

result from cutting off large branches. The practice has been to remove large branches by sawing them off as close to the trunk as was convenient and to let the stump heal over as best it could. Even under the most favorable circumstances, it takes several years for a large pruning wound to heal over. In the meantime, the wound is open to infection by gummosis and other diseases.

All pruning wounds three-quarters of an inch in diameter or larger should have a wound disinfectant applied to them. For this purpose few materials are as satisfactory as Avenarius or Red Arrow carbolineum. In addition, any wound $1\frac{1}{2}$ inches or larger should have a coating of water-emulsified asphalt applied to the carbolineum dressing one week afterwards. Such treatment will maintain the wound surface in a dry, fungus-repellant state until the bark has healed over it.

Painting the surface of an old wound will not eradicate gummosis from deep in the wood. Old infections will have to be excavated with a chisel or gouge. All the discolored diseased wood should

be removed and, after several days of drying, the surface should be treated with carbolineum and asphalt emulsion as in the treatment of new wounds. When gummosis disease has been established a long time the grower will have to determine whether the tree is worth the expense of treatment. Young lesions are easily excavated and heal over in a short time if proper dressings are applied. However gummosis lesions that have apparently healed over without adequate treatment are still alive and will break out with renewed activity at a later date. The proper treatment of wounds is an excellent example of the adage that an ounce of prevention is worth a pound of cure.

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PRESENT STATUS OF SPREADING DECLINE

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The investigation of spreading decline of citrus in Florida has been in progress for the past five years. During that time information on the varieties of citrus and the rootstocks on which the decline was found has been reported (1). In addition, the effect of the disease on the tree (1) and the rate at which the decline spreads in the grove have been discussed (1,2). At one time it was considered that the citrus nematode (*Tylenchulus semipenetrans* Cobb) might be

associated with spreading decline (1) but subsequent results showed that the citrus nematode was not the causative agent for typical spreading decline (2). In the experimental work on virus transmission, no evidence was found to indicate that the disease was caused by a virus (1,2). Although preliminary investigations did not indicate that a fungus was responsible (1), it appears that the trouble may be the result of a fungus infection of the fibrous roots that gradually spreads through the grove from root to root (2). Numerous experiments with various types of possible control measures were conducted but no

successful method for the control of the disease was found (1,2).

Considering all of the information already obtained and the additional results accumulated since the last report in 1949, what is the present status regarding spreading decline?

Spreading decline occurs on all varieties of oranges, grapefruit and tangerines budded on rough lemon, sour orange, sweet orange or grapefruit rootstock. The presence or absence of the disease on other kinds of rootstocks has not been determined. The number of groves in which typical spreading decline is present are located as follows: Polk County—74, Orange County—8, Highlands County—5, and Hillsborough County—2, making a total of 89 groves. There are a number of groves in which spreading decline occurs that we do not have on our list.

Those trees which have spreading decline show sparse foliage and reduced growth but do not die. The trees within the decline area all show the same degree of decline and a distinct margin is evident with the decline trees on one side and the healthy trees on the other. The disease gradually spreads from the declining trees to the adjacent healthy trees.

Rate of Spread

To determine the rate of spread of the disease, the groves are mapped each year after the spring flush of growth. The yearly maps are then compared to obtain the number of trees that become diseased during any given year. Since the decline spreads at the margin of the diseased area, the rate of spread is obtained by dividing the number of trees that become diseased by the number of trees on the margin of the decline area. The results obtained from 25 selected groves are presented in Table 1. These data show that considerable variation occurred in the rate

of spread in the various groves from 1945 to 1950. The marginal rate of

TABLE 1.
Yearly Variation in Rate of Increase of Spreading Decline in 25 Groves.
Rate of Spread¹

Grove	1945- 1946	1946- 1947	1947- 1948	1948- 1949	1949- 1950	Average
1	1.6	1.1	0.3	2.5	out	1.4
2	1.2	1.4	0.6	2.8	1.0	1.4
3	1.0	0.8	0.7	0.9	2.7	1.2
4	1.1		1.4	2.3	2.3	1.8
5	0.2	1.7	1.8	2.7	1.3	1.5
6	0.7	1.5	0.9	1.5	1.6	1.2
7	1.0	2.0	1.0	3.0	0.5	1.5
8	0.3	0.8	0.7	1.3	out	0.8
9	0.5	1.1	1.3	2.8	0.8	1.3
10	0.6	0.6	1.6	3.8	2.3	1.8
11		1.1	0.7	2.0	1.5	1.3
12		2.3	0.7	2.0	out	1.7
13		1.9	1.9	2.2	0.6	1.7
14			1.1	4.7	0.5	2.1
15			1.1	5.2	1.5	2.6
16			2.0	3.0	1.8	2.3
17			1.8	1.1	0.5	1.1
18			0.9	2.7	0.5	1.4
19				3.2	8.3	5.7
20				4.1	0.9	2.5
21				2.6	1.0	1.8
22				2.7	0.6	1.7
23				1.5	1.0	1.3
24				1.6	0.6	1.1
25				4.3	0.1	2.2
Average	0.8	1.4	1.1	2.7	1.5	1.6

¹ Increase in number of diseased trees per tree on the margin of the declining area.

TABLE 2.
Increase in Number of Diseased Trees in Affected Groves During a Five Year Period.

Grove	No. Diseased Trees		Increase
	1945	1950	
2	13	138	125
3	77	244	167
4	164	511	347
5	121	296	175
6	21	199	178
7	29	142	113
9	51	152	101
10	66	249	183

spread varied from 0.1 to 8.3 trees with an average of 1.6 for all groves throughout the five years. Six groves showed an average rate of spread of over 2.0 for the five-year period. The greatest average yearly spread was in 1948-49 when the rate was 2.7. In general, spreading decline can be expected to move outward 1 or 2 trees per year.

To demonstrate the total number of trees that may become diseased over a period of years, the data obtained from 8 groves was examined. These groves had been mapped six times and complete records for the five-year period were available. As is shown in Table 2, the number of diseased trees in the groves varied from 13 to 164 when they were mapped in 1945. By 1950, the number of diseased trees varied from 138 to 511. Grove No. 4 showed the largest increase but also had the most diseased trees in 1945. However, the increase in number of diseased trees was not always greater in the groves that had more diseased trees at the beginning of the experiment as illustrated by comparison of the data from groves 5 and 6. Apparently the conditions in some of the groves were more favorable for the development of spreading decline.

Causal Agent

Spreading decline appears to be the result of a disorder of the fibrous roots of the tree. No evidence has been found to indicate that the disease is caused by a virus. Although the citrus nematode is present in a number of groves in Florida, it was not found in groves which have typical spreading decline. Two kinds of fungi can be consistently isolated from the fibrous roots of the diseased trees. One is a *Fusarium* sp. and the other has not yet been identified. It is probable that the spreading decline is caused by a fungus infection of the fibrous roots. A number of experiments are in progress to determine whether

either of these two fungi may be the causal agent.

One characteristic of a *Fusarium* disease is the ability of the fungus to produce a toxic wilt-inducing material when grown in Richard's solution. This toxic material adversely affects the host when the disease occurs under natural conditions. In the case of spreading decline, a toxic material was obtained from water extracts of the fibrous roots, the woody portion of larger roots and the leaves from diseased trees. This toxic material caused the wilting of citrus cuttings within 48 hours and of tomato cuttings in 24 hours. Extracts from healthy trees did not cause a wilting of the cuttings. *Fusarium* cultures No. 16, 29 and 35 obtained from diseased trees were grown in Richard's solution for two weeks and the filtrate tested for wilt inducing ability. The filtrate from culture 29 was more toxic than that from the other two cultures in causing a wilt of citrus cuttings. Since a wilt inducing material can be extracted from the diseased trees and is produced by the growth of the fungus in Richard's solution, it is indirect evidence that the spreading decline may be the result of the infection of the fibrous roots by a *Fusarium*.

Experiments have been conducted and are in progress to determine the effect of soil from a spreading decline area, healthy grove soil and virgin soil on the growth of rough lemon seedlings and young Duncan grapefruit trees on rough lemon rootstock. In one series of tests, the seedlings in the decline soil show a reduction in growth compared to that of the seedlings in the other soils. It has also been found that Tendergreen beans and sunflowers develop a greater amount of root rot when grown in soil from a spreading decline area than occurs when they are grown in soil from the healthy part of the grove. Both of the previously men-

tioned fungi have been isolated from the diseased bean and sunflower plants. In one instance, velvet beans were used as a cover crop in the spreading decline area of a grove. The stand was poor and about 50 percent of the plants showed root rot. A number of other kinds of plants will be tested for their susceptibility to root rot when grown in soil from a spreading decline area. If a satisfactory test plant can be found, it will be possible to evaluate the effectiveness of the various soil treatments more rapidly than can be done by growing citrus seedlings.

Control Measures

Considering the evidence obtained, it is doubtful if a treatment can be found which will rejuvenate those trees that have spreading decline. Therefore, to control spreading decline, two problems should be considered. How can we stop the spread of the decline in a grove? What soil treatment should be used before the area is replanted? In some cases, growers have attempted to control spreading decline by removing those trees which were visibly diseased. Within a few months those trees at the margin, which appeared healthy when the other trees were removed, began to show typical decline symptoms.

Before decline can be properly controlled it will be necessary to know the number of trees affected with the disorder which are located in advance of those trees showing visible symptoms. Since plant pathogens often affect plant metabolism a measurement of the rate of respiration of citrus leaves or roots should show whether differences exist in metabolic activity between apparently healthy trees in advance of the decline margin. A difference in metabolic activity might be indicative of the spread of the pathogen.

The rate of respiration of fibrous roots from healthy trees and decline

trees was measured using 40 root tips from each tree. The root tips were suspended in a 2 percent glucose solution and placed in a Warburg respirometer at 33° C. where oxygen measurements were made at 10 minute intervals for a period of one hour. The rate of oxygen absorption by the roots secured from three typical groves is shown in Table 3. It was evident in every measurement that the rate of respiration of the decline trees was lower than the respiration rate of the healthy trees in the same grove. The data also indicate that in the majority of the groves tested there was a successive increase in respiration rate from the decline area up to and including the third healthy tree beyond the decline margin. In every grove the respiration rate was highest for the third or fourth healthy tree beyond the decline margin. The rate of respiration of healthy trees beyond the fourth tree was slightly lower than the third tree but usually higher than the first or second healthy tree. It was also interesting to note that the respiration rate was practically the same for all healthy trees in the same grove located more than 4 trees beyond the visible margin of spreading decline. Although these data are preliminary in nature it would appear that the decline

TABLE 3.
Respiration Rate of Fibrous Roots from Decline Trees
and from Consecutive Healthy Trees in
Advance of the Margin.

Tree No.	Condition of Tree	Microliters of Oxygen per Hour		
		Grove 1	Grove 2	Grove 3
0	Decline	15.3	23.4	22.5
1	Healthy	18.6	26.6	30.3
2	Healthy	22.5		
3	Healthy	34.3	38.4	41.2
4	Healthy	33.1		
5	Healthy	31.2	31.8	30.6
6	Healthy	29.2		
7	Healthy	28.8	33.8	30.8
8	Healthy	28.8		
9	Healthy	31.3	29.1	29.0

casual factor had an initial stimulating effect on the respiration rate of the third or fourth healthy tree. It would seem logical, therefore, that as the invasion became more severe the respiration rate was reduced as illustrated by the lower metabolic activity of the first and second healthy tree.

Respiration studies are being continued and additional indices evaluated as an aid in the interpretation of the significance of metabolism in the third and fourth healthy trees beyond the decline area.

The activity of the catalase enzyme in the leaves has been used occasionally as an indication of the rate of metabolic activity. Sixty leaf discs were selected from each tree and ground while fresh with a mechanical mortar. Catalase was determined in Heinicke tubes rotated in a constant temperature water bath. The amount of catalase was expressed as the cubic centimeters of oxygen generated in 90 seconds when the sample was mixed with hydrogen peroxide. The catalase activity of the leaves secured from one grove is shown in Table 4 although the same general relation held for other groves that were tested. In general, there were greater variations in catalase measurements of the leaves than were apparent in the respiration rate of the roots. These differences may have been due to the greater experimental error in the catalase procedure. However, it is significant that in every grove tested the third or fourth healthy tree beyond the decline margin had the most catalase present.

Since a preliminary study of the physiology of the citrus tree has indicated some variation up to the fourth visibly healthy tree ahead of the margin of the decline area, it is probable that, if all of the diseased trees plus four or five good trees around the area were removed, the disease could be elimi-

nated. Assuming that this procedure would be effective, what would be the result if this had been done in 1945 in the eight groves which we have studied? As is shown in Table 5, the number of diseased trees in every grove is greater now (1950) than the number

TABLE 4.
The Catalase Activity of Grapefruit Leaves from Consecutive Trees Across the Margin of the Decline Area.

Tree No.	Condition of Tree	Catalase as cc. of O ₂ Released in 90 Sec.
1	Decline	15.7
2	Decline	20.2
3	Decline	17.1
4	Healthy	30.7
5	Healthy	34.0
6	Healthy	35.7
7	Healthy	30.4
8	Healthy	29.9
9	Healthy	29.3
10	Healthy	30.2
11	Healthy	28.3
12	Healthy	28.5

TABLE 5.
Hypothetical Loss of Trees by Pulling to Prevent Spread Compared to Actual Loss by Unchecked Spread of Decline.

Grove	Trees in 1945			Trees in 1950
	Decline	Pulled	Total	Decline
2	13	99	112	138
3	77	97	174	244
4	164	174	338	511
5	121	133	254	296
6	21	105	126	199
7	29	68	97	142
9	51	82	133	152
10	66	88	154	249

of decline trees plus a margin of four trees that would have been removed in 1945. Arrangements have been made to try this procedure in three groves this winter. It will be two or three years before definite conclusions as to its effectiveness can be obtained.

It is possible that a chemical barrier

maintained in a grove might stop the spread of the decline. Such a barrier would need to kill the roots to eliminate root contact and have some disinfecting action on the soil. Preliminary tests with cyanamid, formaldehyde and D-D (dichloropropane-dichloropropene) indicated that a formaldehyde solution should be effective as a barrier. The barrier would be examined periodically and when the roots started to grow back into the treated soil, the chemical would be applied again. A system of barriers at different distances ahead of the margin has been established in nine groves. Formaldehyde at 3 gallons to 100 gallons of water was injected into the soil at the rate of 2 gallons of solution per 5 feet of barrier. It is possible that some results will be obtained by 1952.

If either or both of the above mentioned measures of pulling marginal trees or using a chemical barrier will stop the spread of the decline, then the problem remains as to a satisfactory treatment for the soil so that replants will grow properly. In February, 1948, two blocks of spreading decline trees were removed and the soil treated with D-D at 400 pounds per acre. The treated areas were replanted with budded trees and records on growth are being obtained. After two years, the trees planted in the treated soil are making better growth than those in the non-treated soil. The data from one of the blocks are shown in Table 6. The D-D is not a good fungicide, but at the rate used has some fungicidal effect.

In another experiment rough lemon seedlings were planted in decline and virgin soils which had been treated with D-D, formaldehyde and ethylene dibromide in December 1948. In October 1950, 6 out of 18 seedlings in the non-treated decline soil had died and the remaining plants had grown about two-thirds as much as the seedlings in

the treated decline soil or in the non-treated or treated virgin soil. Final records have not been made but there does not appear to be any significant difference in the growth of the seedlings whether in treated decline soil, or in the non-treated or treated virgin soil.

TABLE 6.
EFFECT OF SOIL TREATMENT WITH D-D
ON GROWTH OF YOUNG TREES.

	Treated Soil	Non-Treated Soil
Caliper	1.91 in.	1.75 in.
Height	5.80 ft.	4.93 ft.
Spread	5.69 ft.	5.18 ft.

To obtain additional information on various materials that might be effective as a soil treatment, a series of tests were started in May 1950. A total of 56 materials are in the test. It may be possible to obtain some information by the spring of 1951.

Summary

Groves in which spreading decline is present in Florida are located in Polk, Orange, Highlands and Hillsborough counties. Over a five year period, the average rate of spread of the decline in all groves mapped was 1.6 trees per tree on the margin of the decline area. During the same period, the number of trees with the disease increased from 2 to 9 times in different groves.

Spreading decline appears to be the result of a fungus infection of the fibrous roots. A *Fusarium* sp. and an unidentified fungus have been consistently isolated from the roots of diseased trees. Indirect evidence obtained by means of the "wilt test" has indicated that a *Fusarium* may be the casual agent.

Tests on the respiration and catalase activity of rootlets and leaves of diseased and healthy citrus trees indicated that the disease may extend to the third or fourth healthy tree ahead of the

margin of the decline area. Any attempt at controlling spreading decline by removal of the trees should also include at least four healthy trees ahead of the margin.

Rough lemon seedlings have made better growth when the decline soil was treated prior to planting with D-D, formaldehyde or ethylene dibromide in

pot experiments. Field tests with D-D at 400 pounds per acre appear promising.

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THE PURPLE MITE AND ITS CONTROL

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During the past two years purple mite *Paratetranychus citri* McG. infestations have been general throughout the whole citrus area, and they have persisted even during the summer months. As a rule, purple mites are difficult to find in August and September but during these months in 1949 and 1950, light to medium infestations were observed in many groves. Purple mite populations were at a higher level during the summer of 1950 than during any other summer period on record.

Although no particular cause has been determined for the unusually heavy and widespread infestations, favorable weather conditions for mites have existed. Purple mites are often more numerous during and following periods of dry weather and it may be significant that in 1950 the rainfall at Lake Alfred was below normal each month from January to August inclusive.

Spray and cultural practices are factors of considerable importance in the development of purple mite infestations. Thompson (2) reported in 1938 that purple mites increased following copper sprays, and in 1942 Holloway (1) stated that the citrus red mite (pur-

ple mite) in California was more numerous following sprays containing compounds of copper, zinc and lime than where no sprays were applied. In 1944 Thompson (3) also reported that purple mites were more numerous following sprays containing lime-sulfur or compounds of copper or zinc, than where no sprays of any kind had been applied. In fact, the infestations were as heavy where lime-sulfur had been applied as a dormant spray as where either zinc or copper was used in the spray mixture.

Copper residues on the foliage bear a relationship to purple mite infestations as shown by the data in Table 1. The purple mite infestations were heavier in November where a neutral copper-oil emulsion combination was applied in April than where a neutral copper-wettable sulfur was applied at the same time. Analyses (5) of the copper residues* on the leaves showed that there was significantly more copper on the leaves where the copper-oil combination was applied and a higher mite population resulted. It should be emphasized here that the figures in the table represent only external copper. In the opinion of the authors, it is this external copper residue which influences purple mite infestations.

* Made by C. R. Stearns, Associate Chemist, Citrus Experiment Station.