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TEAR-STAIN OF CITRUS FRUITS.

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CONTENTS.

DESCRIPTION OF THE DISEASE.

Florida citrus fruits are subject to two distinct types of tear streaking, namely, wither-tip tear-stain and melanose tear-streak. The former has been attributed to *Colletotrichum gloeosporioides* Penz., while the latter, which will not be discussed in this bulletin, is doubtless due to a peculiar distribution of the spores of the causal organism (*Phomopsis citri* Fawcett) in trickling waters.

Wither-tip tear-stain is a smooth, more or less brownish discoloration of the surface which occurs typically in fingerlike patterns about one-fourth of an inch in width, extending longitudinally toward the stylar or blossom end of the fruit. (Pl. I.) These streaks may or may not be confined to one side of the fruit, and frequently they appear to arise in areas russeted by rust mites (*Eriophyes oleiveros* Ashmead). The streaks are usually few in number, seldom more than six or eight; occasionally two or more merge and form a rather wide discoloration, in which event the injury is generally attributed to rust mites. (Pl. II, fig. 1.)

Wither-tip tear-stain as it occurs in Florida is one of the minor diseases of citrus fruits, and for that reason it has received little attention from investigators. Its effects are principally observed on the round orange and grapefruit, more noticeably and frequently on the latter and to a less extent upon other economic species of citrus.

The economic importance of this blemish is chiefly due to the financial loss which the grower sustains as a result of the lowered market value of the affected fruit, which is rendered unsightly and unattractive. However, tear-stained fruit seems to possess flavor and keeping qualities essentially equal to fruit which is free from these markings.

REVIEW OF THE LITERATURE.

A careful survey of the literature reveals the fact that very little has been published on the subject. The cause of tear-stain was investigated and first reported on by Rolfs (7),[1] who is quoted as follows:

> This peculiar form of russeting manifests itself by streaks running over the fruit from the side that hangs uppermost to the point which is nearest the ground. The cause of this peculiar form of russeting is that somewhere above the fruit a twig occurs which has been infested by the fungus [2] and which contains the hold-over spores. Whenever sufficient rain or dew occurs to cause a dripping from the disease-infected twig on to the fruit, the disseminating spores are liberated from the twig and carried with the rain or dew over the epidermis of the fruit. Many of the spores are left along in different places, and these produce sufficient irritation to the epidermis to cause russeting along in streaks; hence we have the peculiar form of tear staining or tear streaks.

Again in 1911 Rolfs (8) discussed the subject as follows:

> Russeting and tear streaking can nearly always be traced back for their beginning to a small dead spur or sprig. The fungus [2] lives in the dead spur or sprig. Water from rains and moisture following heavy dews collect in drops on these sprigs or spurs and the drops when they fall carry with them numerous fungous spores. These spores come in contact with the epidermis of the fruits and germinate, causing minute lesions on the epidermis, too small for complete infection and the production of anthracnose.

This explanation as presented by Rolfs, to the effect that *Colletotrichum gloeosporioides* is the responsible agent, seems to have been accepted by several investigators at various times, among them Fawcett (2–5), Stevens (9), Stevenson (10), and Matz (6).

On the other hand, Earle and Rogers (1) recognized in the Isle of Pines two types of russeting or tear staining said to be easily distinguishable, the one attributed to rust mites and the other thought to be caused by a fungus. In discussing wither-tip they make the following statement:

> One of the serious troubles attributed to the wither-tip fungus in Florida is that water washing down from infected twigs will cause the serious discoloration of the fruit known as tear streaking. The discoloration is often confused with the work of the rust mite, though it is easily distinguished. It frequently occurs here and can be always traced to some dead twig, but we are still uncertain whether it is due to the secretions of the wither-tip fungus or the Diplodia.

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[1] The serial numbers in parentheses refer to "Literature cited" at the end of this bulletin.

[2] *Colletotrichum gloeosporioides*, mentioned in the context.

The publications cited contain practically all the more important references to wither-tip tear-stain in Florida or near-by sections where the round orange and grapefruit are grown in large quantities, and no presentation of actual experimental data is made therein on which the fungus theory regarding the cause of tear-stain is based. However, it can not be denied that this theory, first stated by Rolfs, is so plausible that it has been accepted readily without the support of published experimental data. It was not until results very strikingly inconsistent with this theory appeared in experimental spraying tests that its validity was questioned, and this has led to a thorough investigation of the causation of the type of injury commonly known in Florida as "wither-tip tear-stain." The evidence presented in this bulletin indicates that *Colletotrichum gloeosporioides* is not responsible for tear staining in Florida. If this fungus ever produces such an effect, it must be extremely rare in that section.

SPRAYING EXPERIMENTS.

During the season of 1917, in connection with spraying experiments which were conducted in a bearing grapefruit grove for the control of citrus scab, it was observed that tear staining was almost entirely absent from those plats that received several applications of lime-sulphur solution, though it was quite prevalent where Bordeaux mixture had been applied during the scab-spraying season. Since this first observation, many similar manifestations of the lack of control of tear-stain by copper sprays have appeared in the experimental plats. Such a failure has been proportional to the severity of rust-mite attack.

Ordinarily the scab-spraying season in Florida begins in February when the spring growth starts and extends over a period of two or three months. It is followed rather closely by the period of very severe attack by rust mites. The latter are usually most abundant during May and June and are readily controlled by two or three applications of lime-sulphur solution diluted 1 to 66. With this explanation the data in Table I can be readily interpreted.

In general, the results of this spraying experiment show (1) that tear-stain was controlled on plats 3 and 5, which received applications of lime-sulphur solution shortly before the period of maximum abundance of rust mites; (2) that tear-stain was materially reduced on plat 4, which received applications of lime-sulphur solution considerably in advance of the period of maximum numbers of rust mites; (3) that tear-stain was not reduced on plats 1 and 2, which received spray applications of copper mixtures. Assuming that tear-stain is caused by a species of Colletotrichum or other fungus, it would be reasonable to expect that plats 1 and 2 would show at least as good control as plat 4. The fact that control of tear-stain was so closely

associated with rust-mite control is strong evidence that rust mites might be largely concerned in the causation of the condition that is generally recognized as wither-tip tear-stain.

TABLE I.—*Effect of various sprays on the control of tear-stain on grapefruit.*

Date.	Plat designation and spray application.					
	No. 1, Bordeaux mixture.	No. 2, Burgundy mixture.	No. 3, Bordeaux mixture.	No. 4, lime-sulphur.	No. 5, lime-sulphur.	No. 6.
1917.						
Jan. 29	3–4–50	3–3½–50	3–4–50	1–40	1–40	Not sprayed.
Mar. 23	3–4–50	3–3½–50	3–4–50	1–40	1–40	Do.
Apr. 7	3–4–50	3–3½–50	3–4–50	1–40	1–40	Do.
May 2 and 22	3–4–50	3–3½–50	Lime-sulphur, 1–40.	Not sprayed.	1–40	Do.
June	Not sprayed.	Not sprayed.	Lime-sulphur, 1–66.	....do....	1–66	Lime-sulphur, 1–66.
1918.						
February (final count of 1,000 fruits), tear-stained, per cent.	70.39[a]	38.2	1.3	7.5	1.25	25.9.

[a] The excessive number of tear-stained fruits on the plat sprayed with Bordeaux mixture may be explained by the fact, quite frequently noted, that rust mites become unusually abundant on trees sprayed with that material. The injury did not resemble Bordeaux russet, but was similar in every respect to the tear staining on plat 6.

A part of a grove of grapefruit was sprayed experimentally in June, 1919, using 3–4–50 Bordeaux mixture for plat 1 and 1–66 lime-sulphur solution for plat 2. Plat 3 was an unsprayed check. This grove had not been sprayed previously that season. At the end of July the fruit in plat 2 was free from tear staining, but numerous tear-stained specimens were observed in plats 1 and 3. The fruit on these plats ripened and was harvested in February, 1920, with no increase of tear-stain over that observed in July, 1919. This indicates that one application of weak lime-sulphur solution applied at a time suitable for rust-mite control in that grove was equally effective for the control of tear-stain, while the standard strength of Bordeaux mixture applied at the same time did not reduce this blemish. (Pl. II, fig. 2.)

Other experimental data obtained during the past four years show without exception similar results.

The conclusion of Yothers (11), based on numerous extensive experiments to control rust mites in various parts of Florida, is that bright fruit—i. e., fruit free from rust-mite injury—is invariably free from tear-stain as well. (Pl. II, fig. 1.)

Growers generally throughout the Florida citrus belt have accomplished commercial control of tear-stain whenever they have controlled rust mites by following the spraying schedules established for these pests.

Definite observations in commercial groves show clearly the following facts: (1) Where rust mites are naturally absent tear-stain is not observed; (2) where rust mites occur and are successfully controlled tear-stain is also controlled; (3) where rust mites are

GRAPEFRUITS SHOWING THE RESULTS OF INFESTATION BY RUST MITES.

These grapefruits show the variant forms in which tear-stain appears. At the left is an example of what may be termed "multiple tear-stain," while at the right is shown a fruit illustrating a form more distinctly marked. These variant types of injury are frequently observed. One side of the fruit may be severely russeted while the other side is bright or tear stained.

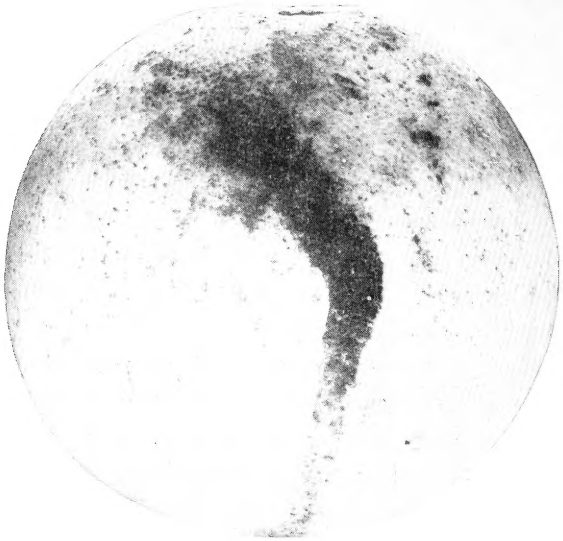

FIG. 1.—DAMAGE TO GRAPEFRUIT BY RUST MITES.

Dark tear stains are found frequently on areas more or less russeted by rust mites, especially where the applications of rust-mite sprays were delayed. For all practical purposes, both tear-stain and russeting are absent from fruit kept free from rust mites. Note the zone of faint russet.

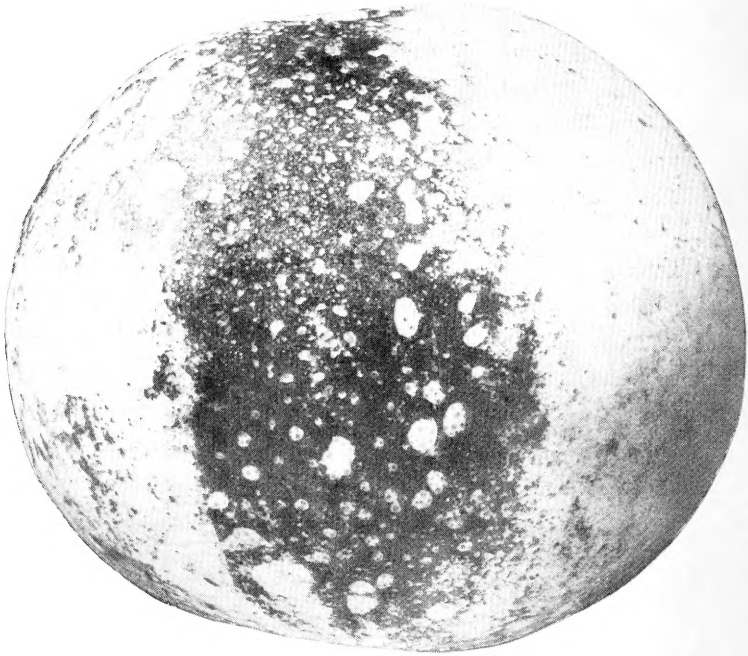

FIG. 2.—THE WORK OF RUST MITES ON GRAPEFRUIT SPRAYED IN JUNE WITH BORDEAUX MIXTURE.

In the zone of rust-mite activity injury is confined to the area not covered with spray. Near-by grapefruit trees sprayed on the same date with lime-sulphur solution (1-66) matured fruit entirely free from russeting or tear-stain.

present and are not controlled tear-stain occurs and usually varies proportionately with the severity of the rust-mite attack; (4) rust-mite russet and wither-tip tear-stain develop simultaneously.

Usually in Florida a small proportion of late citrus blooms occur in June. Fruits from such blooms develop after the normal rust-mite period and almost invariably ripen free from tear staining unless rust mites happen to become abundant during the following winter or early-spring months.

CULTURAL WORK.

Fungi are admittedly capable of producing tear-streak patterns on the host; for example, *Phomopsis citri* is known definitely to produce melanose injury in distinct tear streaks on various citrus fruits. Consequently a careful study was made to determine the frequency of the occurrence of *Colletotrichum gloeosporioides* or other fungi in typical wither-tip tear-stain areas.

In preliminary tests made during the fall of 1918 oranges and grapefruit were selected that showed typical wither-tip tear-stain, as well as fruit affected with rust-mite russet which shaded off into tear-stain. Usually 30 cultures were made from each fruit, 10 each from areas selected as follows: (1) Typical rust-mite russet, (2) typical tear-stained streaks or slightly discolored areas on fruits showing no streaking, and (3) normal areas not discolored. The fruits were washed 1 minute in 1–1,000 mercuric-chlorid solution and afterwards thoroughly rinsed in sterile water. Bits of tissue were then removed with flamed instruments to sterile Petri dishes and covered with melted corn-meal agar. These cultures were allowed to grow five days at room temperature. The results of this preliminary culture test are found in Table II, the percentages being based on the number of the bits of tissue used in each test.

TABLE II.—*Culture tests for the isolation of Colletotrichum gloeosporioides* [1] *from normal and affected parts of orange and grapefruit rind.*

Series.	Number of fruits.	Bits of tissue cultured.	Colletotrichum gloeosporioides.	Miscellaneous organisms.	No growth.
A. Russeted	17	170	38.2	18.8	43.0
B. Tear stained	16	160	21.1	19.3	59.6
C. Normal	19	190	6.2	15.7	78.1

[1] The use of the name *Colletotrichum gloeosporioides* in the culture data of this publication merely follows established custom and is without prejudice as to the proper designation of the organism. Any Colletotrichum showing characteristic growth on corn-meal agar was recorded as this species.

It thus appears that *Colletotrichum gloeosporioides* is recovered in rather low percentage from tear-streaked areas, that it is present to some extent in normal as well as badly russeted areas, and that the frequency of occurrence varies directly with the degree of injury.

TABLE III.—*Isolation of Colletotrichum gloeosporioides, a saprophytic type of Cladosporium, and miscellaneous fungi and bacteria from orange and grapefruit rind having various degrees of russeting and tear staining.*

Lot and number of fruits in group (98 fruits in all).	Variety.	Remarks.	Not disinfected.[1]									Disinfected.[1]								
			Series A, russet.			Series B, tear-stain.			Series C, bright.			Series A, russet.			Series B, tear-stain.			Series C, bright.		
			C. g.	Clad.	Misc.	C. g.	Clad.	Misc.	C. g.	Clad.	Misc.	C. g.	Clad.	Misc.	C. g.	Clad.	Misc.	C. g.	Clad.	Misc.
Lot A, 4 fruits	Grapefruit	Average tear stains				31	23	17							5	4.2	3.7			
Do	do	Average bright fruit							17	12	19							4.5	4.7	11
Do	Orange	do							5.5	11	11							6	1.7	1
Lot A, 3 fruits	do	Average russet fruit	12	13	139							3.6	4.3	3						
Lot A, 2 fruits	do	Average tear-stained fruit				14	20	113	7	5	17				6.5	6.5	13	3	2	.5
Lot B, 3 fruits	Grapefruit	Dark-colored tear stains				38	29	89	22	18	36				27	7.3	3.3	6.6	2.3	3.6
Do	do	Medium-colored tear stains				36	14	32	22	16	24				28	14	4	5.8	1.6	1.3
Do	do	Faint tear stains				31	29	209	14	8.6	69				28	13	6	5.6	5	5
Lot B, 2 fruits	do	Typical rust-mite russet, with accompanying tear streaks.	37	32	120							13	5	4.5						
Do	do	Very dark russeted area	47	26	45							17	7.5	3.5						
Lot B, 4 fruits	do	Typical rust-mite russet[2]	33	15	20							9.5	6.7	4.7						
Lot C, 2 fruits	do	Medium tear stains on one side only; other side bright.				43	20	28	24	25	25				5.5	1	1.5	2.5	3	1.5
Lot D, 4 fruits	do	Tear stains on one side only				46	38	200							23	10	5			
Do	do	Russeted on one side only	40	17	200				7.7	7.7	23	7.5	3	3.5				5.7	6.2	3.7
Lot F, 4 fruits	do	Medium-colored tear stains; one side bright.				27	16	33	21	9.2	92				.5	.5	1	2.5	0	.8
Do	do	Medium rust-mite russet	42	42	51							2.7	1.2	1.7						
Lot G, 2 fruits	do	Fruits entirely russeted	36	16	36							35	7.5	2						
Lot H, 4 fruits	Orange	Black (severe) rust-mite russet	1	2.3	58							1.2	.5	3.2						
Do	Grapefruit	Dark (severe) rust-mite russet	38	33	72							20.8	4.7	5						
Lot H, 2 fruits	do	do	37	28	47							24	8.5	5						
Do	do	Free from visible blemishes							36	26	50							24	17	2
Lot I, 4 fruits	Orange	Medium russets	17	17	33							4	1	5						
Lot J, 4 fruits	do	Severe rust-mite russet	20	11	20							1.7	.3	.3						
Lot K, 4 fruits	Grapefruit	Free from visible blemishes							14	10	22							17	8.5	7.7
Do	do	Medium rust-mite russet	34	28	128							20	11	4.3						
Lot L, 2 fruits	do	Free from visible blemishes							19	22	18							.5	.5	1
Do	do	Medium rust-mite russet	46	29	20							32	13	30						
Lot M, 2 fruits	do	Free from visible blemishes							0	1.5	16							0	0	.5
Do	do	Dark tear stains				5.5	1.5	13							19	4.5	1			
Do	do	Medium rust-mite russet	8	.5	39							30	20	38						
Lot N, 2 fruits	do	Medium-dark tear stains on part of fruit.				58	28	99	39	35	77				15	5	3.5	12	4.2	3
Lot O, 4 fruits	do	Medium tear stains				42	21	40	35	16	35				28	9.5	7.2	20	3.5	3.2
Average for all lots			32	20	65	34	22	78	19	14	36	15	6.2	7.6	17	6.8	4.4	7.7	4	3

[1] Symbols: C. g. = *Colletotrichum gloeosporioides*, Clad. = *Cladosporium*, Misc. = Miscellaneous.

[2] No streaking.

In the fall of 1919 a more extensive test was conducted. Fifteen lots of fruit were involved. Five of these lots were selected by the writer, and the remaining ten lots were selected in various parts of Florida by persons specially chosen for their competence to select typical rust-mite injury and typical wither-tip tear-stain. Each of these lots was sorted into several groups of one to four fruits each according to the variety of fruit, the particular type of effect, and the intensity of it. For a comparative study, cultures were made from fruits in the same lot that were free from blemishes or from unblemished areas on the russeted or tear-stained fruits. One hundred bits of tissue, approximately 1 square millimeter in surface area, were cultured from each test area of each fruit, using 10 Petri dishes, each with 10 bits of tissue. The results when reduced to a percentage basis, as in Table III, also represent the average numbers of occurrence per fruit. Corn-meal agar was used as a culture medium and the plates were held six days at room temperature. Counts were made of the common saprophytic type of Cladosporium as well as of Colletotrichum colonies. Bacteria and fungi other than these were reckoned as miscellaneous. Two parallel series were made, one for undisinfected tissue and one from similar areas on the same fruits washed with a disinfectant. Bichlorid of mercury solution (1 to 1,000) was used for 1 minute with subsequent rinsing on all disinfected lots except K, L, and M; on these three lots undiluted fresh hydrogen peroxid was used without rinsing. Table III gives the results, with fractions omitted for the higher percentages.

Table III shows that *Colletotrichum gloeosporioides* is practically universally distributed on citrus-fruit surfaces and that it escapes to a considerable degree the surface disinfection process ordinarily practiced in culture work. It is present about equally on the average in tear-stained and russeted areas. The amount varies in different lots of fruits, but seems to be more abundant where the visible effects are most pronounced. A saprophytic type of Cladosporium is isolated with the same constancy as *Colletotrichum gloeosporioides*, but with less frequency. These extensive culture tests show, therefore, that it would be about as reasonable to ascribe the blemishes to one of these organisms as to the other, if constancy of isolation from lesions is to be the deciding consideration. However, neither fungus reaches a frequency of occurrence high enough to justify holding it to be the causative organism on this evidence alone.

Certain fruits having the melanose type of tear-streak were selected, and cultures were made from these in the manner already described, comparative tests being made from unblemished areas, from melanose tear-streak, and from diffused melanose areas; and cultures from the surface blemish known as "shark skin" were also made. The results are given in Table IV.

TABLE IV.—*Isolation of Colletotrichum gloeosporioides, Cladosporium sp., and miscellaneous organisms from orange and grapefruit rind affected with melanose or with "shark skin."*

Lot and number of fruits in group.	Variety.	Remarks.	Series A, diffused patches.			Series B, tear-streak pattern.			Series C, unblemished.		
			Colletotrichum gloeosporioides.	Cladosporium.	Miscellaneous.	Colletotrichum gloeosporioides.	Cladosporium.	Miscellaneous.	Colletotrichum gloeosporioides.	Cladosporium.	Miscellaneous.
Not disinfected:											
Lot E, 4 fruits.	Grapefruit.	Melanose tear streaks.	.....	.....	.....	40	41	51	.....	.....	.....
Do.	....do....	Bright area on melanose fruits.	.....	.....	.....	.....	.....	.....	27	23	40
Lot G, 2 fruits.	....do....	Melanose tear streaks.	.....	.....	.....	8	11	45	12	5	15
Lot L, 2 fruits.	....do....	....do....	.....	.....	.....	5	2	1	.....	.....	.....
Lot N, 1 fruit.	....do....	Mud-caked melanose.	32	19	67	.....	.....	.....	.....	.....	.....
Lot J, 4 fruits.	Orange....	Decided "shark skin"	31	17	49	.....	.....	.....	.....	.....	.....
Lot K, 4 fruits.	Grapefruit.	....do....	25	15	11	.....	.....	.....	.....	.....	.....
Disinfected:											
Lot E, 4 fruits.	....do....	Melanose tear streaks.	.....	.....	.....	5	1	.3	.....	.....	.....
Do.	....do....	Bright area on melanose fruits.	.....	.....	.....	.....	.....	.....	.7	.5	.3
Lot G, 2 fruits.	....do....	Melanose tear streaks.	.....	.....	.....	36	7	3	6.5	2	2
Lot L, 2 fruits.	....do....	....do....	.....	.....	.....	5	1	1	.....	.....	.....
Lot N, 1 fruit.	....do....	Mud-caked melanose.	8.5	2	1	.....	.....	.....	.....	.....	.....
Lot J, 4 fruits.	Orange....	Decided "shark skin"	6	1	1	.....	.....	.....	.....	.....	.....
Lot K, 4 fruits.	Grapefruit.	....do....	19	3.7	3.5	.....	.....	.....	.....	.....	.....

It appears from Table IV that *Colletotrichum gloeosporioides* is recovered with about the same frequency as is shown in Table III, thus indicating that no positive conclusion as to the causation of tear-stain can be reasonably based on isolation tests.

All the cultural work shows that *Colletotrichum gloeosporioides* has an isolation percentage from tear-stained areas rather too low for it to be the active pathogen and that this organism is present to some extent on normal as well as badly russeted areas, the frequency varying with the degree of injury.

The fungi in various types of miscellaneous lesions on citrus fruits were examined by similar cultural methods. These lesions included spray-burn scars, hail bruises, thorn scratches, and old citrus-scab lesions. The fungous flora was very similar to that obtained from tear-stained and russeted areas. Colonies of Colletotrichum predominated, the saprophytic Cladosporium ranked next, while colonies of bacteria and various other fungi occurred in smaller numbers.

HISTOLOGICAL EXAMINATION.

To the unaided eye, rust-mite russet with its several patterns and the so-called wither-tip russet or tear-stain intergrade imperceptibly. Ordinarily the grower calls the streak tear-stain and the solid area rust-mite russet.

It was deemed important to make careful microscopic examinations to determine whether distinctive features exist in the rind tissue of the affected parts. Fruits were examined showing typical patterns of rust-mite russet as well as those showing several degrees of the so-called wither-tip tear-stain. Under the microscope there appears to be no material difference between these types of injury. The examination of the injured parts in both instances indicates that the cuticle and epidermal cells appear to be punctured, and beneath, depending upon the degree of russeting, one to three layers of cells, together with their contents, are of a rusty brown color. Quite frequently mycelial threads and spores of fungi are found adhering to the affected parts. These fungi prove to be the types commonly found on citrus, such as species of Colletotrichum and Cladosporium.

The histological examinations have not revealed any feature that would serve to distinguish between the rust-mite russet and the so-called wither-tip tear-stain.

The presence of punctures in the epidermal cells of the tear-stained areas would strongly suggest the work of sucking parasites rather than that of parasitic fungi. This suggestion is further substantiated by the following observation: In July, 1919, by the aid of a hand lens, numerous tear-stained immature grapefruits were examined while still hanging on the tree in an unsprayed grove near Orlando, Fla. The rust mites and their castings were more or less generally distributed over the fruits, but were present in especially large numbers over the tear-stained areas. This condition was very noticeable early in July. By August 2 such a marked segregation of mites in streaks was not particularly evident, and the mites themselves, as well as their castings, were nowhere present in very large numbers, but the tear stains, presumably caused by rust mites, were quite evident.

INOCULATION EXPERIMENTS.

An inoculation experiment was conducted the last week in June, 1919, on immature grapefruits which were about 2 inches in diameter and so far as could be determined free from blemishes. Fifty fruits were used in this experiment. The inoculum was derived in part from dead sweet-orange twigs which had been held in moist chambers and on which developed a copious growth of the wither-tip fungus and in part from pure cultures of *Colletotrichum gloeosporioides*,

which had been isolated from an injured grapefruit leaf. The spores were washed off the twigs and mixed with those from the culture tubes. This wash water, which was clouded with fresh viable spores, was used in saturating wads of absorbent cotton, which in turn were placed on the fruit. The inoculated fruit was covered with two or three layers of waxed paper for 48 hours. At the expiration of this time the paper and wet cotton were removed and the fruit left unprotected. To serve the purpose of a control, other fruits were similarly treated, except that the cotton was wetted with sterile water. These inoculations gave negative results.

Similar inoculation tests were made during the fall of 1919, using as inoculum a mixture of a number of strains of *Colletotrichum gloeosporioides* isolated from typical tear stains on grapefruit and from dead grapefruit twigs, as follows:

On October 20, 1919, on almost fully grown grapefruit; on October 27, on grapefruit showing faint yellowing; on November 7, on grapefruit approximately one-half colored; on November 15, on grapefruit almost fully colored; and on December 3, on mature grapefruit. This test was repeated during the late spring, summer, and fall of 1920, using mixed inoculum from the same strains of *Colletotrichum gloeosporioides*. Inoculations were made on May 15, on grapefruit averaging 1 inch in diameter; on May 21, on grapefruit averaging 1½ inches in diameter; on June 15, on grapefruit averaging 1⅝ inches in diameter; on June 30, on grapefruit averaging 2 1/16 inches in diameter; on July 15, on grapefruit averaging 2½ inches in diameter; on July 30, on grapefruit averaging 3 inches in diameter; and on November 1 and 6, on grapefruit just beginning to color.

The results of all these tests were negative. Not the slightest symptom of tear-stain in any of the fruits inoculated during 1919 could be detected as late as February, 1920, when the crop was harvested. The fruit inoculated during 1920 was free from tear-stain when final observations were made in November.

Observations were made to determine the frequency of association of tear-stain with dead twigs that might harbor Colletotrichum or other fungi. Unsprayed groves with more than the average proportion of dead wood present were examined carefully during the past four years. Among the properties inspected in Florida 4 are in Lee County, 10 in Polk County, 2 in Hillsboro County, 3 in Pinellas County, 3 in Osceola County, 10 in Orange County, 2 in Volusia County, 4 in Brevard County, 3 in St. Lucie County, and 4 in Dade County. The data obtained indicate that dead twigs, spurs, etc., are found immediately over not more than 10 per cent of the tear-stained fruit, and that in damp, densely shaded, low-hammock properties, where trees have an unusually large number of dead twigs and where environmental factors would appear to be especially favorable for the development of fungi, tear-stained fruits are very seldom found. On the other hand, tear-stain is most abundant in higher and drier locations where light and moisture favor the greatest rust-mite development.

CONCLUSIONS.

While the foregoing evidence is to the effect that tear staining of Florida citrus fruits is caused by rust mites rather than by the fungus *Colletotrichum gloeosporioides*, as claimed by Rolfs, it must be admitted that the writer may not have seen all types of this injury. However, if a special type of tear staining caused by this fungus occurs in Florida it must have been exceedingly rare during the past four years to have escaped detection by the writer. The experimental and observational data on the control of what has been regarded as wither-tip tear-stain by investigators and Florida growers seems to be definite enough to warrant the conclusion that practically all of the so-called wither-tip tear-stain in Florida is caused by rust mites and can be readily controlled by controlling these pests.

SUMMARY.

(1) Pure cultures from typically tear-stained fruit show that *Colletotrichum gloeosporoides* is not confined characteristically to the tear streaks, but is even more prevalent on the larger russeted areas and is almost invariably found on normally colored areas.

(2) Inoculations on grapefruit in various stages of development with cultures of the fungus *Colletotrichum gloeosporioides* failed to produce tear streaks or other positive reaction.

(3) Dead wood that might harbor *Colletotrichum gloeosporioides* was found associated with not more than 10 per cent of the affected fruit.

(4) It is impossible to distinguish by histological methods between the ordinary rust-mite russet and so-called wither-tip tear-stain.

(5) Rust mites were found very abundant in recently developed tear-streak patterns on grapefruit.

(6) Spring-bloom fruit on unsprayed trees usually becomes seriously tear stained; June-bloom fruit on such trees usually ripens free from these markings.

(7) Copper sprays applied in the spring and not followed by rust-mite applications of sulphur sprays tend to increase tear staining, but when followed by rust-mite applications no appreciable amount of tear staining develops.

(8) When sulphur sprays are applied during the early spring, which is usually too early for the best rust-mite control, and are not followed by the regular rust-mite application tear staining is greatly reduced.

(9) When only the rust-mite applications are made, tear staining is practically eliminated.

(10) The same local and seasonal conditions of moisture and light that favor rust-mite injury also favor tear-stain.

(11) Practically all of the so-called wither-tip tear-stain in Florida is associated with rust mites and can be readily controlled by controlling these mites.

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