SOME IMMEDIATE EFFECTS ON ALMOND LEAVES OF FEEDING BY *BRYOBIA RUBRIOCULUS* (SCHEUTEN)

BY

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ABSTRACT

Brown mites, *Bryobia rubrioculus* (Sch.), on almonds feed chiefly on upper leaf surfaces. Stylet punctures through the uppermost cuticle of epidermal cells are described. It is estimated that the protoplasts of about 200 palisade cells were destroyed in 30 minutes. When almond trees infested with brown mites were disinfested, they appeared to recover thereafter some of the lost green color. Apparent recovery after mite control related to normal increase in chlorophyll concentration during the period from bloom to June 1. Concentration of chlorophyll *a* and *b* (mg/gm of leaf tissue) increased in undamaged palisade cells while other, nearby palisade cells were being destroyed. The rate of gain in chlorophyll concentration appeared to be similar for both damaged and undamaged almond leaves. Replacement of damaged cells was not demonstrated. Reduction in leaf area due to brown mite feeding from the beginning of the growing period until June 1 averaged 29.8 %.

Brown mites, also known locally as brown almond mites, *Bryobia rubrioculus* (Scheuten), are spider mites which frequently infest trees of stone fruit varieties. They are especially troublesome on almonds in California during the period between petal fall and mid-June. Mites of this species are transient leaf-feeders on this host. During the non-feeding periods they congregate and lay eggs on rough twigs or fruit spurs, mostly on the under surfaces — surfaces which face the ground. An actively feeding portion of the population stabilizes on almond leaves within approximate temperature limits of 70° to 85°F. The mites browse principally on the upper surfaces of almond leaves, feeding in one place for a short time and then moving to another.

In one recent observation, 8-10 almond mites were confined in a small plastic cell applied to the upper surface of a picked almond leaf upon which no mites had fed. One of the individuals wandered briefly and then fixed upon a place to probe. Several strong up and down motions of the stylophore occurred before its pedal motions ceased. The body then tilted slightly downward in front, as though positioned to use leg muscles as aids in the probing of leaf tissue. The relatively stationary phase persisted for about 30 minutes. During this static period the major stylophore movements were infrequent, as signalled by the flapping of the external lobules of the peritremes. Near the end of this phase the body began to pivot and rock slightly but no change of leg posture was made. The frequency of stylophore motions increased in amplitude and frequency. At this instant the churning of the gut contents also accelerated. The restless phase continued for only several minutes before the gnathosoma was lifted from the leaf surface.

The mite then moved forward a fraction of its body length and repeated the entire operation. At the end of 90 minutes the feeding at this general location ended and the mite moved away.

This particular mite appeared to be a teneral adult female, light brown in color, depressed or flattened dorsally. When it finished feeding at this place on the leaf, after 90 minutes, its body had plumped up and the coloration changed to dark greenish brown, allowing little visibility of midgut movements. This individual tolerated the touch of other almond mites without interrupting its feeding. Contact between its mouthparts and the leaf surface was not visibly altered when other brown mites scrambled over its back or repeatedly touched its legs.

When the mite moved away, the locus of feeding on the leaf appeared as a chlorotic blotch, irregular in outline, approximately 0.15 mm in diameter. The affected spot showed no tendency to bleed or show surface wetness and, at this time, it could not be determined that the affected leaf cells were air-filled or the surface depressed. Almond mites similarly observed at other times have followed this general pattern of activity, with differences principally in duration of feeding and areas of leaf tissue involved.

Leaves on infested almond trees in mid-spring have a mottled appearance when the continued feeding of almond mites causes confluence of the chlorotic blotches. The epidermis covering each old feeding site is slightly depressed and there is a silvery sheen over the affected areas of the leaf. The patina is due to the presence of air in empty palisade cells and possibly also in dead epidermal cells. That air occurs in the palisade cells of apple leaves attacked by European red mite has been reported by BLAIR (1950).

Leaves attacked by almond mites are spotted with their dried excrement. The excretion may be a clear, viscous droplet which dries very quickly to a glossy, varnish-like deposit. Mostly, however, the excreted droplets contain few to many dark colored, ovoid pellets of almost uniform size. The drying of the pellet-laden droplets produces shiny black surface spots which become especially obvious when leaves begin to bleach or yellow during May and June.

Brown mites attack the palisade parenchyma, principally from the upper leaf surface (Figs. 2 and 3), and mottling shows first on this side of the leaf. There are no stomata in the upper epidermis of the almond variety studied (PEERLESS). This mite feeds on the lower leaf surface to a limited extent. The chlorotic blotches are less sharply defined and damage to the spongy parenchyma is difficult to demonstrate histologically.

The tissue-piercing mouthparts are a pair of long cheliceral stylets which anchor in a fleshy stylophore. The latter slips up and down in a trough on the anterior face of the conical rostrum and the needle-like stylets slide in a deeper, narrow groove beneath the overlying stylophore (BLAUVELT, 1945). The mouth is believed to open on the apex of the rostrum. The tip of the latter is applied to the leaf surface and sometimes held fast, possibly by the powerful pharyngeal pump. As far as now known, the ends of the pedipalps and the apex of the rostrum are applied to a place to be probed and the stylophore protracts to cause the stylets to penetrate the tissue. The nature of the perforations in the leaf epicuticle seem to affirm the supposition that the leaf cell contents are sucked out through a few small perforations. We have been unable to observe concisely how the feeding operation proceeds, whether probing and food uptake occur together, or whether the stylets must be retracted before the mouth can be applied to the surface puncture. Brown mites which climb the vertical walls of plastic cages sometimes apply the rostral cone to this surface and use it to hold tightly by suction when the front legs are palpating.

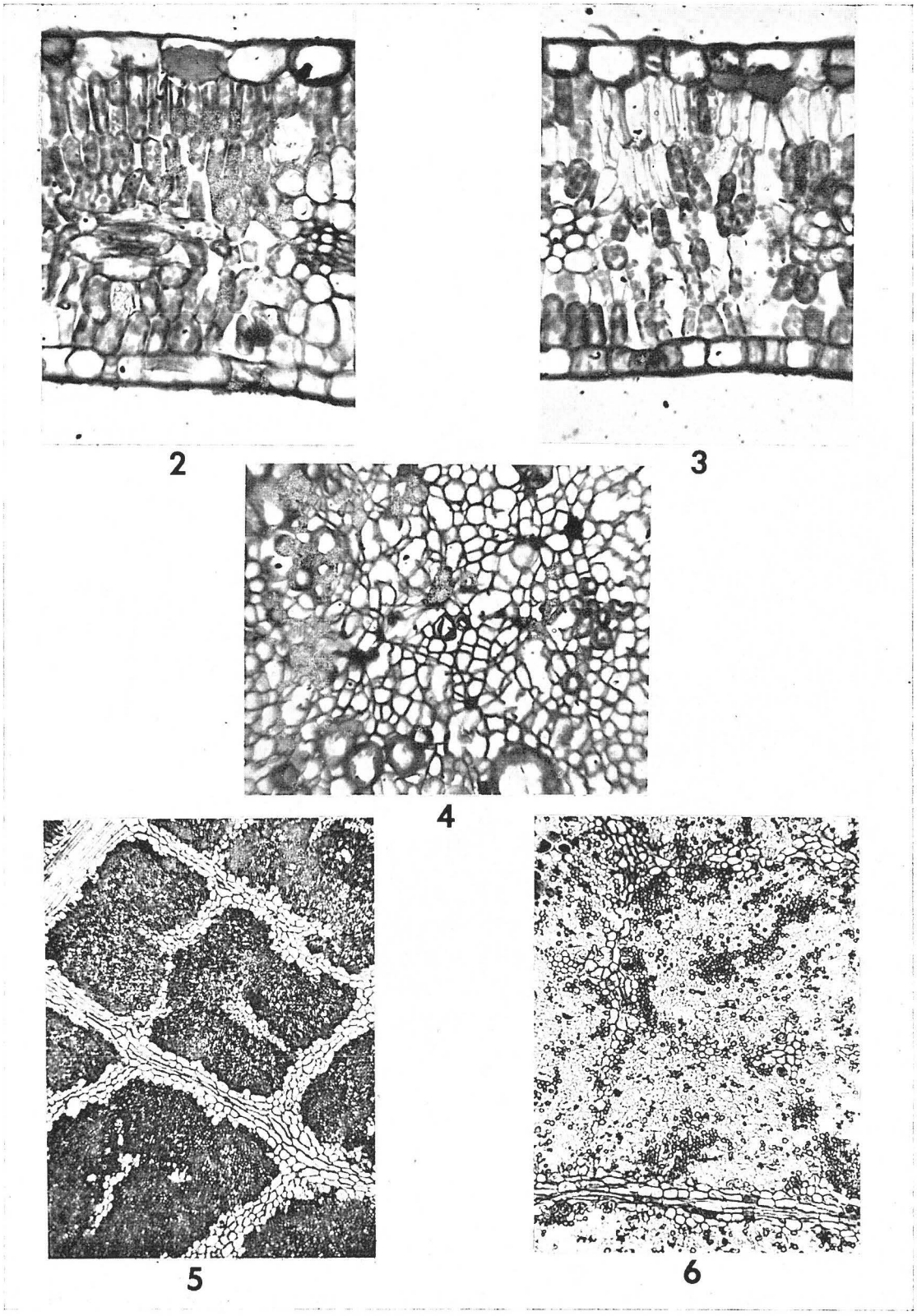

FIG. 2. — Cross section of a portion of almond leaf having no mite damage ; FIG. 3. — Cross section of a portion of leaf seriously affected by brown mites. Note numerous empty palisade cells in first and second tiers ; FIG. 4. — Small area of photo in Fig. 6 enlarged to show breakages of cell walls in badly damaged leaf tissue ; FIG. 5. — Paradermal section of leaf through upper palisade layer. From a mite-free tree, ca. × 100 ; FIG. 6. — Paradermal section of seriously mite-damaged leaf. Intact palisade cells (dark circles) occur singly or in small clumps, ca. × 100.

Structural damage to leaf tissue.

Histological study indicates that at least two tiers of palisade cells can be destroyed where as the spongy mesophyll cells appear to be unaffected by upper surface feeding. Measurements of the cheliceral parts of slide-mounted specimens of this spider mite suggest that its stylets may be capable of penetrating to a maximum depth of 95 μ. This is a rough estimate based on the length of the unsheathed distal part of a stylet which projects beyond the foremost end of the stylophore in repose plus the length of the basal piece or fulcrum by which the stylet is exserted. Transverse sections of mature Peerless almond leaves, in central areas, show that the total leaf thickness approximates 175 μ and the thickness from upper cuticle through two layers of palisade cells approximates 90 μ. In some cross sections of the leaf, it is frequently evident that only the uppermost tier of palisade cells is affected. In other cross sections sometimes only the second tier of cells shows involvement in mite feeding whereas cells in the overlying sheet are intact. Lateral flexions of the stylets are thus indicated. Evidence of mite feeding in the vascular tissue is lacking at present.

Microtome sections made parallel to the upper leaf surface and which include the uppermost cuticle reveal the punctures made in the outer epidermal wall by the stylets of the mite. Paraffin sections of leaves fixed in Craf and dehydrated with tertiary butyl alcohol show each perforation to be a minute, flattened ellipse approximately 3.5 microns across its greatest diameter (Fig. 7). Its outline does not show the imprint of the separate stylets. Other preparations freeze dried and gold-shadowed for scanning electron microscopy show a perforation to be a narrow slit (Fig. 8).

Perforations in the upper epidermal surface are thinly dispersed, usually not more than one per epidermal cell and not demonstrable in every epidermal cell, even of extremely damaged leaves. We have found very few instances where there were 2 or 3 perforations in one epidermal cell. One cell of the upper epidermis of this almond variety covers the ends of approximately 10 palisade cells. The number of palisade cells affected by the feeding in one area of a leaf for a few minutes is surprisingly great. In the observation described above it was possible to fix and stain the affected leaf tissue (Fig. 9). From this preparation it is estimated that more than 200 chlorophyll-bearing cells were affected by the feeding at one site, i.e., during 30 minutes (Fig. 10). The two-spotted spider mite on bean leaves is able to puncture and empty 18-22 cells per minute (Liesering, 1960).

We suppose that the stylets are churned up and down within a surface puncture and that the stylets splay outward to cut obliquely into the sidewalls of the palisade cells. It now appears that few punctures are made in the upper epidermis at each feeding site. The fate of the pierced epidermal cells is conjectural at present.

Leaf tissue fixed immediately after being fed upon shows that some cells in the chlorotic area are empty whereas others contain collapsed or disorganized protoplasts. The latter probably represent an antemortem state because preparations of severely damaged leaves contain empty cells almost exclusively. The walls which separate some of the old empty cells ultimately break down to create larger empty or air-filled lacunae.

We can present no positive information about salivary injections vis-a-vis leaf tissue hystolysis. The short time required to produce an injury comprising so many empty cells suggests that the greater immediate effect of the bite of the brown mite is mechanical rather than chemical. Other observers have reported that tetranychid mites do inject secretions into the plant tissue

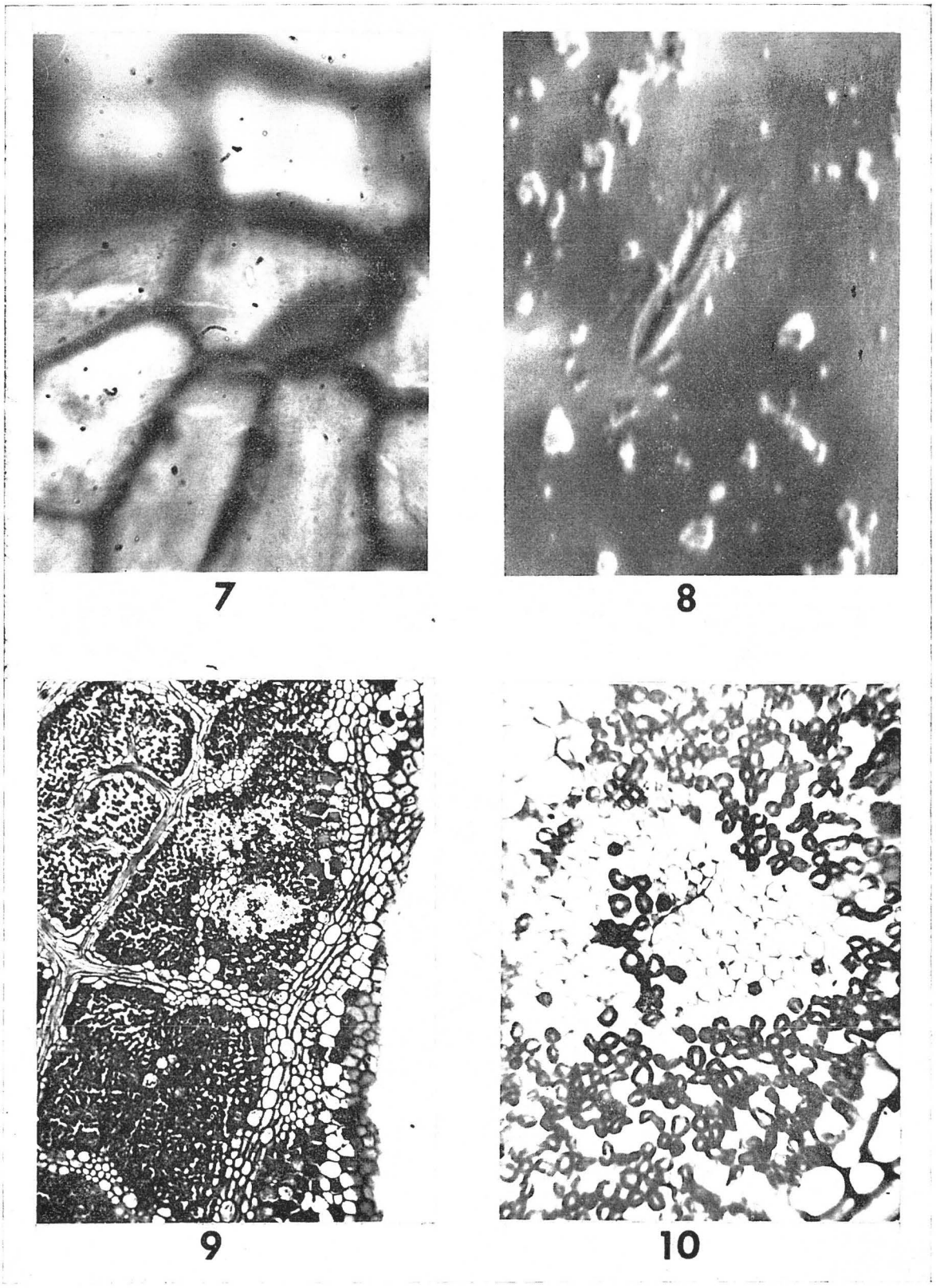

FIG. 7. — Photomicrograph of mite puncture in outer wall of upper epidermis. Small puncture located near center of picture. Conventional microscope ca. × 850 ; FIG. 8. — Scanning electron microscope photograph of mite puncture in upper epidermis, ca. × 9,000 ; FIG. 9. — Paradermal section of leaf showing a chlorotic blotch attributed to feeding of one brown mite in 30 minutes (light area near center) ; FIG. 10. — Greater enlargement of the chlorotic blotch to show absence of chloroplasts in affected area.

(LIESERING, 1960 ; AVERY and BRIGGS, 1968) and that the protoplasts of many of the affected cells are sucked out. There may be a residue of amber-colored coagulum in the cells of apple leaves damaged by the European red mite (BLAIR, 1950). Such residues are not conspicuous in the palisade cells of almond leaves.

EFFECT OF MITE INJURY ON LEAF AREA.

Almond is a suitable host plant for measuring differences in leaf area attributable to the feeding of mites. The leaves are fairly small, nearly flat, stiff blades having almost no marginal serrations. Leaves which accumulate a considerable amount of mite damage from the outset of the growing period until the attack nears its peak or crisis during June fail to develop normally. They are smaller in area and abnormal in shape. On trees of the Peerless variety completely disinfected with prebloom sprays, the leaves tend to be elongate, gradually tapered to prominent points, and heavy enough to hang in a near vertical position. Trees of the same variety which nurture heavy populations of brown mites are apt to have leaves which are broad in relation to length, rounded to blunt points, and tending to project laterally from the shoot axes. The mite damage on or near the growing tip probably retards axial growth so that eventually the matured blade is deficient in length and somewhat altered in form.

Since there is no established method for obtaining reproducible counts of brown mites on the foliage of almond trees, the number of mites on the untreated trees involved in this experiment can be described as sufficient to have damaged 35 % of the mature leaves to " Grade 5 " on a grading scale of 1 (clean) to 5 (bleached). This estimate is based on the classification of 500 mature leaves randomly picked from one check tree on June 1. Mites-per-leaf counts as commonly used for defining populations of tetranychine mites are unreliable indicators of brown mite populations (SUMMERS and BAKER, 1952).

A fairly simple method was improvised to give data on differences in area of leaves on treated and untreated trees. A random sample of 500 full size leaves was picked from one untreated tree and another 500-leaf sample was taken from an adjacent tree on which no almond mites were allowed to develop. Each leaf was measured for greatest width and length. The sum of the length × width products was determined for the 500 leaves of each lot. One hundred leaves were randomly picked from each of these two 500-leaf samples and their outlines imprinted on paper with an office copy machine, about 20 per page. Rectangles having the length and width dimensions of each were carefully pencilled around each image. The rectangles were cut from the copy sheets, 100 rectangles for each subsample. These were then further trimmed to the outline of each leaf. The pieces of paper representing leaf areas and non-leaf areas were collected and weighed. The ratio of the two weights was then used to convert the total length × width to approximate leaf areas. The method of calculating the leaf areas was adapted from a procedure described by MCNAIR and BONELLI (1969).

According to data given in Table 1, the mite-free leaves which matured before June 1 had an average surface area of 792 mm^2. Those which were taken from an adjacent but severely damaged tree had an average surface area of 556 mm^2. The loss of area attributable to prolonged attack by *B. rubrioculus* — no other phytophagous mites present — was approximately 29.8 %.

Somewhat comparable stunting effects of European red mites on plum tissues have been recorded by BRIGGS and AVERY (1968) ; heavy infestations of ERM reared on potted plum rootstocks consistently decreased the length of new shoots by about 10 % and the dry weight of

new shoots and dry weight gains of old stems and roots by about 20 %. Increased yields of fruit and increments in trunk girth were obtained in apple orchards in which European red mites were controlled (CHAPMAN *et* AL., 1952).

TABLE 1. Estimate of reduction in leaf area attributable to severe attack by brown mites. Samples taken 6/1/71, Peerless var., Davis, California.

	ΣL × W 500 Lvs. (mm²)	Weight of Paper Cut-outs — 100 Lvs. (gms)		Average Area per Leaf (mm²)
		Leaf Areas	Non-leaf Areas	
Undamaged.....	554,839	6.466	2.601	792
Damaged.....	381,756	4.697	1.758	556

EFFECT OF MITE FEEDING ON CHLOROPHYLL CONCENTRATION.

Near the peak of attack by brown mites, some of the leaves on experimental trees were so badly damaged that they appeared to be bleached, or without obvious green color. However, extraction with acetone demostrated that, on June 15, such leaves retained about 0.9 mg chlorophyll a and b per gram of whole leaf homogenate. Paradermal microtome sections of severely damaged leaves which still retained a slight green color demonstrated that the few remaining palisade cells were irregularly scattered, singly or in small clumps, throughout a matrix of cell wreckage (Fig. 6). We have not been able to distinguish histologically the old and new " bites ". It is probable, however, that the breaking of cell walls (Fig. 4), and the formation of pathological lacunae occur within the oldest damaged areas.

One of the principal objectives of this study was to investigate the nature of the color change in formed leaves after they are disinfested. Leaves on moderately infested trees, in mid-spring, appear to become greener and the chlorotic blotches partly fade away in time after an effective acaricide is applied. Is this partial restoration of green color attributable to tissue regeneration or possibly to some kind of reorganization of the surviving cells ?

An experiment to measure chlorophyll at several intervals after trees are disinfested was established on Peerless trees known to be heavily infested with brown mites. Three conditions were set up. One plot of 2 trees (A) was winter-sprayed (February 9) to destroy the eggs of the mites before the onset of the growing season. A second plot of 2 trees (B) was not sprayed until most of the leaves displayed mite damage and moderately damaged leaves were abundant. At this date, May 4, the trees had accumulated brown mite injury for approximately two months. No chemicals of any kind were applied to 2 trees of a third plot (C). These sustained the brunt of the attack and were seriously damaged when the attack peaked, about June 15. The population of active almond mites declined rapidly there-after. One tree in each plot was sampled for chlorophyll five times at 14-day intervals beginning immediately after the second plot (B) was sprayed. Leaf samples for analysis were picked from the same tree each time. One sample comprised 200 randomly selected full-size leaves. Four replicate samples per tree were taken each time. The leaves were bagged in paper, held at 40°F overnight and extracted the following

day. Each sample of leaves was shaken to mix in its original bag, then passed though an ordinary household meat grinder equipped with its coarsest cutter. The chopped leaf bits were collected in a plastic bag of small volume and then shaken manually to provide a homogenate of crude leaf bits. Immediately thereafter a subsample of 5.0 gm of leaf particles was weighed and then macerated in 50 ml 80 % acetone in a Virtis " 45 " homogenizer. The subsequent extraction of chlorophyll in 80 % acetone and the spectrophotometric analysis were done according to the method described by ARNON (1949).

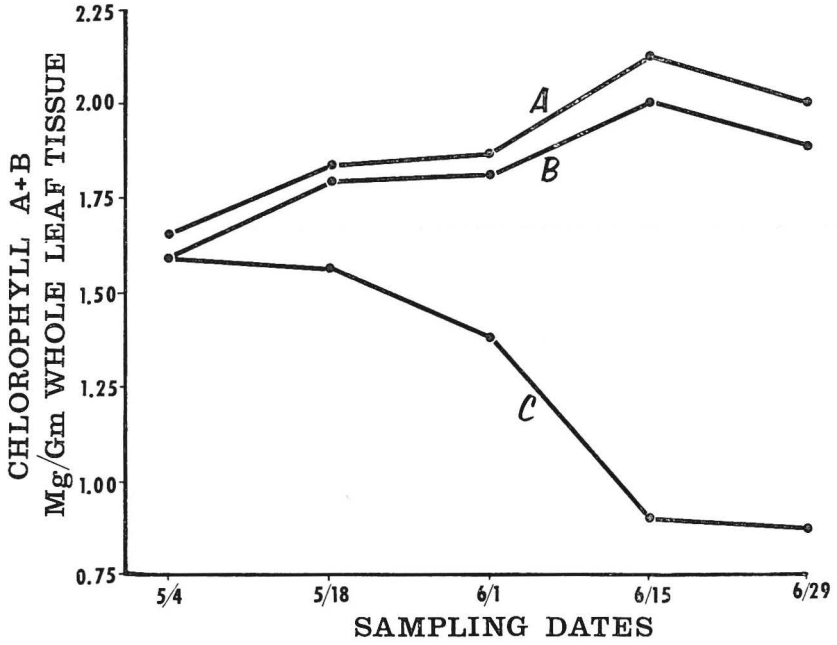

FIG. 1. — Amount of chlorophyll a and b extracted from Peerless almond leaves at five 14-day intervals. A = trees having no brown mites ; B = trees disinfested on May 4 ; C = untreated trees.

The concentration of chlorophyll changed appreciably after May 4, the date on which the trees of plot B were sprayed (Fig. 1). Although the differences between trees of plots A and B were readily apparent by visual inspection, their initial differences in chlorophyll concentration was much less than anticipated. This small differential between treatments A and B and the limited number of replicate samplings do not permit a critical conclusion about post-treatment regeneration in the mite-damaged foliage. However, the data show unmistakably that leaves on this almond variety continue to gain chlorophyll throughout the period of almond mite attack. The rate of gain in partly damaged leaves (Plot B) does not appear to be greater than in completely undamaged leaves (Plot A).

The post-treatment fading of the chlorotic blotches in moderately damaged leaves seems to be attributable principally to normal development rather than to reparative processes. The question of whether damaged green parenchyma may have a regulative or compensative capability requires more sophisticated methods than employed in this study.

When the mite infestation abated naturally after mid-June, the untreated trees in plot C were badly bleached. Not yet known is how much damage the leaf tissues can sustain before the normal chlorophyll gain begins to decline.

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