INFLUENCE OF SPRAY MATERIALS ON THE STRUCTURE OF SOUR CHERRY LEAVES (*PRUNUS CERASUS* L. VAR. MONTMORENCY)

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Table of Contents

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Introduction

The influence of insecticides and fungicides on the function of leaves of fruit trees has been a subject of much experimental work. In general it has been found that certain of these materials at various concentrations and in various combinations may markedly affect the size, structure and photosynthetic activity of leaves. Most studies have dealt, however, with fruits other than the cherry.

The purpose of this study has been to determine the influence of certain spray materials on the leaf growth of the sour cherry (*Prunus cerasus* L. var. Montmorency), using the longitudinal diameter or length of the palisade cells as a measure of this influence.

Review of Literature

The concentration at which a chemical compound or spray material is applied to a plant has been, in many cases, the determining factor in the response of the plant to the material. Talts (19) demonstrated that a weak solution of zinc compounds mixed with a growth-substrate for the germination of spores stimulated spore growth. Stronger solutions showed either no change in the growth, decreased growth, arrested growth, or showed death of the spores, depending upon the anion and the concentration. Menzel (11) found no influence on foliage leaves of Pelargonium from a weak solution (less than 1 per cent) of a copper compound. However, for stronger solutions (1 per cent), a microscopic study of the internal structure showed shriveling of the cell wall, decreased length of the palisade cells in some places, increased size of cells in the spongy mesophyll; while the intercellular spaces were much decreased, and in many places absent. Although there was no visible influence on the epidermis, the protoplasm in the cells, both of palisade and spongy mesophyll, tended to collect on the inside of the cell wall and showed an artificially green discoloration. The thickness of the leaves was decreased.

These influences on the internal leaf structure of Pelargonium became more pronounced as the concentration of the solution was increased. Furthermore, a relatively high concentration (3.5 per cent) caused the death of some cells. More cells were killed in the epidermis than in the spongy mesophyll, and fewer were killed in the palisade tissue than in the spongy mesophyll. Of the plants used by Menzel (11) the influence of chemical compounds on internal structure was more pronounced with Pelargonium, but was similar on the leaves of apple and other plants investigated.

Menzel (11) also demonstrated by localized treatments that the effects of chemical solutions was not restricted to the regions of the leaf directly below the area treated. From his work there appeared to be an absorption and translocation of the chemical, which brought about physiological changes in untreated areas of the leaf. Roberts, Southwick and Palmiter (17) showed that the epidermal layer was continuous, and connected with the vein extensions, but that the cell walls and cutinized layer was not continuous. Since the cutinized layer and cell walls were not continuous the absorption of spray materials takes place very readily.

Heinicke (3), Hoffmann (4,5), Hyre (9), and others have reported findings of reduced photosynthesis following spray applications, and greater reduction from strong spray materials than for mild spray materials. Mills (12), Rassmussen, Toenjes and Strong (16) have shown that the cummulative effect of reduced yield is greater for lime sulfur than for elemental sulfur sprays. Dutton (2) showed that a spray injurious to the foliage leaves of the apple reduced growth of wood, and the rate of reduction was different for lime sulfur and Bordeaux mixture. Horsfall and Turner (8), Horsfall, Harvey, and Suit (7), Bonde, Folsom, and Tobey (1), and others have shown that spray materials may reduce the growth of plants. According to Southwick and Childers (18) the influence of Bordeaux mixture on the photosynthetic process of apple leaves was primarily physiological rather than mechanical.

Pickett and Birkeland (13, 14, 15) believed that the reduction in photosynthetic activity brought about by some spray materials on apple foliage may be explained, in part, by the reduction of the amount of the internally exposed cell wall surface. Kenworthy (10) found a highly significant correlation between the total length or depth of the palisade mesophyll and the ratio of the internally exposed cell wall surface to the external surface. This was later confirmed by Pickett and Birkeland (14) who found that the so-called "stronger" or more caustic spray materials, as lime sulfur, inhibited the development of the palisade cells more than the so-called "mild" or less caustic spray materials such as elemental sulfur. Also, repeated applications inhibited the development of palisade cells more than single applications. According to these reports a measurement of the depth of the palisade tissue or longitudinal diameter of palisade cells becomes a practical test of the physiological effects of spray materials that should save investigators considerable time in evaluating new spray materials.

Procedure

Four-year-old cherry trees grown on the Horticultural farm of Michigan State College, at East Lansing, Michigan, were used in this study. The trees were grafted on seedlings of *Prunus mahaleb* L. Eight different spray treatments were used. There were four replications for each treatment; each replication having two trees.

The treatments were applied five times during the season, using 4 preharvest and 1 after-harvest application. The treatments used may be outlined as follows (amounts listed are for 100 gallons of water):

1. Basic copper sulfate--3 lbs.*
   Hydrated lime--4 lbs.
   Arsenate of lead--2 lbs.

2. Basic copper sulfate--3 lbs.*
   Hydrated lime--4 lbs.
   Benzene hexachloride--3 lbs.**

3. Basic copper sulfate--3 lbs.*
   Hydrated lime--4 lbs.
   Parathion (15%)--1 lb.

4. Ferric dimethyldithiocarbamate--1 1/2 lbs.
   Arsenate of lead--2 lbs.

5. Ferric dimethyldithiocarbamate--1 1/2 lbs.
   Benzene hexachloride--3 lbs.**

6. Ferric dimethyldithiocarbamate--1 1/2 lbs.
   Parathion (15%)--1 lb.

7. Liquid lime sulfur--2 gals.
   Arsenate of lead--2 lbs.

8. Liquid lime sulfur--2 gals.
   Parathion (15%)--1 lb.

On August 7, 1948 sixteen leaves were collected from each treatment, except for treatment No. 6 where only 12 leaves were collected. Two leaves were collected from each tree. The leaves selected were removed from the southern periphery of the tree and near the middle of shoots of average vigor.

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*The basic copper sulfate was known as Tennessee 26 which contains a metallic equivalent of 26% copper.

**The benzene hexachloride contained 6% gamma isomer.

The portion of the leaf used was located midway between the base and the apex of the leaf. From each leaf, one 3/4 centimeter square piece of leaf, including half of the midrib, was placed in a formalin-acetic acid-alcohol killing and fixing solution, dehydrated and embedded in paraffin.

The sections were cut eight microns thick and were stained with haemotoxylin and safranin. One slide of several sections was made from each leaf piece, with the sections being made across the midrib as a means of orientation. The total length of the three layers of palisade cells was measured at ten different places near the midrib and in areas devoid of smaller veins.

Results and Discussion

The results of the measurements are shown in Table 1. Table 2 shows the occurrence of significant differences between the mean values for treatments. The slides showed dark spots of materials that were formed in the epidermal cells, and in some sections this material moved out over other parts of the leaf sections during the staining process (see Figure 1). These dark spots could not be associated with any certain treatment, and may be the gum-like substance found commonly on cherry leaves.

The data given in Table 1 show that sprays containing copper are more injurious to leaves of cherry trees than sprays containing ferric dimethyldithiocarbamate, and that sprays containing liquid lime sulfur were less injurious than sprays containing ferric dimethyldi-

Table 1. The average length of the three layers of palisade cells as influenced by spray treatments (microns)

Leaf Number	(1) Basic $CuSO_4$ Hydrated lime Arsenate of lead	(2) Basic $CuSO_4$ Hydrated lime Benzene hexachloride	(3) Basic $CuSO_4$ Hydrated Lime Parathion (15%)	(4) Ferric dimethyldithio-carbamate Arsenate of lead	(5) Ferric dimethyldithio-carbamate Benzene hexachloride	(6) Ferric dimethyldithio-carbamate Parathion (15%)	(7) Liquid lime sulfur Arsenate of lead	(8) Liquid lime sulfur Parathion (15%)
1	114.8	108.2	130.4	118.4	143.2	138.8	122.4	148.9
2	103.7	93.6	128.1	98.1	140.3	147.0	146.4	140.1
3	98.5	99.5	117.8	119.9	133.4	129.6	125.8	146.0
4	96.0	110.2	113.6	111.7	121.2	139.7	151.0	143.6
5	103.7	115.7	126.8	107.1	130.0	135.7	142.6	154.1
6	102.1	95.6	115.7	101.0	134.2	134.4	135.7	152.5
7	92.9	114.4	122.0	101.6	131.9	132.1	127.3	132.5
8	109.0	97.9	112.8	124.3	136.9	130.6	128.1	138.4
9	105.4	102.9	122.2	112.8	138.8	130.4	123.1	139.2
10	113.8	97.0	122.0	123.9	130.4	142.2	140.7	151.2
11	106.3	112.3	130.4	128.3	139.4	167.4	136.5	133.8
12	106.7	99.3	128.9	122.2	134.2	139.0	131.9	138.6
13	114.2	99.5	125.2	118.0	154.1		130.4	148.9
14	107.7	91.1	123.5	129.6	146.4		136.3	157.7
15	111.5	105.2	124.5	103.5	136.1		126.4	149.1
16	110.9	107.9	128.1	126.6	128.1		131.5	139.7
Total	1702.2	1650.3	1971.8	1847.9	2187.8	166.9	2136.1	2314.8
Average	106.4	103.1	123.2	115.5	136.2	138.9	133.5	144.6

Difference required for significance	1%	5%
Between treatment 6 and all others	8.5	6.3
Between all treatments except 6	7.8	5.8

To accompany Page 6

Table 2. The Occurrence of Significant Differences between Treatments

Treat. No.	Treatment Number 1	2	3	4	5	6	7	8
1			**	**	**	**	**	**
2			**	**	**	**	**	**
3				**	**	**	**	**
4					**	**	**	**
5								**
6								
7								**
8								

thiocarbamate (Figures 1, 2, and 3). The different insecticides likewise showed different influences on the leaves. Sprays containing parathion were less injurious than sprays containing benzene hexachloride. Benzene hexachloride in combination with ferric dimethyldithiocarbamate, or parathion was less injurious than arsenate of lead with the same fungicides. However, benzene hexachloride in combination with basic copper sulfate was slightly more injurious than when basic copper sulfate was used with arsenate of lead. Apparently benzene hexachloride has an undesirable reaction with copper compounds. The greatest thickness of the palisade layers was associated with the use of parathion in combination with liquid lime sulfur, while the smallest thickness of the palisade layers was associated with the use of benzene hexachloride in combination with basic copper sulfate.

The results obtained in this study show that all of the spray materials used had an effect upon the development of palisade cells, and that certain combinations may have a greater influence than sprays containing only one insecticide or fungicide. Since the development of the palisade cells is correlated with the photosynthetic activity of the leaves, the changes in their development brought about by the use of spray materials should eventually be reflected in the growth and fruitfulness of the tree. The length of time required for this influence to become measureable in the field will depend upon the magnitude of the influence. Many of the differences reported here may appear small in numerical value, but are statistically significant, and, although their influence on tree growth could not be measured within a single year, the trees should eventually show a correspondingly significant difference in growth and production.

Summary

Three commonly used fungicides and three insecticides were studied in combination with one another, such as may be used in commercial orchards. Their effect upon the structure of sour cherry leaves (*Prunus cerasus* L. var. Montmorency), using the depth of the palisade mesophyll as an index, was studied.

The fungicides were found to have a very marked effect upon the palisade development. The palisade development associated with the use of basic copper sulfate was less than that associated with either ferric dimethyldithiocarbamate or liquid lime sulfur when the same insecticide was used. Likewise the palisade development associated with the use of ferric dimethyldithiocarbamate was less than that associated with the use of liquid lime sulfur.

The insecticides were found also to have a marked effect upon palisade development. Arsenate of lead resulted in less palisade development than parathion when the same fungicide was used.

Benzene hexachloride with basic copper sulfate resulted in less palisade development than either arsenate of lead or parathion. When benzene hexachloride was used in combination with ferric dimethyldithiocarbamate the palisade development was greater than when the latter was used with arsenate of lead, but less than when parathion was used in combination with ferric dimethyldithiocarbamate.

The magnitude of the physiological effects that spray materials may have is illustrated by comparing the palisade development associated with the mixture of liquid lime sulfur and parathion with that associated with the use of benzene hexachloride and basic copper sulfate. There was 28.7 per cent less palisade development in the leaves sprayed with benzene hexachloride and basic copper sulfate than when the leaves were sprayed with liquid lime sulfur and parathion.

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Figure 1. Comparative effects of spraying leaves with basic copper sulfate in combination with:

A. Arsenate of lead. The total length of palisade cells--106.4 microns

B. Lime, and benzene hexachloride. The total length of palisade cells--103.1 microns

C. Lime, and parathion (15%). The total length of palisade cells--123.2 microns

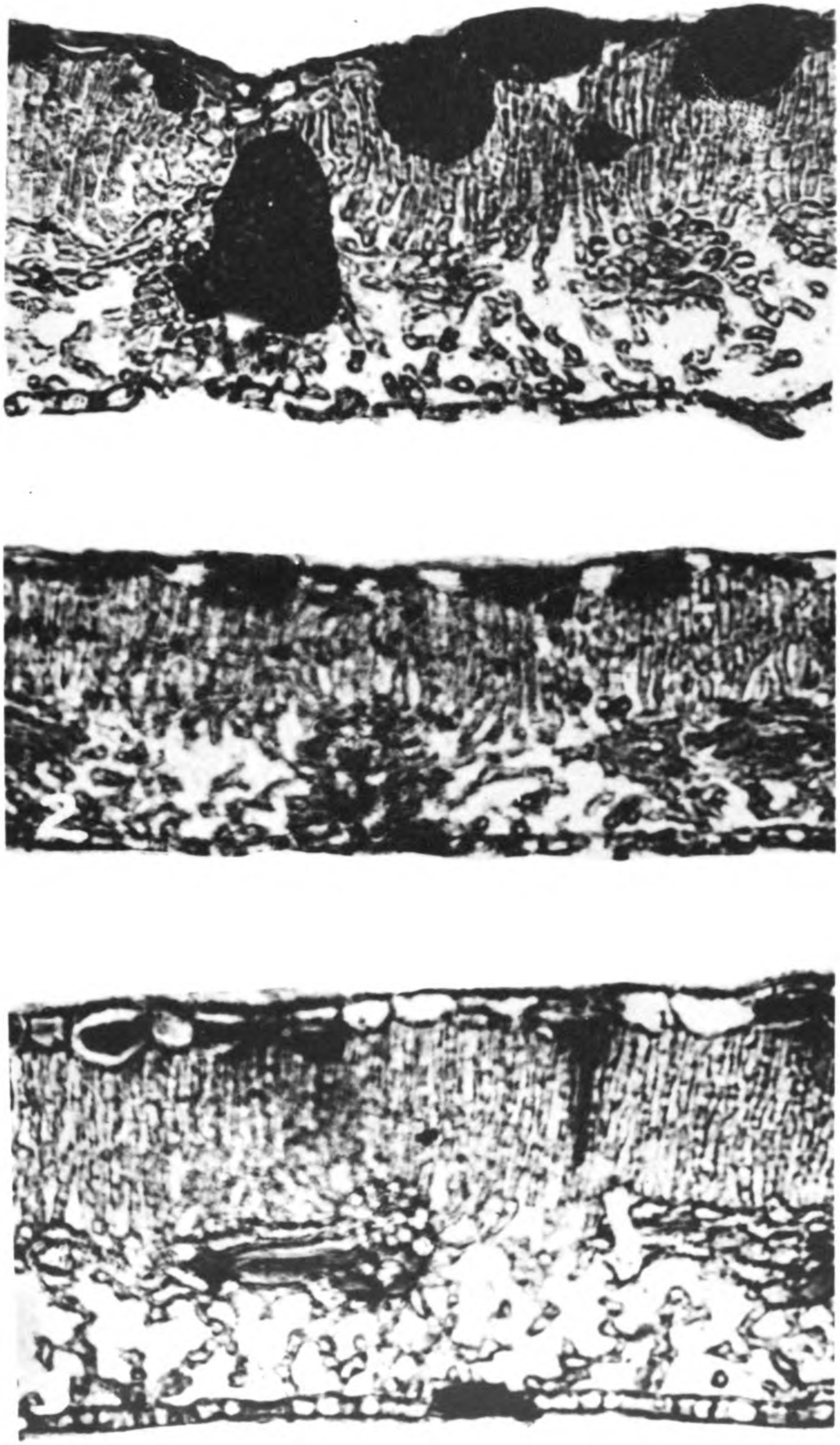
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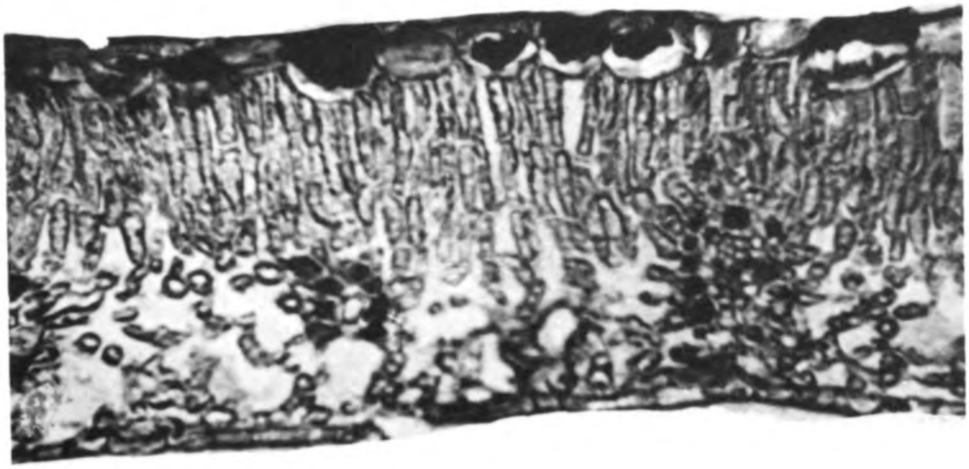
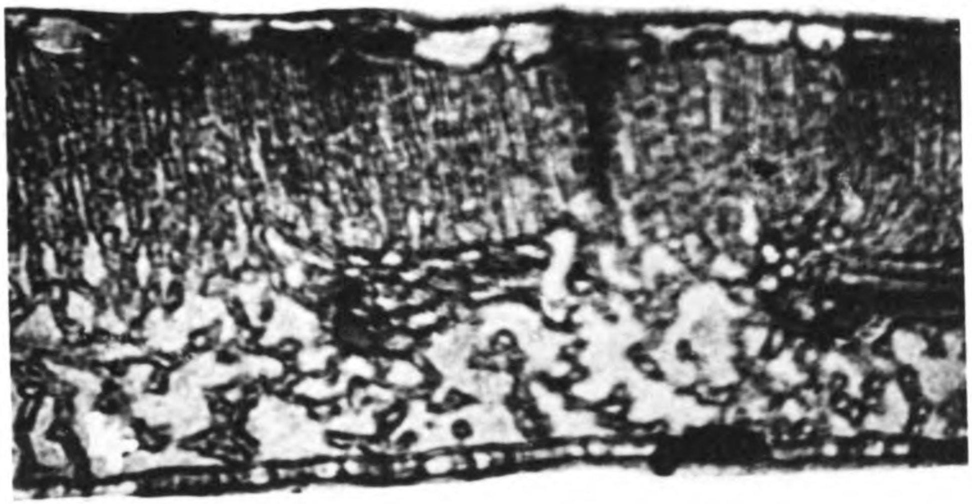
Figure 2. Comparative effects of spraying leaves with ferric dimethyldithiocarbamate in combination with:

A. Arsenate of lead. The total length of palisade cells--115.5 microns

B. Benzene hexachloride. The total length of palisade cells--136.2 microns

C. Parathion. Total length of palisade cells--138.9 microns





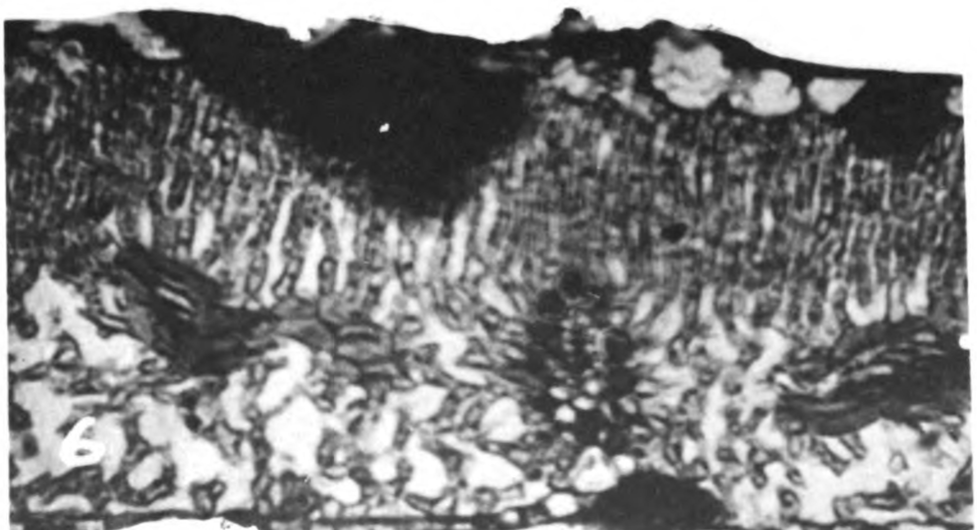

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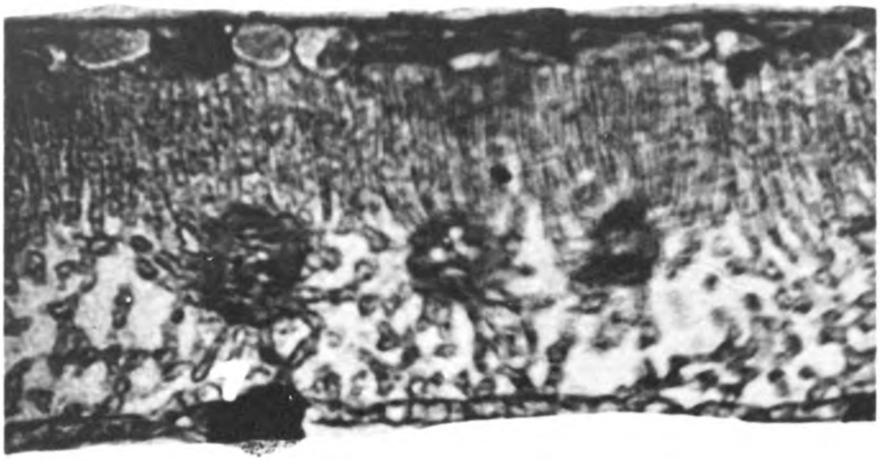
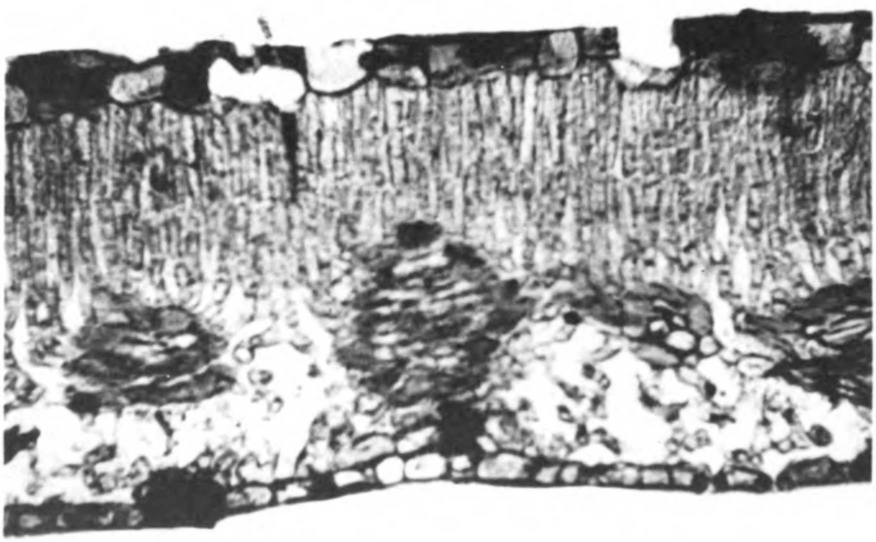
Figure 3. Comparative effects of spraying leaves with liquid lime sulfur in combination with:

A. Arsenate of lead. The total length of palisade cells--133.5 microns

B. Parathion. The total length of palisade cells--144.6 microns





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