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Interrelations between citrus rust mite, Hirsutella thompsonii and greasy spot on citrus in Surinam

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Abstract

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Counts of citrus rust mite *(Phyllocoptruta oleivora* (Ashm.)*)* on leaves and fruit of citrus rose to a peak in the two dry seasons, the build up taking 4-5 weeks. It then decreased partly through infection by the entomogenous fungus *Hirsutella thompsonii* Fisher and partly through a decline in feed quality. The low counts in the wet seasons were associated with rain rather than humidity, temperature or infections by *H. thompsonii*. Spraying with suspensions of fragmented mycelium of *H. thompsonii* (mass concentration 0.5-1.0 g $litre^{-1}$) prevented the build up of citrus rust mite.
The severity of greasy spot *(Stenella* sp.*)* was positively correlated with counts of citrus rust mite. Defoliation of citrus trees after greasy spot infection was associated with high counts of mite.
Control of citrus rust mite (with chlorobenzilate: mass concentration of a.i. 2 g $litre^{-1}$ at 500 litre ha^{-1}) was warranted when 25% of fruit or 15% of leaves bore at least one mite per lens field (1.5 cm^2). Greasy spot could be controlled by preventing build-ups of citrus rust mite.

Contents

1 Introduction

1.1 CITRUS CULTURE

At present citrus ranks third among the important crops of Surinam, being exceeded only by rice and bananas. The principal citrus varieties are oranges (Valencia and Kwata varieties) and grapefruit (Marsh Seedless). Lemons, limes, mandarines and other varieties are also grown, but are not important commercially. Most of the citrus trees are graftings on sour orange rootstock. In 1972, the total citrus area was estimated to be 1 821 ha, 76% of it was covered with oranges, 21% with grapefruit and 3% with lemons, limes, mandarines and other citrus. In the same year, about 10 000 tons of oranges, 6 000 tons of grapefruits and 200 tons of fruits of other citrus varieties were produced, of which about 20%, 13% and 70% was exported, respectively. A great part of the production was not fit for export, because of fruit blemishes, particularly those resulting from infestation by the rust mite *Phyllocoptruta oleivora* (Ashm.).

Citrus is grown mainly in the low coastal region where the great majority of the Surinam population lives. The soils of this region are mainly clay, with a pH ranging from 4-4.5. They are far from optimum for citrus cultivation. Therefore the growing of citrus on higher situated sandy loam soils in the interior of the country has been studied since 1960 (see below). In the coastal area, citrus, like coffee, cacao and sugar-cane, is found chiefly as a plantation crop. Besides, small numbers of trees can be found in many a private garden. The citrus plantations are characterized by an intricate system of irrigation canals and drainage ditches. Sluices are in operation to drain off excess water to the river at low tide. In most plantations, citrus trees are grown on narrow, closely spaced, single or double row beds and consequently the use of tractor-operated weeders, sprayers and harvesters, is hampered. In general, the trees are in a moderate to poor condition. Besides negative factors such as inadequate bedding and drainage, pests and diseases can most seriously hamper normal plant growth.

Rust mites, scale insects and aphids are the more important animal pests in Surinam (van Dinther, 1960; Samson, 1966). However, the detrimental effects of

Fig. 1. Orange leaf partially infected with greasy spot.

these pests are often ignored and control measures are omitted. Rust mite injury to the leaves results directly in weakening of the trees and indirectly in increased defoliation (Chapter 4). Young trees are sometimes killed after an outbreak of scale insects. Flushes are attacked by aphids and leaves remain small and shrivelled.

Among the diseases which contribute to the suboptimum and often poor condition of the citrus trees, greasy spot is most important. This disease, commonly known as a leaf disease, can also infect the branches of some citrus species (Fisher, 1961) and the fruits (Whiteside, 1970). Symptoms of greasy spot found in Surinam do not differ from the descriptions of greasy spot symptoms in the American literature (Knorr et al., 1957; Fisher, 1961). Infected leaves exhibit yellow-brown to black spots with a greasy gloss. The spots occur more frequently on the lower surface of the leaves, where they look slightly raised (Fig. 1). Individual spots often join, thus forming irregular zones. The top leaves of a shoot were more severely infected, while the infection decreases towards the base of the shoot. Symptoms have never been observed on immature leaves.

That successful cultivation depends on the control measures against pests and diseases has so far been demonstrated by the growing of citrus on a 300 ha cleared sandy loam soil in the somewhat more elevated wooded interior of Surinam at 'Baboenhol'. These organisms may damage the foliage considerably, thus affecting assimilation and causing retarded and poor growth.

1.2 CITRUS RUST MITE – HIRSUTELLA – GREASY SPOT COMPLEX

Since 1966, I have studied the phenomenon that the citrus rust mite, *Phyllocoptruta oleivora*, in Surinam builds up its population in a short time and that soon after the maximum infestation is reached, mite numbers drop almost to zero. This phenomenon proved to be caused by a complex of factors, one of which is the entomogenous fungus, *Hirsutella thompsonii* Fisher.

When studying the citrus rust mite, I was confronted almost simultaneously with the occurrence of greasy spot, an important leaf disease of citrus, first described in the United States in 1961 under the name *Cercospora citri-grisea* Fisher (Fisher, 1961), which was superseded by *Mycosphaerella citri* Whiteside (stat. con. *Stenella* sp.) in 1972 (Whiteside, 1972). Although several workers (Pratt, 1958; Fisher, 1961) were aware that greasy spot was influenced by rust mite infestations, the relationship between rust mite and greasy spot had never been fully unravelled and proved.

To elucidate the total biotic complex 'rust mite – *Hirsutella* – greasy spot', I started an ecological study of the rust mite (Chapter 2). I investigated *Hirsutella thompsonii*. Its effects on rust mite populations were tested with laboratory reared fungal material (Chapter 3). Finally experiments were carried out to elucidate whether rust mites play a role in greasy spot infection (Chapter 4).

1.3 CLIMATE

Surinam, situated on the north-eastern coast of South America between 2 and 6 ^{o}N, has a tropical rainy climate. During a great part of the year the trade-wind blows inland from the ocean so that hot spells are few. Strong winds are rare; the mean wind speed at Paramaribo is 1.4 (Beaufort scale).

The annual mean temperature is about 27 ^{o}C. The warmest month is September, averaging 31.6 ^{o}C and the coolest January, averaging 25.6 ^{o}C. In Paramaribo, the mean daily maximum temperature is 30.9 ^{o}C, the mean daily minimum 22.7 ^{o}C. Individual minimum temperatures below 21 ^{o}C are rare.

For Paramaribo, the annual mean percentage of sunshine is 57%. In August, September and October, the percentages of sunshine are highest, averaging 72%, 78% and 76%, respectively. The relative humidity of the air is generally rather high. For Paramaribo, daily averages range from 78% in October to 87% in June, with minima ranging from 51-62%. The annual precipitation is about 2 200 mm. The year can be divided into four seasons: a main rainy season from April to the middle of August, a main dry season from the middle of August to the end of November, a minor rainy season during December and January, and a minor dry season from the beginning of February to the end of March. The main seasons are fairly reliable, the minor seasons on the contrary are highly variable. The wettest months are May and June, with a mean precipitation of 232 and 321 mm, respectively. The driest months are September and October, averaging 86 and 87 mm, respectively. The greater part of Surinam belongs to Köppen's Class of tropical rain-forest climates which are characterized by a rainfall of at least 60 mm in the driest month.

2 The citrus rust mite

2.1 ORIGIN AND GEOGRAPHIC DISTRIBUTION

The original habitat of the citrus rust mite is thought to be South East Asia, where citrus is indigenous. The species has been introduced – in all probability with imported fruit or planting material – in many citrus-growing countries. It is now reported as a serious pest from all citrus growing areas in the world. In some countries, citrus rust mite has long been known, e.g. in Florida, before 1879 (Yothers & Mason, 1930). Other records are: Jamaica, 1916 (Ritchie, 1916); Cuba, 1918 (Johnston & Bruner, 1918); Australia, 1926 (Newman, 1926) and Brazil, 1929 (Bondar, 1929). More recent records are: Cyprus, 1940 (McDonald, 1945); Israel, 1944 (Swirski & Amitai, 1958); Tanganyika, Kenya and Mauritius, 1955 (Hall, 1956); Bulgaria and Yugoslavia, 1962 (Tsalev, 1963; Kosač, 1964); Paraguay, 1969 (Aranda & Flechtmann, 1969) and Nepal, 1971 (Knorr & Moin Shah, 1971).

In Surinam, citrus growers and researchers of the Agricultural Experiment Station became aware of citrus rust mite in 1945 when the export of fresh fruit began. The rust mite occurs throughout the country wherever citrus grows, including the new experimental grove at Baboenhol, in the hinterland. Here the mite was introduced with planting material originating from nurseries in the coastal region.

2.2 TAXONOMIC HISTORY

The citrus rust mite, belonging to the family Eriophyidae (Order Acarina), was first mentioned by Ashmead in 1879 as *Typhlodromus oiliivorus*. However, in 1880, Ashmead in his 'Orange Insects' emended his first spelling into *Typhlodromus oleivorus*. This emended name was overlooked by several workers who used specific names as: *oliivorus*, *oilivorus* and *oil-livorus*. Generically it has also been referred to *Eriophyes* (Ewing, 1923). Banks (1907), in his 'Catalogue of the Acarina of the United States', was the first to mention it under the name of *Phyllocoptes oleivorus* (Ashmead). In 1938, Keifer erected a new genus, *Phyllocoptruta*, and since then the citrus rust mite has been called *Phyllocoptruta oleivora* (Ashm.).

2.3 ECONOMIC IMPORTANCE

Citrus rust mite infests every *Citrus* species growing in Surinam. When climatic conditions are favourable, a rapid population increase results in a serious damage to foliage and fruits in a short time. Infested fruits have discoloured skin (fruit-russeting), which make them unfit for export.

Yothers & Mason (1930) reported that in Florida rust mite infestations, besides russeting (lowering of the fruit-grade), result in reduction of the size and increase of evaporation of the water content of the fruits, sunburn, more rapid decay and retarded ripening of the fruits, reduction in size of the leaves and considerable devitalization of the tree as a result of damage to the branches that have not yet reached maturity.

In grapefruit orchards in Surinam, 10-80% of the fruits become russeted, 40% being a rough mean. Since fruit damaged by rust mite is not suitable for export and since grapefruit is grown in Surinam mainly for export, the 40% fruit injury equals a 40% loss. Since 15 million grapefruit were exported in 1972, assessed at 895 000 Surinam guilders, the loss due to rust mite injury can be estimated to be some 600 000 Surinam guilders annually. Russeted oranges, however, can be sold on the local market. Actually, the loss is much greater, because apart from the damage to the fruit, the damage to the foliage weakens the trees. Furthermore, greasy spot infections, which may defoliate the trees, are promoted by the presence of the rust mites on the foliage (Chapter 4). Undoubtedly citrus rust mite is the most important animal pest of citrus in Surinam.

2.4 HOST PLANT PREFERENCE

Wild species of the genus *Citrus* (fam. Rutaceae, subfam. Aurantiaceae) and related genera of the same subfamily occur throughout India, Malaya, South China, South Japan and the Malayan Archipelago; a few plant species extend to Africa and Australia (Bodenheimer, 1951). I could not trace data on host preference in those countries. Therefore differences observed only refer to cultivated citrus species.

Yothers & Mason (1930), who listed many citrus species and varieties grown in Florida, observed the following order of severity of infestation: lemon > lime > citron > grapefruit > sweet orange. They reported that the nearer varieties and hybrids are related to a 'true' *Citrus* species, the more favourable these plants are for mite development. They found no rust mites in the following species of Rutaceae: *Severinia buxifolia, Chalcas exotica, Toddalia lanceolata, Glycosmis pentaphylla* and *Aeglopsis chevalieri.*

In Surinam I repeatedly found that in grapefruit orchards higher overall mite populations develop than in orange orchards. The effect of developmental stage of the host tree or part of the host tree on rust mite preference is described in Section 2.7.6.

2.5 INJURY

2.5.1 Injury to the fruit

Rust mites, having piercing mouthparts, damage fruits by puncturing the epidermal cells of the peel. Cell layers beneath the epidermis can also become injured in a later phase of infestation. Figure 2 shows a rust mite population build-up on a grapefruit growing on a potted tree, placed outdoors but well protected from showers. The first symptoms of mite attack, visible to the naked eye as a brownish stipple, mainly located in the small depressions of the skin, appeared about three weeks after the beginning of the increase in the mite population. Gradually, symptoms become more pronounced, and after about four to six weeks the infested parts show the characteristic rusty-brown coloration. Discolouring, caused by the mite-piercing activities, is generally called

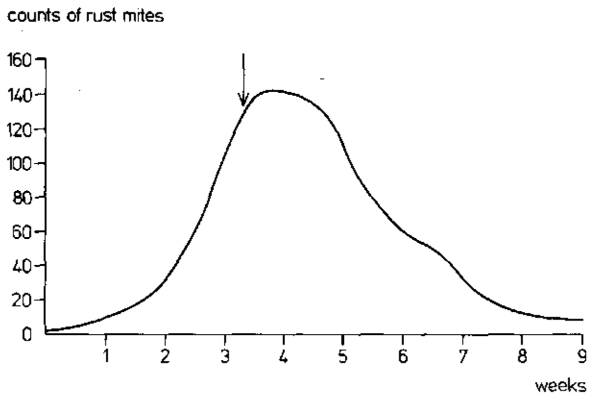

Fig. 2. Citrus rust mite population on a grapefruit (2 August – 30 September 1967), growing on a potted plant in relation to the appearance of russeting (see arrow). The plant was kept in the open, protected from rain. Weekly counts of mite on 1.5 cm^2 orange skin averaged for all quadrants of the fruit.

Fig. 3. Grapefruits with lesions caused by citrus rust mite known as 'tear stain'.

'russeting'; this term holds for injury to the fruits as well as for injury to the leaves. As a rule, infested grapefruits manifest a yellow-brown and oranges a chocolate-brown to black colour, whereas lemons show a silvery-grey gloss. A peculiar symptom, designated by Yothers & Mason (1930) as 'tear stain' has also been observed in Surinam (Fig. 3). Fruits developing on a heavily mite-infested tree may be invaded by so many mites that they look dusty because of the countless numbers of cast mite-skins that remain on the fruit after each moult.

Fig. 4. Grapefruits partially infested with citrus rust mite.

A typical aspect of rust mite injury is that on a mite-infested tree only a certain number of the fruits become heavily attacked, whereas the other fruits are only slightly damaged, if at all. Even on a single fruit, the rust mite tends to infest only a portion of the fruit, leaving the rest undamaged (Fig. 4). Such partial infestation is most probably due to the presence of dew over a certain region of the fruit during the night and early morning. Mites were absent on such dew-covered portions.

I tried to determine the number of mites needed to produce russeting within a certain time. Mite numbers of 2, 5, 10 and 20 were transferred respectively to the fruits of potted grapefruit plants, where they were placed inside small paper rings (diameter 1.5 cm; Section 2.7.1) fixed on the peel. A minute brown stippling could be observed under a binocular microscope (20x) only in the groups of 10 and 20 mites after 5 weeks. After 3 months symptoms visible to the naked eye were still lacking. Since the foregoing method failed to produce the wanted information, the two following field trials were carried out in a grapefruit orchard 15 years old.

(1) Four trees, where rust mites were counted on 50 fruits per tree twice a week, were treated with chlorobenzilate, which stopped further mite development, when there were mean counts of 5.9, 15.5, 24.5 and 49.2 per x10 lens field per fruit. Percentages of russeted fruits were determined a month later, at fruit ripening. Figure 5 shows the correlation between rust mite density and percentage infested fruit. This correlation turns out to be linear for mite densities up to about 25.

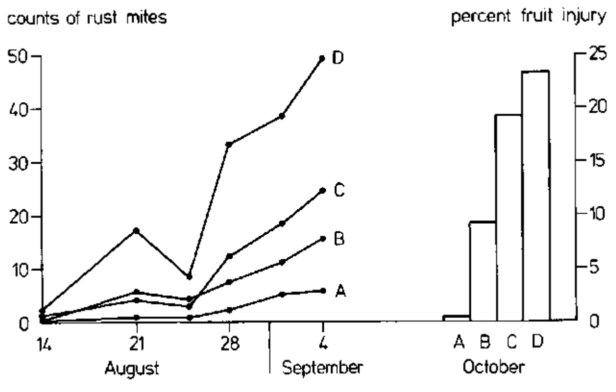

Fig. 5. Relation between counts of rust mite on fruits and percent fruit injury for 4 grapefruit trees (A, B, C, and D). Counts of mite every 3 to 7 days on 1.5 cm^2 orange skin, averaged for all quadrants of 50 fruits. Trial at Geyersvlijt, 1970.

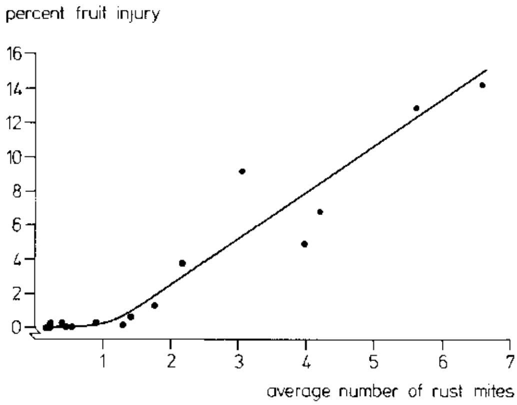

Fig. 6. Relation between counts of rust mite on fruits of grapefruit trees and percent fruit injury. Counts of mite represent the average number of mites per x10 lens field (1.5 cm^2) per fruit per observation (50 fruits per tree and 16 observations in 8 weeks). Trial at Geyersvlijt, 1970.

(2) On 15 trees, chosen at random, rust mite was counted as described under 1. However, mites were left developing till about 4 weeks after they had reached a peak. Rust mite counts thus covered a period of 8 weeks (16 observations). From the 16 observations, the average number of mites per lens field per observation was determined for each tree. Percentages of infested fruits were determined for each tree during picking. The results are given in Figure 6. The percentages of infested fruits increased with increase of mite densities. Figure 6 also shows how fruit injury may vary from tree to tree (each point represents a tree).

2.5.2 Injury to the leaves

Leaves on which rust mites are present in large numbers, show the same dusty appearance as mentioned for mite-infested fruit. The first symptoms of injury are characterized by a rough brown to black speckling which may occur on each of the two leaf surfaces. Russeting also occurs on the leaves, but this type of injury is less common there than on fruits. Partial russeting, as described for fruits, also occurs on leaves (Fig. 7). This type of leaf infestation seems not to be restricted to the citrus rust mite, since partial leaf infestation was also found for a not yet identified rust mite species infesting West Indian cherry, *Malpighia punicifolia* (Fig. 8).

Figure 9 shows a cross section through a healthy leaf (above) and through a leaf with russeting symptoms (below). Epidermal cells of the healthy leaf

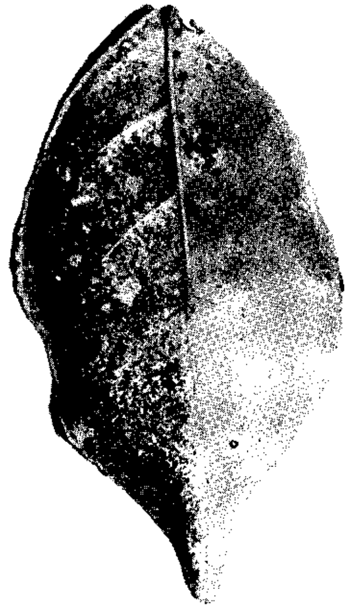

Fig. 7. Orange leaf partially infested with citrus rust mite.

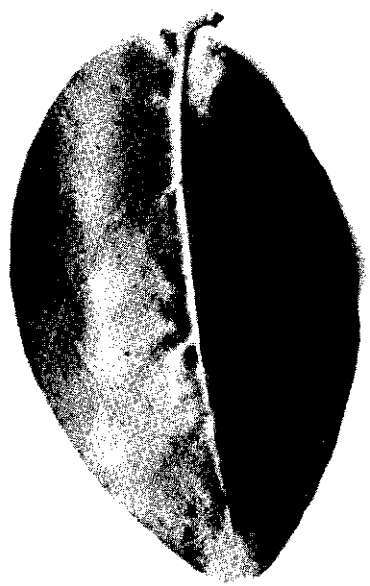

Fig. 8. Leaf of West Indian cherry *(Malpighia punicifolia)* partially infested with an unidentified rust mite species.

Fig. 9. Above: Cross-section through a healthy orange leaf with rectangular epidermal cells. Below: Cross-section through an orange leaf damaged by citrus rust mite; epidermal and subepidermal cells have been destroyed.

are rectangular, whereas in the injured leaf the epidermal cells and also some of the layers of cells situating directly under the epidermis, are destroyed. Even on leaves, which did not show symptoms of russeting, but had a dusty appearance only, almost all the epidermal cells were found to be destroyed.

Infested leaves had a stunted growth and assimilation would have been affected considerably. Because injured leaves lost much of their waxy covering, evaporation increased. Moreover, the presence of rust mites on the leaves leads to severe greasy spot infections, which result in defoliation of the trees (Chapter 4).

2.6 DEVELOPMENTAL STAGES

2.6.1 The egg

The spherical, smooth citrus rust mite egg, of large diameter of about 25 µm, is semitransparent when freshly laid. It becomes less hyalinous soon afterwards. In general no more than two well developed eggs occur in the abdomen of the female at one time. Eggs are deposited, predominantly in groups, on the fruits as well as on both leaf surfaces. An adhesive substance firmly fixes the egg to the plant. Oviposition site preferred were depressions in leaf and fruit surface and to a lesser extent areas adjacent to leaf veins. In the experiments mites inside small paper rings that were attached to the fruits (Section 2.7.1) laid their eggs mainly near the paper wall.

2.6.2 Larval stages 1 and 2

The newly hatched semitransparent larva 1 starts feeding almost immediately. Only after some time does it commence wandering. Gradually the colour turns paler yellow. The dorsal shield design, so characteristic in the second instar larva and the adult form, is obscure. Genitalia are poorly developed and genital setae are absent. Body size increases considerably during development, from a length of 70 µm to 110 µm (average 95 µm). A few hours before molt, the larva enters a motionless state until the skin cracks anteriorly. The posterior end of the skin remains adhered to the substratum, which facilitates the emergence of larva 2. Fixation of the posterior end is probably brought about by an adhesive, as the exuviae are not easily washed off by rainshowers.

Larva 2 has a lemon-yellow colour. Its body length is 100-150 µm (average 130 µm). The larva resembles the adult superficially; the dorsal shield design becomes distinct, and the genitalia and genital setae can be distinguished.

2.6.3 The adult

Like the larvae, the adult mite is elongated and wedge-shaped. Its length is 135-170 µm (average 155 µm). Young adults are lemon-yellow, older mites are darker-yellow to brown. Larvae or young adults of brownish colour are attacked by the entomogenous fungus, *Hirsutella thompsonii* (Section 3.1). The dorsal shield design proves to be constant and seems characteristic for the species (Fig. 10). The chelicerae, about 25 µm in length, are modified as stylets, each being

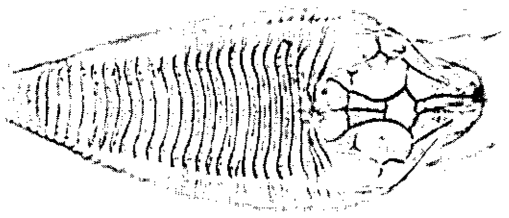

Fig. 10. Adult citrus rust mite; dorsal view.

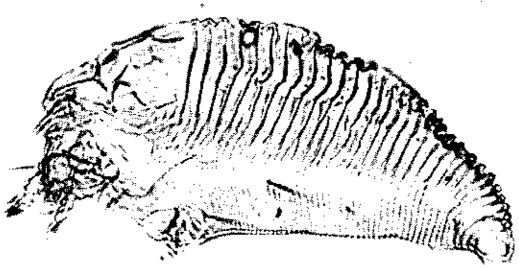

Fig. 11. Adult citrus rust mite; lateral view.

inserted on a knoblike base. During wandering, the chelicerae are raised vertically. The idiosoma (body posterior to mouth region) consists of 28 tergites and about 60 sternites, each of which grows smaller toward the posterior end (Fig. 10 and 11). The tergites and sternites are furnished with microtubercles, along the folds. Six pairs of setae are present on this body part (Fig. 11); for a detailed description of their positions, see Keifer (1938). Each of the 4 legs bears four setae. A pair of suckers is sited on the last abdominal segment. These lobe-like organs become fixed onto the substratum during feeding and so facilitate piercing. Moreover, this method of anchoring the body prevents the mite from being washed off by rain.

Keifer (1938), in his 'Eriophyid Studies' gives a clear description of both males and females, with an illustration of the differences in genitalia. In Surinam, males have never been observed (see also Section 2.7.3).

2.7 LIFE HISTORY

2.7.1 Life cycle on the fruit

The earliest attempts to rear the citrus rust mite in the laboratory were made by Yothers & Mason (1930) in Florida. A small open capsule of gelatin secured to a fruit with hot paraffin formed the most satisfactory cage for confinement of the mites. The stem was placed in water to keep the fruit in good condition. Adult mites were transferred to fresh fruit as soon as the older fruit began to dry. In Israel, Swirski & Amitai (1957) used the same method. They used celluloid cells that were attached to the fruits of rooted twigs or small branches of lemon. In some cases, several generations could develop on a single fruit. Reed et al. (1964) reared the citrus rust mite in conditioned rooms in the United States. Mites were confined inside a ring of lanolin placed on a leaf or fruit.

In Surinam, I found a mini-cage made of parchment-like paper attached to the fruit with melted paraffin around the outside most satisfactory (Fig. 12). In accordance with Yothers & Mason (1930), the cage, measuring 1.5 cm in diameter and 0.5 cm in height, was left open, as the citrus rust mite cannot live in a closed chamber without ventilation. In the laboratory, rust mites were reared on freshly picked, almost mature, grapefruits, whose skin was still green. Besides, mites were also reared on oranges of potted graftings (sour-orange rootstock) that were placed outdoors well protected from showers. These plants received direct sunlight

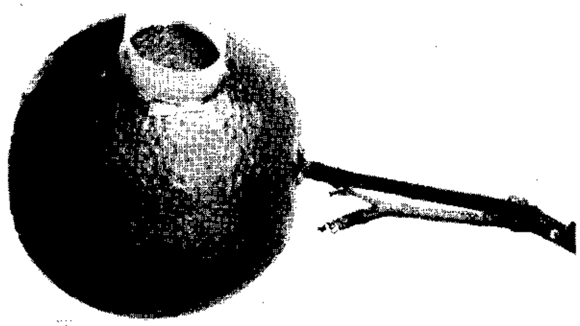

Fig. 12. Paper ring cage, fixed on a fruit of a potted plant, for rearing citrus rust mite.

till about 10 h 00 and remained the rest of the day in the shade. Mites were reared during the rainy and during the dry season. All observations, with a binocular microscope, were based on the individual development of one mite per cage. For this purpose, 3 full-grown mites were initially transferred into the cage on a human hair. As soon as one or more freshly laid eggs were detected the mites were removed and one egg was permitted to develop.

Table 1. Length of the various life stages (in days) of the citrus rust mite on picked grapefruits in the laboratory. Mean daily temperature: 27°C – rainy season.

	Egg	Larva 1	Larva 2	Egg-adult	Preoviposition period	Egg-egg	Adult longevity
Mean	3.2	1.4	1.4	6.0	1.8	7.8	5.2
S.E.	0.100	0.096	0.094	0.163	0.187	0.136	0.649
n	28	28	28	28	16	16	16

Table 2. Length of the various life stages (in days) of the citrus rust mite on oranges growing on potted trees at Paramaribo (Surinam) and on picked oranges at Orlando (Florida) and on lemon fruits growing on rooted branches at Rehovot (Israel). Meteorological data at Paramaribo, temp., mean 27, mean max. 30.9, mean min. 22.7°C; at Orlando, temp. for May-July (summer), mean 27, mean max. 33.5, mean min. 20.5°C; and for January (winter), mean 17, mean max. 25.1 and mean min. 8.9°C.

	Egg	Larva 1	Larva 2	Egg-adult	Preoviposition period	Egg-egg	Adult longevity
Paramaribo – rainy season							
Mean	3.1	1.5	1.3	5.9	3.0	9.3	15.2
S.E.	0.146	0.125	0.125	0.193	0.577	0.750	0.479
n	17	17	14	12	4	4	4
Paramaribo – dry season							
Mean	3.1	1.4	1.3	5.7	2.2	8.0	10.1
S.E.	0.078	0.076	0.078	0.144	0.222	0.289	1.752
n	40	40	32	21	9	9	9
Orlando – summer (from Yothers & Mason, 1930)							
	2-4	1-3	1-3	–	1-4	–	–
Mean	3.1	1.82	1.34	6.17	2.66	8.83	6.89
Orlando – winter (from Yothers & Mason, 1930)							
	4-8	3-6	4-13	–	3-7	–	–
Mean	5.53	4.30	6.40	16.23	5.0	21.23	11.30
Rehovot – mean temp. 26°C, experimental conditions (from Swirski & Amitai, 1958)							
	2-4	2-4 (L_1+L_2)		–	–	–	–
Rehovot – mean temp. 17°C, experimental conditions (from Swirski & Amitai, 1958)							
	5-9	4-8 (L_1+L_2)		–	–	–	–

The duration of the various developmental stages on picked grapefruit in the laboratory is given in Table 1, and on oranges growing on potted trees during the rainy season or the dry season in Table 2. These potted trees, kept in the open, were well protected from rain. In several cases reared adults did not survive to egg-laying and often died prematurely. No reproduction was recorded on picked fruits that were used longer than two weeks. This was probably due to withering of the fruits. Variation in developmental duration of the egg, larva 1 and larva 2 was similar for the mites reared either on picked grapefruits in the laboratory or on oranges growing, protected from rain, in the open. The difference between the life cycle on oranges during the rainy season (9.3 days) and the dry season (8.0 days) was not significant (Wilcoxon test; $P > 0.05$). A life cycle of about 8 days can be accepted as optimum, i.e. shortest period. Table 2 gives data on the duration of the various mite stages on oranges in Surinam, and in Florida and Israel. The effect of temperature is distinct.

2.7.2 Life cycle on the leaf

There seemed to be no data in the literature on the duration of the developmental stages of the citrus rust mite on leaves. Yothers & Mason (1930) mentioned that leaves and stems were unsuitable for rearing the rust mite in confinement, since these plant parts soon withered. Swirski & Amitai (1958) reported that citrus rust mites were reared on fruits and on leaves of lemon. Although a description of the rearing technique on leaves was given, data on the life cycle of the mite were not published. In Surinam, mites could not be reared on leaves in a similar way to that used with fruits. Cages were difficult to attach and fell off easily. Although another method, by which fluon was pencilled with a fine brush on the orange leaf in the form of a barrier ring, was far from satisfactorily, a few mites could be reared from egg to adult in this way. The duration of the developmental stages of egg, larva 1 and 2 and egg → adult are 3.3 ± 0.213, 2.7 ± 0.211, 2 ± 0.316 and 7.8 ± 0.200, respectively.

The two larval stages are both longer than for mites living on fruits. Therefore mites can develop more rapidly on fruits than on leaves. Indeed, when inspecting a citrus orchard, one always finds more mites on fruits than on leaves.

2.7.3 Reproduction

In Surinam, the citrus rust mite reproduces parthenogenetically as an obligate thelytoky; males being absent. From Israel, Swirski & Amitai (1959) report the coexistance of a bisexual reproduction, giving rise to both sexes, and parthenogenesis in which only males developed from unfertilized eggs. Parthenogenesis has also been noticed among citrus rust mites in Lebanon (Nasser, 1954).

In Surinam, I determined egg-production capacity in young adults kept individually in small cages (Section 2.7.1) on picked grapefruits under laboratory conditions and on oranges on potted plants growing outdoors well protected from rain (Table 3). Maximum egg-production was 26 over a period of 16 days.

In Florida, daily egg-production also amounted to 1 or 2 as a rule; exceptionally up to 5 were laid (Yothers & Mason, 1930). In California 2-3 eggs were laid per female per day (Binney, 1934). A maximum egg-laying capacity of 29 eggs over a period of 20 days was observed in Florida (Yothers & Mason, 1930). For Yugoslavia, Kosač (1964) reported a maximum of 30 eggs. Swirski & Amitai (1959) mention a maximum production of 26 eggs during 20 days in Israel. They also noticed that at 26 °C, half the eggs were laid during the first quarter of the life of the female.

In Israel, Swirski & Amitai (1958) found that the incubation period of the egg and the total larval time were greater when mean temperature was less than 24 °C. The slowing of development at lower temperatures was also reported by Reed et al. (1964). They found 27 °C to be optimal for rearing the rust mite in the United States. In Surinam, the mean daily temperature is 27 °C, Since temperatures fluctuate only slightly during the year, this factor would not have much effect on the development of the rust mite.

Under the tropical conditions of Surinam there are about 40 generations of

Table 3. Reproduction of the citrus rust mite on picked grapefruits in the laboratory during the rainy season (A), and on oranges on potted plants growing outdoors under a shelter during the rainy (B) and during the dry season (C).

	Longevity of adult (days)			Number of eggs		
	A	B	C	A	B	C
	5	14	16	7	18	21
	6	6	13	11	8	14
	4	16	14	7	21	15
	6	16	18	9	26	18
	3	15	6	3	21	5
	8	-	7	14	-	9
	8	-	5	16	-	10
	8	-	-	15	-	-
Average	6.0	13.4	11.3	10.2	18.8	13.1
Average per day				1.7	1.4	1.2
S.E.				0.110	0.062	0.145

rust mite annually. In subtropical countries the period of the life cycle increases towards the winter and therefore fewer generations can be expected. In Israel, 28 generations were recorded for the Rehovot area; in those regions where the temperature does not drop below 9.2 °C – the threshold of development for eggs and larvae – at least 30 generations can be expected yearly (Swirski & Amitai, 1958).

2.7.4 Effect of rainfall and relative humidity

Counts of citrus rust mite at different times of year were compared with fluctuations in rainfall and relative humidity. Figure 13 shows how the population build-up of the rust mite coincides with the beginning of the dry seasons (September, February). In Surinam, two periods of maximum mite activity can be distinguished each year. Maximum numbers are reached in 4-5 weeks, and numbers drop to a low level also in 4-5 weeks. Mites are scarce during the rainy seasons.

In general, dry weather is favourable for the development of the rust mite (e.g. Johnston & Bruner, 1918; Yothers & Mason, 1930; Watson & Berger, 1932), though some authors mentioned that dry weather adversely affected rust mite populations (Dean, 1959; Vergani & Valsangiacomo, 1961). In Florida, Muma (1955) found that the increase in rust mite almost coincided with increase in rainfall. Most data from Surinam agree with those of Yothers & Mason (1930) for the summer in Florida. The only difference is that Yothers & Mason observed the quick decline after the maximum coincided with the rainy season, whereas in Surinam the decline occurred in the dry season.

Relative humidity has been mentioned by some of the foreign workers to encourage citrus rust mite. Thus, Dean (1959) and Reed et al. (1964) reported that high relative humidity favours mite development. Puzzi & Veinert (1968) noted from Brazil that rust mite increased in number with high relative humidity. Since these observations are contradictory to those from Surinam (Figure 13), I performed an experiment to elucidate the role of the relative humidity.

Four potted fruit-bearing orange plants were placed in the open under a rain shelter and a group of 4 similar plants was put nearby but outside the shelter. Rust mites on the sheltered trees show a rapid population build-up in contrast to the mites on the trees in the open (Fig. 14, below). Since differences in relative humidity between the two experimental areas were small (Fig. 14, above), rust mite populations were not affected by relative humidity under the tropical conditions of Surinam. Fluctuations in population density are therefore very likely the result of rainfall.

To resolve the indirect or direct role of rainfall in keeping down mite popu-

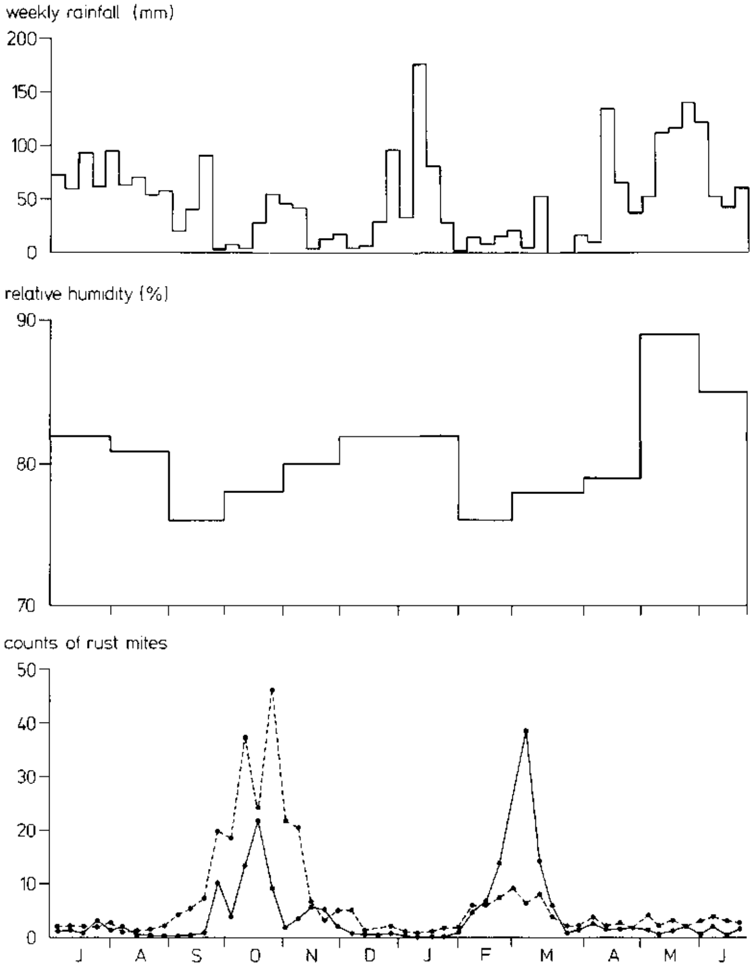

Fig. 13. Rainfall, humidity and counts of citrus rust mite on grapefruit, July 1966 – July 1967.
Top. Weekly rainfall (mm) at Geyersvlijt.
Middle. Relative humidity, monthly averages, recorded at Paramaribo (about 8 km from Geyersvlijt).
Bottom. Counts of rust mite on 5 cm^2 skin of each quadrant of 25 fruits (solid line) and on the whole undersurface of 25 leaves (broken line) from 5 trees in a grapefruit orchard at Geyersvlijt.

lations during the rainy seasons, the following hypotheses were considered:

- rainfall and moist conditions favour the development of *Hirsutella thompsonii* (Chapter 3), a parasitic fungus of the citrus rust mite
- mites are washed off by rain
- mites drown in rain drops

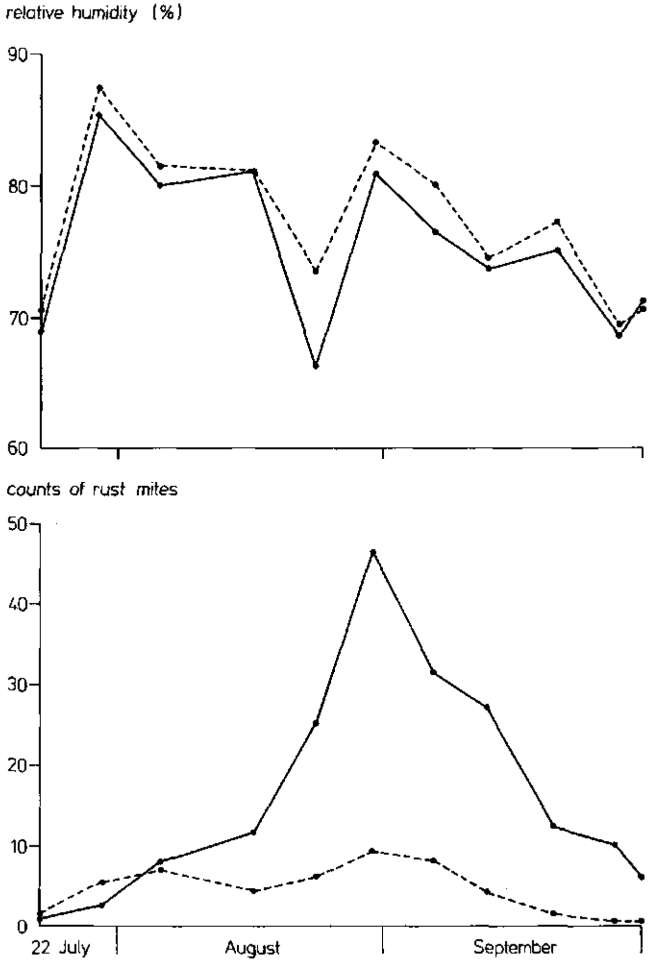

Fig. 14. Relative humidity and counts of citrus rust mite on groups of 4 orange plants in a shelter (solid lines) and exposed to rain (broken lines) in a trial at Paramaribo, 22 July – 30 September 1974.
Above. Daily relative humidity averaged from readings at 08h00, 13h00, 18h00 and 21h00.
Below. Weekly counts of mite on 1.5 cm^2 orange skin averaged for all quadrants of 9 oranges.

- rain increases larval mortality
- rain prevents oviposition.

To evaluate 'rain favours *Hirsutella*', the effect was studied of artificial rain on populations of the citrus rust mite that developed on copper-treated and untreated plants. Two series (A and B) of 8 potted orange plants about two years old were placed outdoors under a rain shelter. Plants were sprayed for about 2 min from an 8-litres plastic bucket, with 19 evenly distributed small holes (diameter 0.8 mm) in the bottom (diameter 18 cm). The bucket was hung about 2 metres above the tops of the orange plants. The spray corresponded to rainfall of about 15 mm.

Figure 15 (above) shows the highly depressing effect of spraying on the mite

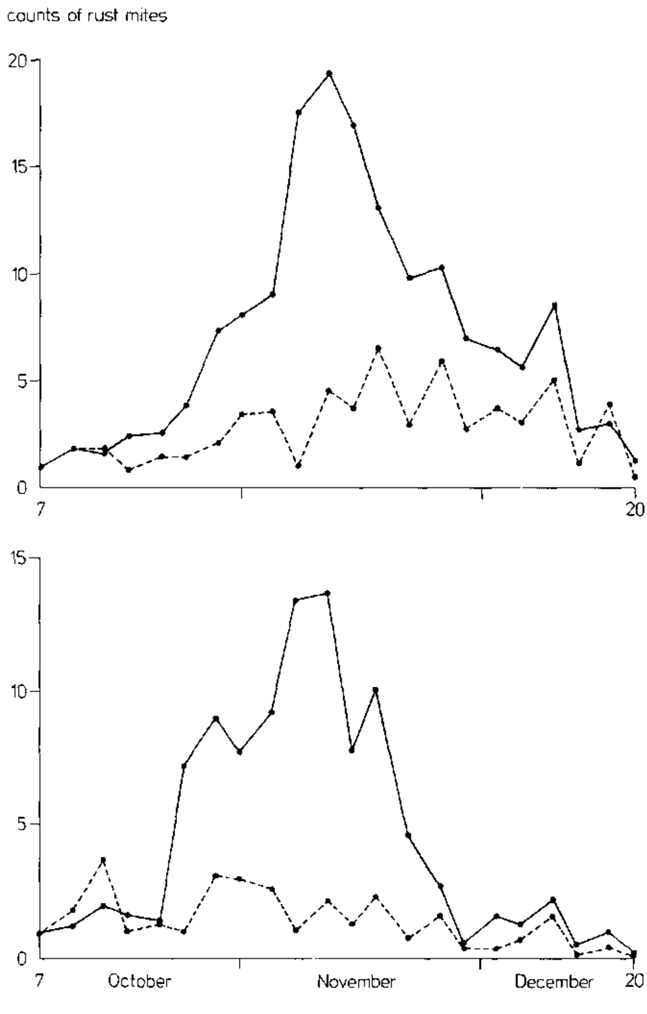

Fig. 15. Counts of citrus rust mite on 1.5 cm^2 leaf surface of potted young orange plants exposed 3 times a week to artificial rain, 15 mm, (broken line) or unsprayed (solid line).
Counts were on both surfaces of 10 leaves of each of 4 plants per group. Trial at Paramaribo, 7 October – 20 December 1974.
Above. Series A: plants not treated with fungicide.
Below. Series B: foliage of plants dipped in tribasic copper sulphate, mass concentration of a.i. 2 g $litre^{-1}$, in the 2nd week of the trial and again half way through the trial.

population and the distinct population build-up in unsprayed plants. Broadly, both curves are like those for mite population in the field during the rainy and dry seasons (Fig. 13). The same trial included copper-treated plants, since citrus rust mite explosions occur after application of a copper fungicide (copper eliminates *H. thompsonii*). If the low population density during the rainy season could be attributed to infections of the rust mites by the entomogenous fungus, *H. thompsonii*, rather than to the direct effect of rain, a rust mite population increase would be expected on those sprayed plants that had also been treated with fungicide. The lower graph shows that this was not so. The curve for the sprayed plants that had also received a copper-dipping was similar in shape to that for the sprayed plants that had not been treated with the fungicide (above). Therefore the low mite counts during the rainy seasons are not brought about by the action of the entomogenous fungus, *H. thompsonii*.

No indication has been found as to a washing-off effect of rains, either in the field where mite-infested leaves were inspected before and after rainfall, or in the water-spraying experiments. These observations are in accordance with the findings of Yothers & Mason (1930), who reported that rust mites were not washed off from the leaves and fruits by a heavy shower. Whether or not rain has a direct effect in reducing mite numbers by drowning I studied as follows:
Drops of water were pipetted onto adult mites on leaves. Mites under water made no effort to escape but remained anchored, creeping away only when the water was removed with a filter paper. In Test 1, 21 rust mites were submerged for 9 h; 20 mites survived. In a similar Test 2, 25 out of 28 mites survived 12-h submersion. Since the vast majority of mites withstand submersion for at least 12 h, drowning by rain is of little importance.

To check whether rain increases larval mortality, the following test was made. Three fruit-bearing potted orange plants were placed in the laboratory where a mini-cage (Section 2.7.1) was attached to one fruit of each plant and 100 adult mites were introduced per cage. After 2 days, many eggs had been deposited and the mites were removed. Once the larvae started hatching, the caged surface of 2 fruits was kept moist by regular spraying with an atomizer. The remaining cage was not treated. A week later, 43 mites were present in the control, against 13 and 5 mites in the treated cages, respectively. Therefore larval mortality increases when larvae are wetted and a water film is present on the food plant. A moist substrate could interfere with molting, since the larva needs firm anchorage of its posterior to the plant.

There is evidence too that rain interferes with oviposition. Citrus rust mites avoid egg-laying on parts of a plant that are frequently covered with dew

(Section 2.5.1). Rain may markedly increase larval mortality and decrease egg-laying.

2.7.5 *Effect of light*

To determine the effect of light on the distribution pattern of the citrus rust mite on fruits, young mite-infested fruit-bearing orange trees in pots were placed outdoors under a roof well protected from showers. The side of fruits facing east received direct sunlight till about 10 h 00. The 'quadrant', facing west, remained permanently in the shade. Two series (A and B) of 9 fruits were inspected during the main rainy season (July-September), and another series (C) of 6 fruits during the main dry season (October-November). The number of mites within one x10 lens field were counted twice a week for each of the four quadrants of a fruit (quadrants are defined by the points of the compass NW-NE, NE-SE, SE-SW and SW-NW) (Table 4 for Series C). The summarized data for the three series in Table 5 show that mite counts are lowest on the east quadrant. The nul hypothesis that the east quadrant was as attractive as the average of the three other quadrants was tested (Sign test) against the alternative hypothesis that the east quadrant was

Table 4. Effect of light on the distribution of citrus rust mites on orange fruits. Fruit divided into 4 quadrants; the west quadrant was permanently shaded. Trial at Paramaribo, 7 Oct.-25 Nov. 1974. Counts are totals of a lens field (1.5 cm^2) from a quadrant of 6 fruits. Series C.

Date (month-day)	Counts of mites			
	north	east	south	west
10-07	12	10	10	14
10-11	24	15	18	16
10-15	25	7	23	39
10-18	30	26	45	42
10-22	36	22	44	58
10-25	57	42	62	50
10-29	133	113	122	107
11-01	174	182	181	177
11-05	143	155	145	148
11-08	230	183	270	220
11-12	305	252	290	255
11-15	238	210	215	185
11-18	195	203	190	161
11-22	121	149	131	121
11-25	152	128	153	131
Total	1875	1697	1899	1724

Table 5. Effect of light on the distribution of citrus rust mites on orange fruits. Fruit divided into 4 quadrants; the west quadrant was permanently shaded. Trial at Paramaribo, 22 July-30 Sept. 1974 for Series A and B. For Series C, see Table 4.

Series	Number of fruits	Number of counting dates	Totaled numbers of mites			
			north	east	south	west
A	9	21	3197	3086	3191	3103
B	9	21	3124	2550	3407	3237
C	6	15	1875	1697	1899	1724

less attractive. The probability, P, was 0.03. The quadrant exposed to sun was less attractive to mites than the others.

In citrus orchards in Surinam it has commonly been observed that on most of the mite-infested leaves, many mites are present only on a part of the leaf. Furthermore mites living in such a limited area remain there even when the hot sun is vertical to them. Hely (1947) reported from Australia that citrus rust mite, living on those parts of the trees that were most exposed to the sun (48 ^{o}C), were not obviously affected by the heat. Therefore the citrus rust mite can endure the hot sun, but does not prefer direct sunlight. The reported habit of the citrus rust mite to infest sun-exposed parts of a tree, including fruits, more severely than the unexposed parts (Binney, 1934), outer trees of an orchard more than inner ones (Bodenheimer, 1951), exterior leaves more than interior ones, and thinned orchards more than unthinned ones (Swirski, 1962) is more likely to be an indirect effect. Dense and shaded leaves and fruits remain wet longer after being moistened by dew or rain, which consequently may interfere with the molting of the mites (Section 2.7.4).

2.7.6 Effect of feeding site and age of the food plant

To find out whether citrus rust mites develop in larger numbers on the upper or lower leaf surface and on leaves of young or older plants, a group of 4 one-year-old orange graftings (on sour-orange rootstock) were placed outdoors under a shelter and 4 others were exposed to rain, and another group, composed of 4 orange seedlings about three months old installed outdoors under a shelter.

Table 6 shows that on orange graftings one year old, under a shelter, 2.4 times as many mites were found on the upper as on the lower surface of leaves. On the orange seedlings (3 months old) the reverse was observed: 3.3 times as many

Table 6. Counts of rust mite on leaves of orange graftings one year old, 4 sheltered and 4 exposed to rain. Counts are totals for a lens field (1.5 cm^2) on 20 leaves of each of the 4 plants.

Date (month-day)	Sheltered plants		Exposed plants	
	upper surface	lower surface	upper surface	lower surface
07-22	9	1	2	0
07-26	34	9	39	10
07-29	21	10	20	7
08-02	46	12	4	0
08-05	176	90	24	20
08-09	479	248	158	74
08-12	376	165	36	25
08-16	612	229	27	11
08-19	573	236	209	81
08-23	1020	469	354	248
08-26	1512	807	93	56
08-30	1171	373	107	26
09-02	1378	541	67	24
09-06	956	291	39	7
Total	8354	3481	1179	589

Table 7. Counts of rust mite on leaves of 4 sheltered orange seedlings 3 months old. Counts are totals for a lens field (1.5 cm^2) on 4 leaves of each seedling. The seedlings were about 6 cm high and bore 4-6 leaves.

Date (month-day)	Upper surface	Lower surface
11-18	109	497
11-26	222	676
12-03	198	723
12-10	112	353
12-17	134	342
Total	775	2591

mites were counted on the lower as on the upper surfaces (Table 7). Similar results have been obtained from other observations. On mature orange and grapefruit trees in the field there were twice as many mites on the upper as on the lower leaf surfaces; on young orange seedlings, mites were 3 or 4 times as many on the lower as on the upper surface. Therefore the count of citrus rust mite on the upper or lower leaf surface is markedly influenced by the age of the host plant.

Rains do not eliminate this difference with age between host plants. Table 6 shows that mites were twice as numerous on the upper as on the lower surface of leaves of exposed trees. When orange trees were sprayed with water (Section 2.7.4), mites were also more abundant on the upper than on the lower surface. Contradictory are data from Yothers & Mason (1930) and Swirski (1962). They found that mites were more abundant on the lower leaf surfaces of orange trees that were at least one year old. Yothers & Mason (1930) also reported that during periods of rain, the rust mites crawl to the lower surfaces of the leaves, to shelter from rain.

The relation between fruit age and mite density was studied at the Geyersvlijt citrus orchard. Differences in mite counts on young and full-grown oranges were slight (Fig. 16) and proved insignificant (Rank sign test; $P > 0.05$). However, mite counts differed distinctly between young and full-grown grapefruits and the differences were significant. Therefore counts of citrus rust mite on grapefruit are distinctly influenced by the age of the fruit; full-grown grapefruits become more severely infested than young fruits. I have never noticed that young grapefruits become russeted after an outbreak of rust mite. Yothers & Mason (1930), in listing the host plants of the citrus rust mite in the order of severity of infestation, rate both leaves and fruits of grapefruit higher than

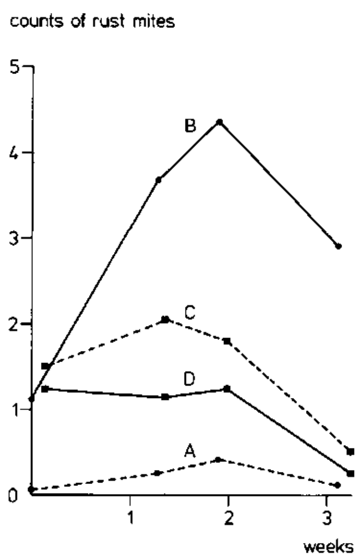

Fig. 16. Counts of citrus rust mite on young (A) and full-grown (B) grapefruits, diameter 3-4 and 8-12 cm, respectively, and on young (C) and full-grown oranges (D), 2-3 and 6-7 cm.
Observations on 6 trees of each species at Geyersvlijt orchard, 14 September – 7 October 1971, at weekly intervals. Counts per lens field (1.5 cm^2) were averaged for 4 quadrants on 20 fruits per tree.

leaves and fruits of orange. Since they give no information on the age of the leaves and fruits of the different *Citrus* species, their rating sequence is not very meaningful.

2.8 NATURAL ENEMIES

2.8.1 *Predatory mites*

Several mite species, predacious on the citrus rust mite, have been recorded. McMurtry & Scriven (1964, 1965), in studying *Amblyseius hibisci* Chant. and *A. limonicus* Garman & McG. (Phytoseiidae) in the United States, observed that the citrus rust mite, though eaten by these predatory mites, did not form a satisfactory food source for their development. Similar results were obtained with *A. largoensis* Muma in Florida (Kamburov, 1971), with two other phytoseiid species in Israel, *Typhlodromus athiasae* P.& S. and *Metaseiulus occidentalis* Nesbitt (Swirski et al., 1967; Swirski & Dorzia, 1969), and finally with the introduced species *A. hibisci*, *A. limonicus* and *A. chilenensis* Dosse in laboratory studies in Israel (Swirski & Dorzia, 1968; Swirski et al., 1970).

In citrus orchards in Surinam, a few of the following predatory mites have been detected: *Amblyseius* sp. and *Iphiseius quadripilis* Banks (Phytoseiidae), *Cheyletia* sp. (Cheyletidae) and *Agistemus* sp. (Stigmaeidae) (identified by E.W. Baker, United States Department of Agriculture). Since the results of published work on predation on mites were not encouraging, they were further ignored.

2.8.2 *Insect predators*

In Surinam, adults of the ladybird beetle *Pentilia castanea* Muls. were found to reduce the number of rust mites in cages that had been attached to citrus leaves. Since this beetle normally feeds on scale insects, the tiny citrus rust mites would probably not be attractive under natural conditions. From Florida, the ladybird beetle *Stethorus nanus* Lac., the lacewing *Chrysopa oculata* Say and larvae of Cecidomiidae have been listed as predators of the citrus rust mite. None of them was sufficiently common to be significant in reducing mite populations (Yothers & Mason, 1930). Adults of *Coniopteryx vicina* Hagen have been reported to feed voraciously on citrus rust mites under laboratory conditions, but no effect on counts of rust mite was observed in the field (Muma, 1955; 1958).

Recently a predacious thrips species (Fig. 17), belonging to the family

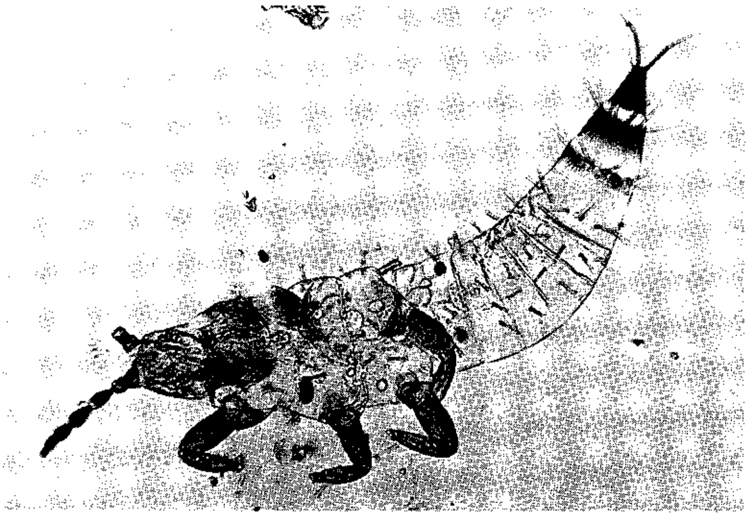

Fig. 17. Unidentified thrips species (Phlaeothripidae), a predator of the citrus rust mite.

Phlaeothripidae, has been detected feeding voraciously on the citrus rust mite in Surinam. This predator is an active wanderer. The thrips recognizes prey at short distance only. Once a rust mite is spotted, its body contents is imbibed within half a minute. Under laboratory conditions, I have observed one thrips to destroy some 300 adults and larvae of the citrus rust mite in one night. In view of this feeding capacity, the thrips might help to regulate mites. Further research into the importance of this thrips species is merited.

2.8.3 Fungal parasites

The only fungus known to be parasitic on citrus rust mite is *Hirsutella thompsonii* Fisher. It is the most important density-dependent mite-regulating factor. For details, see Chapter 3.

3 Hirsutella thompsonii Fischer

3.1 REVIEW OF LITERATURE

Speare & Yothers (1924), who studied the citrus rust mite in citrus orchards in Florida, were the first to suggest that the marked decrease in mite numbers – a phenomenon which occurs annually with the onset of the rainy season at the end of June or early July – was probably due to a fungal disease. High counts of mite per grapefruit of some hundred thousands dropped to almost zero by the end of September. They observed hyphal bodies in abnormally darker-coloured mites that also moved more sluggishly. Furthermore they noticed mycelium on dead mites with hyphae protruding from the bodies. Rust mites were always more abundant on trees sprayed with fungicides than on unsprayed trees. Yothers & Mason (1930), in reporting similar data, concluded that the reduction in mite numbers could not have been the result of food scarcity, since on average only half the untreated fruits were severely infested with the rust mites. Fisher et al. (1949) confirmed the findings. They especially studied the citrus rust mite during the period of maximum population. The fungus, which was regularly associated with dead mites was tentatively identified as a *Hirsutella* species, and was later described as *Hirsutella thompsonii* (Fisher, 1950a). Muma (1955) studied the decline in the population of the rust mite in Florida. At maximum population, about 70% of the mites were infected with *H. thompsonii*. The severity and duration of the fungal outbreak was proportional to mite density (Muma, 1958).

A criterion of whether a mite is infected is the brown colour of the infected mite. However ageing adults may also adopt a brownish hue (Swirski & Amitai, 1958).

Since 1969, research has started on the culture of *H. thompsonii* and on its use in the biological control of the citrus rust mite. McCoy & Kanavel (1969) found that the fungus grew rapidly on potato dextrose agar, V-8-juice agar, Sabouraud dextrose agar and modified soil fungus medium. Maximum production of conidia was after 12 days on potato dextrose agar, Sabouraud dextrose agar and agar-agar (Bacto). For large-scale production of mycelium, they used a submerged culture (McCoy et al., 1972). In Florida, McCoy et al. (1971) applied fragmented mycelium of *H. thompsonii* as a foliar spray on heavily mite-infested orange trees. The mycelium sporulated in about 48 h. The count of mites per leaf decreased and the incidence of infected mites increased a week after treatment. Mite populations remained low for 10-14 weeks. They concluded that fragmented mycelium applied as a spray would sporulate and reduce a large mite population but doubted whether similar field results can be obtained with low mite populations. Therefore, the usefulness of the fungus in biological control of the citrus rust mite needed further study.

H. thompsonii has been isolated from the citrus rust mite in the Chekiang Province of China, where its culture on various synthetic media had been studied and inoculation experiments had been carried out in the laboratory and in the field (Anonymous, 1974). The mite inoculated in the laboratory showed a mortality of 90.5% within 3 days, against 18.5% in the control. Field inoculation resulted in 90% mortality within 3 days under dry conditions and in 70% within 3 days with heavy rainfall. The inoculation effect was still noticeable in the field 2 months after treatment.

Several other *Hirsutella* species have been recorded as fungal parasites of insects and mites, viz.: *H. fusiformis* from the curculionid *Brachyderes incanus; Hirsutella* sp. from the lace bug *Leptopharsa heveae; H. besseyi* from the scale insect *Lepidosaphes beckii* and *Hirsutella* sp. from the saddleback mite *Hemitarsonemus* sp. (de Fluiter & Blijdorp, 1935; Charles, 1937; Fisher, 1950b; Fisher, 1953).

3.2 TAXONOMY AND MORPHOLOGICAL CHARACTERISTICS

The taxonomy of the perfect state of *Hirsutella thompsonii* (Fungi Imperfecti: Moniliales) is uncertain. It was formerly considered as to belong to the Basidiomycetes (Polyporales) (Gäuman, 1926). A relationship with some *Cordyceps* spp. (Ascomycetes, Hypocreales), as suggested by Speare (1920), was confirmed by Petch (1923) and since then various *Hirsutella* spp. turned out to be the conidial state of *Cordyceps* spp. Besides, some other genera of the Hypocreales proved to be related to *Hirsutella* (Steinhaus, 1949).

In Surinam, the typical conidiophore of *H. thompsonii*, as described by Fisher (1950a), are found on the dead bodies as well as on living infected mites.
Figure 18 displays the characteristic forms and deviants from mycelium, growing on synthetic media.

Mycelial growth in an infected adult citrus rust mite is depicted in Figure 19. Hyphae spread from the anterior and posterior region as well as from the side. The hyphae arise from small oval bodies. This symptom has also been reported by Bucher,

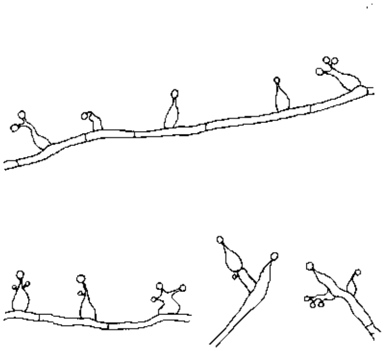

Fig. 18. Conidiophores of *Hirsutella thompsonii*.
Above. Typical. Below. Deviant forms on synthetic media.

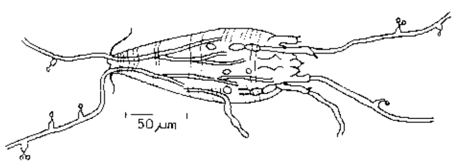

Fig. 19. Citrus rust mite infected with *Hirsutella thompsonii*.

who noted that hyphae penetrate the susceptible host, and usually fragment into unicellular forms (hyphal bodies), which multiply by division or by budding and which later give rise to filamentous hyphae (Bucher, 1964).

For a morphological description of the genus *Hirsutella*, see Barnett (1956).

3.3 ISOLATION AND CULTURE

To obtain pure inoculum for the culture of *H. thompsonii*, several techniques were tried with living and dead fungus-infected citrus rust mite. The simple method was to transfer hyphae protruding from the bodies of dead mites with a sterilized needle to a medium of Sabouraud maltose-peptone agar, and proved the most satisfactory. The best results were obtained when hyphae about 1 mm long with spore-bearing conidiophores were used.

Four synthetic media were tested to find a good method for the culture of *H. thompsonii*:

- Sabouraud maltose-peptone agar. Composition: 4 g maltose, 1 g peptone, 2 g agar-agar and 100 cm^3 water. Sterilization for 45 min at 115 ^oC.
- Sabouraud dextrose agar. See McCoy & Kanavel (1969).

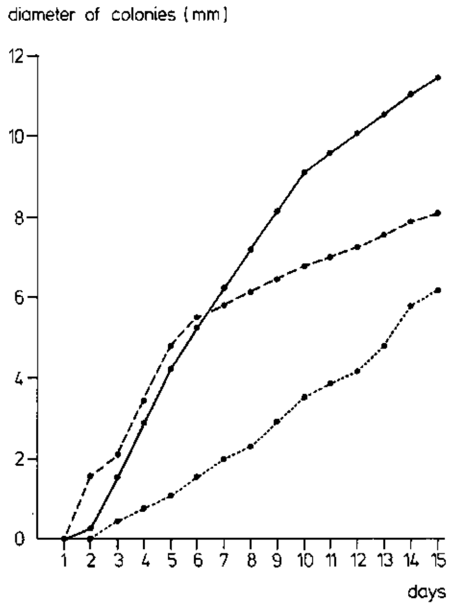

Fig. 20. Diameter of colonies of *Hirsutella thompsonii* as a function of time after inoculation onto Sabouraud maltose — peptone agar (solid line), egg yolk agar (broken line) and grapefruit agar (dotted line).

- grapefruit agar. Composition: 60 cm^3 grapefruit extract, 6 g agar-agar and 60 cm^3 water. Sterilization: 15 min at 150 ^oC. The extract was obtained by boiling 125 g of a finely sliced peeled grapefruit in 200 cm^3 water for 30 min, and filtering.
- egg yolk agar. See Müller-Kögler (1959).

After inoculation, the diameter of 5 growing colonies was measured almost daily. As growth on Sabouraud dextrose agar turned out to be very poor, that medium was abandoned.

The most rapid growth takes place on the Sabouraud maltose-peptone agar; development on grapefruit agar was rather poor (Fig. 20). Spore production was greatest on Sabouraud maltose-peptone agar, and was very poor on egg yolk agar.

I attempted to grow *H. thompsonii* in a liquid medium by the submerged culture technique of McCoy et al. (1972). Erlenmeyer flasks, containing the liquid medium and inoculated with *H. thompsonii*, were placed on a rotary shaker. After 10 days, little mycelium had been produced. This disappointing result was attributable to insufficient aeration of the medium.

3.4 COUNTS OF RUST MITE IN RELATION TO INCIDENCE OF HIRSUTELLA THOMPSONII

To find out how *H. thompsonii* interferes in the development of a rust mite population, healthy and *Hirsutella*-infected mites were counted on fruits of grapefruit plants. Figure 21 shows a distinct coincidence between the increase in incidence of *Hirsutella*-infected mites and the decrease in mite counts. However, the increase in *Hirsutella* incidence cannot fully account for the rapid decrease in population. Moreover, it is not clear why population growth stops so abruptly at a *Hirsutella* incidence of only 15%. At least one other regulatory factor is involved.

Since epidermal cells of the leaf and the fruit rind are seriously damaged at high mite counts, food quality was examined to establish whether this factor may help to stop population growth and to promote its decline. Mite development on mite-damaged fruits was severely depressed (Fig. 22); maximum count remained far lower than for mites that developed on initially healthy fruits. The reduced quality of the substrate seems to decrease population growth and helps to determine the moment of maximum density. Food quality also affects the decline in the mite population.

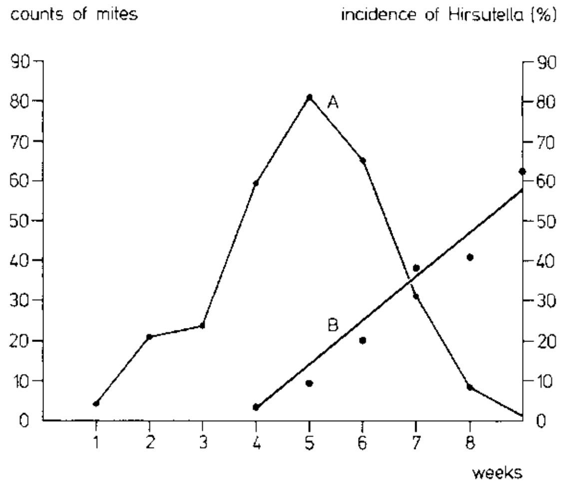

Fig. 21. Counts of citrus rust mite (A) and incidence of *Hirsutella thompsonii* (B) in the mites on grapefruit. Observations on 5 grapefruits growing on 5 potted plants, sheltered from rain, Paramaribo, 5 August – 23 September 1967. Weekly counts are numbers per lens field (1.5 cm^2) averaged for the 4 quadrants of the fruits.
Incidence is the average proportion of mites with at least one hypha protruding in samples of 50 mites (mounted in chloralphenol) from each fruit.

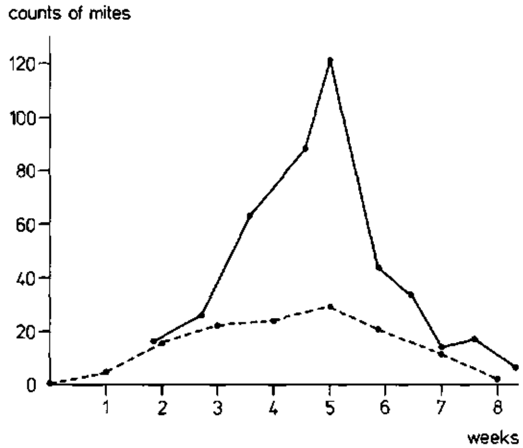

Fig. 22. Weekly counts of citrus rust mite on 4 unblemished oranges on 4 potted plants sheltered from rain (solid line) and on the same oranges after russeting by mite (broken line). After 8 weeks the fruits were cleared of mites by treatment with fungicide (tribasic copper sulphate, mass concentration of a.i. 2 g $litre^{-1}$) and wiping with a wet cloth; a day later they were rinsed and reinfested with 50 healthy mites. Trial at Paramaribo, 8 August – 5 October 1974.

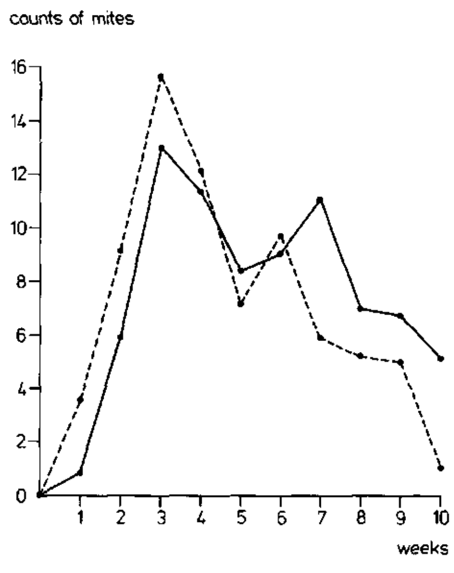

Fig. 23. Weekly counts of citrus rust mite on leaves of 3 grapefruit seedlings exposed to *Hirsutella thompsonii* (broken line) and 3 protected against fungal infection (solid line). Seedlings 8 cm high with 5 leaves. Initially 5 healthy mites were placed on each leaf and a week later 2 infected mites on leaves of the exposed group. Counts represent numbers per lens field (1.5 cm^2) averaged for upper and lower surfaces of leaves. Trial at Paramaribo, 9 October – 18 December 1974.

In a third experiment, rust mite was studied on the leaves of grapefruit seedlings. One group was placed in a plastic cage (1 m x 1 m x 0.5 m) and was kept free from *Hirsutella* infection as much as possible. Fresh air was sucked through a charcoal filter into the cage by means of a ventilator. A second group of 3 seedlings was placed in a cage with its front open.

Though mites decreased faster in the group exposed to *Hirsutella*, the population trends under either experimental conditions were similar (Fig. 23). Therefore *H. thompsonii* and food quality simultaneously affect populations of citrus rust mite.

3.5 EFFECT OF HIRSUTELLA-MYCELIUM SUSPENSION ON RUST MITE

Having developed a good technique for culture of *H. thompsonii* on Sabouraud maltose-peptone agar, laboratory and small-scale field trials were started to assess the use of this parasitic fungus in citrus rust mite control. Suspensions in rainwater, after blending in a mixer to fragment the mycelium, were sprayed on citrus leaves and fruits at different mass fractions with an ordinary hand sprayer ('atomizer').

3.5.1 Laboratory tests

Experiment 1

Experiment 1 tested whether early application of *Hirsutella* could prevent a build-up of rust mite. Figure 24 shows that mite populations were kept low when mites come into contact with *Hirsutella* within 2 days after its application. Since the other data indicate that mite were not affected by the parasitic fungus when contacting *Hirsutella* material 8 days after spraying, *H. thompsonii* apparently loses its infectivity after 3-8 days under the experimental conditions. In the field, the fungus probably maintains itself inside or on the rust mite, also in periods when counts are low.

Experiment 2

Experiment 2 examined the effect of a *Hirsutella* treatment against a mite population that had already reached a certain density. Figure 25 shows that in the *Hirsutella*-treated plants, the counts of rust mites decreased from 54.6 to 7.6 in five days. In the control, numbers increased from 46 to 61.2 during that period.

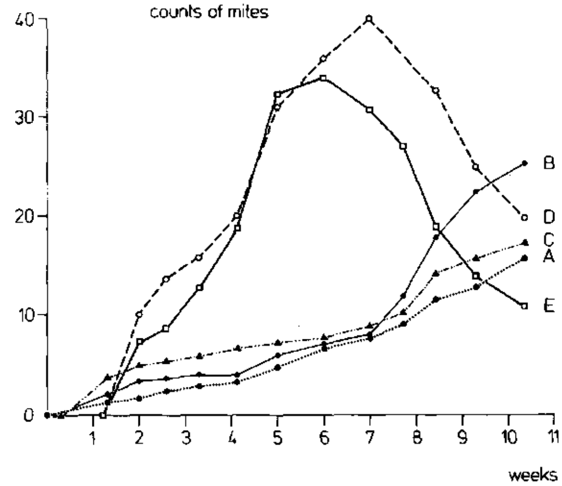

Fig. 24. Counts of citrus rust mite on leaves of sheltered potted grapefruit plants 2 years old after spraying (with an atomizer) with suspension of fragmented mycelium of *Hirsutella thompsonii*, mass fraction of mycelium in rainwater 0.05% (Treatments A-D) or with rainwater only (Treatment E), and transfer of 40 mites to the lower surface of each leaf. A. On the day of the spraying. B. The day after spraying. C. Two days after spraying. D and E. Eight days after spraying. Counts every 4-7 days are numbers on the whole undersurface averaged for 5 leaves of each of 4 plants. Trial at Paramaribo, 4 December 1972 – 14 February 1973.

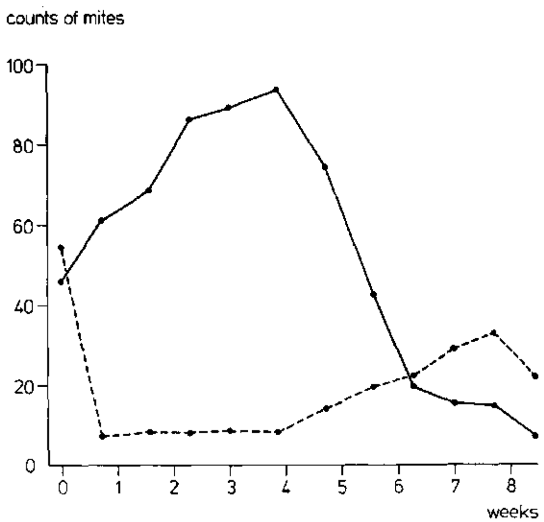

Fig. 25. Counts of citrus rust mite on leaves of grapefruit plants sprayed at time 0 with a suspension of *Hirsutella thompsonii* (broken line) or with rainwater (solid line). Counts every 5-6 days are numbers on the whole undersurface averaged for 5 leaves of each of 2 plants. The 4 plants were selected as having almost equal counts of mites about 2 weeks after artificial introduction of mites onto 8 potted plants 2 years old under a shelter. Other experimental details as in Experiment 1 (Fig. 24). Trial at Paramaribo, 2 January – 17 March 1973.

Four weeks after *Hirsutella*-spraying the average number of mites per lower leaf surface was only 8.2 in the treated plants, against 93.8 in the control. Thereupon mite population density slightly increased in the treated plants, whereas in the control the mites rapidly decreased in numbers.

These data demonstrate that under shelter a developing rust mite population can be controlled for four weeks after one application of the suspension of fragmented *Hirsutella* mycelium, applied as a foliar spray.

Experiment 3

Experiments 1 and 2 showed that counts of citrus rust mite on leaves could be kept low with foliar application of *Hirsutella* suspensions. In Experiment 3, the effect was tested of a *Hirsutella* treatment on fruits of orange trees 2 years old. The trees were growing outdoors, well protected from rain. Fruits were cleaned with a wet cloth, were artificially infested with 50 rust mites per fruit and were sprayed with a *Hirsutella* suspension, mass fraction 0.075%, five days after mites had been transferred.

Regular fruit inspection proved that the mite population failed to build up, whereas in the control infestation followed a normal course. Results were similar to those of the leaf treatments (Experiments 1 and 2).

3.5.2 Field tests

To study the effect of *Hirsutella thompsonii* suspensions on citrus rust mite under natural conditions, two exploratory field trials were made on fruits of grapefruit trees 15 years old in the Geyersvlyt citrus orchard during the dry season. Mycelial suspensions were applied with a hand-sprayer ('atomizer').

Field trial 1

Trial 1 tested the effect of spraying fruits with suspensions of mycelium of different mass concentrations. Figure 26 shows that sprays with 0.5-1 g $litre^{-1}$ of *Hirsutella* effectively suppress development of a small mite population for about 2 weeks. Thereafter the population increases only to a much smaller maximum.

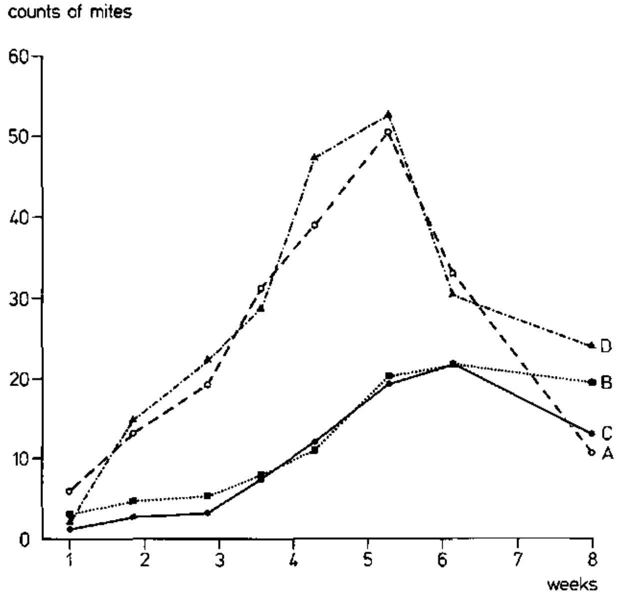

Fig. 26. Counts of citrus rust mite on grapefruit sprayed with suspensions of *Hirsutella thompsonii*, mass fraction of mycelium 0.025% (A), 0.05% (B) or 0.1% (C), or with rainwater (D). Counts are per lens field (1.5 cm^2) averaged for the 4 quadrants of 10 fruit of Trees A-C. D is the average of 5 fruit on each of 2 trees. The fruits were cleaned with a wet cloth and infested with 50 adult mites 5 days before spraying them at time 0 with an atomizer. Field trial 1 at Geyersvlijt orchard in the dry season, 20 January – 20 March 1973.

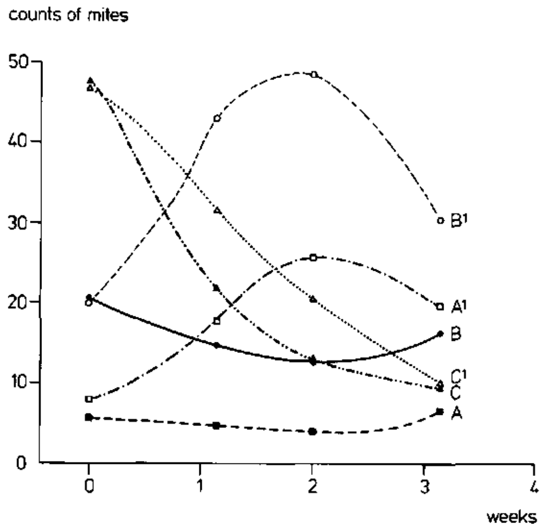

Fig. 27. Counts of citrus rust mite on grapefruit sprayed with suspension of *Hirsutella thompsonii* when mite counts reached 1-10 (A), 10-30 (B) and 40-60 (C) at time 0. Controls (A', B', C') were sprayed with rainwater. Weekly counts are numbers per lens field (1.5 cm^2) averaged for the 4 quadrants of 10 fruits. Each treatment was on 1 tree. Mass fraction of mycelium in the spray 0.05%. Field trial 2 at Geyersvlijt orchard in the dry season, 26 February – 20 March 1973.

Field trial 2

In contrast to Trial 1, Trial 2 tested the effect of *Hirsutella* spray against infestations of mite of different severity. Figure 27 shows that small and moderate populations were inhibited for some 3 weeks after spraying with *Hirsutella*. For heavy infestations both the *Hirsutella*-treated mite population and the untreated population declined sharply right from the start, i.e. from the moment of the natural population decrease. On the *Hirsutella*-treated fruits, the population decline seemed to be simultaneously governed by a reduced food quality and by food deficiency (Section 3.4). *Hirsutella* treatment came too late.

3.5.3 Conclusion

A spray of *Hirsutella thompsonii*, mass fraction of mycelium in rainwater 0.05-0.1%, controls citrus rust mite for at least 3 weeks in dry conditions either under a rainshelter or in the dry season in the open. Counts decrease rapidly within 5 days of treatment of small populations building up.

4 Citrus rust mite-greasy spot relationship

4.1 REVIEW OF LITERATURE

Greasy spot has been known among citrus growers in the United States of America since the end of the 19th Century. Stevens (1918), reporting on this disease which he described under the names 'black melanose' and 'greasy melanose', mentioned that the causative agent is unknown.

Betancourt (1940), in studying citrus rust mite in Brazil, recorded that the mites preferred those areas on the leaves (yellow spots), where chlorophyll was partly damaged by disease. He considered that rust mite was responsible for greasy spot. From field observations in the United States, Thompson (1948) concluded that greasy spot was the direct result of rust mite feeding. He found much less greasy spot in citrus fields where rust mite numbers were reduced by sulphur treatments, than in the untreated plots. Fawcett & Klotz (1948) reported that greasy spot symptoms were seen in certain areas of California where rust mite was not known to occur.

Tanaka & Yamada (1952), carrying out inoculation experiments in Japan, were the first who proved that greasy spot was caused by a fungus, *Cercospora* sp., of which *Mycosphaerella horii* Hara is the perfect state. In greenhouse trials, Thompson et al. (1955) found no greasy spot on grapefruit plants that were severely infested by rust mite. However, they did observe greasy spot on leaves of other test plants that were kept free from rust mite by aramite treatments. They concluded that the rust mite was not an important factor, if any, in causing greasy spot and that therefore the disorder could be prevented by applying oil as a fungicide. Griffiths (1955), in control trials with oil and sulphur in the United States, concluded that a low incidence of greasy spot was rather due directly to fungicidal action of both chemicals on the causative agent of greasy spot than to reduction of the rust mite population (sulphur acts as a fungicide as well as an acaricide). He stated that more research was needed to determine whether the rust mite had a part in the total greasy spot syndrome. Pratt (1958) believed that greasy spot was caused by a fungus that, during favourable weather, penetrated the leaves through injuries, provoked by rust mites or other organisms. He advised to control the disease with copper fungicides and to aim at a good control of the rust mite as well.

The findings of Tanaka & Yamada (1952) were confirmed by Fisher (1961) in Florida. She isolated a *Cercospora* species from greasy spot lesions. The fungus differed from the one described by Tanaka & Yamada and she named it *Cercospora citri-grisea*. As to the rust mite – greasy spot relation, Fisher (1961) remarked that 'although greasy spot was known to be a fungus disease, the frequent rust mite – greasy spot association remains to be adequately explained'.

Whiteside (1972) found the name *Cercospora citri-grisea* to be incorrect. As a result of a renewed study into the nature of its imperfect state and the finding that *Mycosphaerella* in Florida differed greatly from the description of *M. horii*, he proposed the name *Mycosphaerella citri*, with *Stenella* as the imperfect state. Later, Whiteside (1974) reported the association of greasy spot with honeydew-excreting insects on citrus in a greenhouse. He supposed that the insects encouraged extramatrical fungal growth by their excreta and perhaps also by decomposition of their bodies.

In Surinam, greasy spot had always been considered a leaf disease, i.e. as a purely pathological problem. The imperfect state of the fungus corresponds with Whiteside's *Stenella* sp. (identification by G.S. de Hoog, Centraal Bureau voor Schimmelcultures, Baarn, NL). Childs (1964, 1966), who visited Surinam, recorded greasy spot as the most important disease of citrus, seriously reducing production. He observed grapefruit trees that were more than 50% defoliated as a result of greasy spot.

4.2 SCOPE OF THE RESEARCH

In view of the contradictory data in the literature and the lack of information from Surinam, experiments were needed to elucidate the following questions:

- Is defoliation of citrus plants the result of rust mite or greasy spot or of both? (Field trial 1).
- Does a relationship exist between rust mite infestation and greasy spot? (Field trials 2, 3 and 4).

4.2.1 Field trial 1

4.2.1.1 Materials and method

Eighteen grapefruit grafts 1 year old on sour-orange rootstock 2 years old were set out 1 m apart on 3 adjacent beds on shell-containing loamy sand. They were defoliated on 9 March 1967, and started to flush uniformly 2 weeks later. Further flushes were removed so that at the end of the trial all leaves were of uniform age. Leaf drop began in July. The crowns of one group of 6 plants were dipped in a fungicide (tribasic copper sulphate, mass concentration of a.i. 2 g $litre^{-1}$), which was effective against greasy spot, on 4 April, 9 and 30 May, 7 and 30 June, 7 and 30 July, 19 August, and 9 and 30 September. Since trees in natural orchards would usually bear many infected leaves, sprigs of citrus infected with greasy spot were put in the crowns of the test plants to encourage infection. Leaves with greasy spot were counted at the end of August and were expressed as a percentage of all leaves per tree averaged per treatment.

4.2.1.2 Results

Counts of mite were highest on the plants that had been treated with tribasic copper sulphate (Fig. 28, above). Differences were slight between plants not treated with copper, on which rust mites were transferred initially and those on which rust mites settled naturally. The higher mite peaks of copper-treated plants can in all probability be attributed to the elimination of the entomogenous fungus, *Hirsutella thompsonii* (Chapter 3).

Although rust mites were far more numerous on the copper-treated plants than on the plants not treated with copper, defoliation was only observed in the untreated controls (Fig. 28, below). Leaf drop, therefore, could not have been the consequence of leaf-damage by the rust mites. When recording the percentage of leaves infected by greasy spot, I found a very low percentage for the copper-treated plants and a very high percentage for both controls. On 29 August 1967, these percentages were 0.92 and 100, respectively. Therefore defoliation of citrus plants is very likely the result of greasy spot infection.

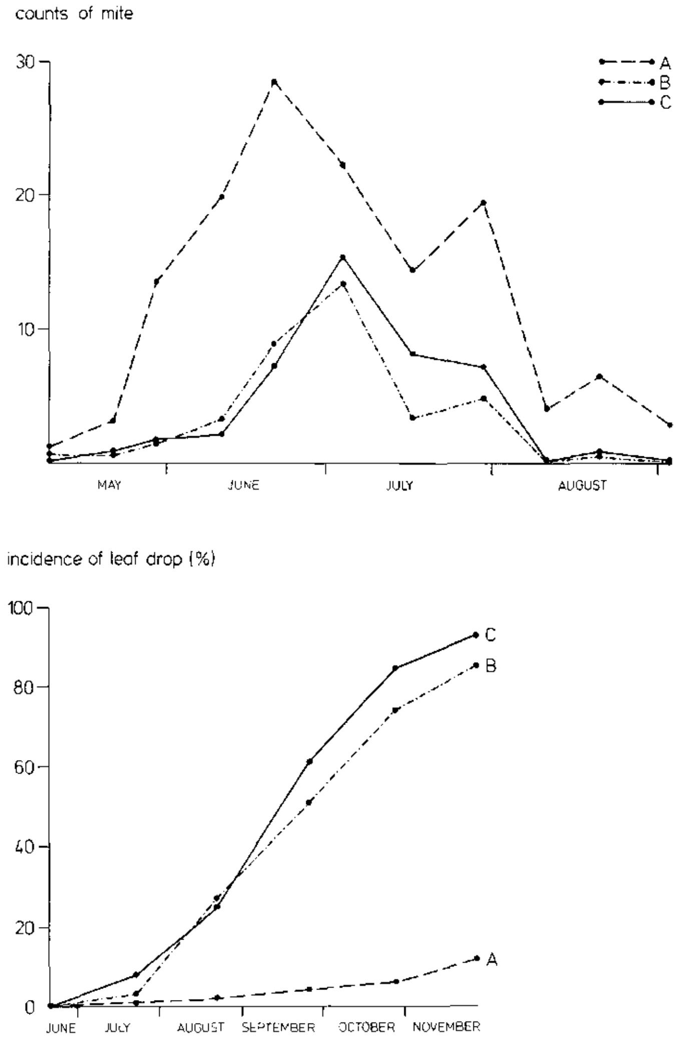

Fig. 28. Counts of citrus rust mite (above), and incidence of leaf drop (below) on citrus plants. Crowns of 6 plants were dipped in fungicide and had both natural and introduced mite (A). Other groups were not dipped and had only natural mite (C) or both natural and introduced mite (B). Counts of rust mite are numbers per lens field (1.5 cm^2) averaged for undersurfaces of 10 leaves for each plant. Incidence of leaf drop is percentage of initial number of leaves averaged for the 6 plants of each group. For other details, see Section 4.2.1. Field trial 1 at Paramaribo, March – November 1967.

4.2.2 Field trial 2

4.2.2.1 Materials and method

The trial compared the effect on greasy spot of an acaricide and an insecticide effective against citrus rust mite and of a fungicide. Grapefruit plants (see Section 4.2.1) were set out and defoliated early in September 1968. Flushing started on 21 September and subsequent new shoots were removed, so that only leaves formed then were later examined for greasy spot. Plastic bags were left initially on Control Group D, which was transferred to a greenhouse on 14 October and 50 rust mites were introduced on each plant. They were replaced in the field 4 weeks later, when counts of rust mite were high. Dry weather in October favoured mite. At the end of October, many mite were observed in Groups C (fungicide) and E (control), and moderate numbers in Group D (control) where the population was declining. Groups A and B (acaricide and insecticide) remained free from mite. In January 1969, one plant looked yellowish, perhaps because of a nutritional deficiency and all plants were given 50 g of an NPK dressing. To encourage greasy

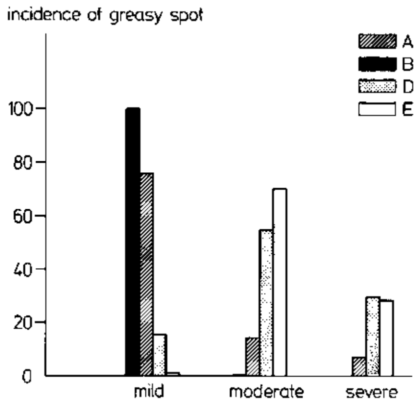

Fig. 29. Incidence of mild (1-3 spots per leaf), moderate (4-10) and severe symptoms (> 10) of greasy spot on leaves of citrus plants. A. Dipped in an acaricide (chlorobenzilate; emulsifiable concentrate; vol. fraction 25%, diluted to mass concentration of a.i. 0.5 g $litre^{-1}$). B. Dipped in an insecticide (dipterex; wettable powder; mass fraction 80%, diluted to mass concentration of a.i. 1 g $litre^{-1}$). C. Dipped in a fungicide (tribasic copper sulphate; diluted to mass concentration of a.i. 2 g $litre^{-1}$). D. No pesticide; natural and introduced citrus rust mite. E. No pesticide; natural infestation only. Latin-square arrangement set out September 1968; 5 plants in each group. Crowns of plants were first dipped on 16 October and subsequently at intervals of a week for Groups A and B, and of 3 weeks for Group C. Field trial 2 at Paramaribo. Observations on 7-10 May 1969. Group C had no greasy spot and is therefore not depicted.

Plants of Group III were immediately treated with the acaricide chlorobenzilate and transferred to the field, where treatment was repeated every 10 days. Plants of Group II remained protected against rain until 27 May, those of Group I until 17 July, before similar treatment with chlorobenzilate as for Group III. Rust mite counts of Group I and II continued until the dates of the first acaricide treatment. They were as follows:

Date (month-day)	I	II	III
05-05	2.85	1.04	0.26
05-15	5.76	2.75	treated
05-27	19.36	8.34	
06-07	26.89	treated	
06-17	28.32		
06-26	12.82		
07-06	9.75		
07-16	4.94		
	treated		

4.2.4.2 Results

On plants of Group I, with heavy mite infestation, greasy spot was much more severe than in Group II, with moderate mite infestation (Table 9), which in turn was more severely infected than Group III, where there were few rust mites. Therefore count of rust mites affects the severity of greasy spot.

Table 9. Frequency distribution (%) of different classes according to severity of greasy spot for high (Group I), moderate (Group II) and low counts (Group III) of citrus rust mite. Severity was assessed on 20 November 1974 on a scale according to proportion of leaf surface infected: Class 0, 0%; 1, >0-2%; 2, 3-20%; 3, 21-40%; 4, 41-60%; 5, 61-80%; 6, 81-100%. For each class, percentages were averaged per treatment. Other details in Section 4.2.4. Field trial at Paramaribo, March-November 1974.

	Class						
	0	1	2	3	4	5	6
Group I	2.20	11.87	37.50	25.13	16.40	6.90	0
Group II	31.82	30.05	25.00	10.33	2.80	0	0
Group III	79.73	12.35	7.17	0.75	0	0	0

4.3 DISCUSSION

The field trials provide conclusive proof that citrus rust mite is associated with the severity of citrus greasy spot.

Citrus rust mites frequently infest one part of a leaf, leaving the other part uninfested (Fig. 7 in Section 2.5.2). This phenomenon is not limited to citrus, having been observed in West Indian cherry, *Malpighia punicifolia* (Fig. 8 in Section 2.5.2). We may then expect that leaves partially infected by greasy spot would also occur and indeed did occur (Fig. 1 in Section 1.1). Knorr et al. (1957) published a similar picture of a citrus leaf on which greasy spot was restricted to one portion of the leaf blade, and stated that they had often observed this characteristic of greasy spot. The coincident distribution of rust mite and greasy spot gives a clear evidence of the relationship between the two.

Rust mites and greasy spot coincide in other ways too. The citrus rust mite infests grapefruit much more severely than orange (Yothers & Mason, 1930). Fisher (1961) and Stevens (1918) mentioned that grapefruit was more severely infected by greasy spot than orange. Summerville (1933) reported that rust mite preferred younger trees to older ones. According to Fisher (1961), young trees were more susceptible to greasy spot than older trees. Lemon was the most preferred food plant of the rust mite (Yothers & Mason, 1930; Swirski, 1962). Fisher (1961) noted that lemon may be more damaged by greasy spot than any other *Citrus* species. Rust mites were found more abundantly on the foliage near the tops of the trees (Yothers & Mason, 1930). Fisher (1961) stated that in most citrus groves, regardless of age or spacing, branches in tree tops were more readily defoliated by greasy spot than lower branches. I have noticed the same. According to Binney (1934), rust mite was almost restricted to those parts of the tree that were directly exposed to the sun. Swirski (1962) wrote that the rust mite usually preferred the exterior leaves to the interior ones. Fisher (1961) mentioned that young trees, widely separated and exposed to sun, were generally more seriously affected by greasy spot than older, crowded trees. There is clearly close relationship between citrus rust mite and greasy spot.

A way in which rust mite could interfere in greasy spot will now be discussed in the light of more recent knowledge about greasy spot etiology. Whiteside (1974) found the following: (1) the potential penetration of the greasy spot fungus into stomata can be increased considerably, under favourable conditions, by the development of a ramifying extramatrical mycelial growth; (2) a spray of sucrose before inoculation greatly increases disease severity because of its nutritional effects in promoting extramatrical mycelial growth and the number of stomatal penetrations;

(3) the observed association of greasy spot with honeydew-excreting pests in a greenhouse, in which the atmosphere was otherwise too dry for infection, can be attributed to the hygroscopicity and sucrose content of the honeydew or, more likely, to the additional source of nutrients for fungal growth formed by the excreta of the insects and perhaps also to the decomposition of the insect bodies.

Outbreaks of rust mite occur in the dry season and populations are very low during the rainy season. The leaves of the experimental plants (Sections 4.2.2-3; Field trials 2 and 3) reached maturity about two months after the flush started, when the weather was still dry. According to Cohen (1959), young unexpanded leaves remain free from greasy spot; only mature leaves are susceptible (Cohen, 1959). However, dry weather is unfavourable for greasy spot (Whiteside, 1974). In the light of Whiteside's other findings, it is therefore likely that the severe greasy spot on the control plants (Field trials 2 and 3) had been promoted during the dry season by dying and dead rust mite, which formed a food-supply for extramatrical fungal growth. Moreover, plants treated with chlorobenzilate and dipterex (Field trials 2 and 3), on which rust mites were not permitted to develop, showed only mild greasy spot, despite the favourable weather (rainy season), about a month after the leaves had reached maturity.

Germination of spores and mycelial growth could well be favoured by the tissue fluid that appears superficially as the result of puncturing by the rust mites. Whiteside (1974) stated that results of experiments gave evidence that stomatal guttation fluid might in some manner be involved in stimulating appressorium formation.

Another possibility is that leaf surfaces damaged by rust mites and speckled with their dead bodies, cast skins and excrements, may trap spores which would not be washed off by rain as easily as off healthy clean leaves. During dry weather, spores may accumulate on such leaves to germinate subsequently in the wet season.

On leaves with epidermal and subepidermal cells destroyed by rust mite (Fig. 9 in Section 2.5.2), hyphae do not necessarily have to penetrate through stomata (penetration through stomata was reported by Whiteside, 1972). I have noticed that methylene-blue solution, applied to a mite-infested leaf by dipping, readily penetrates the tissue.

The poor condition of the citrus trees in Surinam is largely due to leaf-damaging organisms that affect assimilation considerably and consequently interfere with the physiology of the plants. Figure 31, summarizing the results of Field trial 1 (Section 4.2.1) in which 6 plants (A) were copper-treated and free from greasy spot and 12 plants (B) were not copper-treated and were highly infested by rust mites, clearly shows that plants of Group A had a well developed crown and

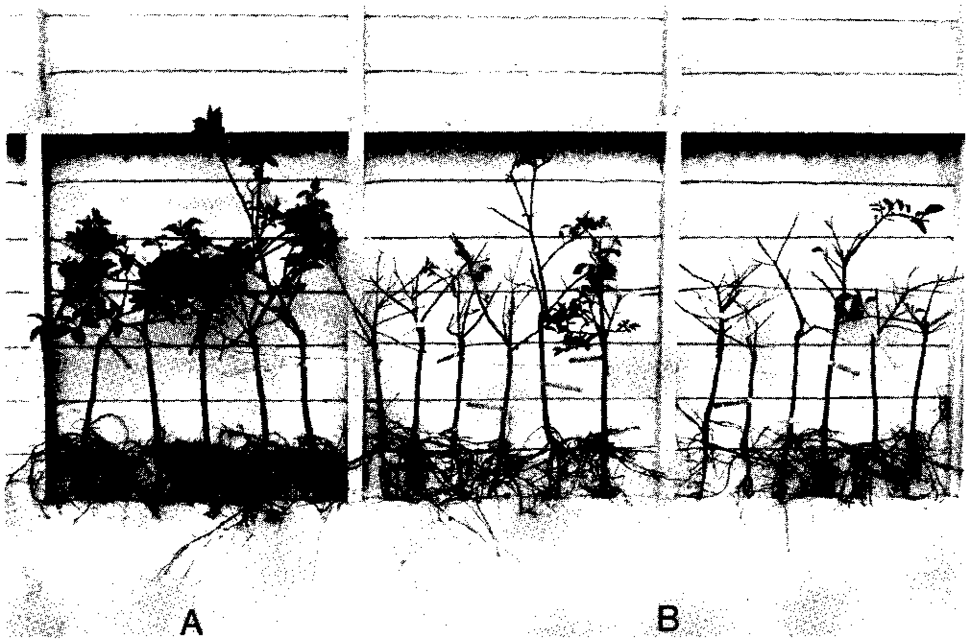

Fig. 31. Grapefruit grafts. A. Treated with tribasic copper sulphate: highly infested with citrus rust mite but free from greasy spot. B. Not treated with copper: highly infested with mite but severely affected by leaf drop as a result of greasy spot.

root-system, whereas plants of Group B had a poor crown and poor root development. Root growth seemed to stop immediately on defoliation. This would maintain a physiological balance between leaf and root volume.

5 Control of citrus rust mite and greasy spot

5.1 CHEMICAL CONTROL OF CITRUS RUST MITE

Chemical control of citrus rust mite dates back to the 1880s in the United States, when sulphur was found to form a satisfactory remedy against this mite (Hubbard, 1885; cited in Yothers & Mason, 1930). During the first half of the 20th Century, other chemicals were tested, e.g. lead arsenate, nicotine and mineral oils. These chemicals, however, proved to be unsuccesful in rust mite control (Yothers & Mason, 1930). In many countries a distinct preference was given to sulphur, being the most effective product.

Since the 1950s, numerous new chemicals have come on the market, several effective against rust mite: chlorobenzilate (Yeppson, 1955; Johnson, 1966a), zineb, ethion, azinphos-methyl, dicofol, quinomethionate ('morestan') and hexachlorophene (Johnson, 1960; Johnson, 1966a,b).

In Israel effective control was obtained with chlorobenzilate, phenkapton (Swirski, 1958), maneb, mancozeb, quinomethionate (Swirski et al., 1967) and phenisobromolate ('neoran')(Swirski et al., 1969). From Egypt, Attiah et al., (1967) mentioned zineb and Rasmy et al., (1972) reported phosalone, dicofol and ethion as giving satisfactory control of the citrus rust mite. In Taiwan, Tao & Wu (1969) tested 11 systemic insecticides against citrus insects and mites, and obtained a good control of the citrus rust mite by bark treatments with monocrotophos.

In Surinam, the use of chlorobenzilate, sulphur and zineb was first reported by Samson (1966). To date, chlorobenzilate is the preferred means of control. I have confirmed its favourable acaricidal action (Fig. 32). Low-volume spraying (500 litre ha^{-1}) at a mass concentration of a.i. of 2 g $litre^{-1}$ is advised.

Since chlorobenzilate is an acaricide with little insecticidal action, it is suitable for a future integrated control program. In contrast, sulphur and zineb have a fungicidal action and would counteract application of *Hirsutella thompsonii* against citrus rust mite. Sulphur and zineb also kill fungi that are parasitic on scale insects infesting citrus.

Recently I tested disulfoton ('disyston'; granules; mass fraction of a.i. 10%),

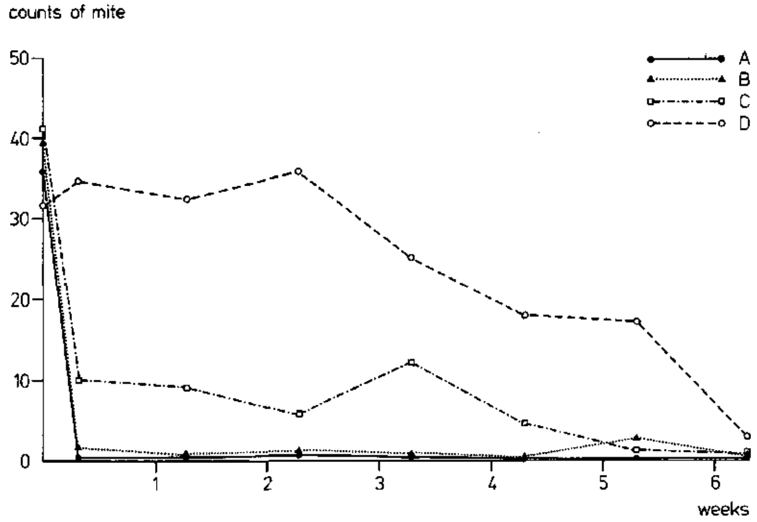

Fig. 32. Effect of Chlorobenzilate (A), wettable sulphur (B) and zineb (C) on counts of citrus rust mite, compared with an untreated control (D). Treatment at time 0 was on 5 randomly selected grapefruit trees about 15 years old on double row beds, with 4 rows of untreated trees as buffers between treatments. Counts of mite immediately before and 1 day after treatment and then every week on 10 fruits per tree. Counts are numbers per 5 cm^2 for each quadrant of a fruit, averaged per treatment. Application with low-volume motor sprayer at about 500 l/ha. Mass concentration of active ingredient were for chlorobenzilate 2.5 g $litre^{-1}$, sulphur 10 g $litre^{-1}$ and zineb 10 g $litre^{-1}$. Trial at Geyersvlijt, 20 April – 3 June 1966.

a systemic insecticide and acaricide. Soil treatment with 2 g granules per tree 2 years old every 3 months effectively controlled aphids and scale insects. However, the citrus rust mite was not affected at all. The substance seems to be well transported to the vascular tissues of the leaves, but not into the epidermal cell layers, where feeding of the rust mite occurs.

5.2 CHEMICAL CONTROL OF GREASY SPOT

Copper fungicides and mineral oils are the most effective chemicals against greasy spot (Fisher, 1954; Thompson et al., 1956; Pratt, 1958; Cohen, 1959; Fisher, 1961). However, they may have several undesired side-effects. Copper sprays can accumulate to toxic levels in soil (Reuther & Smith, 1952), can cause star melanose (Knorr et al., 1957) and can start outbreaks of citrus rust mite and scale insects, by elimination of parasitic fungi (Spencer, 1939; Thompson, 1939; Griffiths & Fisher, 1949). Mineral oils reduce the transpiration rate of the plant (Merrin, 1929). According to Cohen (1959), application of oil emulsions may reduce production.

In Surinam, effective control of greasy spot was obtained with tribasic copper sulphate (Fong Poen & Raktoe, 1971). Its adverse effect on scale insects and citrus rust mite has been noticed. Since the association citrus rust mite-greasy spot has been more elucidated (Chapter 4), attention is nowadays paid to the 'indirect control' of greasy spot by preventing outbreaks of citrus rust mite.

5.3 TIMING OF CITRUS RUST MITE CONTROL

The rapid build-up of the citrus rust mite population – generally within a period of 4 to 5 weeks – necessitated the development of a sampling technique by which the grower could easily decide when to spray. Although counts of mite with a hand-lens can be a criterion for the start of control, this method was too laborious if used on various trees per orchard to produce a reliable average. Therefore observations were made to determine whether the number of rust mites per lens field (1.5 cm^2) on fruits could be correlated with the percentage of fruits or leaves infested with one or more mites per lens field. If such a correlation did exist, a quick separation of infested and uninfested leaves or fruits – based on the presence of at least one mite per lens field as a criterion – would suffice as an indication of mite numbers.

To check this correlation, 15 trees in a grapefruit area of 1 ha in the Geyersvlyt citrus orchard were selected at random. From the beginning of the dry season onwards, 50 fruits and 50 leaves were inspected per tree almost weekly. Figure 33 summarizes the results. Highly significant correlations existed between:

- the number of mites per lens field of fruit and the average percentage of fruits infested with one or more mites per lens field ($r = 0.93$; $P < 0.005$).
- the number of mites per lens field of fruit and the average percentage of leaves infested with one or more mites per lens field ($r = 0.62$; $P < 0.01$).

A final step was to assess the economic threshold of citrus rust mite damage. To ascertain this threshold, grapefruit trees bearing fruits were sprayed with chlorobenzilate immediately after counting mites. Percentage of fruits with russeting was counted at harvest. It turned out that an average mite count per lens field of 2.1 on fruit corresponded with a russeting of 0.4%, which is the economic threshold of damage. Control is therefore warranted when rust mite counts per lens field reach 2, at which count 25% of fruit and 15% of leaves are infested (Fig. 33).

More research is required to establish the minimum sample needed for a reliable count on fruits or leaves infested with citrus rust mite. Sampling of

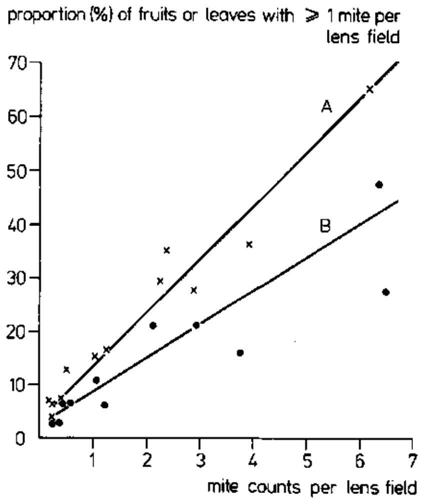

Fig. 33. Correlation between counts of citrus rust mite per lens field (1.5 cm^2) on grapefruit and proportion of fruits (A) and leaves (B) with one mite or more per lens field. Counts are averaged for 50 fruits and 50 leaves on each of 15 trees about 15 years old in a 1-ha plot. Trial at Geyersvlijt, August – September 1970.

15 trees per ha is too laborious for large orchards. A similar technique can be developed for orange groves where mites are fewer than in grapefruit groves.

5.4 BIOLOGICAL CONTROL

Small experiments and field trials show that the entomogenous fungus *Hirsutella thompsonii* can control citrus rust mite (Chapter 3), if applied as a dilute mycelial suspension (mass concentration 0.5-1 g $litre^{-1}$) to small infestations of mite.

In Florida, McCoy et al. (1971) obtained good results with *H. thompsonii* suspensions against the citrus rust mite, with high concentrations of mycelium (10, 50 and 100 g $litre^{-1}$) applied to large infestations of mite.

Further field trials are needed before biological control of citrus rust mite becomes feasible in Surinam. Its ease of culture and use, its short incubation period and its effectiveness during the dry season are promising aspects of *H. thompsonii*.

Summary

In Surinam, citrus ranks third among the economically important crops. The citrus rust mite, *Phyllocoptruta oleivora* (Ashm.), forms the most important pest. During 1966-1974 I studied the citrus rust mite at the Agricultural Experiment Station in Paramaribo and at Geyersvlijt, a citrus grove 7 km away. *Hirsutella thompsonii* Fisher is a parasitic fungus of the citrus rust mite. Trees infested with rust mite are often simultaneously infected with the greasy spot fungus, *Stenella* sp., a disease mainly of the leaves. Citrus rust mite plays a role in infection of leaves with greasy spot fungus and rust mite infestations are associated with subsequent defoliation of the trees.

These studies were made to find out more about the citrus rust mite – *Hirsutella* – greasy spot complex. The results form a basis for devising an integrated pest control program in citriculture of the humid tropics.

Citrus rust mite on fruits discolors the skin (fruit-russeting). In Surinam, about 40% of the grapefruit could not be exported because of the blemishes resulting from rust mite injury. The incidence of russeting was positively correlated with rust mite counts on trees. Fruits were often partially infested. These partial infestations seemed related to the presence of dew on the fruits. Many epidermal cells of the leaves are destroyed by the piercing activities of the rust mites. Like fruits, leaves were also often partially infested.

A mini-cage (1.5 cm diameter, 0.5 cm high), made of parchment-like paper and attached to the fruit with melted paraffin, was satisfactory for rearing the rust mite. On the leaf, mites were confined within a ring of fluon. Variation in time of development of the egg, larva 1 and larva 2 was similar for mites reared either on picked grapefruits in the laboratory or on oranges growing, protected from rain, in the open. The length of the life cycle averaged 8 days. The differences between the total life cycle on orange potted plants during the rainy season and the dry season was not significant. The larval stages took longer on leaves than on fruits.

Reproduction was entirely parthenogenetic (obligate thelytoky). The daily egg production averaged 1.7 on picked grapefruits (rainy season) and 1.4 and 1.2 on oranges on potted plants during the rainy and dry season, respectively. The

greatest number from one female was 26, deposited over a period of 16 days. In Surinam, a good 40 generations could be expected in a year (annual mean temperature: 27 °C).

During the rainy seasons, counts of rust mite were low. The mite population increased at the beginning of the dry seasons. Maximum counts were reached in 4-5 weeks, and dropped to a low level in a similar period. Dry seasons coincided with lowest mean relative humidity. However rust mite populations were not much affected by relative humidity under the tropical conditions of Surinam. Low mite counts during the rainy seasons were not entirely attributable to the entomogenous fungus *Hirsutella thompsonii*, despite the favourable moist conditions for fungal growth. They were neither the result of a washing-off, nor of a drowning (adult mites can survive 12 hours in water). They seemed to be the result of larval mortality, which increased when larvae were wetted and a water film was present on the food plant. A moist substrate seemed to interfere with molting. Rain also interfered in oviposition; rust mites avoided egg-laying on wetted parts of the food plant.

The parts of the fruits exposed to sunlight were less attractive to the rust mite than the others. The citrus rust mite could withstand the hot sun.

Counts of rust mite on the upper or lower leaf surface were markedly influenced by the age of the host plant. On young orange seedlings (about 3 months old), the mites preferred the lower surface of leaves, but on trees (grafts) older than one year the upper surface. Rains do not seem to eliminate this difference. Counts of rust mite on fruits of grapefruit were distinctly influenced by the age of the fruit. Many mites developed on full-grown fruits (resulting in fruit-russeting), but small to moderate numbers occurred on young fruits (diameter up to about 4 cm). No effect of age of the fruit on counts of mites was recorded for oranges.

I have just found an unidentified predator thrips (Phaeothripidae), which fed voraciously on rust mites under laboratory conditions and thus proved to have a high predator value.

The parasitic fungus *Hirsutella thompsonii* could be cultured most rapidly and produced most spores on Sabouraud maltose-peptone agar. The rapid decrease in rust mite after maximum infestation seemed to result from infection by *H. thompsonii* and from reduced quality of the mite-food at peak counts. In the absence of rust mites, *H. thompsonii* lost its infectivity within 3-8 days under dry conditions. In orchards, the fungus probably maintains itself inside or on the rust mite.

A developing but still low rust mite population was suppressed for at least 3 weeks with one application of a suspension of fragmented *Hirsutella* mycelium of mass concentration 0.5-1 g $litre^{-1}$. Rust mite decreased rapidly to a low level within 5 days of *Hirsutella* treatment.

In Surinam, greasy spot infections result in defoliation of the trees. The fungus could infect a leaf in the absence of rust mites. The citrus rust mite alone could not produce symptoms like greasy spot. Greasy spot was considerably promoted by rust mites; when they were absent, the leaves became only slightly infected. The incidence of lesions on leaves was positively correlated with counts of rust mite. Because of the rust mite – greasy spot relationship, partially mite-infested leaves may result in leaves partially infected with greasy spot.

Direct rust mite injury (decreased assimilation) and indirect influence of the rust mite on defoliation of the trees (greasy spot) reduced the leaf and root volume of the tree.

For the chemical control of the citrus rust mite, low-volume spraying (500 litre ha^{-1}) with chlorobenzilate solution (mass concentration of a.i. 0.2%) can be advised. Chlorobenzilate is an acaricide with little insecticidal action. Since this chemical was not fungicidal, it will be suitable for a future integrated control program.

Greasy spot was controlled effectively with tribasic copper sulphate. The use of copper compounds, however, increased counts of rust mite and of scale insects by eliminating the parasitic fungi of these arthropods. Therefore, control of greasy spot should concentrate on preventing build-ups of citrus rust mite, which promote greasy spot.

The economic threshold of citrus rust mite damage on grapefruit is when 15% of the leaves or 25% of the fruits contain one mite or more per lens field (1.5 cm^2).

Samenvatting

In Suriname, en in vrijwel alle subtropische en tropische landen waar citrus geteeld wordt, behoort de roestmijt *Phyllocoptruta oleivora* (Ashm.) tot de belangrijkste plagen van dit cultuurgewas. En gezien het feit dat de citruscultuur in Suriname, naar economische belangrijkheid, momenteel de derde plaats inneemt, betekent dit een groot verlies voor de citrusexport. Veelal blijken door roestmijt aangetaste bomen gelijktijdig geïnfecteerd te zijn door greasy spot, een door de schimmel *Stenella* sp. veroorzaakte ziekte, die voornamelijk op de bladeren voorkomt en die bevorderd wordt door het optreden van de roestmijt.

Gedurende de jaren 1966-1974 heb ik in Suriname op het Landbouwproefstation en in een citrusboomgaard op de plantage Geyersvlijt een studie gemaakt van de roestmijt, waarbij met name aandacht werd besteed aan *Hirsutella thompsonii* Fisher, alsmede aan de samenhang tussen roestmijt en greasy spot en aan de relatie tussen roestmijt-aantastingen en bladval. De ontwikkeling van de roestmijtpopulatie wordt in sterke mate beïnvloed door de aanwezigheid van de parasitaire schimmel *Hirsutella thompsonii*. Na de snelle opbouw van een mijtenpopulatie volgt een daling tot ongeveer het nulniveau.

Het onderzoek over het 'roestmijt-*Hirsutella*-greasy spot' complex had tot doel leemten in kennis, die vooral in de tropen te signaleren zijn, aan te vullen. De onderzoek-resultaten vormen een basis voor de ontwikkeling van een geïntegreerde bestrijding van plagen in de citruscultuur in de humide tropen.

Ten gevolge van de aantasting van de vruchten door de roestmijt ontstaat bruinverkleuring van de schil ('fruit russeting'). Gemiddeld wordt 40% van de vruchten van grapefruit afgekeurd. Het percentage aangetast fruit blijkt positief gecorreleerd te zijn met de mijtdichtheid. Vaak wordt slechts een deel van een vrucht aangetast. Deze partiële aantastingswijze staat mogelijk in verband met dauwvorming op de vrucht. Aantasting van de bladeren leidt tot ernstige beschadigingen van de epidermis en van de onmiddellijk daaronder gelegen cellagen. Ook het blad wordt vaak slechts gedeeltelijk aangetast.

De duur van de verschillende ontwikkelingsstadia werd bepaald door de mijt te isoleren in een mini-kooitje van perkamentachtig papier (doorsnede 1,5 cm,

als insekticide. Het middel doodt noch *Hirsutella thompsonii*, noch de parasitaire hoogte 0,5 cm) dat met behulp van paraffine bevestigd werd op een citrusvrucht; op het blad werden mijten geïsoleerd binnen een ring van fluon. De duur van de verschillende ontwikkelingsstadia op geplukte grapefruits in het laboratorium blijkt nauwelijks te verschillen van die op sinaasappelvruchten aan potplanten.

schimmel van schildluizen op citrus.

Greasy spot kan afdoende bestreden worden met driebasisch kopersulfaat. Toepassing van koperbespuitingen resulteert echter in een toename van citrusroestmijt en schildluizen, aangezien de parasitaire schimmels van deze arthropoden worden geëlimineerd. Meer aandacht dient dan ook besteed te worden aan de indirecte bestrijding van greasy spot; voorkomen moet worden dat de citrusroestmijt, die het optreden van greasy spot bevordert, zich sterk kan ontwikkelen.

De economische schadedrempel voor roestmijt op grapefruit wordt bereikt als op 15% van de bladeren of op 25% van de vruchten één of meer mijten per loupeveld (1,5 cm^2) worden waargenomen. Onderzoek naar de mogelijkheid van een praktijk toepassing van *H. thompsonii*-mycelium suspensies als biologische bestrijdingsmethode is gewenst.

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