The Production of a Noninfectious Lettuce Root Rot Under Controlled Environmental and Soil Conditions

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ABSTRACT

A noninfectious root rot of lettuce (*Lactuca sativa*) was produced by placing 10-week-old lettuce plants in soil-crop residue mixtures for 3 weeks (exposure period) which previously had been incubated for 5, 7, or 9 weeks (incubation period). Vascular discoloration and corkiness of the root surface were the two characteristic symptoms produced. The mixtures contained either (i) 200 g organic soil; (ii) 200 g organic soil and 500 ml water; (iii) 200 g organic soil and 300 g chopped fresh lettuce leaves; or (iv) 200 g organic soil, 300 g fresh lettuce leaves, and 500 ml water. Only the last mixture produced vascular discoloration in the roots of the plants during the exposure period. Production of the corky root symptom was not specifically related to any of the four incubated mixtures, but occurred whenever plants were subjected to flooded soil conditions for 1 week, followed by a 2-week drying period. Vascular discoloration occurred when the 300 g of fresh lettuce leaves in the fourth mixture were replaced by either 20 g of chopped dried lettuce leaves or roots, 25 g sucrose, 25 g casein hydrolysate, or 25 g sucrose and 25 g casein hydrolysate. When the length of the incubation period was increased, the amount of vascular discoloration observed after a 3-week exposure period decreased. Increasing the amounts of either lettuce residue or sucrose in the mixtures increased vascular discoloration and root injury. The standard method developed for producing the two symptoms of noninfectious root rot involved transplanting 10-week-old lettuce plants into 2-week-old mixtures of 20 g dried lettuce leaves, 300 g organic soil, and 1 liter water, followed by an exposure period consisting of 1 week under flooded conditions and 2 weeks during which the soil was allowed to dry. Phytopathology 61:1153-1158.

The belowground symptoms of a root rot disease frequently occurring on lettuce (*Lactuca sativa* L.) grown on New York organic soils are yellowing or browning and cracking or corking of the exterior taproot surface. The apical end of the taproot generally becomes necrotic, resulting in a shortened taproot and a reduced number of fibrous roots. The internal symptoms of the diseased taproot range from partial discoloration of the vascular system to total discoloration of the vascular system accompanied by the disintegration of the stele. The aboveground symptoms are related to the degree of root injury. Slightly damaged roots produce little or no change in the foliage; severely damaged roots may result in wilting, stunting, and/or commercially unacceptable heads.

Past investigators reported that (i) *Pythium* spp. were the causal organisms of root rot (5, 6); (ii) bacteria were responsible for plugging and discoloring the xylem of the roots (12); and (iii) the addition of different types and amounts of nitrogen fertilizers produced different degrees of the disease (7, 8). Therefore, field experiments were conducted during 1964 and 1965 on organic soil in Oswego County, N. Y., to investigate if *Pythium* spp. or nitrogen fertilizers were the cause of lettuce root rot. The effect of controlling *Pythium* spp. on lettuce root rot was investigated by applying *p*-dimethylaminobenzenediazo sodium sulfonate (Dexon, Chemagro Corp., Kansas City, Mo.) at planting time. The relationship between lettuce root rot and nitrogen fertilizers was investigated by side dressing 10-day-old lettuce plants with either ammonium nitrate, urea, or calcium nitrate. Bacteria isolated from rotten lettuce roots were also investigated as a possible cause of lettuce root rot by the inoculation of aseptically grown lettuce seedlings. All three studies failed to indicate that lettuce root rot was caused by either *Pythium* spp., bacteria, or nitrogen fertilizers. The field experiments showed that: (i) The incidence of root rot was higher in a poorly drained soil than in a well-drained soil; (ii) the incidence of root rot was less during dry years than during wet years; (iii) root rot could occur on virgin soil; (iv) decomposition of lettuce residue in the field was so rapid that partially decomposed residue never was observed near diseased roots; (v) root rot usually was more prevalent in the spring and early fall than in the summer; (vi) continuous lettuce cropping and especially the production of two lettuce crops on the same field in a single year generally tended to increase the severity of lettuce root rot the following year.

The similarity of these field observations with those of Cochrane (3, 4), Patrick et al. (11), and Amin & Sequeira (1, 2) indicate that toxic lettuce residue decomposition products might be causing the root rot. The experiments reported herein were conducted in the greenhouse and controlled environment chambers, and were designed to reproduce the symptoms of root rot by subjecting the roots of lettuce plants to lettuce residue decomposition products while the soil was flooded with water. This paper reports on those residue-incubation mixtures which produced noninfectious root rot under controlled environmental conditions.

MATERIALS AND METHODS.—*Test plants.*—Lettuce test plants (Great Lakes 659) were germinated in ver-

miculite, transplanted into flats after 2 weeks, and transplanted again after 3 weeks into 6-inch pots containing field-collected organic soil. The plants were moved from the greenhouse to a growth chamber with a 14-hr photoperiod, 22-C day, 16-C night, 75% relative humidity, and 2,000 ft-c. After 5 weeks in the growth chamber, the plants were transplanted into crocks containing different incubation mixtures.

Incubation mixtures.—Different amounts of fresh lettuce leaves or dried lettuce roots or sucrose and/or casein hydrolysate were combined with either 200 or 300 g organic soil. These mixtures were placed in 8-inch-diam glazed crocks and flooded with either 500 ml or 1 liter of water. The crocks were covered with aluminum foil or aluminum foil and plastic wrap, and held at 18 C for incubation periods of 1-9 weeks. Three additional incubation mixtures consisting of: (i) organic soil; (ii) organic soil and either 500 ml or 1 liter water; and (iii) organic soil and lettuce residue prepared to test individual components of the complete mixtures in the production of lettuce root rot. The composition of the mixes and the amount of water added is given for each experiment described.

The exposure period.—After the incubation period, 10-week-old test plants were placed in the crocks to initiate the exposure period. Injury to the root systems of the test plants due to transplanting was minimized by placing the intact root system and soil into the crock (Fig. 1). To secure the test plant in the crock, organic soil was added to the crock. The soil moisture level in the crocks then was brought to saturation. During the exposure period (2-3 weeks), the soil either was maintained at saturation by daily irrigation or allowed to dry during the last 2 weeks of the 3-week exposure period. At the termination of exposure, the roots and leaves (including stems) were weighed and the roots rated for vascular discoloration and corkiness.

Root rot rating system.—The amount of taproot vascular discoloration was used to rate the disease intensity: 1 = less than 25%; 2 = ca. 25%; 3 = ca. 50%; 4 = ca. 75%; 5 = 100% with no apparent hollowing of the taproot; 6 = 100% plus partial or complete hollowing of the taproot.

RESULTS.—*Requirements for production of lettuce root rot.*—Four different incubation mixtures were prepared as follows: (i) 200 g organic soil (S); (ii) 200 g organic soil and 500 ml water (SW); (iii) 200 g organic soil plus 300 g chopped fresh lettuce residue (SL); (iv) 200 g organic soil, 300 g fresh lettuce residue, and 500 ml water (SLW). The mixtures (four replications each) were placed in 8-inch-diam glazed crocks covered with aluminum foil and incubated for 5, 7, and 9 weeks. No additional water was added during the incubation period. Following incubation, lettuce plants were placed in the crocks for a 3-week exposure period. A saturated soil moisture condition was maintained by the daily addition of water. The SLW mixture incubated for 5 weeks produced the greatest degree of leaf wilting, vascular discoloration, and root necrosis (Fig. 2-A). The root and leaf weights (including stems) of the SLW plants were consistently lower for all three incubation periods than the weights of the plants exposed to the other three incubation mixtures (Fig. 2-B, C). The 7- and 9-week SLW incubation mixtures were less toxic to the plants than the 5-week SLW mixtures.

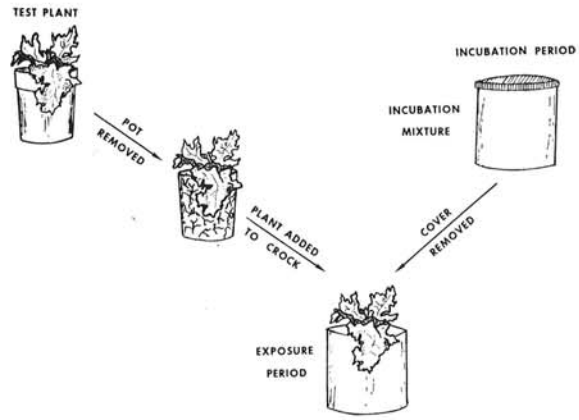

Fig. 1. Procedure for transplanting lettuce test plants to incubation mixtures initiating the exposure period. A 10-week-old plant (Great Lakes 659) was removed from its 6-inch pot and placed in a glazed crock containing the incubation mixture. The plant was secured in the crock by adding additional organic soil from the same source as that used in the incubation mixture. The soil moisture level in the mixture was brought to saturation, and the exposure period begun.

Because SLW toxicity decreased as incubation time increased, an incubation period of less than 5 weeks was tested. To promote more uniform and pronounced disease symptoms, the contents of the incubation mixtures were increased. The incubation mixtures were as follows: (i) 300 g organic soil (S); (ii) 300 g organic soil and 1 liter water (SW); (iii) 300 g organic soil and 450 g fresh lettuce residue (SL); (iv) 300 g organic soil, 450 g fresh lettuce residue, and 1 liter water (SLW). The incubation times were 3, 5, and 7 weeks. Test plants were exposed to the incubation mixtures for 2 weeks.

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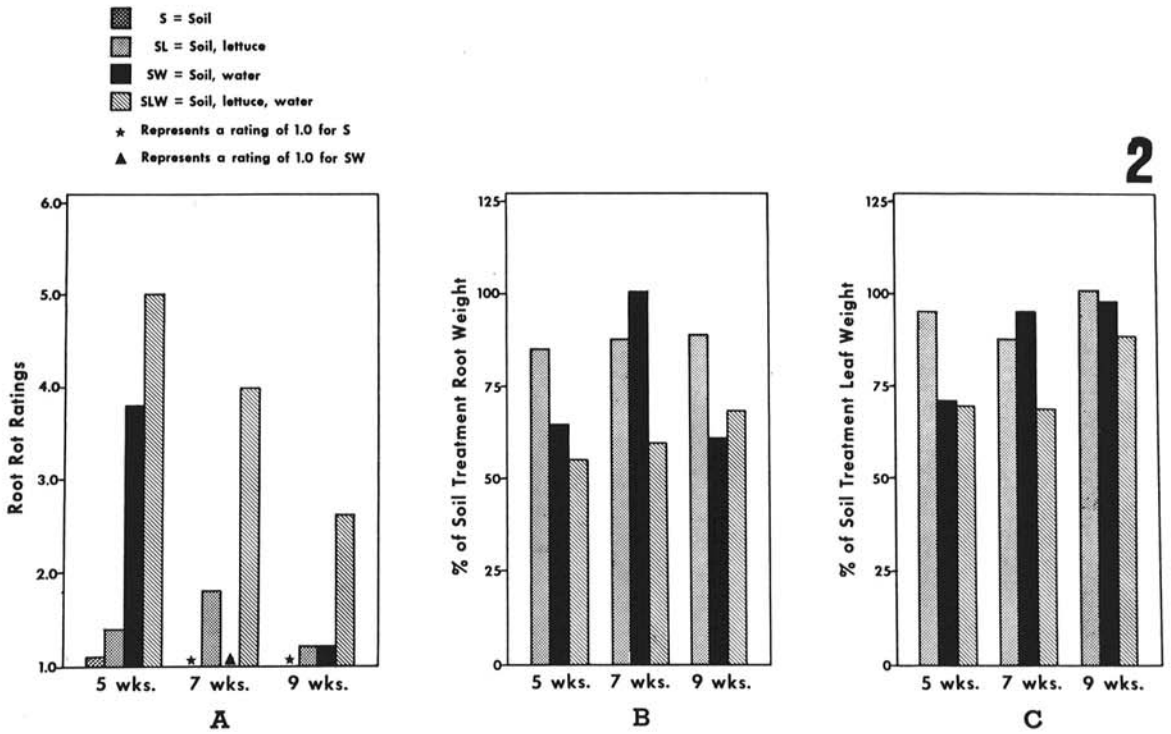
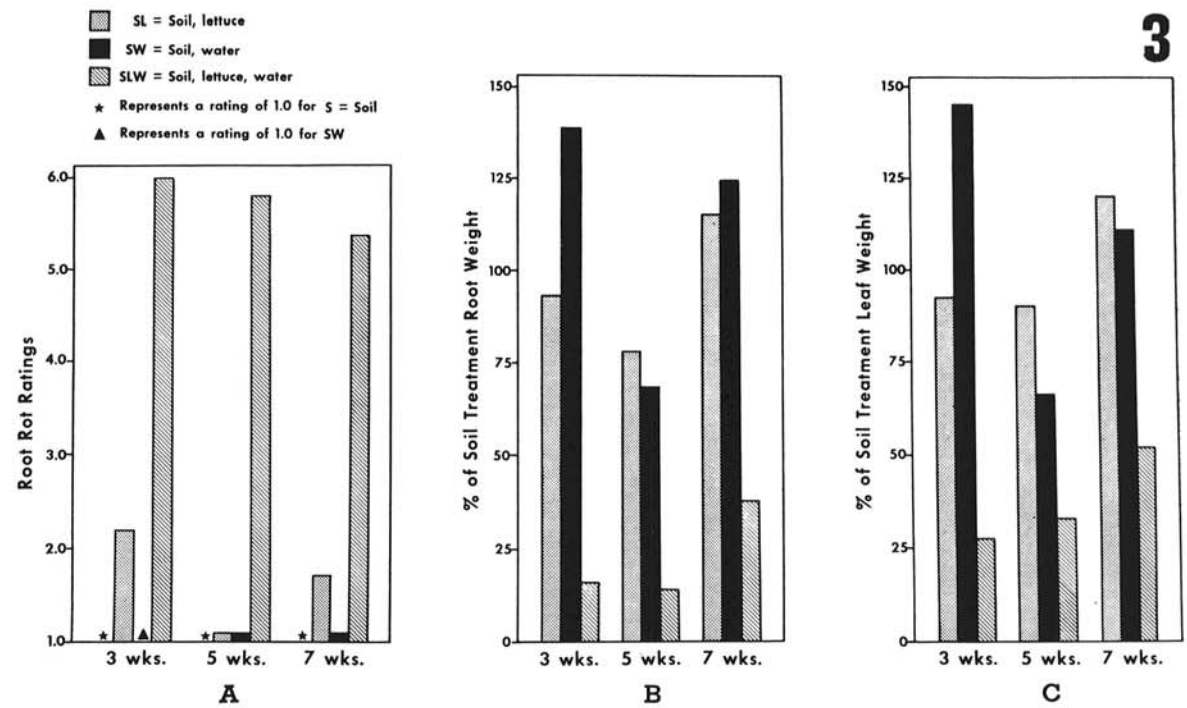
Fig. 2-3. **2)** Root rot ratings for lettuce plants exposed to 5-, 7-, and 9-week incubation mixtures consisting of: **(i)** organic soil (S); (ii) organic soil and lettuce residue (SL); (iii) water-saturated organic soil (SW); and (iv) water-saturated organic soil and lettuce residue (SLW). The ratings are an average of the four plants in each treatment. The figures for the per cent of soil treatment root weight (or leaf weight) were calculated by comparing the weights of test plants exposed to each of the three incubation mixtures (SL, SW, SLW) to those of plants exposed to the S incubation mixture. **3)** Root rot ratings for lettuce plants exposed to 3-, 5-, and 7-week incubation mixtures consisting of: (i) organic soil (S); (ii) organic soil and lettuce residue (SL); (iii) water-saturated organic soil (SW); and (iv) water-saturated organic soil and lettuce residue (SLW). The ratings are an average of the four plants in each treatment. The figures for the per cent of soil treatment root weight (or leaf weight) were calculated by comparing the weights of tested plants exposed to each of the three incubation mixtures (SL, SW, SLW) to those of plants exposed to the S incubation mixture.


S = Soil
SL = Soil, lettuce
SW = Soil, water
SLW = Soil, lettuce, water
★ Represents a rating of 1.0 for S
▲ Represents a rating of 1.0 for SW
2
Root Rot Ratings
6.0
5.0
4.0
3.0
2.0
1.0
5 wks.
7 wks.
9 wks.
A
% of Soil Treatment Root Weight
125
100
75
50
25
0
5 wks.
7 wks.
9 wks.
B
% of Soil Treatment Leaf Weight
125
100
75
50
25
0
5 wks.
7 wks.
9 wks.
C


SL = Soil, lettuce
SW = Soil, water
SLW = Soil, lettuce, water
★ Represents a rating of 1.0 for S = Soil
▲ Represents a rating of 1.0 for SW
3
Root Rot Ratings
6.0
5.0
4.0
3.0
2.0
1.0
3 wks.
5 wks.
7 wks.
A
% of Soil Treatment Root Weight
150
125
100
75
50
25
0
3 wks.
5 wks.
7 wks.
B
% of Soil Treatment Leaf Weight
150
125
100
75
50
25
0
3 wks.
5 wks.
7 wks.
C

The 3-week SLW incubation mixture was the most toxic to the lettuce plants (Fig. 3-A). The roots were 100% discolored and severely hollowed. The SLW mixture incubated for 3 weeks (Fig. 4-A) produced more intense disease symptoms than the 5-week SLW incubation mixture (Fig. 4-B), which produced more intense disease symptoms than the 7-week SLW incubation mixture (Fig. 4-C). Within each incubation period, the SLW incubation mixture always was more toxic than the other incubation mixtures. The root and leaf weights (including stems) of plants exposed to the SLW incubation mixtures again were consistently lower than those of plants exposed to the other three incubation mixtures (Fig. 3-B, C).

By increasing the volume of the incubation mixtures while maintaining the same fresh lettuce residue to soil ratio (3:2), we produced more pronounced disease symptoms. In the 3-week incubation mixtures, the fresh lettuce in the SL mixtures contained enough water to saturate the soil. The moisture provided by the lettuce was sufficient to produce lettuce root rot in the 3-week SL mixtures. The 5- and 7-week SL mixtures appeared much drier at the time the test plants were added to the crocks, as the excess moisture from the lettuce had more time to evaporate than in the 3-week SL incubation trials. The root disease levels of plants exposed to the 5- and 7-week SL treatments were comparable to those of plants exposed to the SW and the S treatments.

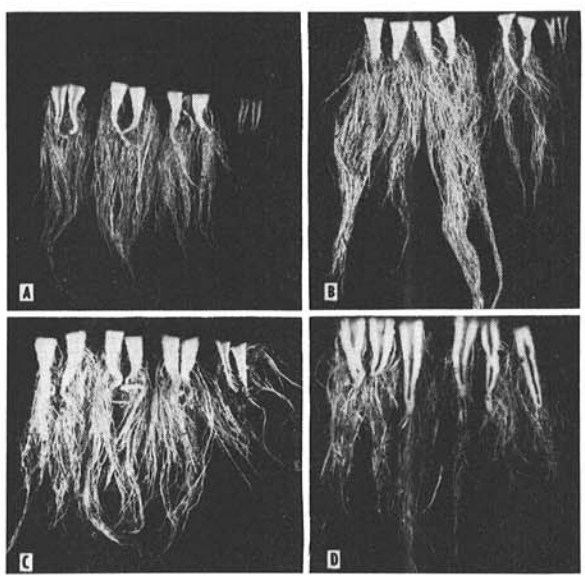


Fig. 4. Lettuce root rot. **A)** Roots from plants exposed to 3-week incubation mixtures of (left to right) organic soil and lettuce (SL); organic soil (S); water-saturated organic soil (SW); water-saturated organic soil and lettuce residue (SLW). **B)** Roots from plants exposed to 5-week incubation mixtures of (left to right) SL, S, SW, and SLW. **C)** Roots from plants exposed to 7-week incubation mixtures of (left to right) SL, S, SW, and SLW. **D)** Roots from plants exposed to 7-week incubation mixtures of (left to right) sucrose and water-saturated organic soil (SucSW); casein hydrolysate, sucrose, and water-saturated organic soil (CasSucSW); casein hydrolysate and water-saturated organic soil (CasSW).

Amino acid and carbohydrate substrates.—Three additional incubation mixtures were prepared: (i) 25 g sucrose, 300 g organic soil, and 1 liter water (SucSW); (ii) 25 g casein hydrolysate, 300 g organic soil, and 1 liter water (CasSW); (iii) 25 g casein hydrolysate, 25 g sucrose, 300 g organic soil, 1 liter water (CasSuc SW). These mixtures were incubated for 7 weeks.

Test plants exposed for 2 weeks to the SucSW, CasSW, and CasSucSW mixtures are shown in Fig. 4-D. All the plants showed complete vascular discoloration and some hollowing of the taproot. The disease index on each plant was similar to that on plants exposed to SLW mixtures in the previous experiments.

Comparison of fresh and dried lettuce leaves as incubation mixture substrates.—Because fresh lettuce leaves in the SL incubation mixtures in the previous experiments contained enough water to saturate the soil, the role of dried lettuce leaves in the production of lettuce root rot was studied. Mixtures consisting of 20 g dried lettuce residue or 45 g fresh lettuce residue, 300 g organic soil, and 1 liter water were incubated for 2 weeks. Both of these mixtures produced 100% vascular discoloration and some hollowing of the roots of test plants (root rot rating of 6.0) during the exposure period. The root weights and leaf weights (including stems) of the plants exposed to either fresh or dried lettuce residue were comparable. However, the weights were far less and the root rot rating far greater than those of plants simultaneously exposed to a comparable SW incubation mixture or those of unexposed plants (S) grown in 6-inch pots.

Production of the external corky symptom.—It was thought that a drier soil environment during the latter part of the exposure period might result in: (i) the recovery of the leaves from the wilt symptom; and (ii) the appearance of the corky symptom on the exterior of the root. Thirty g dried lettuce leaves, 300 g organic soil, and 1 liter water (SDLLW) were incubated for 4 weeks. Thirty g of dried lettuce roots and 30 g of sucrose (SDLRW and SucSW incubation mixtures) also were tested in place of the dried lettuce leaves. A soil incubation mixture (S) served as the check. Half of the crocks in each treatment were maintained at a high soil moisture level during the 3-week exposure period; half were allowed to dry during the last 2 weeks.

When irrigation was withheld after the 1st week of the exposure period, the plants partially recovered from the wilting symptoms. When the water-saturated soil environment was maintained throughout the exposure period, the plants did not recover. The leaf weights of plants exposed to SDLLW and SDLRW treatments were increased somewhat under the drier soil conditions during the exposure period than under the continuously wet soil environment (Table 1). The greater root weights of the plants exposed to the SDLLW and the SDLRW mixtures in the drier soil environment indicated that the roots began to function and grow again. The roots of test plants exposed to the 4-week-old mixture containing 30 g sucrose were

TABLE 1. Lettuce root rot resulting when test plants were transplanted to organic soil amended 4 weeks earlier with 30 g each of three carbon sources, and the influence of reduced soil moisture on root rot[a]

Soil amendment	Root ratings, interior, 1–6[b]	Fresh wt, g	
		Roots	Shoot
Irrigation not withheld			
Dried lettuce leaves[c]	6.0	1.8	27.4
Dried lettuce roots[c]	6.0	2.3	32.2
Sucrose[c]	6.0	1.4	13.6
None, control	1.0	8.2	65.6
Irrigation withheld			
Dried lettuce leaves[c]	6.0	5.4	36.2
Dried lettuce roots[c]	5.0	5.4	35.6
Sucrose[c]	6.0	1.4	13.7
None, control	2.0	12.6	76.6

[a] The root ratings and the weights are an average of the two plants in each treatment.

[b] Root rot rating system based on vascular condition: 1 = less than 25% discolored; 2 = ca. 25% discolored; 3 = ca. 50% discolored; 4 = ca. 75% discolored; 5 = 100% discolored but no hollowing of taproot evident; 6 = 100% discolored plus partial or complete hollowing of taproot.

[c] The organic soil plus the amendment were flooded with water during the 4-week preplant incubation period.

damaged so severely that the plants did not recover during the dry portion of the exposure period.

The wet soil environment followed by a drier one produced the corky symptom on the exterior of the taproot. The appearance of the corky symptom occurred with or without the presence of residue material in the incubation mixture. The taproots of plants exposed to either the SDLLW mixture or the S mixture exhibited corky root exteriors (Fig. 5-A), but only the plants exposed to the SDLLW mixture exhibited vascular discoloration of the root system (Fig. 5-B). The exterior corky symptom is caused by excessive fluctuations in soil moisture (flooding and drying), and apparently is not related to crop residue decomposition products.

The mixtures containing dried lettuce roots (SDLRW) essentially caused the same degree of root rot (rating of 5 or 6) as the ones with dried lettuce leaves (SDLLW) during the exposure period (Table 1). Mixtures containing the high substrate level (30 g) severely damaged the roots. The root systems of test plants exposed to the SucSW mixtures were decomposed almost completely.

DISCUSSION.—Observations in New York (1964-'65) suggested that a lettuce root rot was most prevalent in fields or areas of fields having high soil moisture. Since partially decomposed lettuce residue was never associated with the diseased roots, the situation did not appear completely analogous to that described by Patrick et al. (11) on mineral soils in the Salinas Valley, California. The results of field experiments conducted later by Amin & Sequeira (1, 2) were similar to the situation observed in New York because the incidence of root rot was greater for spring planted lettuce than for summer planted lettuce. They likewise observed the failure of late-season plantings because of root rot.

The objectives of the first experiments in the present study were to corroborate the work of Amin & Sequeira (1, 2) that lettuce residue decomposing under high soil moisture conditions was toxic to lettuce roots, and to determine the optimum time period for disease production. Of the four incubation mixtures tested, only the SLW mixture produced the internal symptoms of lettuce root rot. This proved that high soil moisture alone did not cause the disease. The shortest incubation period tested (3 weeks) was the most injurious to the lettuce test plants during the exposure period (Fig. 3). In subsequent experiments, a 2-week incubation period produced even more severe root rot symptoms than the 3-week period. A 1-week incubation period also produced disease symptoms, but quantities of gas were still evolving after the plants were placed in the crocks. This gas pushed the test plants up and almost forced them out of the crocks during the exposure period. The 2-week incubation period, therefore, was considered to be optimum.

The gas production from the 1-week incubation mixture suggests that highly anaerobic conditions existed during the initial phases of residue decomposition. Although such predominately anaerobic conditions may not occur under field conditions, anaerobic metabolism may be important for the production of toxic residue decomposition products. This possibility is substantiated by the findings of Patrick & Koch (10) that toxic decomposition substances were much more abundant in a water-saturated soil than in a soil at field capacity. Mishustin & Erofeev (9) showed that the anaerobic decomposition products of wheat straw were toxic to ryegrass seedlings; the aerobic decomposition products were not. This suggests that the soil moisture may be high enough in the spring and early fall so that toxic lettuce residue decomposition products would occur under predominately anaerobic conditions, but during the summer the drier soil conditions would provide predominately aerobic nontoxic residue decomposition products.

The observation that root rot could occur on a poorly drained virgin soil indicated that lettuce root rot was not solely dependent on the decomposition of lettuce residue under high moisture conditions. This observation was investigated by subjecting lettuce plants to mixtures of soil and water containing either sucrose, casein, or sucrose plus casein which had been incubated for 7 weeks. Both substrates alone and in combination produced the same results as lettuce residue. These indicate that the decomposition of all plant material in general under predominately anaerobic conditions should result in lettuce root rot.

When the soil in the crocks was allowed to dry during the 2nd and 3rd weeks of the exposure period, the exterior corky symptom was produced on the roots of the test plants. The corky appearance occurred not only on the roots of the test plants exposed to the SDLLW incubation mixtures, but also on the check plants exposed to the soil incubation mixtures (Fig. 5). This suggests that the cause of the interior vascular discoloration may not be related directly to that factor

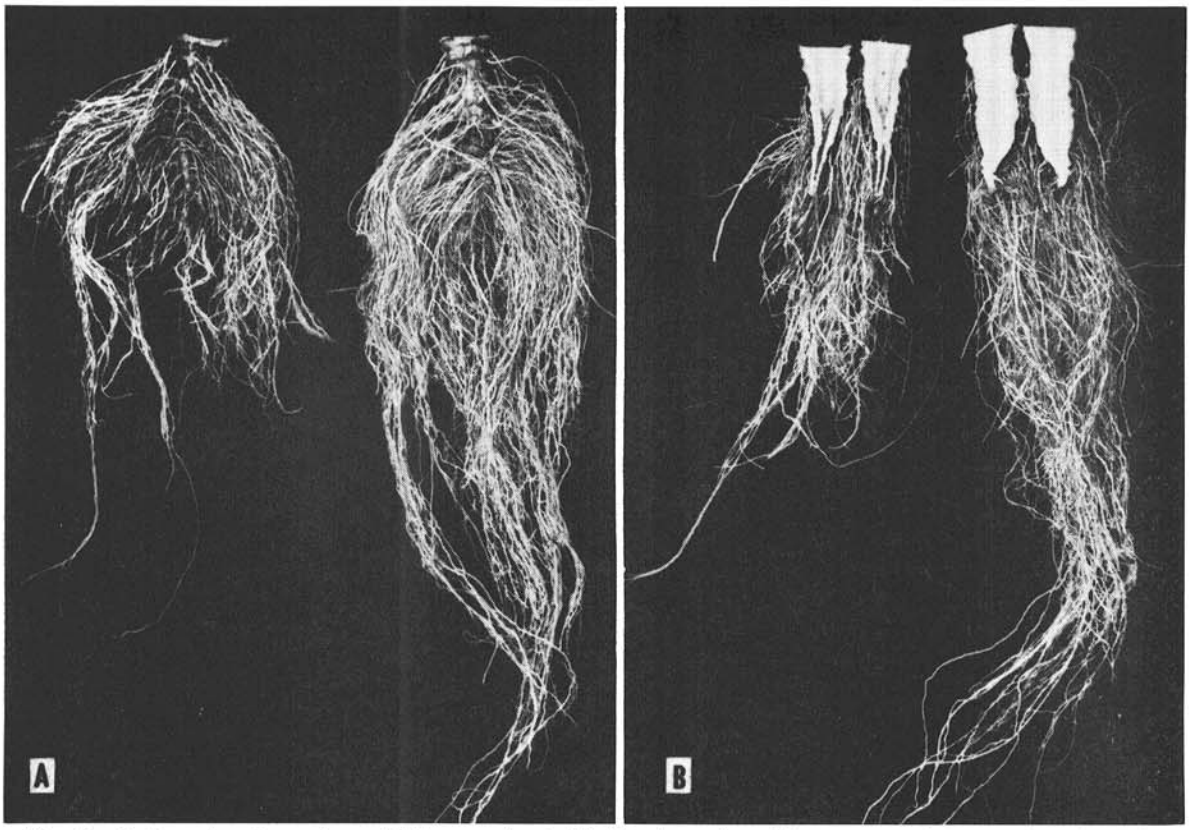


Fig. 5. Corky external symptom of lettuce root rot. **A)** The taproot on left was exposed to a water-saturated organic soil and dried lettuce leaves (SDLLW) incubation mixture. The taproot on the right was exposed to an organic soil (S) incubation mixture. The withholding of irrigation during the last 2 weeks of the exposure period resulted in corky symptoms on the roots of the plants in both treatments. **B)** Longitudinal slices exposing the vascular tissue of the root from the SDLLW incubation mixture (left) and the root from the S incubation mixture (right) in 5-A. Only the root of the plant exposed to the incubation mixture containing lettuce residue had vascular discoloration.

causing the exterior corky symptom. Under field conditions, roots showing vascular discoloration frequently exhibit the corky symptom because the conditions that produce both symptoms often occur simultaneously. Although this is generally true, roots have been observed in the field with all intergradations of both the vascular discoloration and corky symptoms.

Since crocks incubated for 2 weeks with 300 g organic soil, 20 g dried lettuce residue, and 1 liter water consistently produced complete vascular discoloration of the taproots of the variety Great Lakes 659, it is possible that the crock system could be used for testing lettuce breeding lines for resistance to root rot. Breeding lines developed and supplied by Luis Sequeira (Univ. of Wisconsin, Madison) were tested using Great Lakes 659 as a comparative standard. The lettuce selections exhibited comparable reactions of resistance or susceptibility when screened under field conditions or controlled conditions. Only one line exhibited noticeably less vascular discoloration and corkiness than did Great Lakes 659.

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