SOME FACTORS AFFECTING THE PATHOGENICITY OF CORNYNEBACTERIUM SEPEDONICUM (SPIECK. AND KOTT.) SKAPT. AND BURK.

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by

Herman L. Warren

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ABSTRACT

SOME FACTORS AFFECTING THE PATHOGENICITY
OF CORYNEBACTERIUM SEPEDONICUM
(SPIECK. AND KOTT.)
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Corynebacterium sepedonicum (Spieck, and Kott.) Skapt. and Burk., the organism of the bacterial ring rot disease of potato, was studied in relation to: (1) association effects with *Erwinia carotovora* (L. R. Jones) Holland, the bacterial soft rot organism and (2) the influence of soil temperature on symptom expressions in seedling plants in resistant and susceptible potato progenies.

When cells of *C. sepedonicum* were mixed with cells of *E. carotovora* at a ratio of 1 to 1 and 9 to 1 and seeded in nutrient-dextrose broth, the ratio dropped most rapidly during the first 10 days and less rapidly over the following 20 day period. These results were consistent with 10 cultures of *E. carotovora*.

Sterile filtrates of *E. carotovora* inhibited *C. sepedonicum* in nutrient-dextrose broth cultures. Greatest inhibition was obtained with filtrates from 5-day old cultures. As the incubation period was increased the viability of *C. sepedonicum* was decreased.

Symptoms of bacterial ring rot in potato seedlings developed more severely at 24° C than at the other temperatures studied (16°, 20°, and 28° C). The optimum temperature for ring rot infection in tomato plants is 28° C but that for potato seedlings was 24° C.

Ring rot infection developed more severely in susceptible seedling populations than in resistant seedling populations.

I. INTRODUCTION

This thesis is divided into two parts. The first section deals with the influence of *Erwinia carotovora* (L. R. Jones) Holland, the bacterial soft rot organism, on growth of *Corynebacterium sepedonicum* (Spieck, and Kott.) Skapt. and Burk., the bacterial ring rot organism. Ring rot of potato (*C. sepedonicum*) and bacterial soft rot or blackleg of potato (*E. carotovora*) usually occur in association with each other in naturally infected potato tubers, but *C. sepedonicum* does not compete well with *E. carotovora* and the latter eventually becomes dominant. This study was begun to determine factors influencing the growth and survival of *C. sepedonicum* by *E. carotovora*.

The second section is a study of the relation of soil temperature in evaluation of segregating potato seedling populations for resistance to *C. sepedonicum*. The relation of temperature to ring rot infection of potato varieties and of tomato plants has been studied. To date, no studies have been reported on the relation of temperature to potato seedling populations differing in resistance to ring rot.

II. REVIEW OF LITERATURE

Starr and Riedl (1941) observed that soft rot is usually present in advance stages of decay due to ring rot infection. Knorr (1943) indicated that in advance stages of decay, demonstration of *C. sepedonicum* in rotted tissue may be difficult or impossible. Kreutzer and McLean (1943) demonstrated that bacterial soft rot usually over-runs potatoes infected by *C. sepedonicum* and that soft rot progresses more rapidly through ring rot infected tubers than when ring rot is absent. Sherf (1944) demonstrated that the percentage of infection decreased while the length of time required for development of ring rot symptoms increased when inoculum contained equal amounts of pure culture of *C. sepedonicum* and *E. carotovora* as compared with inoculum of *C. sepedonicum* alone. There is strong evidence (Ark 1946) that blackleg disease in the field may follow a strong pathogen such as *C. sepedonicum*.

Potato varieties resistant to ring rot such as Teton and Merrimack (Riedl, *et al.*, 1946) (Akeley, *et al.*, 1955) have been developed. These resistant selections become infected with ring rot to a much lesser degree than do the commonly grown susceptible varieties, even when subjected to drastic inoculation methods (Bonde, *et al.*, 1947).

The influence of temperature on development of ring rot of potatoes and tomatoes has been determined by Larson and Walker (1941), Sherf (1944), Logsdon and Eide (1954), and Logsdon (1955). Larson, Walker and Fogelberg (1941) showed that symptoms in inoculated tomato plants developed most rapidly between 24^{o} and

28^{o} C and that symptom development was supressed at 16^{o} C or below. Knorr (1948) demonstrated that artificially inoculated tomato plants expressed the first symptoms in 12 to 15 days when inoculated with bacterial ring rot ooze from tubers and 17 days were required when inoculated with pure cultures.

Larson and Walker (1941) showed that growth of potato plants varied in soil temperature similar to air temperature except that plants grown in 82^{o} F soil temperature developed the most severe symptoms. Air temperature of 82^{o} F slightly retarded symptom development of potato plants. At air temperature of 75^{o} F symptom development was greatest.

Sherf (1944) showed that the temperature favorable for the development of ring rot in potato stems, stolons and tubers was between 18^{o} and 22^{o} C. Higher temperatures decreased infection and at 30^{o} C little or no infection occurred. Logsdon (1955) demonstrated that soil temperatures of 19^{o} to 28^{o} C were equally favorable for infection, but at 16^{o} C. there was a reduction in symptom intensity and the number of stems infected by _C. sepedonicum_. At 31^{o} C or above symptoms were masked and the movement of bacteria in the potato plants was retarded.

The influence of temperature on development of ring rot in resistant potato seedling populations has not been studied. Bonde, _et al_. (1959) described a method for screening out ring rot susceptible seedlings in potato breeding programs. Roots of selfed seedlings dipped in a heavy slurry of ring rot inoculum developed symptoms of infection 30 to 50 days after inoculation.

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III. INFLUENCE OF *ERWINIA CAROTOVORA* ON GROWTH OF *CORYNEBACTERIUM SEPEDONICUM*

A. Materials and methods.--A culture of *Corynebacterium sepedonicum* (Spieck. and Kott.) Skapt. and Burk. pathogenic to potato and tomato was grown in shaker culture in nutrient-dextrose broth for approximately 5 days. By this time, the suspension had become cloudy. *Erwinia carotovora* (L. R. Jones) Holland which was pathogenic to potato tubers and carrot slices was grown in similar media for 1 day.

The number of cells per ml was determined for each culture by mixing equal volumes of the suspension and a 5% solution of methylene blue and counting with a haemacytometer. After counting, bacterial suspensions were mixed in a ratio of 9 cells of *C. sepedonicum* to 1 cell of *E. carotovora* and incubated as before. At intervals, up to 20 days after mixing, 15 1-ml drops of the suspension were removed from the mixed culture, placed on clean glass slides, dried and stained with Reed's rapid Gram stain (Glick, *et al.*, 1944). The smears were examined under the microscope using the 97X oil immersion objective. One field was selected at random from each of the 15 smears and 100 cells from each field were counted.

Hydrogen-ion concentration was determined in a more extensive trial involving 7 *E. carotovora* isolates alone and in combination with *C. sepedonicum.* These cultures had been obtained from a number of sources and were pathogenic on carrot slices. At the start of the trial, cultures were mixed in the proportion 9 cells of C. sepedonicum to 1 cell of E. carotovora. Relative numbers of each bacterial species were determined as previously described over a period of 36 days.

Toxicity of sterile culture filtrates of E. carotovora was determined by survival of C. sepedonicum in such filtrates. Bacterial soft rot cultures were grown in shaker culture at 24°C for 1, 2, and 5 days in nutrient-dextrose broth and filtered aseptically through a Seitz filter. Cultures of C. sepedonicum prepared as previously described and incubated 5, 6, and 10 days respectively were mixed in proportions of 1 to 1 and 1 to 9 with the bacterial soft rot filtrates. In the controls, nutrient-dextrose broth was used in similar volumes as the culture filtrate from bacterial soft rot. C. sepedonicum-filtrate suspensions were maintained in shaker culture at 24°C. At intervals up to 5 days, 1 ml aliquots of the C. sepedonicum-bacterial soft rot filtrate were transferred to each of 5 plates containing Sniezsko's agar (Snieszko and Bonde, 1943) and incubated at room temperature. In each of 5 plates for each treatment, 5 microscope fields were selected at random and colonies were counted with a microscope using the low power objective. Results of 1 of 2 similar trials are presented.

B. Experimental results.-- Culture studies. When C. sepedonicum and E. carotovora cultures were mixed in the proportion of 9 cells to 1 cell and grown together in nutrient-dextrose broth, the relative number of gram positive C. sepedonicum cells in the mixture dropped steadily over the period of observation. In all, 10 *E. carotovora* cultures were tested. Results of 3 separate trials, each involving one different culture of *E. carotovora*, were in very close agreement (Fig. 1). In another more extensive trial (Fig. 1, test 4) in which 7 isolates of *E. carotovora* were compared, the relative rate of increase of *E. carotovora* cells was remarkably constant between the different isolates.

In the final trial, hydrogen-ion concentration of the medium containing mixed cultures was compared with that from pure cultures of either organism (Table 1). The 7 bacterial soft rot cultures isolated from several different sources were very similar in their influence on hydrogen-ion concentration of the media both in pure culture and in mixed culture. Furthermore the pH of *C. sepedonicum* culture medium was similar to that of *E. carotovora*. During this time, medium containing mixed cultures changed from the original hydrogen-ion concentration (pH 6.9) to a pH of 6.3 - 6.4 at the 36 day reading. The hydrogen-ion concentrations of pure cultures of *E. carotovora* were approximately 0.1 pH unit higher than the mixed culture. There was little change during the first 20 days of incubation and the greatest change occurred between the 20 and 36 day observations.

Filtrate Studies

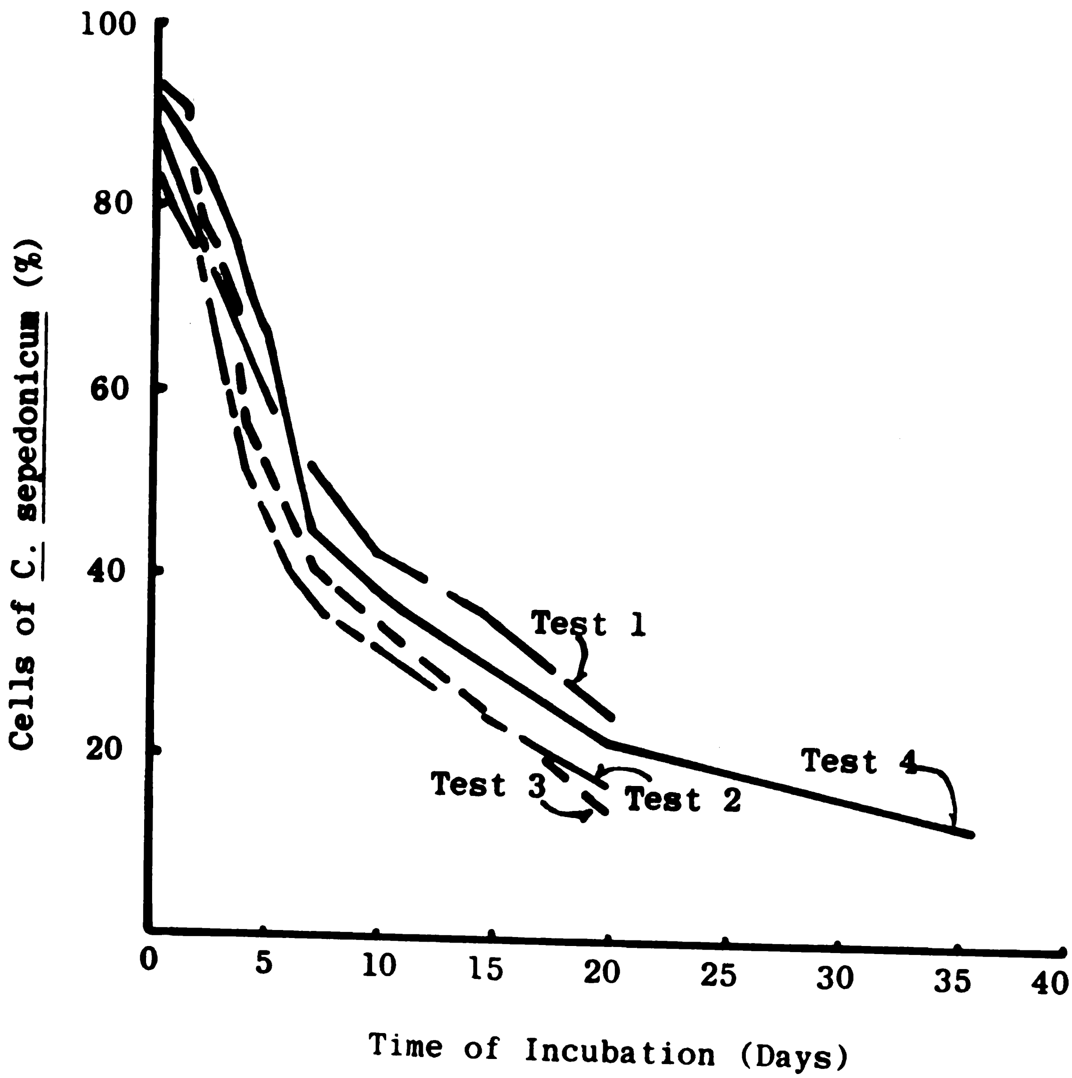
Influence of the age of *E. carotovora* culture filtrates on viability and growth of cells of *C. sepedonicum* was determined *in vitro* in 2 similar trials with essentially similar results. Sterile filtrates were obtained from *E. carotovora* cultures grown in shaker

Table 1. Influence of several *E. carotovora* cultures alone and mixed with *C. sepedonicum* on hydrogen-ion concentration of nutrient-dextrose broth.

Cultures	Source of isolates		Days of incubation 1 pH	5 pH	10 pH	15 pH	20 pH	36 pH
C. sepedonicum	potato tubers	Bay County	6.9	6.9	6.9	6.9	6.8	6.4
E. carotovora (1)	potato tubers	Macomb county	6.9	6.9	6.8	6.8	6.8	6.5
do + *C. sepedonicum*			6.9	6.8	6.8	6.7	6.7	6.3
E. carotovora (2)	potato tuber	Storage bin E. Lansing	6.9	6.8	6.8	6.8	6.8	6.4
do + *C. sepedonicum*			6.9	6.8	6.7	6.7	6.7	6.3
E. carotovora (3)	potato tuber	Montcalm county	6.8	6.8	6.8	6.8	6.7	6.4
do + *C. sepedonicum*			6.9	6.8	6.8	6.7	6.7	6.3
E. carotovora (4)	carrot	Soil Inoculation	6.9	6.9	6.9	6.8	6.8	6.5
do + *C. sepedonicum*			6.9	6.8	6.7	6.7	6.7	6.4
E. carotovora (5)	carrot	Soil Inoculation	6.9	6.8	6.8	6.8	6.8	6.4
do + *C. sepedonicum*			6.9	6.8	6.7	6.7	6.7	6.3
E. carotovora (6)	potato tuber	Muck Farm - Bath	6.9	6.8	6.8	6.8	6.8	6.5
do + *C. sepedonicum*			6.9	6.8	6.8	6.7	6.7	6.4
E. carotovora (7)	carrot	Muck Farm - Bath	6.9	6.8	6.8	6.8	6.8	6.4
do + *C. sepedonicum*			6.9	6.8	6.7	6.7	6.7	6.3

FIGURE 1

Survival of *C. sepedonicum* incubated with *E. carotovora* in nutrient-dextrose broth shaker culture in 4 trials.



100
80
60
40
20
Cells of C. sepedonicum (%)
Test 1
Test 2
Test 3
Test 4
0
5
10
15
20
25
30
35
40
Time of Incubation (Days)

culture for 1, 2, and 5 days. These filtrates from cultures of different ages were mixed with cultures of _C. sepedonicum_ in proportions of 1 to 1 and 9 to 1. Controls were prepared using nutrient-dextrose broth in volumes similar to the culture filtrates.

The inhibitory influence of _E. carotovora_ culture filtrates on multiplication of _C. sepedonicum_ became greater as age of the filtrate was increased (Fig. 2). The greatest amount of inhibition was obtained in filtrate from a 5-day bacterial soft rot culture.

In both trials, 1-day filtrates (Fig. 2 A) mixed in equal quantity with _C. sepedonicum_ (A 2) were non-toxic immediately after mixing as compared with the control (A 1). Even after 1 day incubation in mixed culture, populations of _C. sepedonicum_ were essentially similar. By the end of the 2-day exposure to _E. carotovora_ filtrates, survival of _C. sepedonicum_ (A 2) was considerably reduced as compared to the control (A 1) and this difference was even more marked after 5 days in association. When a 1-day filtrate and _C. sepedonicum_ (B 2) were mixed in the ratio of 1 to 9, differences between this mixture and the control (B 1) were marked after 1 day's exposure and differences became considerably greater on the 2 and 5 day observations. Although the filtrate did not have an immediate toxic effect after mixing (0 day observation), it had a toxic influence (possibly static in nature) on _C. sepedonicum_ after a 1-day exposure in dilute _C. sepedonicum_ suspension (B 2) and after 2 or more days exposure in more dense _C. sepedonicum_ populations (A 2). Viability of _C. sepedonicum_ was relatively constant over the 5-day period exposure to 1-day _E. carotovora_ filtrates.

Two-day filtrates of _E. carotovora_ (Fig. 2 B) of either concentration were not immediately toxic to _C. sepedonicum_ cells. In both trials, after 1 day exposure to filtrates, viability was sharply reduced at either level of concentration. Viability remained low but constant over the 5-day period of observation, suggesting a static influence.

Five-day culture filtrates (Fig. 2 C) were toxic immediately after mixing. In both trials, the high concentration (B 2) of filtrate by the second day of incubation completely destroyed viability. Also, in both trials, at the low concentration of filtrate (A 2) viability after 1 day exposure was reduced by 50%.

FIGURE 2

Survival of C. sepedonicum cells in culture filtrates of E. carotovora.

A One day culture filtrate mixed with a 5-day C. sepedonicum culture.

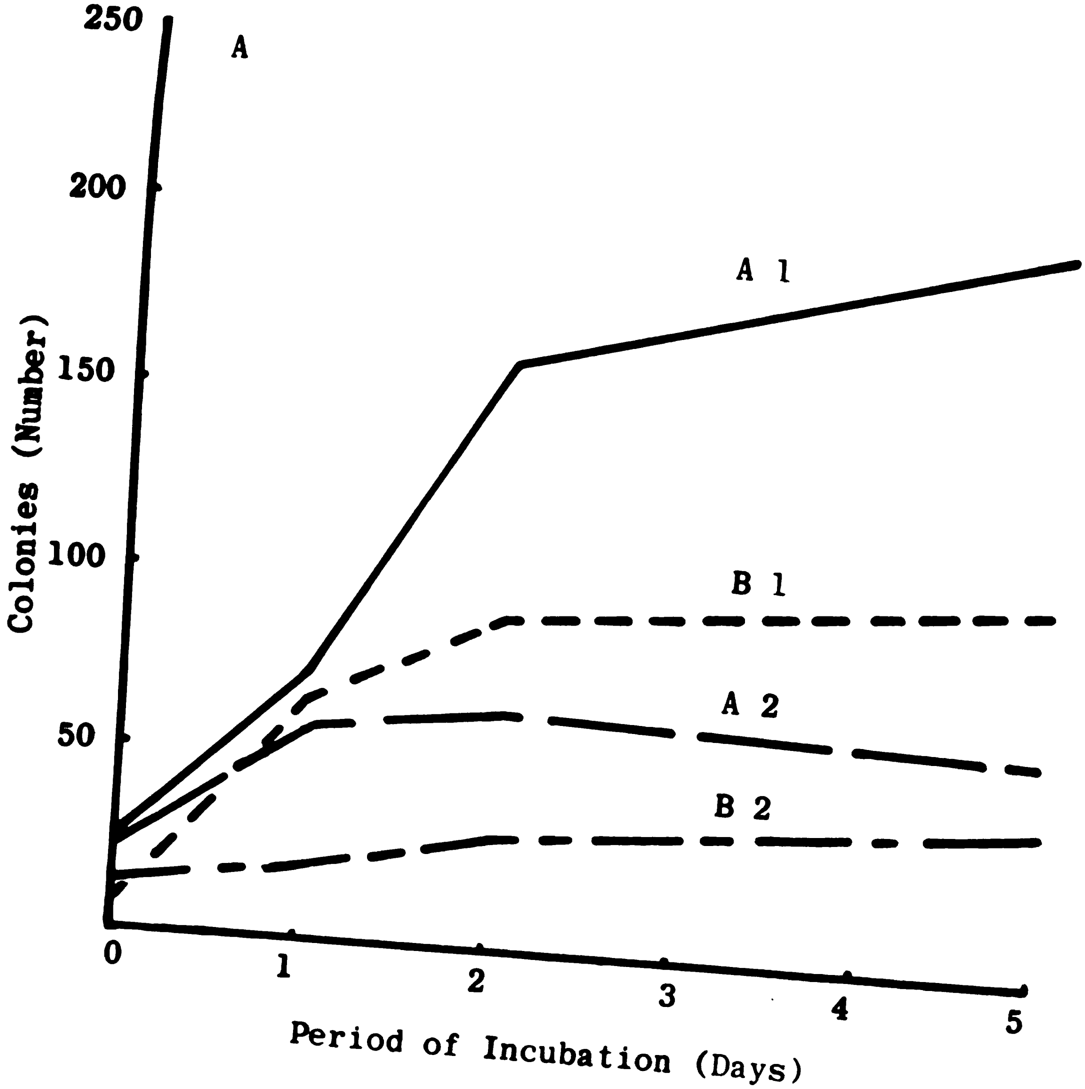
B Two day culture filtrate mixed with a 6-day C. sepedonicum culture.

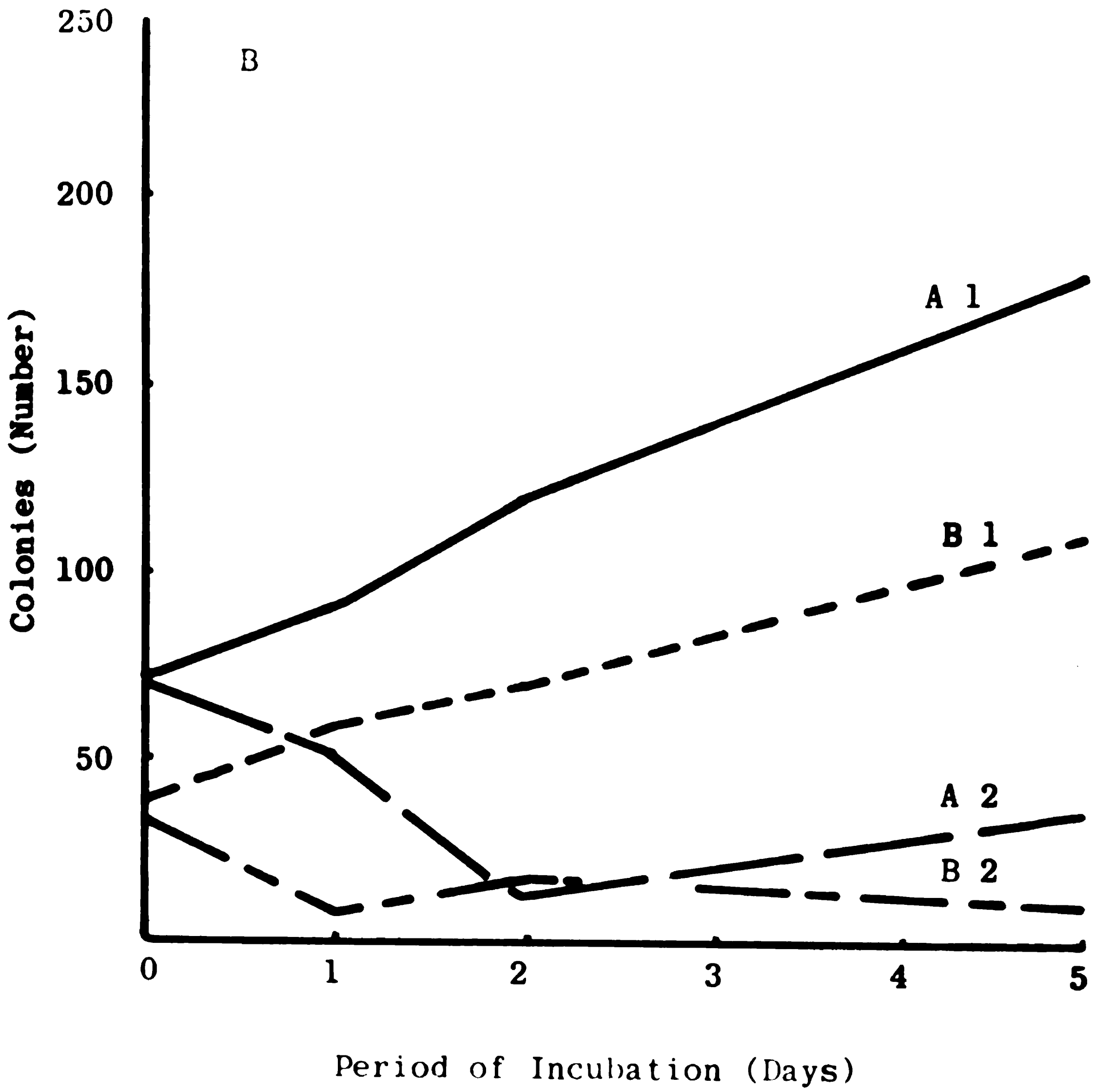
C Five day culture filtrate mixed with a 10-day C. sepedonicum culture.

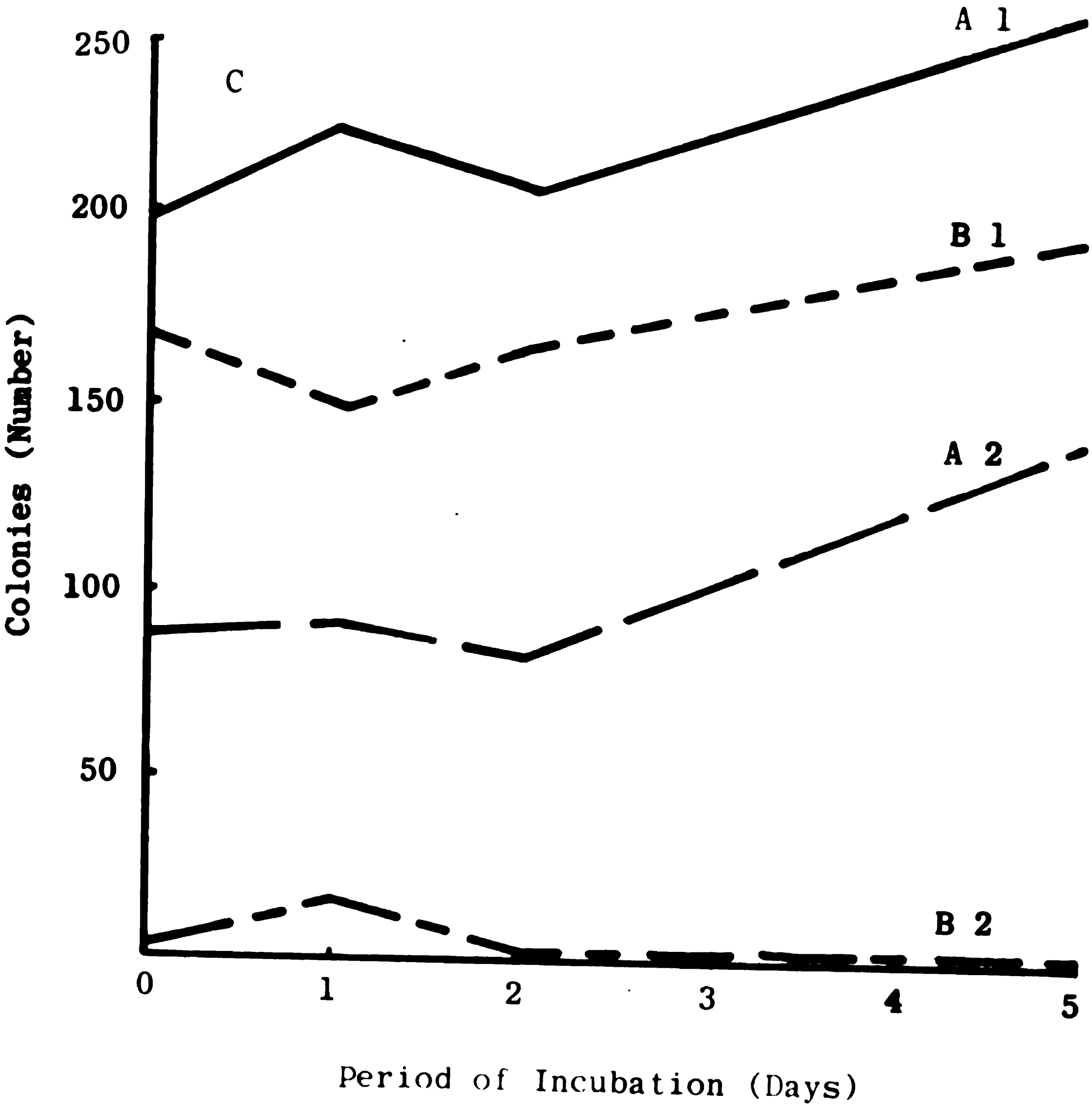
A1 C. sepedonicum culture 1 part and nutrient-dextrose broth 1 part.

A2 C. sepedonicum culture 1 part and E. carotovora culture filtrate 1 part.

B1 and B2 similar to above except C. sepedonicum 1 part and broth or culture filtrate 9 parts respectively.


250
200
150
100
50
0
A
A 1
B 1
A 2
B 2
Colonies (Number)
1
2
3
4
5
Period of Incubation (Days)


250
B
200
150
100
50
Colonies (Number)
A 1
B 1
A 2
B 2
0
1
2
3
4
5
Period of Incubation (Days)



A 1
250
C
200
B 1
150
A 2
100
50
B 2
Colonies (Number)
0
1
2
3
4
5
Period of Incubation (Days)

IV. INFLUENCE OF SOIL TEMPERATURE ON THE DEVELOPMENT OF RING ROT IN POTATO AND TOMATO SEEDLINGS

A. Materials and methods.-- Potato seedling populations were grown from true seed obtained by selfing susceptible varieties such as Katahdin and Onaway and by selfing a resistant potato variety, Merrimack. Seeds were planted in rows in sterilized soil in flats and grown on a greenhouse bench at 20° C.

When the seedlings were approximately 1-inch tall (usually 10 to 14 days later) suspensions of *Corynebacterium sepedonicum* (Spieck. and Kott.) Skaptason and Burkholder were used for inoculation. Inoculum was obtained from pure cultures or from grinding portions of frozen ring rot infected tomato stems. Roots of seedlings were gently rubbed between the thumb and forefinger, then placed in either type of inoculum for 60 minutes. Controls were treated in a similar manner using distilled water.

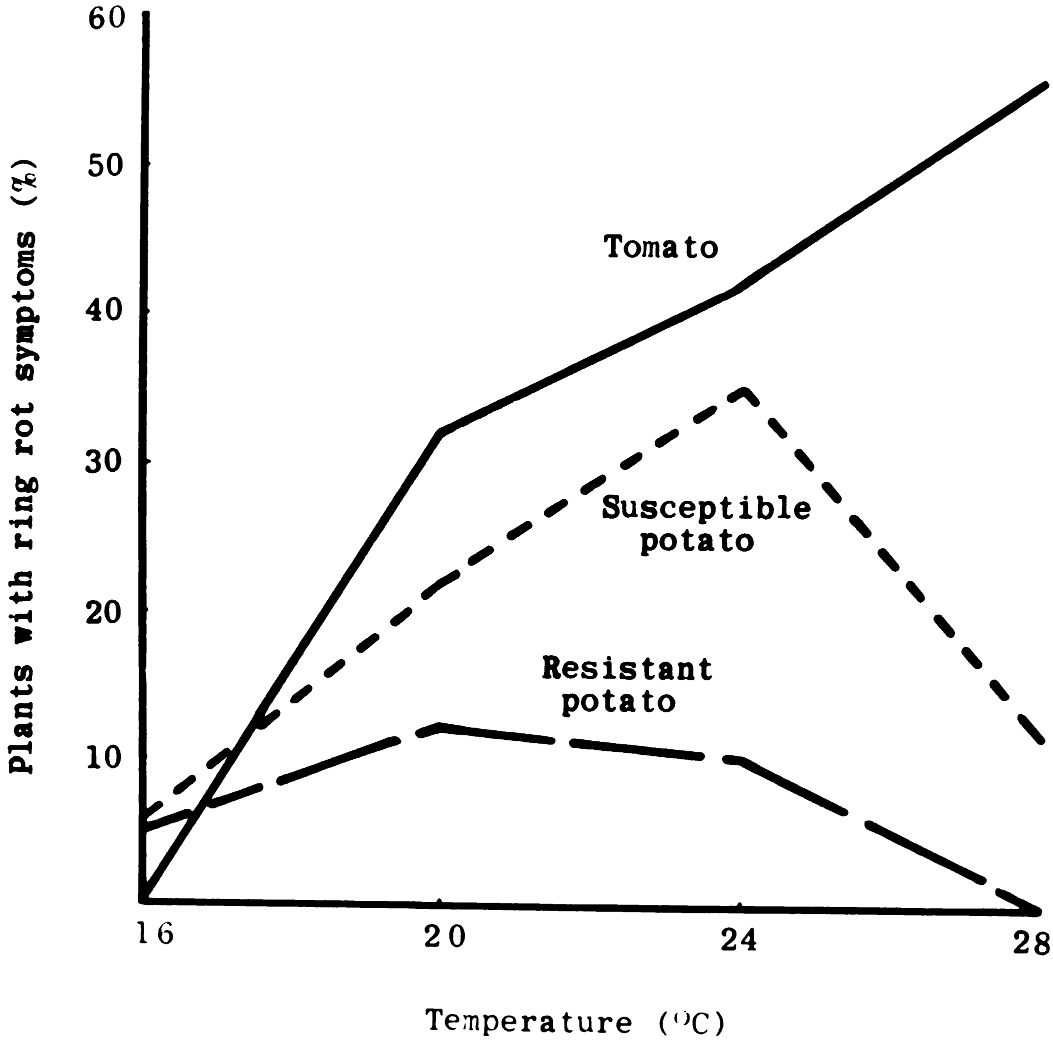
After inoculation had been completed, seedling plants were transplanted into sterilized soil in galvanized metal pans 13½ X 15½ X 4 inches deep. Pans were placed on a greenhouse bench for 5 days in a 20° C house in order to permit recovery from injury and transplanting and then placed in soil temperature tanks at temperatures of 16°, 20°, 24°, and 28° C. Plants were examined at regular intervals and symptoms of individual plants were evaluated and recorded. For distinguishing symptom severity a scale (index number) of 0 to 4 was used (0= no ring rot, 1= slight yellowing of lower leaves, 2= yellowing and slight wilting, 3= yellowing and moderate wilting, and 4= leaves completely wilted or plant dead from ring rot). Within each treatment, the number of plants was multiplied by the index number of the group and the total of each group obtained. This was expressed on a percentage basis by multiplying the total by 100 and dividing by the number of plants in the treatment multiplied by 4 (the highest index number).

At the end of each trial the final readings were made from sections of all stems near ground level after the method of Sherf (1944). Three sections of the stem were triturated in 3 separate drops of water on clean glass slides and stained with Reed's rapid Gram stain (Glick, et al., 1944). Controls and apparently healthy inoculated seedlings were tested in a similar manner. Slides were examined microscopically using the oil immersion objective.

B. Experimental results.-- Temperature studies on reaction of potato seedlings obtained from true seed from susceptible (Katahdin or Onaway) and from resistant (Merrimack) varieties which had been selfed and on Bonny Best tomato plants were made under controlled conditions after root inoculation. Reference to seedling populations will be made by naming the potato variety from which the selfed seed was obtained. Onaway and Merrimack potato seedling populations were compared with tomato in the first trial (Fig. 3). In the trial shown 40 plants of each population were grown at each temperature. In a second trial with essentially similar results, 30 Merrimack seedlings grown at each temperature were compared with an equal number of tomato plants. After 30 days of growth the percentage of infection was determined after smears had been made from the lower stem of each plant and Gram stained.

FIGURE 3

Ring rot symptoms 30 days after root inoculation in potato seedling populations and tomato plants grown at different temperatures.



60
50
40
30
20
10
Plants with ring rot symptoms (%)
Tomato
Susceptible potato
Resistant potato
16
20
24
28
Temperature (°C)

At 16^{o} C. (Fig. 3) tomato plants artificially inoculated with C. sepedonicum developed neither symptoms nor were ring rot cells demonstrated in smears made from sections of the stem. In the second trial there was slight but considerably less infection at 16^{o} C than at higher temperatures. In both trials, as the temperature increased the percentage of infection progressively increased to the highest at 28^{o} C. Potato plants of either the susceptible or resistant seedling population developed few symptoms and were only slightly infected at 16^{o} C. In susceptible potato seedlings the percentage of infection was highest at 24^{o} C whereas in both trials, the resistant seedlings were slightly more severely diseased at 20^{o} C than at 24^{o} C. At 28^{o} C., symptom development and the percentage of infection were suppressed in both susceptible and resistant populations. Resistant seedlings developed considerably less infection than did susceptible seedlings at temperatures of 20^{o}, 24^{o}, and 28^{o} C. In contrast, 28^{o} C was optimum for tomato plants in both trials and plants were much more severely diseased than potato seedlings at this temperature. The optimum temperature for infection of potato seedlings (24^{o} C) contrasted markedly with the optimum (28^{o} C) temperature for tomatoes.

In 2 additional trials, the influence of soil temperature in relation to infectivity of C. sepedonicum to potato seedlings was determined at temperatures of 16^{o}, 20^{o}, 24^{o}, and 28^{o} C with 30 plants per population at each temperature (Fig. 4). In either population of potatoes, susceptible (Fig. 4 A) or resistant (Fig. 4 B) 24^{o} C was optimum for symptom expression. Differences between temperatures were relatively slight at 16^{o}, 20^{o}, and 28^{o} C. At the end of the trial, the number of symptomless but infected Merrimack seedlings was highest at 16^{o} C and the number of symptomless ring rot infected plants in the resistant Merrimack population was considerably higher than the susceptible Katahdin population.

In a second trial, involving 15 plants of each population at each temperature when a susceptible (Onaway) seedling population was compared to a resistant (Merrimack) population of seedlings, differences between resistant and susceptible populations were marked. The soil temperature of 24^{o} C was again optimum and differences between the other temperatures were slight.

At the termination of the tests (Fig. 4), all plants were examined using bacterial smears for the presence of C. sepedonicum in the lower stem. At low temperatures (16^{o} and 20^{o} C) Merrimack seedling populations were infected with C. sepedonicum without showing symptoms as evidenced by the rapid rise between the 34 and the 36 day observations. However, at higher temperatures of 24^{o} and 28^{o} C symptom expression in resistant populations was a reliable indication of infection. In contrast, in susceptible populations of Katahdin seedlings there was little latent infection and visual symptom expression was a fairly reliable means of determining ring rot infection.

In another trial, susceptible Onaway seedlings were similar to the reaction of Katahdin and the amount of latent infection in the resistant population of Merrimack was considerably higher.

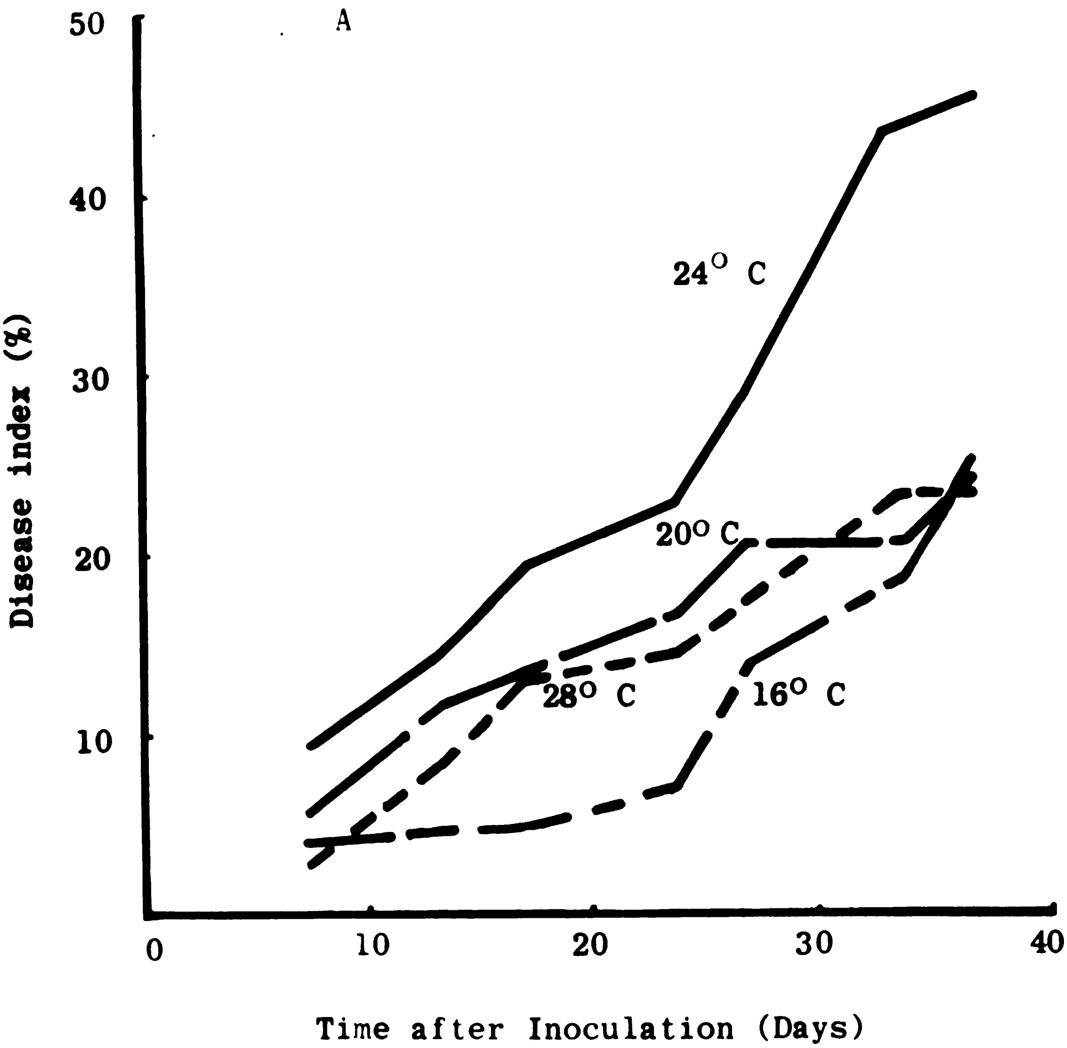
The reaction of susceptible and resistant populations in these 2 tests were compared (Fig. 5). In both trials, the amount of ring rot infection was higher in the susceptible population than in the resistant population. There was evidence that the susceptible Onaway was somewhat more susceptible to ring rot than were the Katahdin seedlings. In both tests differences between resistant (Merrimack) and susceptible seedlings were marked. Even though the susceptible Katahdin and Onaway seedlings were more severely infected, less than 50 per cent of either population became infected.

FIGURE 4

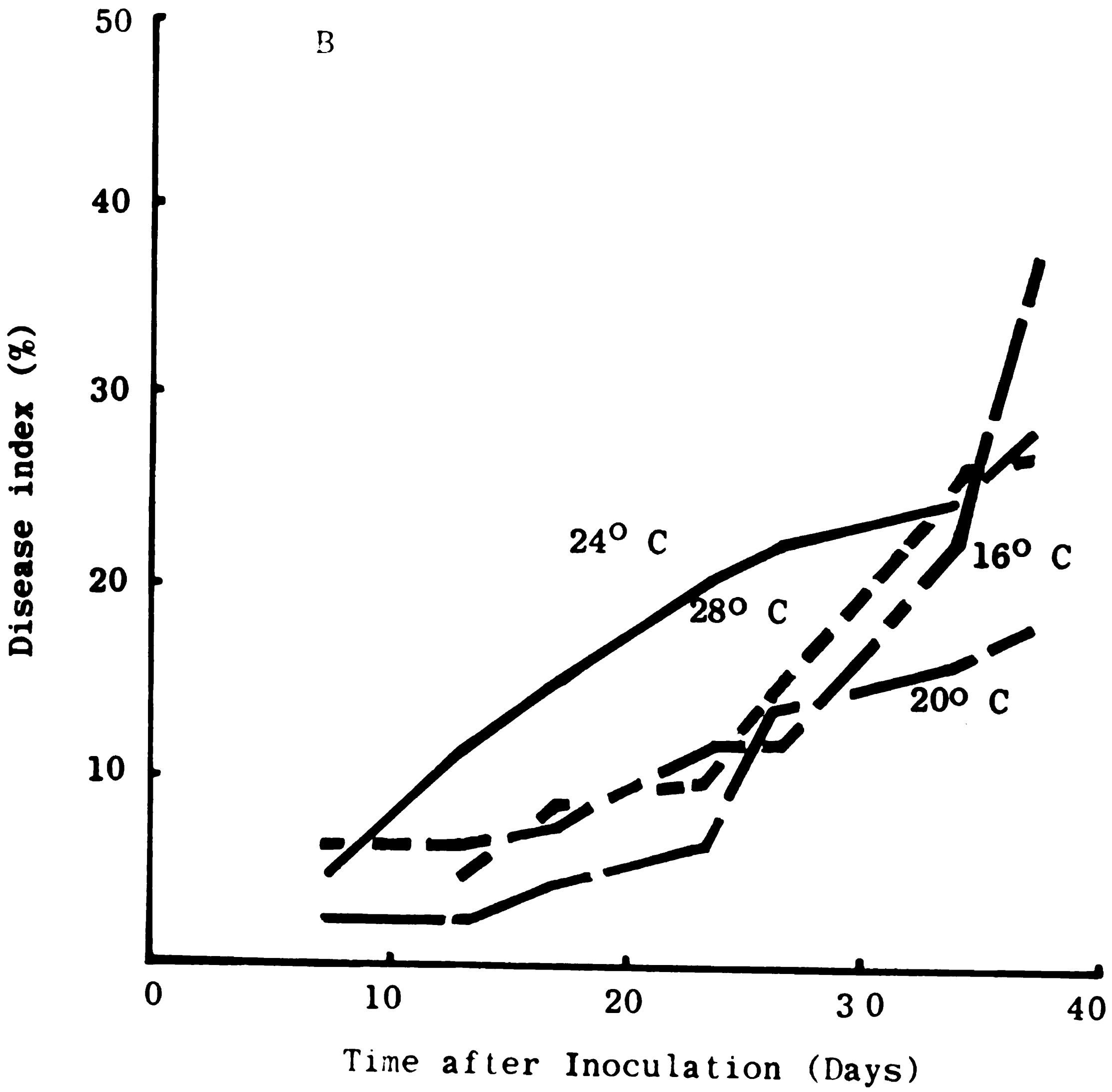
Influence of temperature on ring rot development in two populations of seedling potatoes

A. Population from seed of susceptible Katahdin which had been selfed.

B. Population from resistant Merrimack.



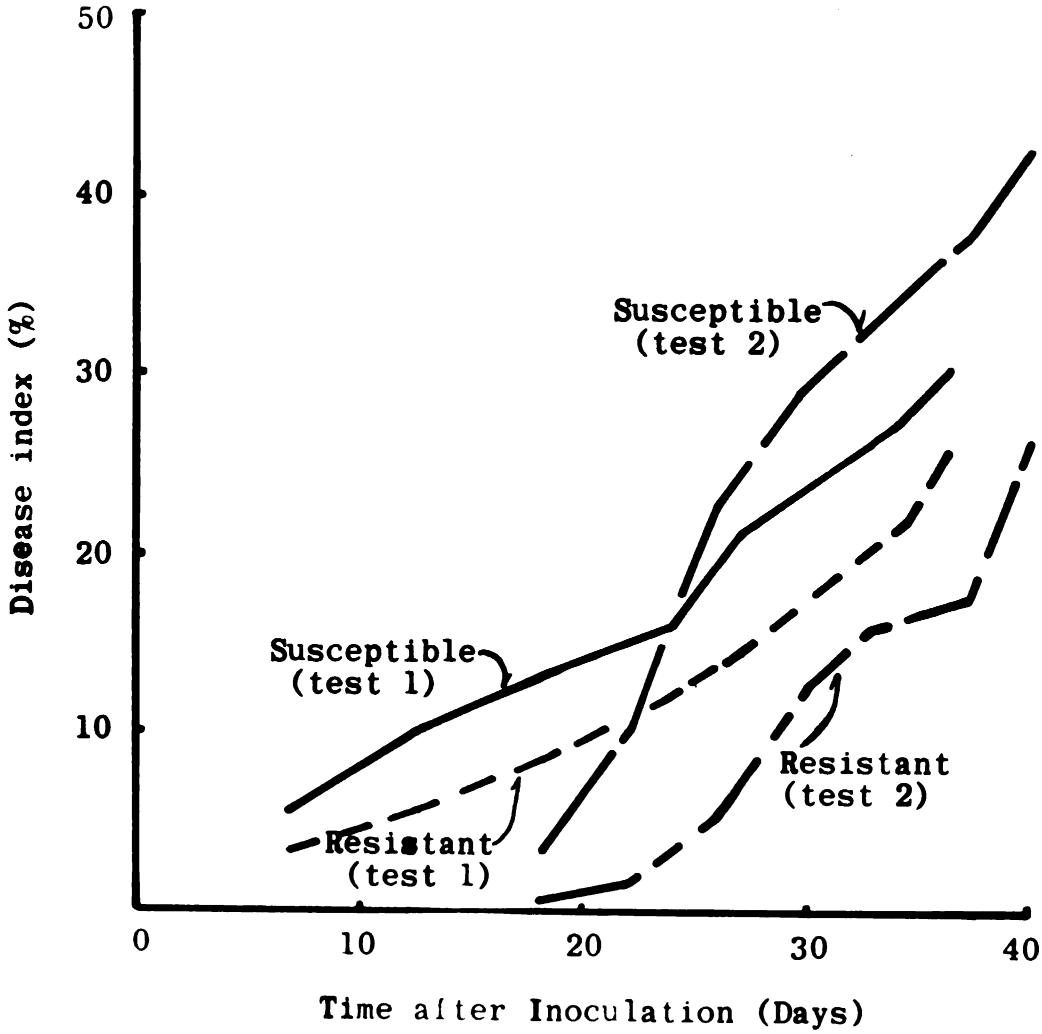
A
50
40
30
20
10
0
10
20
30
40
Disease index (%)
Time after Inoculation (Days)
24° C
20° C
28° C
16° C



B
Disease index (%)
50
40
30
20
10
0
10
20
3 0
40
Time after Inoculation (Days)
24° C
28° C
16° C
20° C

FIGURE 5

Ring rot incidence in susceptible and resistant seedling populations after inoculation with *C. sepedonicum.*



50
40
30
20
10
0
10
20
30
40
Disease index (%)
Time after Inoculation (Days)
Susceptible (test 2)
Susceptible (test 1)
Resistant (test 1)
Resistant (test 2)

V. DISCUSSION

The natural and frequent association of E. carotovora and C. sepedonicum in potato tubers as well as the lack of compatibility between the two bacteria has been pointed out by Sherf (1944) and by Ark (1946). Practically no controlled experiments have been carried out to determine the factors involved. In suspensions of C. sepedonicum and E. carotovora mixed in a ratio of 9 cells to 1 cell respectively the relative concentration of ring rot cells decreased rapidly during incubation, while the relative number of soft rot cells increased. The inhibitory effect or lack of compatibility of these 2 bacterial types was apparently not due to an increase in hydrogen-ion concentration of the medium, since hydrogen-ion levels of either bacterial culture alone or of the mixed cultures did not vary over more than 0.1 unit during the incubation period of 36 days.

The culture filtrates of E. carotovora markedly affected viability of C. sepedonicum during incubation. The filtrate from a 5-day old bacterial soft rot culture was much more toxic to ring rot cells than was the filtrate from a 1-day old culture. Whether the toxic substance or substances were bacteriostatic or bactericidal could not be determined.

A number of varieties of potato are resistant to C. sepedonicum (Bonde, et al., 1942), (Akeley, et al., 1955). It is generally agreed, however, that although the degree of resistance is high, such varieties are not immune to ring rot. In our trials, the influence of soil temperature on ring rot infection of seedling populations and tomato plants was marked. At the high temperature

(28° C) symptom expression was a rather reliable indication of infection, and tomato plants became infected more severely than did potato seedlings. In fact, 28° C was a relatively unfavorable temperature for disease development in seedling potato plants. This is in agreement with the observations of Sherf (1944) but contrasts with the report of Larson and Walker (1941) who obtained most severe symptoms which included dwarfing and stunting in large potato plants grown from tubers at 28° soil temperature. At low temperatures, the resistant Merrimack seedling population in our trials became infected without developing symptoms. In addition to the relatively poor disease development at 16° C, the masking of symptom expression was very undesirable for identifying infected plants.

The value of a screening method in a breeding program lies in its efficiency and in the reliability with which it can be used to distinguish symptom development at a level of precision sufficient to prevent the escape of symptomless but infected seedlings. The development of ring rot symptoms in seedling plants is markedly influenced by soil temperatures. Selection for ring rot resistance in seedling progenies has been reported by Bonde and Snieszko (1944), Bonde, _et al._, (1947), and Bonde, _et al._, (1959). Although these investigators were able to identify resistant segregates they apparently did not determine the optimum temperature for evaluation of resistance in seedling plants. In our trials, the optimum temperature for screening seedling progenies in a potato breeding program was close to 24° C. At this temperature, there was practically no masking of symptoms in either of the susceptible or resistant populations. Furthermore symptom expression was most severe at this temperature.

VI. SUMMARY

Pure cultures of _C. sepedonicum_ and _E. carotovora_ were mixed in a cell ratio of 9 to 1 and incubated up to 36 days. In 4 similar trials involving 10 different isolates of _E. carotovora_ the relative number of _C. sepedonicum_ cells decreased markedly with increased time of incubation.

The hydrogen-ion concentration of 7 isolates of _E. carotovora_ mixed with _C. sepedonicum_ and incubated for 36 days was essentially similar to that of pure cultures of either organism.

Filtrates sterilized by filtration of 1, 2, and 5 day cultures of _E. carotovora_ were mixed with _C. sepedonicum_ cultures in ratios of 1 to 1 and 1 to 9 and incubated for 0, 1, 2, and 5 days. The inhibitory effect of _E. carotovora_ culture filtrates on survival of _C. sepedonicum_ increased with increasing age of filtrate. Inhibition was almost complete when a 5 day filtrate was mixed in the ratio of 1 part of _C. sepedonicum_ to 9 parts of filtrate. Filtrates of 1 and 2 day cultures were markedly inhibitory but permitted relatively good survival of _C. sepedonicum_ when mixed in ratios of either 9 to 1 or 1 to 1.

The optimum temperature for infection of tomato plants was 28^{o} C but symptom development in potato seedlings was greatest at 24^{o} C.

The amount of ring rot infection was higher in susceptible populations than in the resistant populations. At 16^{o} C, latent infection in the resistant (Merrimack) population was considerably higher than in the susceptible (Katahdin and Onaway) seedling populations.

VII. LITERATURE CITED

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