreen as compared to rhizospheres of virus-free peas(table 12).

Table 12. Numbers of colonies of actinomycetes, bacteria and fungi from rhizospheres of PMV-1 infected and healthy Miragreen peas

			No.of colonies/g of oven dried rhizosphere soil[a]		
Test	Days after virus inoculation	Virus[b]	Actinomycetes	Bacteria	Fungi
1	8	PMV-1	5.6	-	-
		None	8.4	-	-
2	16	PMV-1	5.9	530,000	0.16
		None	7.9	750,000	0.25

[a]Each figure is a mean of 6 plates and represents the number of organisms in millions/g in oven dried rhizosphere soil.

[b]PMV-1 is pea mosaic virus, isolate I.

DISCUSSION

Root-rots (*A. euteiches* and *F. solani*) and virus diseases, respectively, were the first and second most important pea diseases in Michigan pea fields in 1955 and 1956 (17). The results of the present study suggest that pea viruses may play an important role in the development of the fungus root-rot complex of peas. Over a wide range of greenhouse environmental conditions, 3 pea varieties were found to be more susceptible to fungus root-rot following infection with each of 4 pea viruses. Similar results were obtained using different strains of two of the viruses, plants of different ages, and different time intervals between virus and fungus inoculations.

The length of virus establishment before fungus inoculation is often reported to have an effect on fungus development (29,7). Generally the longer the virus establishment the greater protection afforded the host (7), or conversely, the greater the increase in a host's susceptibility to fungi (29). In this study the time interval between virus and fungus inoculations was short (0-5 days) and no differential root-rot development was observed. Tests should be conducted with the time interval between virus-fungus inoculations extended.

Attempts to correlate greenhouse results with field observations have not been successful. Since virus-infected plants occurred infrequently in Michigan pea fields in 1962 and 1963, only limited field data are available. Observations were made on plants that were larger and more mature than peas used in the greenhouse tests. More field observations should be made on younger peas in situations where root-rot and virus infections occur together.

When plants previously infected with _A. euteiches_ or _F. solani_ were transplanted into non-infested soil following washing of the roots, the root-rot that developed would mainly be from primary infections that occurred before transplanting. In plants so treated the root-rot severity of virus-fungus-infected plants was not increased over that in plants infected with a fungus only. When plants infected with root-rot fungi were transplanted into infested soil, secondary infections would occur. In these plants root-rot severity of virus-fungus-infected plants was significantly greater than that in peas infected with a fungus only. These transplanting experiments suggested that virus infection influenced the fungus inoculum potential at the root surface.

The pathogenicity (inoculum potential) of several root-infecting fungi has been shown to be increased by the nutrients exuded from plant roots (12,24,25,26,30). Chlamydospore germination and mycelial growth of _F. solani f phaseoli_ were stimulated by materials exuded from bean roots (26). Root regions from which there was heavy exudation were more severely infected with root-rot. Amino acids and sugars have been identified as major constituents of bean root exudate (26).

Sharen has observed that *A. euteiches* oospores, the probable survival unit of this fungus, germinated significantly more in sand containing plant roots than in plant-free sterilized or unsterilized soil (24). Thus root exudations appear to provide nutrients favoring the successful pathogenesis of these two root infecting pathogens.

Root exudations and the consequent rhizosphere effect were increased in a virus-infected *Dolichos* sp. (tropical legume) (personal communication from T. S. Sadasivan). Therefore it seemed plausible that the increased root-rot in virus-infected pea plants might be due to stimulation of root-rot fungi by increased root exudations.

Attempts to collect root exudates of virus-infected plants under aseptic conditions were not completely successful. A more comprehensive interpretation of the transplanting experiments must be withheld until sufficient data on root exudates from uncontaminated plants have been accumulated.

The transplanting experiments suggest that the inoculum potential of *A. euteiches* or *F. solani* is increased at the root-surface. If this interpretation is incorrect, the possibility exists that virus-infected plants may be more susceptible to fungus root-rot because of a decreased formation or increased utilization of a host metabolite responsible for root-rot resistance. That plants infected with a fungus or virus may be more susceptible to infection by other pathogens because of an abnormal accumulation of certain normal or abnormal metabolites, has been suggested in several of the papers reviewed in this thesis (7,20,29). There is evidence that production of Pisatin (an antifungal metabolite produced by peas in response to fungus spores, and to which resistance to non-pathogens is attributed) is lowered in peas weakened by adverse environment conditions (personal communication from I. A. Cruickshank). If Pisatin induces some resistance to root-rots in peas, and if virus-infection decreases the formation of Pisatin in root-rot infected tissues, further research along these lines may be profitable.

SUMMARY

Miragreen peas infected with a virus [pea mosaic virus (PMV) strains PMV-1 or PMV-2, bean yellow mosaic virus (BYMV) strains BYMV 61-35 or BYMV 61-36, alfalfa mosaic virus (AMV), or pea enation mosaic virus (PEMV)] and a fungus, [*Aphanomyces euteiches* of *Fusarium solani* f. *pisi*], developed more severe root disease symptoms than peas infected with a root-rot fungus only. The length of virus establishment (2 or 5 days) prior to fungus inoculation did not significantly affect disease severity in virus-fungus-infected plants. The age of the plant at virus-fungus inoculations (13-, 18-, or 27-days) did not significantly affect the disease severity of virus-fungus or fungus-only-infected plants.

Pea varieties Perfected Wales and P.I. 169604 infected with PMV-1 or BYMV 61-35 and *F. solani* or *A. euteiches* developed more severe root-rot symptoms than plants infected with either root-rot fungus alone.

Virus infection did not appear to increase root-rot development in the field.

When young pea seedlings infected with PMV-1 and *A. euteiches* or *F. solani* or either fungus alone were transplanted into non-infested soil, virus-fungus-infected plants had no more root-rot than fungus-only-infected plants. However, when plants were transplanted to infested soil, virus-fungus-infected peas had more severe root-rot than transplants

infected with fungus only. These results suggested that PMV-1 infection enhanced the infectivity of the fungal inoculum at the root surface.

There was no increase in the numbers of actinomycetes, bacteria or fungi in the rhizospheres of PMV-1-infected Mira-green peas as compared to rhizospheres of virus-free peas.

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