BIOLOGICAL STUDIES ON THE CITRUS TREE SNAIL *Drymaeus dormani* (BINNEY), AND THE CITRUS RUST MITE *Phyllocoptruta oleivora* (ASHMEAD), AS WELL AS THE EFFECT OF DIFFERENT ACARICIDES ON THE CITRUS RUST MITE

By

Michael Edward Bledsoe

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Chairman: D. R. Minnick
Major Department: Entomology and Nematology

An arboreal snail, *Drymeaus dormani* (Binney), and several acaricides were evaluated for their effect on the citrus rust mite, *Phyllocoptruta oleivora* (Ashmead), on citrus in North Central Florida. *D. dormani* was found to have the potential to suppress the citrus rust mite populations by ingestion of the mites and their spermatophore as well as by deposition of mucilage on the fruit surface. Studies using scanning electron microscopy to observe the fruit surface, where snails had grazed, demonstrated the removal of all microbiota. *D. dormani* feces were determined to contain fungi, whitefly pupae, citrus rust mites, citrus rute mite spermatophores, and other mites.

Snail grazing and motion were demonstrated to be dependent on 100% relative humidity. *D. dormani* potential for removal of fungi and citrus rust mites was determined.

Studies of citrus rust mite intrinsic and extrinsic orientation on Valencia orange failed to demonstrate tree orientation, while orientation on the fruit was greatest at the marginal (semi-shaded) areas.

In 1975, control of citrus rust mite by PP199 (0.02%) was comparable to dicofol (0.03%) standard while oxamyl and PP067 (0.04%) gave nonacceptable control. In 1976, PP199 and oxamyl exhibited better control of citrus rust mite than the chlorobenzilate standard. Banex® gave poor control.

Chairman

LITERATURE REVIEW

Citrus Tree Snail

An arboreal snail, Drymaeus dormani (Binney), was first reported in citrus groves in Florida by Sellards (1906). D. dormani has commonly been called "Manatee snail" (Simpson, 1893; Sellards, 1096; Dailey, 1975), "Tree snail" (Sellards, 1906; Norris, 1955), "Citrus tree snail" (Kramer, 1952), and "Bob Norris snail" (Lawrence, 1950).

L. J. Dorman first collected the snail near St. Augustine, Florida, and Binney (1857) described the species as Bulimulus dormani Binney, in honor of Dorman. B. dormani was found to be closely related to B. masculatus and B. floridanis of New Grenada (Binney, 1859; Binney and Bland, 1869).

The genus was changed to Liostracus dormani (Tryon, 1882). Pilsbry (1899) assigned the citrus tree snail to its present genus.

The current hierarchical classification of D. dormani is as follows:

Kingdom	-	Animal
Phylum	-	Mollusca
Class	-	Gastropoda
Subclass	-	Pulmonata (air breathers)
Order	-	Stylommatophora (terrestrial)
Family	-	Bulimulidae

The range of the snail is limited in the U.S.A. to Florida (Griffiths, 1949). Pilsbry (1946) stated that D. dormani was "an

apparent descendent of a Mexican species, and it probably migrated to Florida via the Southern United States in Pliocene times" (p. 24). By 1895, following the description given by Binney (1885), the snail was reported near the Manatee River, at Port Orange, Florida, and Oak Hill, Florida, in Volusia County, and on Florida's West Coast. Simpson (1893) reported finding several hundred dead shells in a heavy hammock north of the Manatee River.

Very little practical interest was accorded the citrus tree snail until Sellards (1906) reported that it was feeding on sooty mold on citrus in Manatee County, Florida. It was brought to Sellards' attention by Mr. F. D. Waite who, in 1904, first noted the cleaned fruit and leaves. The use of the citrus tree snail as a biological agent grew from 1906 until Norris (1946) summarized existing knowledge on the history of the snail from this time.

Biology. Binney (1878), giving a general description of land snails, stated they normally lay their eggs in the soil and were phytophagous. Griffiths (1949) gave an estimate of 40 or more eggs layed in mass by the citrus tree snail. This was later defined as 40 to 400 eggs per egg mass (Muma, 1955). The newly hatched snail measures 2 mm or less (Norris, 1946; Griffiths, 1949).

The snails were believed to take up to two years to reach full maturity (Tryon, 1882) until Muma (1955) recorded mature snails in one year or less. Fully mature shells reach 3 cm in length (Griffiths, 1949).

Lawrence (1950) stated that the snail was hermaphroditic, laying its eggs in the soil and leaf mold at the base of citrus trees during the summer rainy season. Griffiths (1949) placed oviposition in the

early summer months over a period of six to eight weeks, possibly triggered by onset of warm weather.

Morphology of the shell was described by Sellards (1906) as smooth, white or coneous white, with about four bands of brown spots. Old shells often have corroded surface, the bands becoming indistinct or absent.

Physical requirements. The order Stylommatophora or terrestrial air breathing mollusks have learned to control water loss by frequenting areas of high environmental humidities (Edney, 1960). Boycott (1934) found only 12 obligatory hydrophiles and eight species of xerophiles. Hunger (1964) stated that water is primarily lost through general evaporation from the moist skin, but some loss resulted from the continued pedal secretion of mucus for locomotion. The land snails are active at night or just after rain (Binney, 1878). Binney also mentioned the epiphram, a semitransparent membrane-like structure, which is secreted by the snail during hibernation to attach the shell to substrate and help reduce water loss.

Griffiths describes the onset of hibernation in December coinciding with winter cold. Activity is reinitiated in the spring. He also suggested that breaking of dormancy may have to do with tree growth and availability of water.

The "resting stage," that period when the snail is withdrawn into the shell, is generally divided into two categories. The first is for the purpose of hibernation. Hibernation is usually for overwintering and the mouth of the shell is sealed by secretions of calcareous material and hardened mucus termed the "epiphragm" (Hunter, 1964). The second type of "sealing off" occurs daily during unfavorable conditions.

Howes and Wells (1934) referred to it as an estivation period. During estivation, a "mucous veil" of dried mucus seals the opening. The use of the term estivation here is unfortunate since the closing off is short term and may occur at any time of the year.

Mature citrus trees six years old and older are the only ones from which the snail can receive the proper abiotic conditions for survival (Kramer, 1952). Cover crops were suggested by Kramer (1952) as a means of increasing the shade and relative humidity in the grove undergrowth for snail migration. He also suggested this would increase humidity in the trees.

Hunter (1964) discussed the ability of stylommates to maintain temperature homeostasis by withdrawal into the shell. The migration of snails toward a preferred temperature or habitat was discussed by Getz (1959). He showed the snails possessed a temperature and humidity preferendum.

Calcium is necessary for development of snail shells. Experiments on land snails in captivity show that the thickness and weight of the shell is directly dependent on the amount of calcium in food supplied (Boycott, 1934; Robertson, 1941). Some snail species are limited to high calcium content soils (Boycott, 1934).

Predators, parasites, and pathogens. Muma (1955) suggested factors that cause mortality of D. dormani. Egg mortality was due to desiccation and to small earthworms. Newly hatched snails were preyed on by a predatory snail, Euglandina rosen Ferrusac. Additional mortality was attributed to desiccation. Young snails were found to be parasitized by two Diptera, Johnsonia elegans Ald. and Johnsonia sp. probably frontalis Ald. Euglandina rosea also was found to prey on young snails (Muma, 1955).

Predators of mature snails include birds, mice, and again, E. rosea. Two disease-like conditions were described as green body and brown body. Two parasitic flies, Sarcophaga lambens and S. morionella, were associated with mature snails, as were Hippelates dissidens (Tuck) and megaselia sp. (Muma, 1955).

Chemicals. The first study of the effects of chemicals on the citrus tree snail was undertaken by Norris (1946). Zinc sulfate or sulfur was shown safe to snail colonies. Kramer (1952) warns of dusting or spraying snail infested trees with the exception of sulfur. He also states that fertilizer applied to the base of citrus trees during summer months should be avoided because of its toxicity to young snails. The nitrogen portion of chemical fertilizers is toxic to the snail causing immediate withdrawal into the shell and death (Muma, 1955). He indicated that zinc and copper were repellent to the snail because no feeding occurred on leaves dipped in these solutions. Boron and manganese displayed no toxic or repellency effects, while arsenic treated leaves were readily fed on and resulted in death. Muma also recommended sulfur, lime-sulfur, and to a limited extent oil in conjunction with snail culture.

Food sources. Binney (1878) described the land snail as a phytophagous with no mention of any other food source. Algae was reported as a food source by Pilsbry (1946). Sellards (1906) first recorded D. dormani feeding on sooty mold on citrus in Manatee County, Florida. Kramer (1952) stated that the snail consumes neither scales or mites but, due to the deposited slime trail, makes conditions unfavorable to these pests. Large lichens and entomopathogenic fungi on white fly were reported unaffected by the citrus tree snail (Griffiths, 1949).

Melanose infestation serious enough to cause economic damage was not found in snail groves from 1946 to 1948 (Griffiths, 1949). Griffiths speculated as to the possibility that the snails may eat the spore normally found on the tree trunk.

Effects on yield. Griffiths (1949) in a study of the effect of the snail on yield production compared to nonsnail trees was unable to show an increase or decrease in production costs or yield in the groves. Muma and Selhime (1963) considered the purple scale, Florida red scale, Chaff scale, Glover scale, dictyospermum scale, citrus rust mite, Texas citrus mite, and citrus red mite on snail and nonsnail trees. They reconfirmed Griffiths' findings that no difference could be found from snail versus nonsnail trees. Sprayed plots, however, maintained lower infestations than the unsprayed snail plots with exceptions of Florida red scale and citrus red mite. Muma and Selhime (1963) also found that sprayed plots produced a higher quality fruit with a higher yield than the unsprayed acreage.

Citrus Rust Mite

The citrus rust mite, Phyllocoptruta oleivora (Ashmead), is considered a serious pest in all humid citrus growing regions of the world (Delucchi, 1975). This species was introduced into the Western Hemisphere from Southeast Asia (Yothers and Mason, 1930). The citrus rust mite belongs to the family Eriophyidae. It is tetrapodili having only four legs located anteriorly near the mouth. The eriophyids characteristically have elongated bodies annulated with small spines or furrows giving a segmented appearance (Krantz, 1975). A complete generation can be completed in six to eight days during warm seasons in subtropical regions (Delucchi, 1975).

Egg. The egg of the citrus rust mite is round, white or pale yellow, with a smooth surface approximately .03-.04 mm in diameter (Swirski and Amitai, 1958). Yothers and Mason (1930) referred to detection of a folded larvae visible within the egg a few hours prior to hatching. Both Yothers and Mason (1930) and Swirski and Amitai (1958) found the egg incubation period to be 3.1 days.

Larva. Swirski and Amitai (1958) described the first and second stages of the citrus rust mite. The first stage is white and measures about .08 mm in length. The second stage ranges from .10 to .12 mm and is pale yellow. Developmental times varied according to temperature with 3.1 days at 32.6°C and 10.7 days at 25.1°C (Yothers and Mason, 1930).

Adult. The adult citrus rust mite is generally wedge-shaped, pale yellow, and 1/200 in. long (Muma, 1965). The adult becomes light brown to brown with age (Swirski and Amitai, 1960). The female is 0.15-0.16 mm in length and lives up to 16 days at 26°C (Swirski and Amitai, 1959) while the male is only 0.13-0.14 mm in length (Keifer, 1938; Swirski and Amitai, 1959).

Sex determination. Yothers and Mason (1930) described the citrus rust mite as parthenogenetic when they were unable to find any males. Ebling (1959) found 39% males present in units of 144 individuals. Swirski and Amitai (1960) recorded males to be present throughout the year with increase in spring and decrease numbers in autumn.

Independent observations were made by Oldfield et al. (1970), Sternlicht (1970), and Sternlicht and Goldenberg (1971) as to the occurrence of spermatophores in Eriophyidae and the females self-fertilization (laying eggs resulting in offspring of both sexes, as opposed to unfertilized females which lay parthenogenetic eggs bearing only male

offspring). This type of fertilization is common in tetrapodeliform acari (Shevtchenko, 1957; Hall, 1967; Oldfield et al., 1970; Sternlicht and Griffiths, 1974).

The use of spermatophore is a primitive method of insemination where a male deposits a spermatophore (sperm sac) on the substratum (Chapman, 1971). P. oleivora spermatophore consists of a base, a stalk approximately 10 μ in length, with an expanded apical head (12 μ in diameter), and capped with a spherical sperm bearing case approximately 3 μ in diameter (Oldfield et al., 1970; Sternlicht and Griffiths, 1974).

The male P. oleivora produced approximately 16 spermatophore per day (Oldfield et al., 1970). The sperm capsule of the spermatophore of P. oleivora is removed and taken into the female (Oldfield et al., 1970). The attraction of the spermatophores for virgin females of Eriophyes sheldoni Ewing was noted (Sternlicht and Goldenberg, 1971). This was later confirmed with Aculus cornutus (Banks) (Oldfield et al., 1972).

Rust mite dispersion. Bodenheimer (1951) found the citrus rust mite primarily on the outer canopy with the highest numbers near the crown of the tree during the warm season. During winter months these mites concentrate on the undersides of the citrus leaves and the interior portions of the tree (Yothers and Mason, 1930; Swirski, 1962). Hibernation was suggested by Bodenheimer (1951) as a means of overwintering severe conditions.

Positive phototaxis was demonstrated by Yothers and Mason (1930), but the eriophyids avoided the direct sunlight. A semi-shaded preference of the citrus rust mite on citrus fruit was mentioned by Watson and

Berger (1937). They found rings of rust on citrus fruit corresponding to the aforementioned semi-shaded areas.

Citrus rust mite populations are extremely variable from tree to tree and from various parts of the same tree. This variation in population distribution was substantiated by Osburn and Mathis (1944). Swirski (1962) felt that the recorded discrepancies were due to a lack of knowledge of density and its relationship to time and space.

Pratt (1957) reported two peaks of infestations annually during the summer months. He attributed this to the number of hours at dew point. Rasmy et al. (1972) was not able to support his correlation to relative humidity, but was able to show a relation to temperature.

Cultural practices. Cover crops, the cultivation of annual crops in citrus groves, were studied by Osburn and Mathis (1944). They felt cover crops helped maintain a humid condition that supports parasites and especially fungi that attack the citrus rust mite. They were unable to support this hypothesis, finding that cover crops had very little affect on relative humidity, temperature, parasites, and amount of fungi present. However, they did state that clean culture stimulated tree growth and gave an overall impression of a healthier tree.

The condition of the citrus tree has been suspected of affecting rust mite populations. Hamstead (1957) demonstrated a correlation between rust mite populations and high nitrogen levels in leaves. Leaf age was shown to cause variations in populations (Mohamed, 1964). Muma (1965) attributed fluctuations in population to leaf drop and wind.

Cultural practices such as hedging or thinning of citrus trees were found by Swirski (1962) to improve conditions for the citrus rust mite. Overhead irrigation was shown to cause a sixfold mite popula-

tion increase (Rasmy et al., 1972). Tree planting distances, especially relating to tree crown distances, were related directly to rust mite density (Swirski, 1962).

Chemical control. Yothers and Mason (1930) gave an account of tobacco and whale-oil soap being used to eliminate the russeting damage. Sulfur was introduced as a miticide, but was found (Speare and Yothers, 1924; Griffiths, 1950) detrimental to fungicidal activity by reducing entomopathogenic fungi attacking the citrus rust mite. Scheduled treatments were applied in the spring, summer, and the fall. Numerous acaricides such as chlorobenzilate, ethion, sulfur, and dicofol can be used for control of P. oleivora (Florida Citrus Spray and Dust Schedule, 1977).

Injury to citrus fruit. Citrus rust mite has been reported to be responsible for three visable types of fruit injury, namely sharkskin, russet, and bronzing (Griffiths and Thompson, 1957; Albrigo and McCoy, 1974). McCoy and Albrigo (1974) demonstrated that injury to the surface of citrus fruit by P. oleivora is restricted to epidermal cells. Sharkskin which is found on grapefruit, lemons and limes (Yothers and Mason, 1930; Griffiths and Thompson, 1957) is occasionally found on oranges. This is characterized by severe damage at an early age. Further fruit growth results in cracking of the dead epidermis in patches which may slough off, leaving a smooth injured periderm (Albrigo and McCoy, 1974). Russet damage, which occurs prior to fruit maturity, results in additional fruit growth, which breaks up dead epidermis, and subsequent wound periderm formation beneath the epidermis. The cracks result in an unpolishable rough texture, while the oxidized cell content gives the fruit the rust color (McCoy and Albrigo, 1974). Fruit

bronzing is damage to the surface of citrus fruit when little additional fruit growth will occur. P. oleivora feeding causes epidermal cells to die and turn brown, but the cuticle does not crack. These fruit will take a polish because the cutin and waxy layers remain intact (McCoy and Albrigo, 1974).

Methods of sampling. The square method of sampling was described by Yothers and Miller (1934) as a piece of paper with a half inch square hole cut in it. The paper was placed over the surface of the citrus leaf and mites counted by viewing through a 10X hand lens. A linen tester with a defined field of 0.5 in. x 0.5 in. was used to establish P. oleivora infestation by Osburn and Mathis (1944). Pratt (1957), Johnson (1960), and Simanton (1960) used 10X hand lens to count citrus rust mites per field of view. A stereoscopic microscope at 18X magnification was used to count mite populations on leaf samples (Dean, 1959). Later that year, a brushing machine was used to gently brush the mite population off the surface of leaves by Dean and Sleeth (1959), then by Bailey and Dean (1962).

A method of removal of all citrus rust mites from the fruit surface was described by Muma (1965). He washed the fruit in an alcohol bath while still on the tree. Another method of rust mite sampling was described by McCoy et al. (1971). An index for the number of mites per leaf was determined by counting the mites within four microscope fields, two on the upper and two on the lower leaf surface, at 100X magnification.

Allen (1976) has recently developed an attachment which fits a 10X hand lens and defines a 1 cm^2 field.

GENERAL INFORMATION

The citrus industry in Florida prior to 1904 was confined to the Northeastern part of the state. During this period of time D. dormani was believed to have signifiacant importance on the health of the citrus tree. With the advent of synthetic pesticides following World War II, coupled with changes in cultural practices and a general southerly movement of the industry in Florida, a decline in the citrus tree snail has resulted. Along with this decline there have been increases in the citrus rust mite, disease problems, and a greater dependence of the gorwer on the use of pesticides. This study was conducted to determine the role the citrus tree snail has in relationship to the following:

1. The seasonal fluctuations of snails in citrus groves,
2. Effects of relative humidity on snail activity,
3. Effects of snail movement and feeding on fruit microbiota using scanning electron microscopy,
4. Examination of snail fecal content,
5. Determination of the snail feeding potential.

Additional studies of the citrus rust mite were conducted on the following:

1. Method for monitoring citrus rust mites,
2. Extrinsic and intrinsic orientation of citrus rust mite on Valencia orange,

3. The effects of various candidate acaricides on control of citrus rust mite.

CHAPTER I
BIOLOGICAL STUDIES ON THE CITRUS TREE SNAIL

Section 1. Seasonal Fluctuations of Snails in Groves

Introduction

No information is available on D. dormani distribution on citrus, therefore studies were conducted to determine the distribution of D. dormani within a citrus grove in Florida's northern citrus growing region from 1976-77.

Materials and Methods

A map of the grove was made indicating the position of each tree in the grove. During the winter months the snail colonies migrate to the dead wood and protected areas of the trees. Burlap feed sacs, one per tree, were placed in the lowest fork of the tree trunks, during the summer months, to create artificial snail harborages for the hibernating snails. The snails then could move under these sacs during the winter for protection.

On March 18, 1976, and April 7, 1977, while in hibernation, the snail colonies were examined on each tree. Population and percent mortality counts were made under each burlap sac and within dead wood. The number of viable snails and total shell counts were recorded as V/T, that is those viable (V) out of the total (T) shell count.

Results and Discussion

The snail population recorded in 1976 showed that this grove consisted of a pocket of snail trees surrounded by nonsnail trees (Table 1).

Table 1. Drymaeus dormani population counts for 1976. Population determination was made during winter hibernation of the snail during 1975-76. Examination of deadwood and under artificial snail harborages gave indications of snail populations.

	Row								
Column	1	2	3	4	5	6	7	8	9
1		X		41/53	96/97	91/91	X	44/50	
2		X		46/47	X	54/60	13/16	16/20	
3	X	X		89/89	87/88	73/73	50/51	73/73	36/36
4	4/6	X	X	101/101	98/98	104/104	36/40	58/58	X
5	X	X		X	X	130/130	55/55	47/47	40/54
6	X		55/66	54/55		45/50	60/61	50/50	X
7	X	X	X	39/45	54/56	71/77	77/77	66/67	X
8	X		X	56/70	X	86/90	30/31	X	
9				73/73	46/48	70/71	X	X	58/58
10	X		X	49/60	X	111/111	X	111/111	60/60
11	X		X			X	X	69/70	X
12	X	X	X			35/37	16/81	81/81	X
13		X	X	X	X	47/49	15/20	17/19	X
14	X	X	X	15/15	X	X	X	X	X
15	X	X	X	9/9	14/15	X	16/17	X	
16		X	X	14/15	26/27	X	X	X	X
17			X	X	X	X	21/21	18/19	X
18	X	X	X	X	X	X	X	X	10/12
19	X		5/6	X	X		20/21	19/19	X
20			X	X	X	X	X	X	X
21	X	X	X	X	X	X	X	X	
22	X	X	X	X	X	X	X	X	
23	X	X	X	X	X	X		X	X
24	X	X	X	X			X	X	X
25		X	X	X			X	X	X
26	X	X	X	X	X		X		
27		X		X		X		X	
28	X	X	X	X			X	X	
29		X	X	X	X				
30			X	X	X	X		X	

X = Citrus tree present but no snail population
V = Number of viable snails
T = Total number of snails
Space = No tree

Table 1. Continued.

Column	Row 10	11	12	13	14	15	16	17	18
1	45/50	50/53	X	X			X		X
2	X	X		X		X		X	X
3	45/50	15/20		5/5		X		X	
4	X	X	41/50	1/1	X	X	X	X	X
5	58/58	4/9	42/42	14/15			X		X
6	78/81	17/25	X	17/25		X	X		
7	X		X	X	X	X	X	X	
8	X			X	X	X	X		
9	10/12					X		X	
10	18/18	11/19	X						X
11	9/10			X	X	X		X	
12	X	X			X	X		X	
13	33/40	43/43				X	X	X	X
14	X	53/55	X		X	X	X	X	
15	X	40/40	X		X		X		
16	X	X	X	X	X		X	X	
17		X	X	X	X	X	X		X
18	14/15	16/17	X	X	X	X	X	X	X
19	17/19	16/16	X	X	X	X	X		
20	19/19	15/18		X	X	X	X	X	
21	X		X	X	X	X	X	X	X
22				X	X	X	X	X	X
23	X	X		X	X	X	X	X	X
24		5/6	X	X	X	X	X	X	
25			X	X	X	X	X	X	X
26		X	X	X	X	X	X	X	X
27			X	X	X	X	X	X	X
28		X		X	X	X	X	X	X
29						X	X	X	
30			X	X	X	X	X		X

There were 93 snail trees recorded. These trees had a total population of 4,137 viable snails and 283 dead snails. The dead snails represented 6% of the population, and are believed to have died during hibernation.

According to visual observations this snail population in 1976 is believed to have declined from the previous year. In 1975, snail trees could be identified from several feet away by the unusual shiny appearance of the leaves. During 1976, this characteristic was not evident.

The snail population census was taken again in 1977 in the same grove (Table 2). A total of 227 viable snails were located on 74 trees. This represented a 95% reduction in the snail population from the previous year. Mortality within the snail harborages were also greater. Where 6% mortality was recorded in 1976, the 1977 census indicated 20% of those snails reaching the snail harborages died. In 1976, there was an average of 45 snails per snail tree compared to an average of three snails in 1977. This reduction is believed due to two major factors. The first factor was a change in cultural practices. The grove originally was maintained with a ground cover, a summer grass, which was believed by the owner to increase the humidity on the ground and in the tree. This practice was replaced by one of total tillage "clean culture." Snail migration from tree to tree and egg deposition in the soil are believed to depend on the ground cover. Also, a tree pruning program was initiated during this time. All trees were hedged with mechanical hedgers in an east-west pattern. This hedging opened up the trees to the drying effects of wind and sun, as well as made the snails more vulnerable to bird predation.

Table 2. Drymaeus dormani population determination was made during winter hibernation of the snail during 1976-77. Examination of deadwood and under artificial snail harborages gave indications of the snail population.

	Row								
Column	1	2	3	4	5	6	7	8	9
1		X		2/3	6/8	4/7	X	3/6	
2		X		X	X	X	X	X	
3	X	X		3/4	13/15	9/19	X	X	1/1
4	X	X	X	0/4	0/2	13/14	X	X	X
5	X	X		X	X	9/10	5/4	0/1	7/10
6	X		X	X		X	X	X	X
7	X	X	X	2/2	5/2	5/5	2/4	1/1	X
8	X		2/2	X	X	12/14	X		5/7
9			X	X	9/11	2/4	X	5/7	X
10	X		0/1	7/11	X	1/1	X	7/9	4/7
11	X			3/6		X	X	0/2	X
12	X	X	X			8/10	1/1	X	X
13		X	X	X	X	0/2	X	0/3	3/4
14	X	X	X	0/1	X	X	X	X	X
15	X	X	X	X	0/2	X	X	X	
16		X	X	0/1	6/9	X	X	X	X
17				X	X	X	X	X	X
18	X	X	X	X	X	X	X	X	0/3
19	X		X	X	X		X	0/1	X
20			X	X	X	X	0/1	X	0/1
21	X	X	X	X	X	X	X	X	
22	X	X	X	X	X	X	X	X	
23	X	X	X	X	X	X		X	X
24	X	X	X	X			X	X	X
25		X	X	X			X	X	X
26	X	X	X	X	X		X		
27		X		X		X		X	
28	X	X	X	X			X	X	
29		X	X	X	X				
30		X	X	X	X	X		X	

X = Citrus tree present but no snail population

V = Number of viable snails / T = Total number of snails

Space = No tree

Table 2. Continued.

Column	Row 10	11	12	13	14	15	16	17	18
1	9/9	1/1	1/1	X			X		X
2	X	X		0/1		X		X	X
3	4/4	5/5		0/1		X		X	
4	X	X	1/2	1/3	X	X	X	X	X
5	14/16	12/17	0/1	0/3			X		X
6	0/2	1/3	0/3	1/3		0/2	X		
7	1/1		X	X	X	X	X	X	
8			X	X	X	X			
9					X		X		
10	1/1	0/1	X						X
11	X			X	X	X		X	
12	X	X			X	X		X	
13	9/13	0/1				X	X	X	X
14	X	X	X		X	X	X	0/1	
15	X	X	X		X		X		
16	0/3	X	X	1/2	X		X	X	
17	X		X	X	X	X	X		X
18	0/1	X	X	X	X	X	X	X	X
19	X	0/1	X	X	X	X	X		
20	3/3	0/1		X	X	X	X	X	
21	X		X	X	X	X	X	X	X
22				X	X	X	X	X	X
23	X	X		X	X	X	X	X	X
24		X	X	X	X	X	X	X	
25			X	X	X	X	X	X	X
26		X	X	X	X	X	X	X	X
27			X	X	X	X	X	X	X
28		X		X	X	X	X	X	X
29						X	X	X	
39			X	X	X	X	X		X

The second major factor believed responsible for the decline in the snail population was the severe freeze during January, 1977. This freeze resulted in crop losses, and up to 95% defoliation of trees. It is possible that the snail harborages were not sufficient to protect the snail colonies.

A combination of the aforementioned factors is believed to have accounted for this radical population reduction. Within two years, a thriving snail culture was reduced to near extinction. This reduction was accomplished without the use of any pesticide sprays. It simply reflects the fragile balance needed to maintain a snail culture.

Observations made during the summer following the winter population counts of 1977 reflected the population decline. A total of eleven snails were located within the entire grove area. Further population reductions were possible, due to an extremely dry spring. Unless cultural practices conducive to snail growth are resumed, this grove will no longer harbor a viable snail culture.

This survey indicates that the cultural practices once used to help maintain a snail culture played an important role. These practices, however, are now in conflict with modern techniques. Ground cover is no longer used because of water loss and nutritional consumption by the grasses. Dead wood,once used by the snail for overwintering, now is removed and burned. Dense tree canopy is reduced by row pruning to allow for easier picking and grove maintenance. All of these techniques, once common in citriculture and important to snail survival,are no longer practiced.

Section 2. Effects of Relative Humidity on Snail Activity

Introduction

No information has been reported on the effect of environmental conditions on the citrus tree snail's movement. Therefore, studies were conducted to obtain information on the relationship of snail activity and relative humidity. This information is necessary to determine if relative humidity is a limiting factor in snail activity. If snail activity is directly dependent on relative humidity, it would be possible to monitor for periods favorable for the snail and to determine the hours of snail activity.

Materials and Methods

A naturally occurring population of the citrus tree snail, D. dormani was located within a grove in Orange Lake, Florida. The grove was unsprayed and was maintained with a cover crop or ground cover. It was observed that the snails were generally active at night or during heavy rains. Twenty snails were located to determine the snail activity and relationship to relative humidity. Ten quiescent snails on each of two adjacent trees were located and marked on the dorsum of the shell with an identification number using Day-glow^{R} paint. Application was made with a one milliliter tuberculin syringe. A spring type clothespin was placed adjacent to each snail and was marked with the same number as the snail.

A battery operated ultraviolet lamp, model U.L.V.-56, illuminated the Day-glow^{R} pigment which facilitated locating the snails at night. Relative humidity, time, and percent snail activity (percent of snails active per unit time) were recorded every half hour from 9:00 p.m. untill 8:00 a.m. The experiments were terminated at 8:00 a.m. because

of solar light decreasing the effectiveness of the artificial light. Relative humidity was measured with a Bacharach[R] sling psychrometer. The clothespins were placed adjacent to the snail following each reading to help relocate the snail at later time intervals. This experiment was replicated three times over a three year period.

Results and Discussion

The first experiment on August 27, 1975, showed a correlation in line slope between the increasing percent relative humidity and the increasing percent snail activity (Figure 1). In this case a slope (M) of M=2 was found for both items. It is interesting to note that as the relative humidity reached 100%, the first trace of snail activity was noted. The dotted line demonstrates this phenomenon. The accompanying snail activity line then proceeds at the same rate of increase as did the percent relative humidity line, that is the slopes (M) were the same.

A similar relative humidity and snail activity slope correlation can be seen with the April 28, 1976, observation (Figure 2). The first traces of snail activity began with the relative humidity reaching 100%. Again there are the similar slopes of the lines, in this case M=4.3. It would appear that the increase in relative humidity may predispose or effect the ensuing snail colony's activity pattern. Similar line slopes for relative humidity and snail activity were found on two of the three replicates.

On July 18, 1977, the final field observation was made (Figure 3). A unique situation occurred where 96% relative humidity was maintained for seven hours prior to reaching 100%. Even under this extended duration of high humidity the first snail activity did not begin until 100% relative humidity was obtained. This would indicate a need for 100%

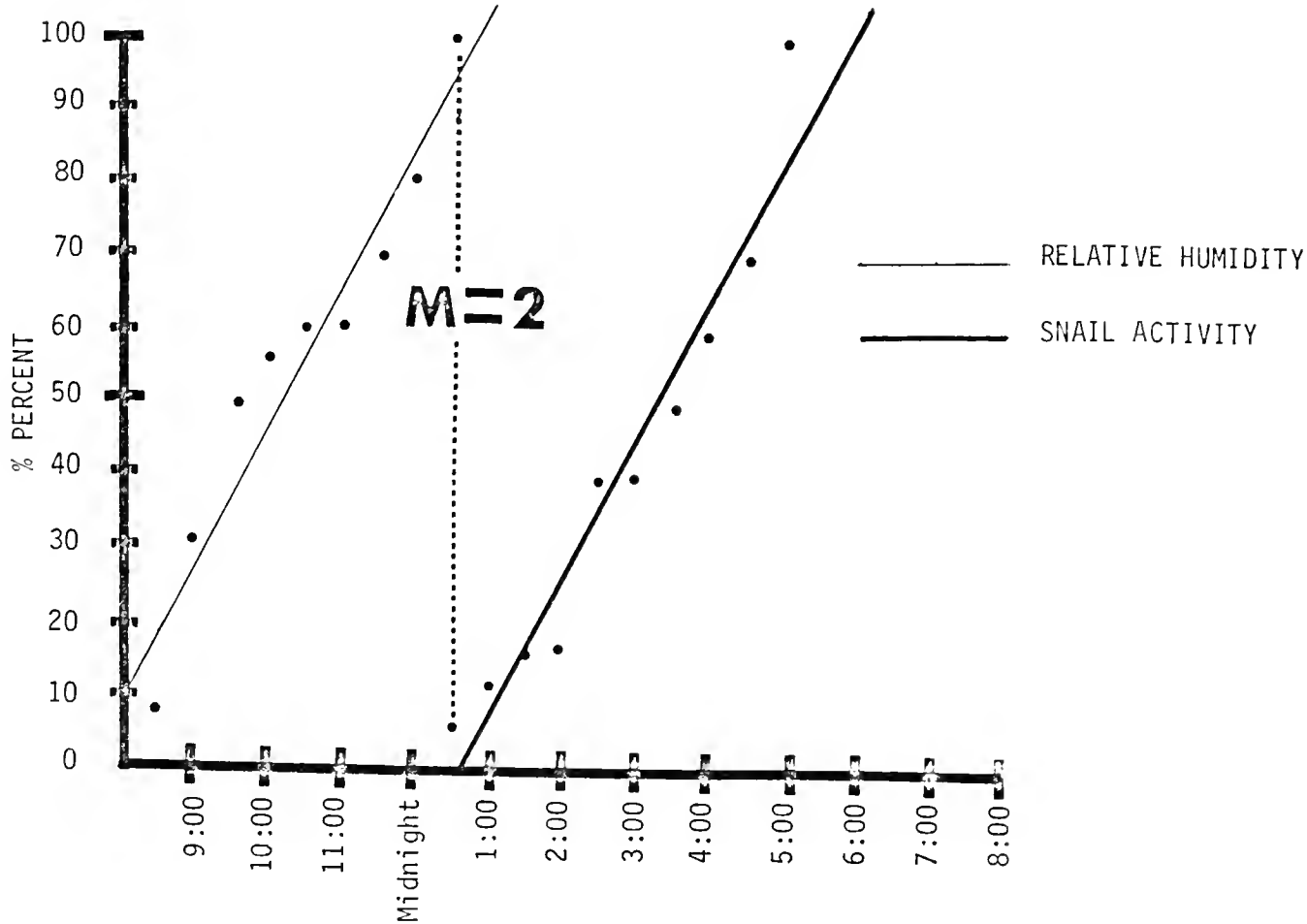

Figure 1. August 27, 1975, percent snail activity and relative humidity. (The dotted line correlates the initiation of snail activity with 100% relative humidity).

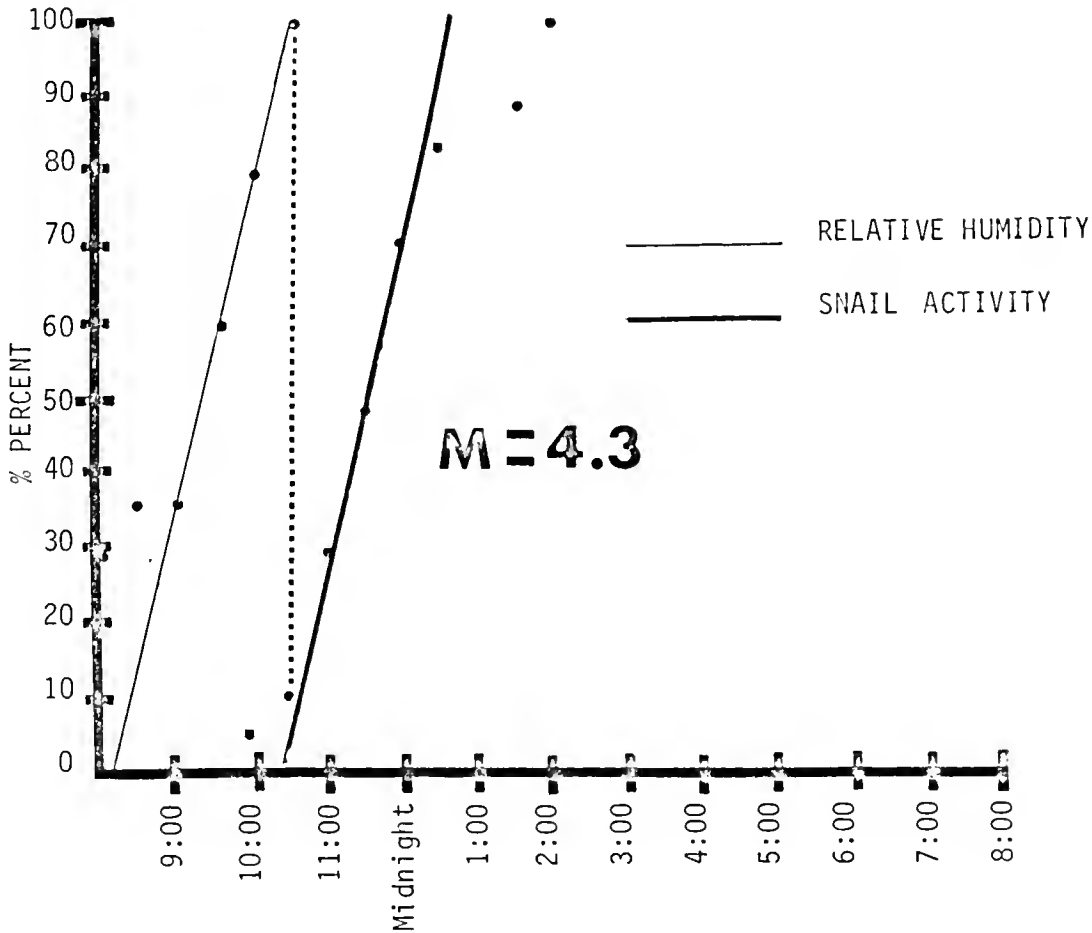

Figure 2. April 28, 1976, percent snail activity and relative humidity. (The dotted line correlates the initiation of snail activity with 100% relative humidity).

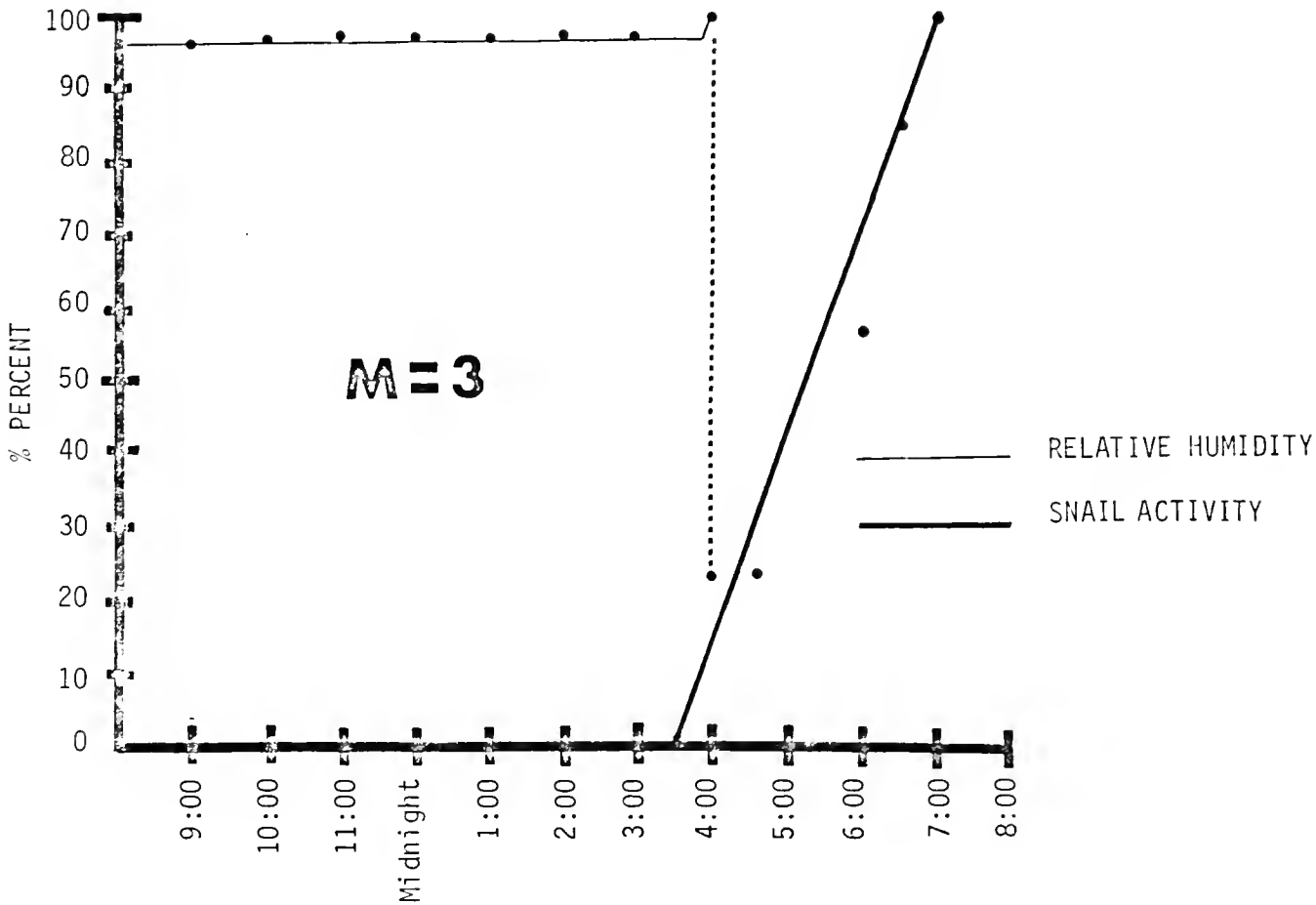

Figure 3. July 18, 1977, percent snail activity and relative humidity. (The dotted line correlates the initiation of snail activity with 100% relative humidity).

relative humidity or saturation for initiation of snail activity. In each of the three observations over the three years, 100% relative humidity was obtained before the initiation of any significant snail activity. The July 18, 1977, observation resulted in a snail line slope of M=3. The resulting line slope of the percent relative humidity was M=0 until 3:30 a.m. when it began increasing. Saturation was reached at 3:45 a.m. The slope formed from 96% to 100% relative humidity was M=3, the same as with the snail activity line slope. Again the correlation of the slopes was demonstrated.

The problem with locating the snails during light hours made snail activity determination extremely difficult. For this reason no attempt was made to determine the relationship of the termination of snail activity with a decreasing relative humidity. Smith, 1976[1], (unpublished data) was able to demonstrate a rapid decline in snail activity when the relative humidity fell below 100%, again supporting the need for 100% relative humidity for activity.

The citrus tree snails were determined to be active only during periods of 100% relative humidity. This was demonstrated in all three replicates. D. dormani, which is dependent on water, has evolved to an arboreal habitat and utilized available water sources found during 100% relative humidity. Locomotion is believed limited to this condition due to excessive pedal secretions used to maintain adhesion to the surface. If sufficient water was not present the snail would quickly desiccate. During showers the snails have been observed to be active throughout the day. This would suggest the dependence of the snail on

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[1] Smith, B. 1976. Graduate Student. Department of Entomology and and Nematology, I.F.A.S., (University of Florida).

water and not on nocturanl conditions. The author wishes to acknowledge the possibility that the snail may be dependent upon free water which develops on the leaf surface. Further studies examining this environmental factor are needed.

Section 3. Effect of Snail Movement on Fruit Microbiota Using Scanning Electron Microscopy

Introduction

No information was available on *D. dormani* effect upon the microbiota of citrus. Therefore, this study was undertaken to characterize the microstrata of the gruit surface and the effect of *D. dormani* on it.

Materials and Methods

Scanning electron microscope work was done at the Orlando location of the U.S.D.A., Horticultural Research Laboratory, Orlando, Florida. All *D. dormani* used were collected in Orange Lake, Florida, from the Parson Brown variety of *Citrus sinensis* (L.) Osbeck. Green fruit, 6.5 to 7.0 cm in diameter, were collected in Lake Alfred, Florida, and were selected on the basis of high citrus rust mite spermatophore counts.

A glass aquarium (1' x 2' x 1 1/2') used to house the snail was maintained at relative humidity (R.H.) of 90% ± 2% by filling the bottom with one inch of water. *D. dormani* were placed on five oranges suspended by cotton strings attached to the top of the aquarium. The string was tied to paper clips partially opened and inserted into the fruit.

Snail trails were recognized easily by a wet silvery sheen left on the fruit surface. Each trail where feeding had occurred was marked with a felt tip marker for identification. Twelve 4 x 4 x .05 mm samples were carefully removed with a single edge razor blade from the surface of the fruit in each of the following three areas: control areas (not

visited by snails); ambulatory areas (areas visited by snails without feeding); and grazing areas (areas visited by snails where feeding had occurred). Each of the labeled samples were placed into a solution of 50% Gluteralaldahyde and 50% phosphate buffer at pH 7.0 for two hours (Anderson, 1966) and then reduced to 2 x 2 x .05 mm samples. Samples were left in osmium for two additional hours, removed, washed, and placed into a phosphate buffer (pH 7.0) for approximately 18 hours.

The following day the samples were subjected to both an acetone dilution series and a Freon TF dilution series which consisted of 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, 100, 100_2, 100_3% washes for ten minutes each. Samples were critically point dried using a Freon 13 critical point dryer.

The specimens were placed on one centimeter cylindrical studs and held in place by a conducive silver glue. A Hummer II sputter coater gold plated the sample's surface. A JEOL/JSM-35 scanning scope was used to review and to photograph the surface. Surface photographs were taken with a polaroid camera horizontally mounted to the scanning electron microscope.

Results and Discussion

Control areas not affected by the citrus tree snal are covered generally with microbiota (Figures 4-9). The ambulatory areas demonstrated the ability of D. dormani to encrust with mucilage the surface of the fruit. Spermatophores were pressed to the surface and their sperm sacs ruptured. Mycelia were encompassed generally within the mucilage (Figures 10-17). Grazed areas, one centimeter wide, were void of microbiota and were covered generally with a thin mucilage veil (Figures 18-22).

Control area. The surface of the fruit is a stratum for the life processes of many mite and fungal species. A careful examination of Figure 4 displays the exuvia of a citrus rust mite, a citrus rust mite spermatophore, a fruit stoma, and some of the mycelia found on the surface. By viewing exuvia and spermatophore together a perspective of size became evident. Stomata were found scattered over the surface of citrus fruit.

Oldfield et al. (1970) characterized the spermatophores of eriophyoidea. As shown in Figure 5, this spermatophore, though not erect, can still serve to exemplify the base, stalk, expanded apical head, and sperm sac. The functional posture of the spermatophore is upright or erect. The base of the spermatophore is shown at 34000X magnification to demonstrate its structure and attachment to the surface of the fruit (Figure 6).

Other areas of the surface of the orange peel (Figure 7) again serve to show the intertwining of mycelia and the presence of spermatophores on the surface. An erect spermatophore and citrus rust mites' eggs are shown as normally associated with the surface of citrus (Figures 8 and 9).

Ambulatory movement. For the context of this work ambulatory movement refers to the translocation of snails from one area to another under its own incentive and energy without feeding.

The terrestrial snails secrete mucilage from several small pores at the ventral portion of the cephalic end of the foot which help form a suction to the surface (Dr. F. Thompson, personal communication). The thin layer of dried mucilage less than 1 μ thick is visible in Figure 10. The mucilage has entirely coated the traveled surface of the fruit.

Figure 4. Surface of a citrus peel, demonstrating the general condition of a complex mycelial matrix and stoma (bottom-center) which are associated with the surface. An immature citrus rust mite's exuvia is viewed to the entire left, and a spermatophore (lower-center). 1000X magnification.

Figure 5. Citrus rust mite spermatophore. Evident in this photograph is the base, stalk, expanded apical head, and a partially removed sperm sac.

Figure 6. Base of a spermatophore. 34000X magnification.

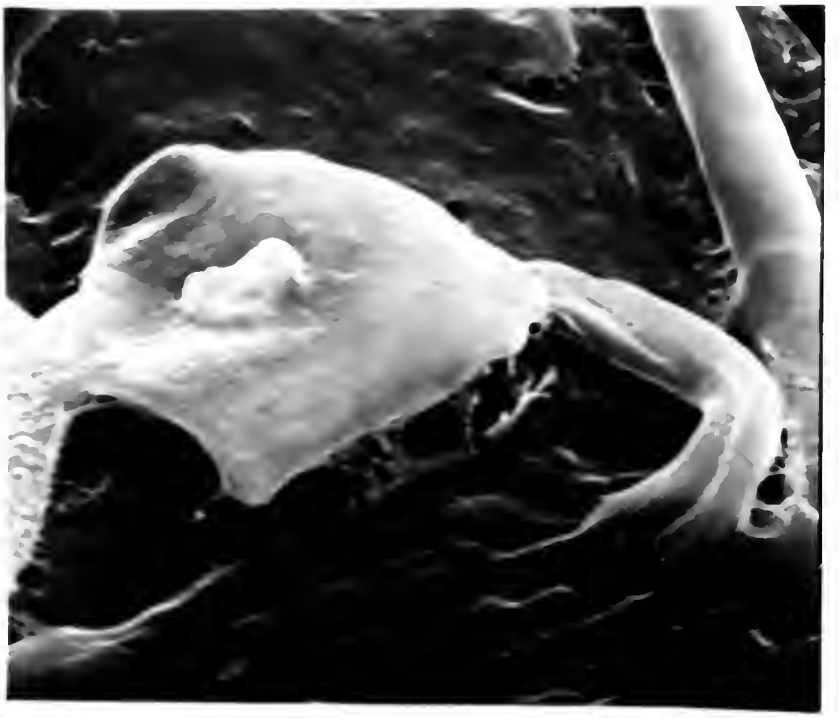
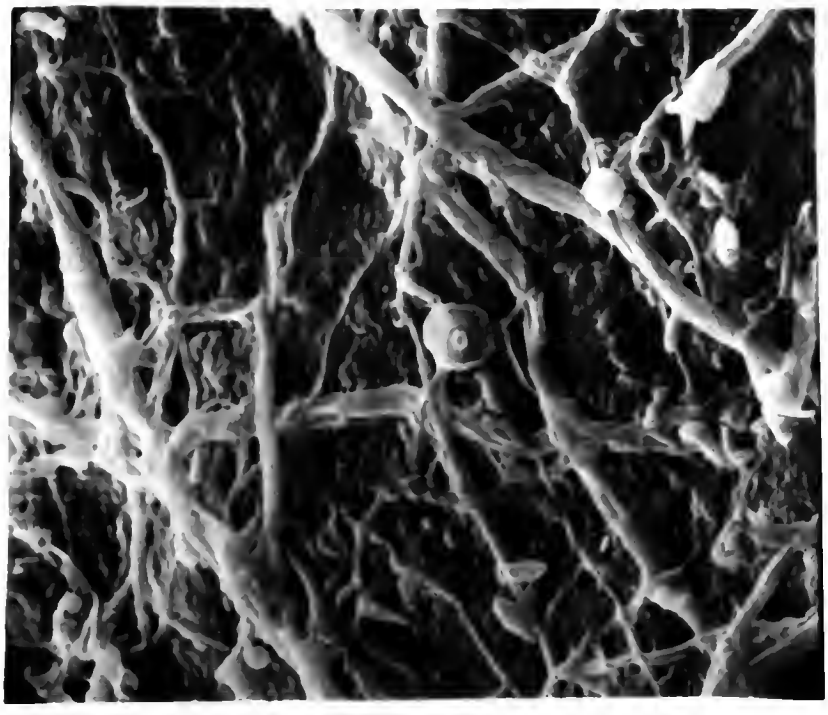


Figure 7. Mycelia covering surface of citrus fruit. The spermatophore (center) is in its erect posture. 1000X magnification.

Figure 8. A close up of Figure 7 displaying the sperm sac atop the expanded apical head of the spermatophore. 7000X magnification.



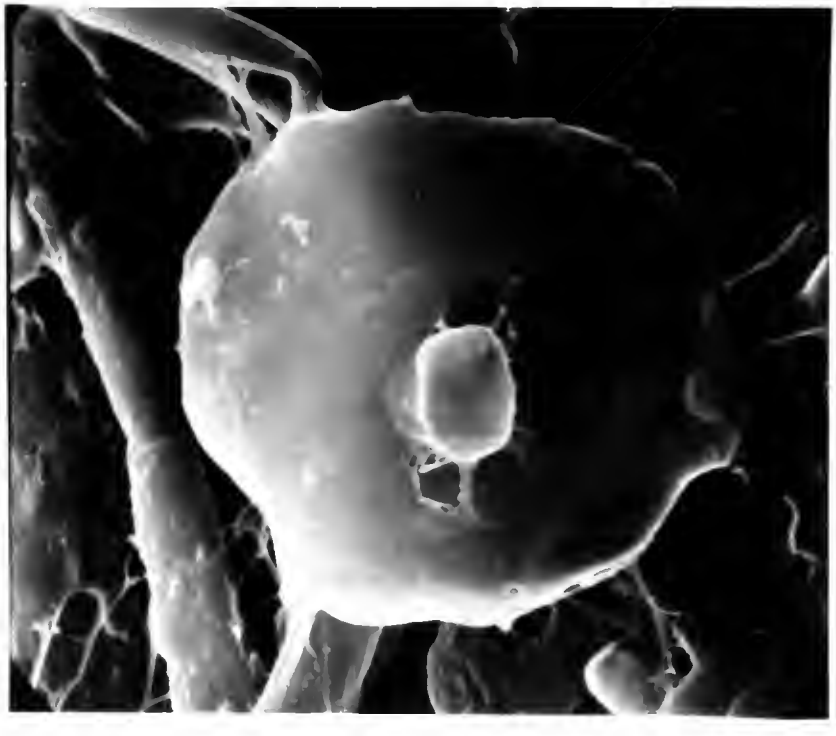


Figure 9. Mycelia normally associated with the surface of the citrus fruit. Three citrus rust mite eggs (top, left and bottom) are shown. 400X magnification.

The mycelia is sealed generally in mucilage while the stomata extend above it. In this photograph a close observation discloses a spermatophore just below the stoma (Figure 10). The spermatophore has been flattened, and slightly elongated by the weight of the snail and embedded in the mucal crust (Figure 11). The sperm sac was ruptured expelling the sperm. The sperm sac was believed damaged by the snail, but the author acknowledges the possibility that it may have been previously damaged.

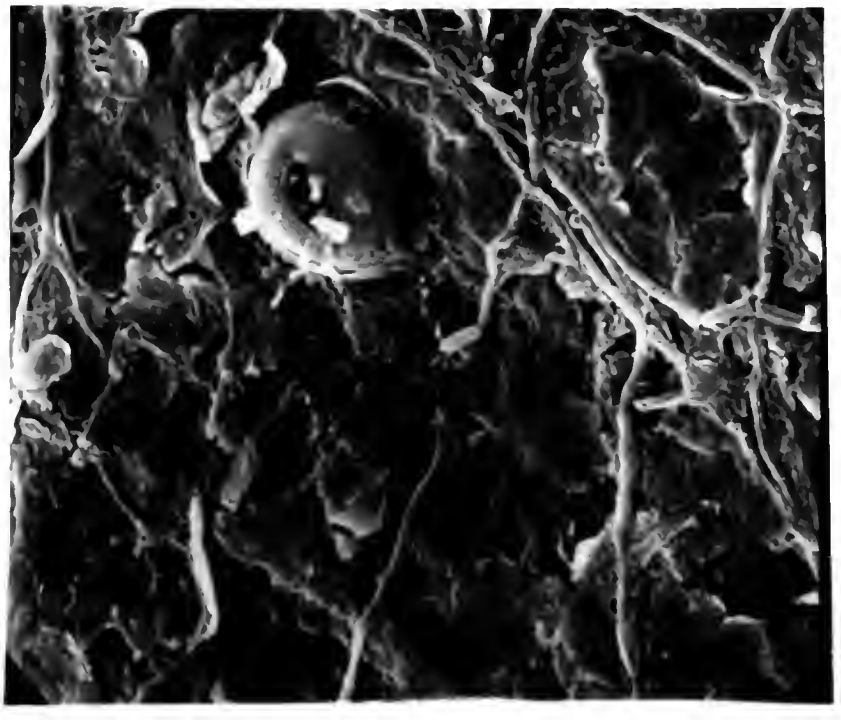
The action of glattening the spermatophore and rupturing the sperm sac are both important, in that either would be sufficient to prevent sperm transfer in the citrus rust mite. This is the first definitive example that the ambulatory movement of the tree snail has any effect on the citrus rust mite sex determination. Should sufficient spermatophore become damaged, there would be an increase in the ratio of males in the next generation.

The slime trail as it hardened often broke up into small irregular shaped platelets ranging from 20 μ to 5 μ or less across (Figure 12). If disturbed the platelets apparently collapsed or fell off taking with it the mycelia and debris. This left a clean fruit surface. The formation of these platelets could possibly be due to stress from the mycelia, surface drying and shrinkage or simply surface stress due to preparation for scanning electron microscope (S.E.M.) work.

The ability of the slime (mucilage) to adhere to various surfaces inherent to the fruit surface were examined. The fruit stoma (Figure 13) was generally free of mucilage adhesion to its surface. The surface adhesion of the mucilage to the citrus rust mite spermatophoe is demonstrated in Figure 14. The sperm sac is ruptured and a

Figure 10. Area where the citrus tree snail moved across the citrus fruit's surface. A thin mucilage veil was deposited on the surface encrusting some of the mycelia. 1000X magnification.

Figure 11. Spermatophore. The structure is flattened and the sperm sac ruptured. 5400X magnification.



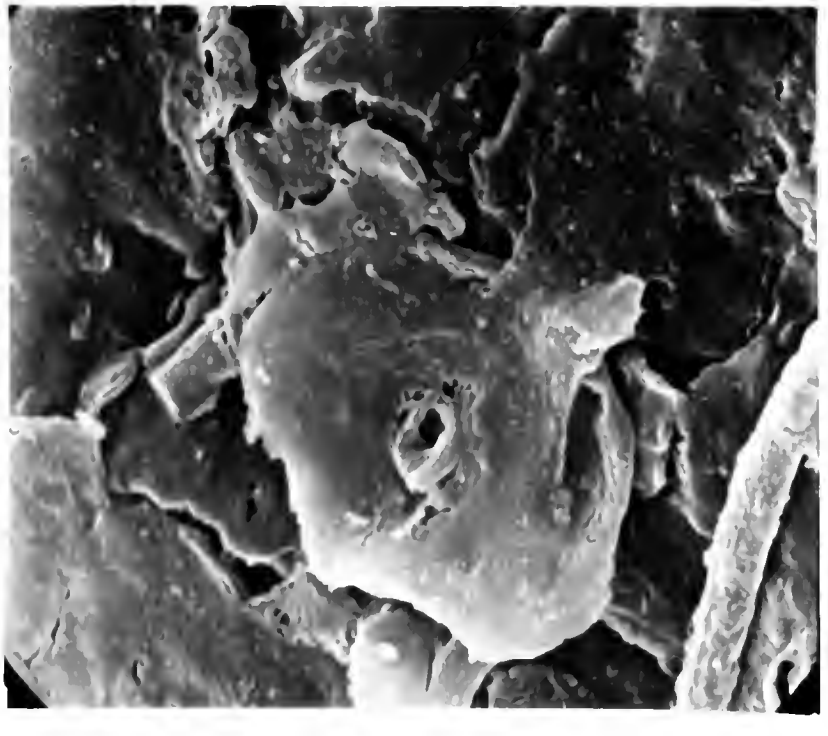
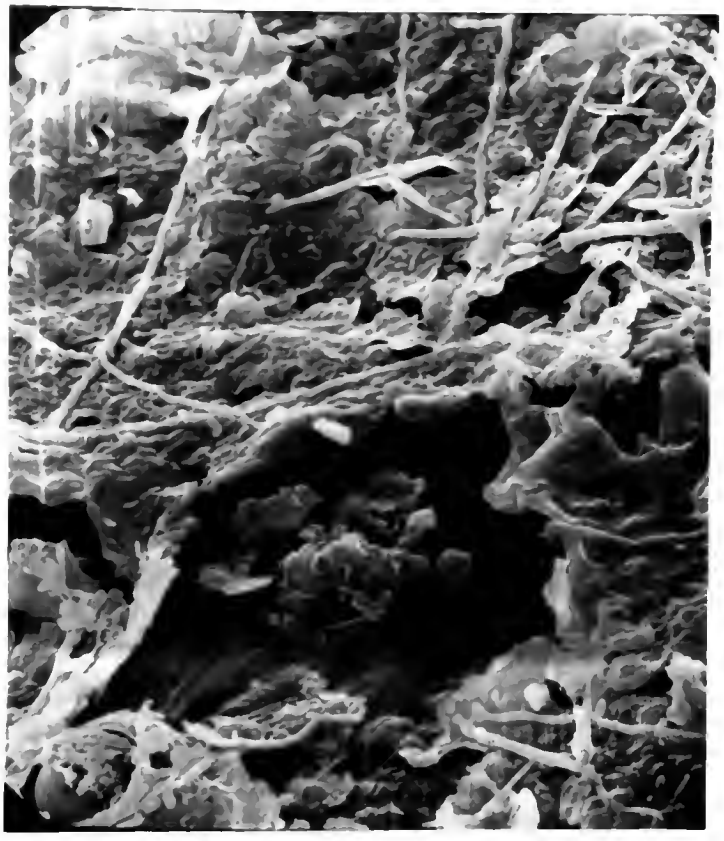


Figure 12. Area traversed by snail not accompanied by grazing. Note the mucilage platelet formation on the surface. 720X magnification.

Figure 13. Fruit stoma and mucilage. 1000X magnification.



continuous layer of material is coating the spermatophore surface. There is a definite affinity of the mucilage to its surface. This adhesion of the slime to the surface of the spermatophore aids in sealing it to the surface, making it inaccessible to the female population. D. dormani mucilage was not found adhering to the surface of citrus rust mites. An immature citrus rust mite was found free of mucilage (Figure 15).

The effect of the snail trails on the citrus rust mite egg was observed. No visible damage or adhesion was detected (Figures 16 and 17). Apparently the mucilage did not adhere to the egg surface. Even more surprising is the fact that the weight of the snail did not seem to damage the eggs, as many of those observed showed no signs of dehydration or compression.

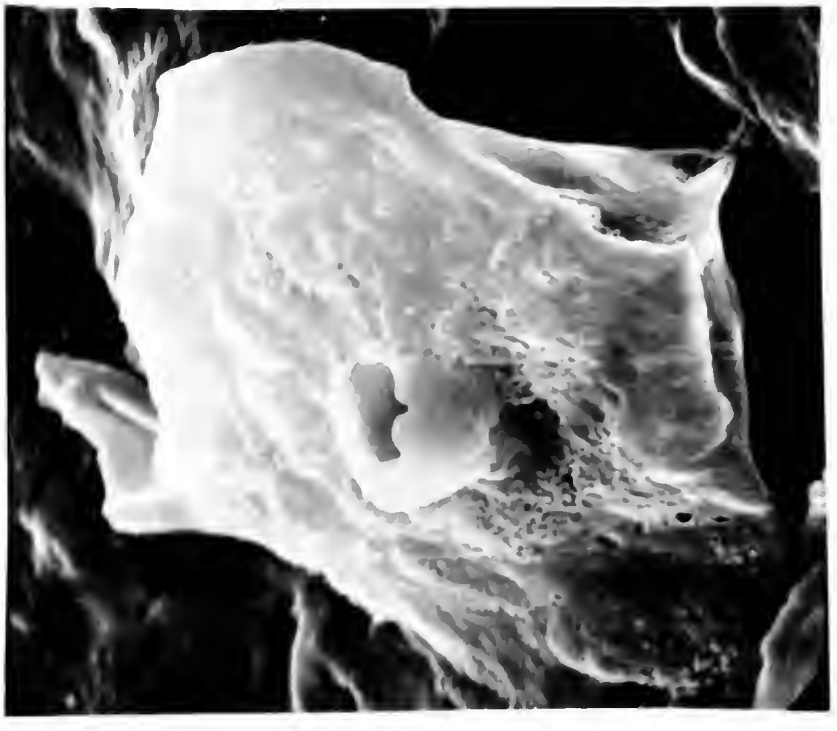
Areas of snail grazing. The surface of the fruit where grazing occurred was found covered with mucilage. This was, as expected, because of the necessity of the slime for adhesion of the foot to the surface (Figures 18 and 19).

If the snail was a selective feeder and removed mycelia only, nonmycelial objects would be expected to be found on the surface following feeding. On the other hand, if the snail was an indiscriminant feeder engulfing everything in its search for fungi, the surface should be clean except for mucilage. As is evident in Figure 18, the surface was totally void of all matter with exception to the deposited mucilage.

Areas where citrus rust mites and their eggs, immature whitefly, spermatophore, as well as other mites and foreign surface material were previously found, now completely were devoid of any microbiota. This would suggest the ingestion of these items. An examination of the

Figure 14. Spermatophore with mucilage encrusted on its surface and its ruptured sperm sac from an area of nonsnail grazing. 11000X magnification.

Figure 15. Immature citrus rust mite. 3000X magnification.



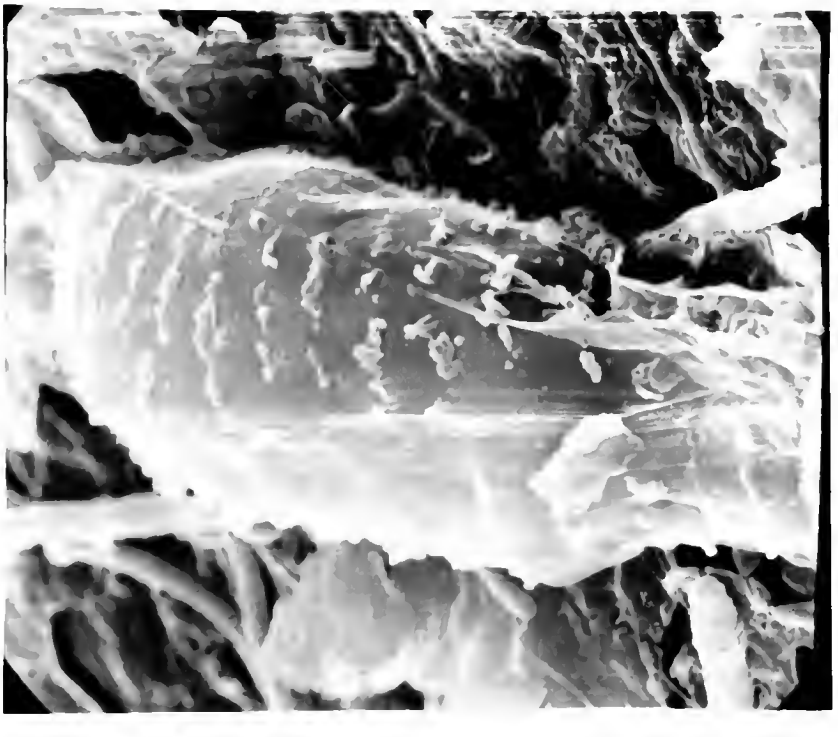


Figure 16. Citrus rust mite egg in area where the snail has traversed without feeding (ambulatory area). Mucilage failed to adhere to the egg's surface. 1000X magnification.

Figure 17. Close up of Figure 16. Citrus rust mite egg. 2400X magnification.

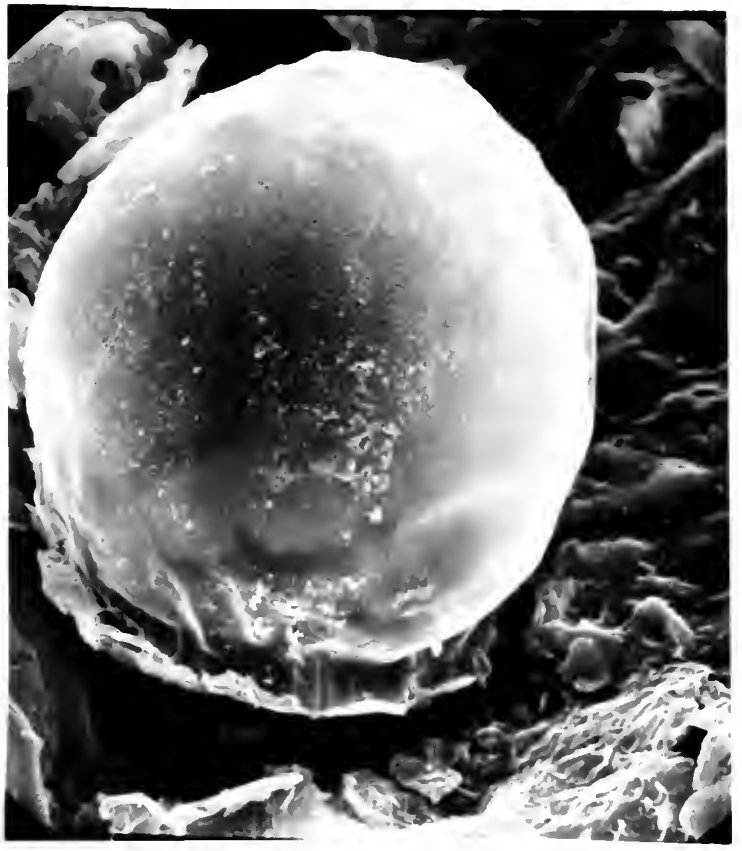
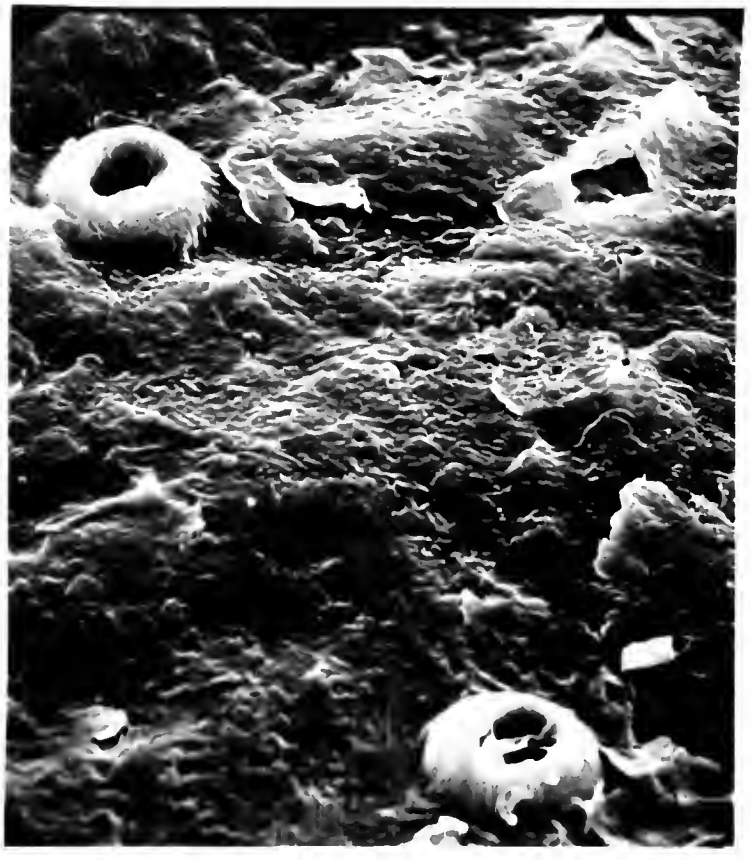
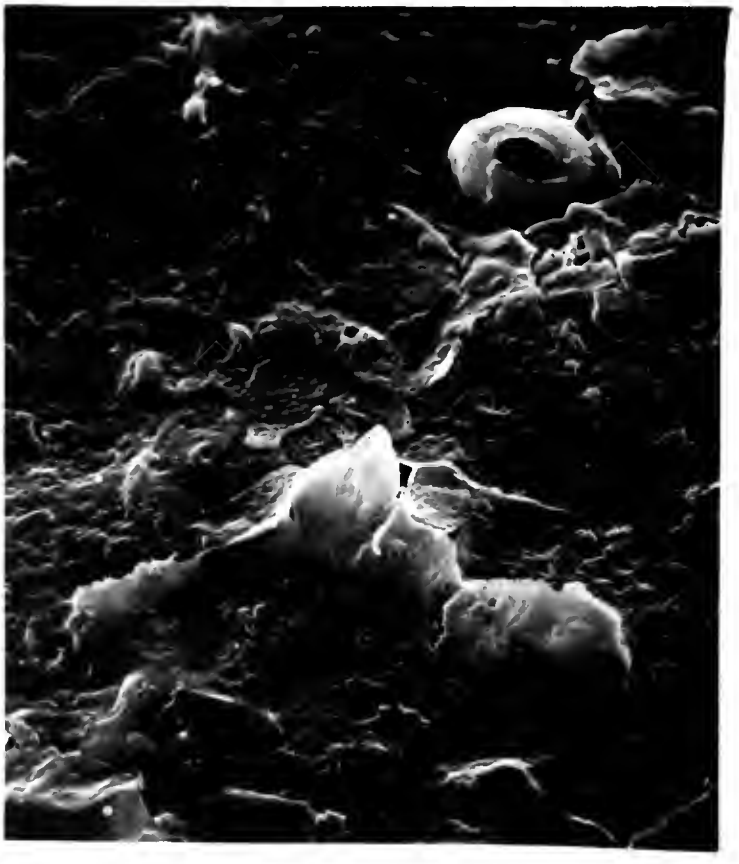


Figure 18. Areas grazed by the citrus tree snail. Absence of microbiota demonstrated the ability of the snail to remove all surface material. 1000X magnification.

Figure 19. Area of snail grazing resulting in removal of all surface material. 1000X magnification.





snail fecal content, discussed in Section 4 of this chapter, was used to determine what material the snail was ingesting.

In several areas, the author observed the absence of the mucilage in areas traversed by D. dormani. This is possibly the normal course of events where, under natural conditions, wind, abrasion, and rain may cause the breaking away of the mucilage (Figure 20) until the entire surface is cleaned and just the waxy surface of the fruit remains (Figures 20 and 21).

The forward movement of the snail easily could provide areas where the foot had traveled while not covered by feeding. Figure 22 demonstrates this phenomena. One-half (the right side) of the photographed surface is clean of surface material with the exception of mucilage. The center left of the figure shows the area covered by the foot not yet grazed. The reduction of mycelia in this area was due to the strings of mycelia being pulled away by adjacent feedings. The remaining surface showed no signs of mucilage and the flora still remained. This is a good example of the transition from grazing to nongrazing and was generally indicative of samples taken.

Close observation of exposed fruit surface, that is those surfaces devoid of mucilage or debris, permitted observations into the effect the snail might have on the fruit's waxy layers. No damage was evident on the waxy layer of any of the surfaces viewed. The only effect the snail had was to remove all surface material and/or to deposit its slimy mucilage. As was expected, there was no damage to the surface of the fruit. If damage had occurred it would have been manifested as scar tissue on the fruit and observed in the field. Studies using the scanning electron microscope failed to detect any surface damage. If

Figure 20. Stoma in area of snail grazing. No surface damage to the fruit was observed. 1000X magnification.

Figure 21. Snail grazed area. 1000X magnification.

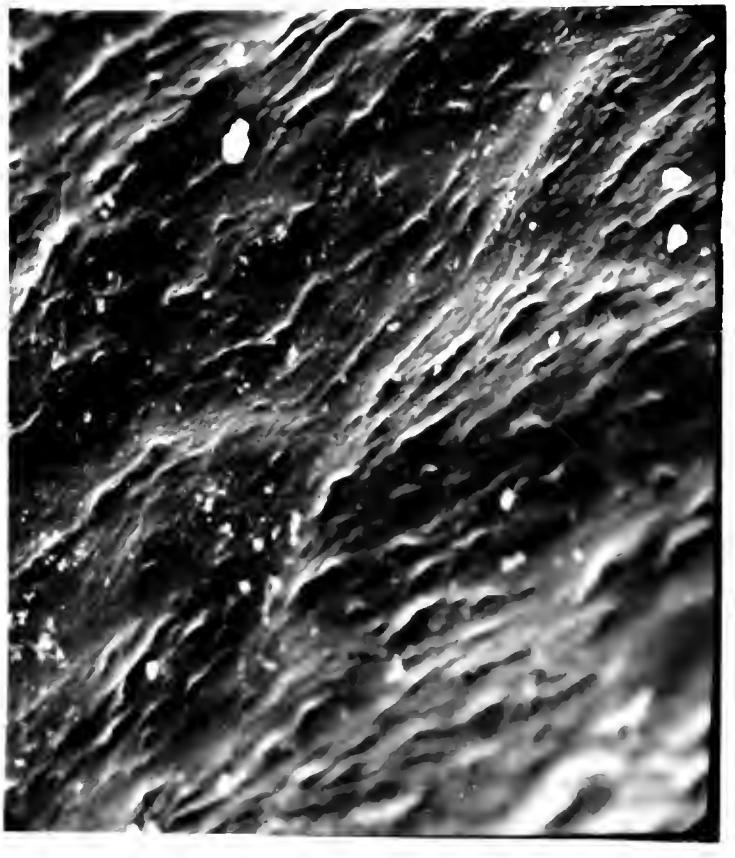


Figure 22. Photograph demonstrating the three conditions covered in earlier figures. Snail grazed the right side removing all surface material and depositing mucilage. Center is where the snail's foot traversed over the surface but no feeding occurred. Left side is normal mycelia found on fruit surface. 220X magnification.

damage had been associated with the feeding, this could negate easily any possible good the snail could have accomplished. Further studies as to the surface effects of the snail on the leaves are needed.

Section 4. Examination of Snail Fecal Content

Introduction

No information is available on the fecal content of the citrus tree snail. To support the scanning electron microscopy findings, this study was undertaken to determine if *D. dormani* ingests insects, citrus rust mites, and their spermatophores.

Materials and Methods

Citrus tree snails were examined for their fecal content. Fecal pellets 2 mm x 3 mm were field collected from a grove in Orange Lake, Florida. Also snails were placed on citrus fruit and leaves for feeding. Fecal pellets were collected from laboratory specimens as they were deposited. The feces were placed first into watch glasses containing distilled water, then 70% isopropyl alcohol. This was repeated three times for each fecal pellet. The baths were used to remove any foreign or living material from the surface of the feces. The fecal pellets then were allowed to air dry prior to disruption by forceps then by a sonic vibrator in 5 ml of distilled water. Suspended materials were placed onto glass microscope slides. All samples first were examined with a binocular microscope, then with a phase microscope and photographed.

Results and Discussion

The preponderance of the fecal content consisted of strands of various length fungal mycelia. Due to its disrupted condition, no

attempt was made to identify the mycelial species. It was assumed that some of the mycelia was from sooty mold.

Further examination of the fecal content showed several species of mites, some of which were still alive, whole white fly pupae, and various parts of insect bodies. Lepidopterous wing scales and parts of chitinous hexapod appendages were among the debris. Several specimens of the citrus rust mite were found, none of which showed movement. Some of the larger Prostigmatid mites were still alive when placed in water for observation. The viability of these mites suggests the inability of the snail to digest them supporting the hypothesis that the snail is feeding on the fungi, but also consumes other foreign matter by chance. However, it seems unlikely that the various mites could escape from the encrusted fecal entrapment.

The mite and insect portion of the feces represented a small fragment of total content. The white fly pupae were the most numerous with citrus rust mites second.

Once it was determined the snail was ingesting material other than mycelia, additional studies were undertaken to locate citrus rust mite spermatophores in snail fecal material. Spermatophores were found in the fecal pellets and demonstrated yet another means for the snail to exert pressure on the rust mite sex determination. The spermatophore removal by the snail could alter the sex ratio increasing the ratio of males in the next generation. Reduction in the ratio of female P. oleivora could be of significance in the suppression of the population especially if other factors suppressing the citrus rust mite populations were present.

Section 5. Determination of the Snail Feeding Potential

Introduction

Quantitatively speaking, no literature was found on the feeding potential of the snail. Occasionally sooty mold was mentioned as a food source but no other mention of the quality and quantity of ingested materials is available. The first part of this study was directed at quantifying the citrus tree snail's ability to clean given areas of citrus. The second part extrapolates this rate to encompass the entire citrus canopy.

Materials and Methods

Specimens for this study were collected at Leesburg, Florida. Due to the severity of the winter only a very small population was located. Twelve citrus tree snails collected were transported in glass mason jars with a relative humidity of 98% $\pm$ 2% to Orange Lake, Florida, where observations were made on Valencia orange.

A suitable tree with both citrus rust mite and sooty mold was located. Nine, one cubic foot areas of peripheral leaves from two feet to six feet off the ground were created by use of hedge shears and a one foot carpenter's square. The shears were used to isolate an area of one cubic foot on the tree.

Once the cubic unit was formed, all leaves from adjacent branches were removed, leaving a six to eight inch leafless barrier around each unit.

A standard 18 mesh metal screen 2" x 4" was wrapped about the stems of the branches included in the cubic unit, to restrict the snails to this area and to keep any other snails from entering. The snails were unable to traverse the screen due to loss of contact with the

tree surface, thus maintaining them within the confines of the cubic foot.

Tie wire was twisted around the screen to secure it. A clip-type clothespin was placed just beyond the screen and outside of the cubic unit to identify the particular cube. Yellow ribbon was also attached to make locating the cubes easier.

Three of the cubes were randomly chosen to have one snail, three with three snails, and three without any as controls. As the snails were placed on the units, yellow Day-glow^{R} numbers representing the particular cube unit were applied to the dorsal surface of the shells as in Chapter I, Section 2. A similar number was placed on the clothespin for identification. For the first twelve hours (8:00 p.m. until 8:00 a.m.) the snails were observed hourly. An ultraviolet light, model ULV-56, was used to see the Day-glow^{R} painted snail and identify it. Temperature, relative humidity, and periods of snail movement were recorded every 15 minutes.

Pretreatment and posttreatment counts on citrus rust mite populations, as well as presence or absence of sooty mold, were collected just prior to snail infestation, using a modified hand lens (Allen, 1976). Ten leaves from nine different cubic feet of citrus were randomly chosen and one 1 cm^2 observation made on each leaf. Numbers of rust mite P. olievora were counted as were positive or negative presence of sooty mold. These observations were continued for six days.

Determination of snail hours each day was initially made by visual observations and relative humidity readings. Later, snail hours were determined by recorded humidity readings on a recording hygrothermograph located near the test tree.

At the completion of this study all of the leaves were removed from each cubic foot area. The total surface area for each leaf, average leaf surface, total leaf surface, and average leaf surface per cubic foot were obtained by use of a portable area meter model Li-Cor L1-3000 in sequence with a Wang computer, model Wang 600 Programmable Calculator.

Results and Discussion

Snail density and its effect on sooty mold. Snail hours and percent of mold present are indicated in relation of mold to the various snail populations in Figure 23. In the controls without snails the percent of mold present was a constant 97.14% level of infestation for the entire six day period. Where one snail was permitted to graze over a one cubic foot area of leaf surface, the entire surface was cleaned at 26 snail hours (Figure 23). This was equal to just over five days in this test. As would be expected, where three snails were placed on a cubic foot of citrus, it was calculated to take 8.5 snail hours, approximately one-third of the time necessary for one snail.

The following calculation shows the average surface ($\bar{S}$) area grazed by the snail per snail hours. Numerical values for these calculations are found in Table 3.

$\bar{S}$ = Surface area grazed per snail hours.

$$\bar{S} = \frac{3415.06 \text{ cm}^2}{26 \text{ snail hours}^*} \text{ (average leaf surface area/ft}^3\text{).}$$

$\bar{S}$ = 131.34 cm^2/snail hours.

The average total leaf surface area (dorsal and ventral surface) of the citrus tested equals 28.8024 cm^2/leaf (Table 3). Determination

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* - Number of hours calculated necessary to clean the surface of one cubic foot of citrus per snail (Figure 23).

Figure 23. The lines, X = 0, X = 1, and X = 3, refer to % mold removed from the surface of one cubic foot of citrus by 0, 1, and 3 snails, respectively.

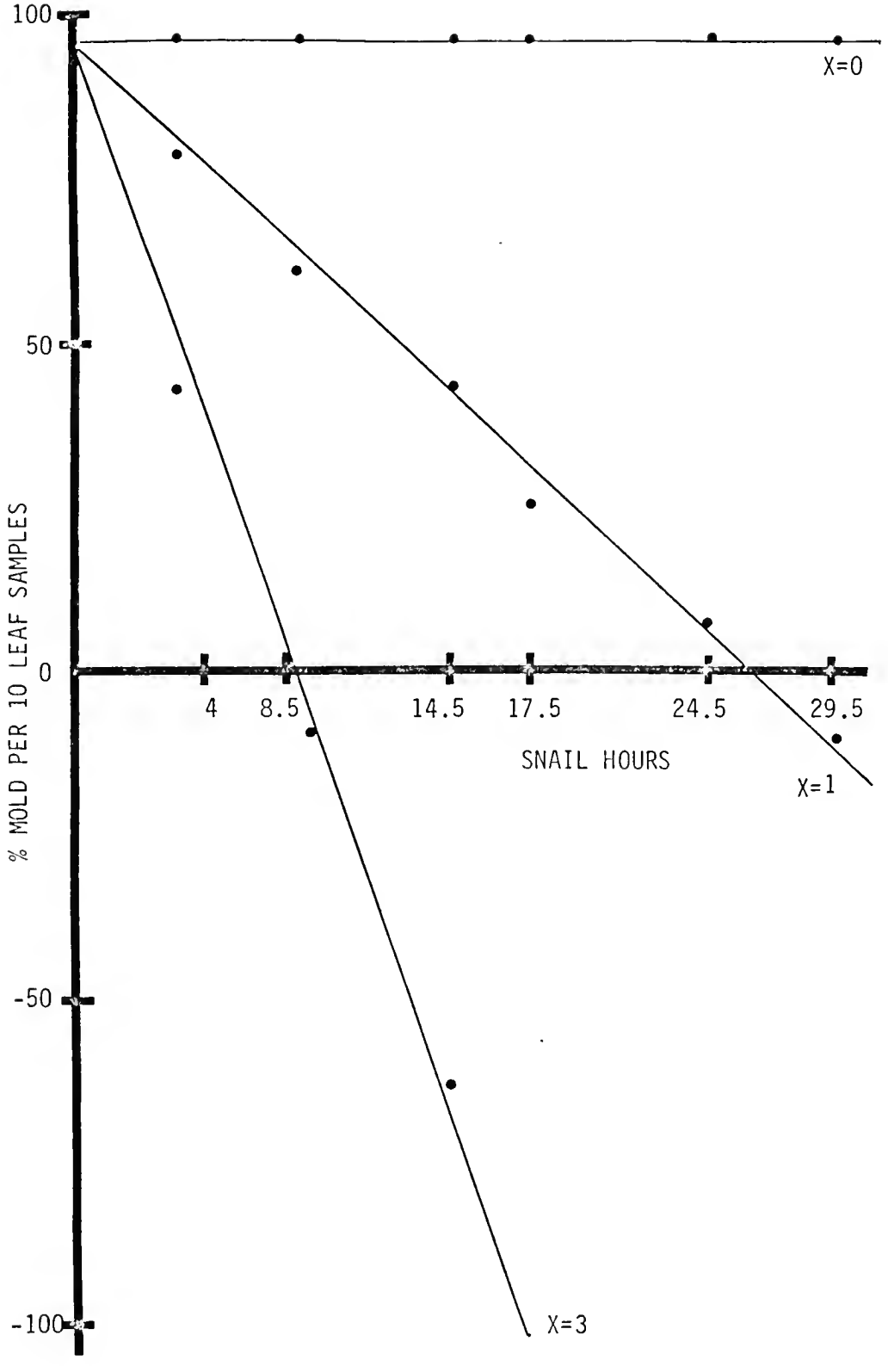

100
50
0
-50
-100
% MOLD PER 10 LEAF SAMPLES
4
8.5
14.5
17.5
24.5
29.5
SNAIL HOURS
X=0
X=1
X=3

Table 3. Citrus Leaf Surface Areas. Measured by Li-Cor Portable Area Meter. Recorded figures represent surface area of dorsal leaf surface. Total surface area can be calculated by multiplying by factor of two*.

Number Snail/ft.^{3}	Replicate	Number of Leaves / ft.^{3}	Total Surface Area (cm^2)	Ave. Leaf Surface Area / ft.^{3}
0	1	141.00	2024.9300	14.3612
0	2	115.00	1863.3900	16.2033
0	3	88.00	1180.4000	13.4136
1	1	108.00	1937.0100	17.9352
1	2	162.00	1974.4200	12.9489
1	3	153.00	1479.3100	9.6686
3	1	115.00	1828.4400	15.8994
3	2	108.00	1603.4000	14.8462
3	3	103.00	1476.4900	14.3348
Total		1093.00	15,367.7900	129.6112
Ave.		121.44	1,707.5322	14.4012
			3,415.0644*	28.8024*

* - Doubled to represent both dorsal and ventral leaf surfaces.

of the average number of leaves grazed by D. dormani per snail hour ($\bar{L}$) is as follows:

$$\bar{L} = \frac{131.34 \text{ cm}^2/\text{snail hour}}{28.8024 \text{ cm}^2/\text{leaf}}$$

$$\bar{L} = 4.56 \text{ leaves/snail hour}$$

Calculations of the quadratic equation describing the relationship of percent sooty mold present by time are given in Table 4.

The original percentage data were transformed by use of Arc-sin tables to allow for computations with percentages (Table 5). An examination of the significance of the various sources of error showed only the intercept and the day by snail (N·X) interactions as being significant (Table 4). Statistically, factors such as time (N) or time^2 (N^2) had little if any effect in accounting for the reduction in the sooty mold on the leaves. As was expected, the number of snails (X) by time (N) interaction was significant. That is to say, by increasing the snail population, over the six days you would get a reduction in sooty mold. There was a reduction as opposed to an increase because the value was negative for N·X interaction. It was possible to make a direct comparison of the different cubic foot units, without having to deal with the variation in leaf numbers (NLVS) because the NLVS interaction was not statistically significant. The calculated values for Figure 23 are displayed in Table 6.

Sooty mold was calculated to be consumed at a rate of 131.34 cm^2/snail hour. The basis for the linear representation was derived from a quadratic equation where only the intercept and N·X interactions were significant.

Snail density and its effect on citrus rust mites. The data followed a Poisson distribution which is found generally when dealing with

Table 4. Quadratic Equation Variables for Sooty Mold Grazing Test. These variables were examined for sources of error in the sooty mold grazing test. Only the intercept (Intercept) and Day by Snail population interaction (N·X) were significant.

Parameter	Estimate	PR ITI
Intercept	97.1404	.0001**
NLVS	- 0.0622	.2315
N	- 0.0000	1.0000
N^2	0.0000	.0000
X	2.9601	.8426
X^2	0.5236	.9099
N·X	-17.8688	.0495*
$N^2 \cdot X$	1.4395	.2896
$N \cdot X^2$	2.4099	.4220
$N^2 \cdot X^2$	- 0.1192	.7757

** - Highly significant (99%) .01

* - Significant (95%) .05

NLVS - Number of leaves per cubic foot

N - Number of days (or nights)

X - Number of snails

R^2 Value = .931642 (Very high accounting for sources of variation)

Table 5. Data and Transformed Data for Mold Grazing Test. Arc-Sin transformation used to allow for computation with percentages.

Observations	Unit	Percent Mold Present	Arc-Sin Transformation	Number of Leaves	Surface Area	Day	Number of Snails
1	1	0.8	63.44	108	1937.01	1	1
2	1	0.8	63.44	108	1937.01	2	1
3	1	0.8	63.44	108	1937.01	3	1
4	1	0.8	63.44	108	1937.01	4	1
5	1	0.6	50.77	108	1937.01	5	1
6	1	0.7	56.79	108	1937.01	6	1
7	2	1.0	90.00	162	1974.42	1	1
8	2	0.6	50.77	162	1974.42	2	1
9	2	0.6	50.77	162	1974.42	3	1
10	2	0.5	45.00	162	1974.42	4	1
11	2	0.5	45.00	162	1974.42	5	1
12	2	0.4	39.23	162	1974.42	6	1
13	3	1.0	90.00	153	1479.31	1	1
14	3	0.8	63.44	153	1479.31	2	1
15	3	0.8	63.44	153	1479.31	3	1
16	3	0.6	50.77	153	1479.31	4	1
17	3	0.6	50.77	153	1479.31	5	1
18	3	0.4	39.23	153	1479.31	6	1
19	4	0.9	71.56	115	1828.44	1	3
20	4	0.7	56.79	115	1828.44	2	3
21	4	0.2	26.56	115	1828.44	3	3
22	4	0.2	26.56	115	1828.44	4	3
23	4	0.1	18.44	115	1828.44	5	3
24	4	0.2	26.56	115	1828.44	6	3
25	5	0.9	71.56	108	1603.40	1	3

Table 5. - Continued

Observations	Unit	Percent Mold Present	Arc-Sin Transformation	Number of Leaves	Surface Area	Day	Number of Snails
26	5	0.6	50.77	108	1603.40	2	3
27	5	0.3	33.21	108	1603.40	3	3
28	5	0.3	33.21	108	1603.40	4	3
29	5	0.4	39.23	108	1603.40	5	3
30	5	0.1	18.44	108	1603.40	6	3
31	6	1.0	90.00	103	1476.49	1	3
32	6	0.5	45.00	103	1476.49	2	3
33	6	0.4	39.23	103	1476.49	3	3
34	6	0.3	33.21	103	1476.49	4	3
35	6	0.3	33.21	203	1476.49	5	3
36	6	0.3	33.21	203	1476.49	6	3
37	7	1.0	90.00	141	2024.93	1	0
38	7	1.0	90.00	141	2024.93	2	0
39	7	1.0	90.00	141	2024.93	3	0
40	7	1.0	90.00	141	2024.93	4	0
41	7	1.0	90.00	141	2024.93	5	0
42	7	1.0	90.00	141	2024.93	6	0
43	8	1.0	90.00	115	1863.39	1	0
44	8	1.0	90.00	115	1863.39	2	0
45	8	1.0	90.00	115	1863.39	3	0
46	8	1.0	90.00	115	1863.39	4	0
47	8	1.0	90.00	115	1863.39	5	0
48	8	1.0	90.00	115	1863.39	6	0
49	9	1.0	90.00	88	1180.40	1	0
50	9	1.0	90.00	88	1180.40	2	0
51	9	1.0	90.00	88	1180.40	3	0
52	9	1.0	90.00	88	1180.40	4	0
53	9	1.0	90.00	88	1180.40	5	0
54	9	1.0	90.00	88	1180.40	6	0

Table 6 . Calculation of points for graphic representation of time by % mold present for 0, 1, 3 snails per cubic foot of citrus.

Number of Snails	Day	Snail Hours/ Day	Y Value
0	1	4.0	97.1404
0	2	4.5	97.1404
0	3	6.0	97.1404
0	4	3.0	97.1404
0	5	7.0	97.1404
0	6	5.0	97.1404
1	1	4.0	79.2616
1	2	4.5	61.4028
1	3	6.0	43.5340
1	4	3.0	25.6652
1	5	7.0	7.7964
1	6	5.0	-10.0724*
3	1	4.0	43.5340
3	2	4.5	-10.0724*
3	3	6.0	-63.6788*
3	4	3.0	-117.2852*
3	5	7.0	-170.8916*
3	6	5.0	-224.4980*

* - Negative values of % mold were used only for determining period of time necessary to reach total sooty mold removal.

Y intercept = 97.1404

random sampling of organisms in some medium, or insect and mite counts in field plots (Steel and Torrie, 1960). For this and all Poisson values in these studies the transformation of $Y = (X' + .5)^{\frac{1}{2}}$ was used (Table 7).

The derived quadratic equation (Table 8) was used to give a representation of the effects the snail populations had on the citrus rust mites (Table 9; Figure 24). Control areas (X=0), after day one, displayed a constant value through the experiment. The reason for the apparent initial decline in mite population was due to the variability in average surface area throughout the experiment. The surface areas of the nine cubic feet of test area (X_1) were averaged as were the averages of all the untransformed initial mite counts (X_2). This allowed for comparison of the various cubic units. Quadratic equations, having basis in all of these factors, are affected by any averaging (Figure 24). Thus by averaging X_1 and X_2 values, a decline in population values is depicted. The average of these two values, X_1 and X_2, were combined with the intercept value to yield the control values.

$$Y \underset{(n = 1\text{-}6)}{(x = 0)} = \text{Intercept} - .003\, X_1 + .0260\, X_2$$

$$Y \underset{(n = 1\text{-}6)}{(x = 0)} = 2.8613 - .5122 + .2503$$

$$Y \underset{(n = 1\text{-}6)}{(x = 0)} = 2.599 \text{ (transformed data)}$$

To untransform the data to original state, the following formula was used.

$$X' = Y^2 - .5$$

$$X' \underset{(n = 1\text{-}6)}{(x = 0)} = 6.2548 \text{ (Table 7)}$$

Test areas using one snail per cubic foot (X = 1) displayed a sharp decline in citrus rust mite populations for the first four days

Table 7. Calculation of points for graphic representation of time by mite population for 0, 1, and 3 snails per cubic foot of citrus.

Number of Snails	Day	Snail Hours/ Day	X' Value	Y Value
0	1	4.0	7.6870	2.5994
0	2	4.5	6.2568	2.5994
0	3	6.0	6.2568	2.5994
0	4	3.0	6.2568	2.5994
0	5	7.0	6.2568	2.5994
0	6	5.0	6.2568	2.5994
1	1	4.0	6.4674	2.6396
1	2	4.5	4.1997	2.1679
1	3	6.0	2.5905	1.7580
1	4	3.0	2.1455	1.6265
1	5	7.0	2.3940	1.7012
1	6	5.0	3.4287	1.9821
3	1	4.0	5.5319	2.4560
3	2	4.5	3.1975	1.9229
3	3	6.0	1.9812	1.5752
3	4	3.0	1.4962	1.4129
3	5	7.0	1.562	1.4360
3	6	5.0	2.2043	1.6445

Y intercept = 2.8613
X' = Untransformed values ($X' = Y^2 - .5$)
Y = Transformed values

Table 8. Calculation of the Quadratic Equation for Snail Feeding on Citrus Rust Mite. Refer to Table 4 for source of quadratic. Table 7 gives calculated values.

$$Y = 2.8613 - .003X_1, + .0266\ X_2 + 1.0896X - .2993X_2 - 1.1446\ (X \cdot N)$$
$$+ .1392\ (X \cdot N^2) + .2914\ (X^2 \cdot N) - .0361\ (X^2 \cdot N^2)$$

X_1 = Average initial surface area per cubic foot = 1707.43

X_2 = Average untransformed initial mite count = 9.4111

X = Number of snails

N = Number of days (or nights)

Table 9. Quadratic Equation Variables for Citrus Rust Mite Grazing Test. These parameters were evaluated after statistical elimination of several unsignificant parameters. All of the parameters proved significant except the (X^2) i.e., (number of snail)^2 factor.

Parameter	Estimate	PR (T)
Intercept	2.8613	.0001**
SFAREA	-0.0003	.0489*
I Count	0.0266	.0050**
X	1.0896	.0413*
X^2	-0.2993	.1368
$X \cdot N$	-1.1446	.0022**
$X \cdot N^2$	0.1392	.0076**
$X^2 \cdot N$	0.2914	.0251**
$X^2 \cdot N^2$	-0.0361	.0468**

** - Highly significant (99%) .01

* - Significant (95%) .05

SFAREA- Source of error due to surface area

I count - Pretreatment mite count

X - Number of snails

N - Number of days (or nights)

R^2 value = .14986

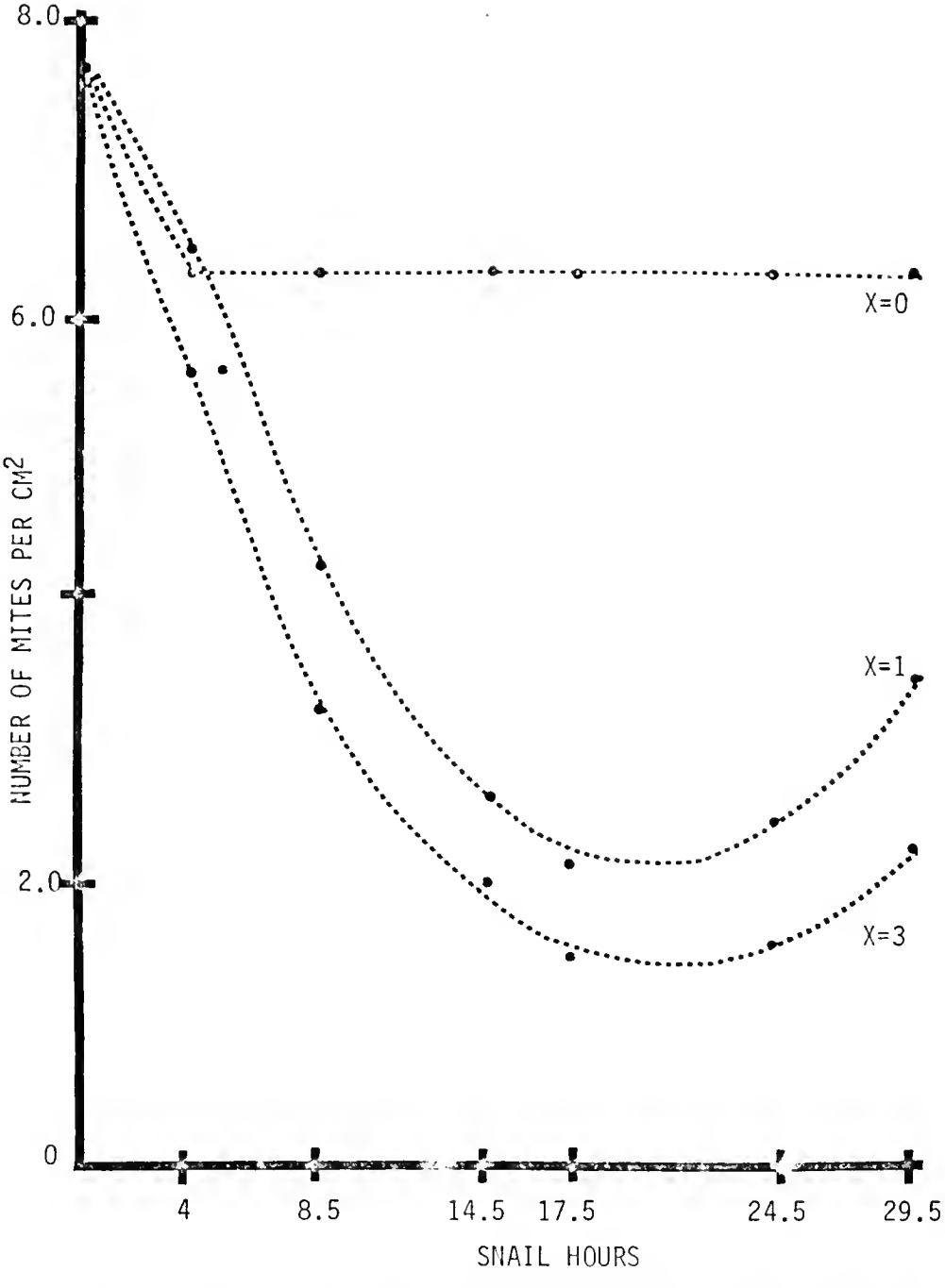


Figure 24. Graphic representation of 0, 1, and 3 snails per cubic foot of citrus and their ability to suppress citrus rust mite populations on citrus.

(20 snail hours) which reduced the population by 83.7%. After this point (Figure 24) there was an increase in the mite population. Field observations indicated that areas cleaned by the snail could become reinfested from immigrating rust mites. The immigration and migration of mites by locomotion, wind dispersal, and rain could account for the reinfestation of previously cleared areas.

This test demonstrated that even in this type of uncontrollable mite infestation a high degree of citrus rust mite population suppression was attained briefly. Greater suppression was displayed by the units containing three mites per cubic foot (X = 3). By day four, as high as 90% reduction in the rust mite population was observed. This again demonstrated the capacity of the snails for rust mite removal.

Having determined the amount of surface area covered by a snail per snail hour (S), it was possible to determine the number of snails (N) necessary to clean the surface of a tree (A) in any given number of days (T).

The snail hours per night (h) were calculated by monitoring periods of 100% R.H. and its daily occurrence. The value for S, surface area cm^2 cleaned by a snail per snail hour, was taken as an average $\bar{S}$ = 131.34 cm^2/snail hour, as calculated earlier.

Calculations of the number of snails (N) that would be needed is as follows:

$$N = \frac{2A \times 10{,}000}{Txhxs}$$

N = Number of snails needed

A = Surface area of tree ($meter^2$)

T = Time (days) to completion

h = Snail hours/night

s = Area cleaned by snail per hour.

To calculate for A, total leaf surface area per tree, the following formula was needed (Turrell, 1961):

$$\text{Log } A = C_2 + N_2 \log a.$$

Constants

C_2 = .994 on Valencia

N_2 = 1.068 on Valencia

Variables

a - age of tree

$$\text{Log } A = .994 + 1.068 \log a.$$

In the calculation, 2A is used because Turrell's formula just measures the dorsal surface area. Using this set of formulas and the calculated averages wherever possible, the author arrived at some estimated number of snails needed per tree (Table 10).

The author estimated that because of the short, four month activity period of the snails, that a T value of 15 to 30 days should be used. This means that the entire leaf surface of the tree should be cleaned within this period. Several factors such as ambulatory movement, and original snail distribution on the tree may tend to make the T period longer than calculated. For this reason the snail values N for 15 days were calculated.

The trees,evaluated in Chapter II, Section 1, dealing with snail population per tree, were 25 year old trees. Reference to Table 10 shows that up to 168 snails per tree would be needed to clean totally the tree surface in a 15 day period. Even if the lower value of 84 snails for 30 days is used, it is quickly realized that the 1976 populations were too low to afford the control needed. An average of only 45 snails per tree were found. At this rate the earliest the

Table 10. Calculated minimal number of snails necessary to clean an entire citrus tree per unit time.

Age of Tree (Years) (a)	Days to Completion (T)	Number of Snails (N)
10	15	64
10	30	32
15	15	98
15	30	49
20	15	134
20	30	67
25	15	168
25	30	84
30	15	206
30	30	103

* - For the above calculations the following averages were derived from the field data.

$S = \overline{S} = 131.34\ cm^2$

$h - \overline{h} = 5$ hours per night

entire surface could be cleaned would be 60 days, about half of the entire active snail season. At this rate the snail grazing may not suppress the biotic potential of the sooty mold and citrus rust mite.

The author believes that the 1975 snail population levels at Orange Lake, Florida, were sufficient to suppress sooty mold and mite populations in accordance with calculated levels, but no quantitative data confirming this are available. Snail trees during that period exhibited a clean shiny gloss which was not seen during 1976 or 1977 observations. The author also wishes to acknowledge that these calculations are based on a small number of snails and that additional testing would be needed to be conclusive.

CHAPTER II
BIOLOGICAL STUDIES OF THE CITRUS RUST MITE

Section 1. Methods for Monitoring Citrus Rust Mites

Introduction

Early researchers used linen testers and 0.5 in. holes cut in paper to help define the field of view sampled in citrus rust mite counts (Yothers and Miller, 1934; Osburn and Mathis, 1944). Later researchers used the entire field of view of a 10X hand lens (Pratt, 1957; Johnson, 1969; Simanton, 1960) to count mite populations. The lack of uniformity of method and surface area examined by earlier researchers dictated the need for a method of monitoring which delineates the field of view. This study defines the field of view and relates samples taken to the entire mite population on the fruit.

Materials and Methods

Valencia orange trees located in Orange Lake, Florida, were used in this study. Fruit size ranged from 3.4 to 4.2 cm, and were chosen randomly from a five acre plot.

Hand lens method. A 14X Bausch & Lomb^{R} hand lens was used to count the numbers of rust mites per cm^2 on the surface of oranges. A rubber stamp was used to stamp a square 1 cm area on the fruit. Three areas were randomly counted on each fruit along its equatorial belt. Diameter was taken with aluminum calipers and a metric rule. Circumference was taken later in the laboratory by placing a fresh red line on the fruit vertically over its equator, then rolling it along the

equator on white paper. A mark was left at two places on the sheet denoting the fruit's circumfrence. The volume of the fruit was taken in laboratory by placing the fruit into a 1000 ml graduate cylinder and recording water displacement. Samples were multiplied by factors derived from diameter, circumference and volume to give total population estimate over the entire fruit.

Alcohol emersion method. At the completion of each set of lens counts, the orange,while still on the tree, was placed into a clear plastic bag 8 x 3 x 15 x .0015 in. containing 50 ml of 95% isopropanol. The bag over the fruit was sealed by twisting it several times around the branch bearing the fruit. The alcohol was vigorously shaken over the orange for ten seconds to remove citrus rust mites. The bag was removed from the fruit and sealed. The fruit was removed from the tree marked for later identification.

In the laboratory the alcohol was agitated in the bag, and three 2 ml samples were pipetted into separate watch glasses. An Olympus^{R} binocular microscope at 25X was used to count the number of mites per sample. The three 2 ml samples were averaged and multiplied by the percent of the sample they represented to give total number of mites from each fruit.

Results and Discussion

The average number of citrus rust mites on the equatorial belt of ten fruit ($\bar{Y}$) are shown in Table 11. These values were multiplied by the estimated surface area calculated from fruit volume (Sv), fruit diameter (Sd), and fruit circumference (Sc), which yielded estimates of 51.25, 48.56, and 55.93 mites per orange, respectively. The alcohol emersion method gave an average of 53.23 mites per orange (Table 12).

Table 11. Ten oranges were sampled for rust mite with a 14X hand lens. Three lens fields ($1cm^2$ each) were viewed per orange and recorded (Sample 1, 2, 3). Volume, diameter, and circumference were used to determine the surface area of the orange using formula for a sphere, (Sv, Sd, Sc). Overall average for the ten samples is given at the bottom of the Sv, Sd, Sc columns (*).

	Hand Lens Method						
Fruit Number	Average Number of Mites $\bar{Y}$	Surface Area (cm^2) By Volume Sv	Mites Per Fruit $\bar{Y}$xSv	Surface Area (cm^2) By Diameter Sd	Mites Per Fruit $\bar{Y}$xSd	Surface Area (cm^2) By Circumference Sc	Mites Per Fruit $\bar{Y}$xSd
1	.11	64.36	7.08	55.41	6.09	65.87	7.24
2	.55	23.00	12.65	36.31	19.97	41.23	22.68
3	.44	56.44	24.83	52.81	23.24	55.43	24.38
4	.33	36.10	11.91	36.31	11.98	39.85	13.15
5	1.22	41.61	50.76	38.48	46.95	39.85	48.62
6	1.11	51.29	56.93	50.26	55.97	60.59	67.25
7	2.11	55.43	116.96	50.26	106.05	58.58	123.60
8	1.00	55.43	55.43	45.36	45.36	56.44	56.44
9	.33	46.13	15.22	45.36	14.96	50.78	16.75
10	3.33	48.27	160.74	45.36	155.04	53.80	179.15
			51.25*		48.56*		55.93*

* - Average of ten samples
Sv - Surface area from volume measurements
Sd - Surface area from diameter measurements
Sc - Surface area from circumference measurements

Table 12. Ten oranges were washed in separate bags containing 50 ml isopropanol. Three 2 ml aliquots were taken from each bag and the number of rust mites counted and recorded (Sample 1,2,3). These were then averaged (Average) and multiplied by the percent of the sample they represented to give (Number of mites per fruit).

Alcohol Emersion Method

Fruit Number	Sample 1	2	3	Average	Number Per Fruit
1	0	0	0	0	0
2	0	1	1	.66	16.50
3	1	0	0	.33	8.25
4	0	2	0	.66	16.50
5	0	2	0	.66	16.50
6	2	4	2	2.66	66.50
7	4	3	4	3.66	91.50
8	3	1	2	3.00	75.00
9	4	0	0	1.33	33.25
10	10	9	6	8.33	208.25
					53.23*

* - Average of 10 samples.

A linear correlation coefficient was used to determine that there was a 99% correlation between the alcohol emersion method and the hand lens data. A X^2 test for expected values failed to demonstrate any statistical difference between the average citrus rust mite populations on the ten fruit.

This test, though lacking in replicates and large populations of citrus rust mites, was carried out early in 1975 as a first indication of the usefulness of the hand lens monitoring method for determining citrus rust mite populations on fruit. This test did indicate a high degree of correlation,but is inconclusive and needs additional replication. It is presented here only as on indication of the relationship between the population of P. oleivora on the equatorial belt and the entire citrus rust mite population which is believed to exist on the fruit. Other factors such as seasonal variation would need to be considered as would greater mite populations. Citrus rust mite orientation on the equatorial belt of the fruit is examined in the next section.

Section 2. Extrinsic and Intrinsic Orientation of Citrus Rust Mite on Valencia Orange

Introduction

Watson and Berger (1937) described rings of russet damage corresponding to semi-shaded areas around the fruit. They speculated that this was due to an aggregative effect of the citrus rust mite and its preference for these areas. Yothers and Mason (1930) demonstrated a positive phototaxis but made mention of the mites avoidance of direct sunlight. It was noted previously (Chapter II, Section 1) that the sample about the citrus fruit equatorial belt was believed to be a

representative sample of the mite population on that fruit. To gain a better understanding of this population an experiment was conducted. This study examined the distribution of the citrus rust mite on both the tree and on the fruit.

Materials and Methods

P. olievora was studied for population distribution patterns on both fruit and tree quadrants at Orange Lake, Florida. All tests were on Valencia variety C. sinensis with east-west row orientation.

Four blocks with two trees per block were monitored. One tree per block was treated with water at 200 p.s.i. to run off while the second tree was left untreated. Counts were made pretreatment (day of application), and at one week intervals for six weeks.

Method of counting. Four fruit were chosen to represent each of the four major compass directions. Citrus rust mite counts were taken as in Chapter II, Section1, along the equatorial belt (a plane through the fruit parallel to the ground) on each of the four major compass directions. This gave a total of 16-1 cm^2 samples/tree.

Results and Discussion

Initial efforts to analyze the data employed the use of an analysis of variance (ANOVA) (Table 13) and tested for the various interactions. There was no significant difference between the blocks and treatments for the trees tested, permitting summation of data of the two treatments (Tables 14-19). The significant difference displayed

Table 13. ANOVA for citrus rust mite orientation on fruit.

Source	Df	Sum Squares	F
Blocks (B)	3	170.71	A
Treatments (T)	1	8.96	A
Error (B*T)	3	330.65	
Tree Direction (TDIR)	3	61.53	A
(TDIR * T)	3	192.55	1.96
Error B(B*TDIR) (B*TDIR*T)	18	590.45	
Orange Direction (ODIR)	3	9.26	1.05
ODIR * T	3	24.34	2.75*
ODIR * TDIR	9	74.85	2.82**
ODIR * TDIR * T	9	19.18	A
Error C	72	212.65	
Week (W)	5	888.70	
TXW	5	50.27	1.13
TDIR * W	15	441.27	3.3**
ODIR * W	15	72.88	A
Residual	600		

* - Significant at .01 level.

** - Significant at .05 level.

A - Less than 1.0 (not significant).

Table 14. Citrus Rust Mite Orientation (Week 1). Values for citrus rust mite orientation on both the citrus tree and on the individual fruit.

Week	Treatment	N				E				S				W				Average
		N #1	E 2	S 3	W 4	N 5	E 6	S 7	W 8	N 9	E 10	S 11	W 12	N 13	E 14	S 15	W 16	
1	11	0	0	0	1	0	2	0	0	1	2	0	0	0	4	0	1	
1	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	
1	11	0	0	2	8	1	0	0	1	0	0	0	0	0	1	1	0	
1	10	0	2	13	4	50	4	2	23	20	32	0	60	67	57	111	29	
1	10	24	5	0	0	2	14	86	16	0	53	1	72	25	11	12	0	
1	11	11	26	16	9	8	0	41	2	0	0	0	0	147	53	83	4	
1	10	1	0	0	0	1	2	0	0	0	0	0	0	1	0	0	1	
1	11	3	6	42	40	7	0	8	12	128	120	0	200	2	92	101	2	
		39				69				149				247				15.6
			39				22				210				218			15.3
				73				137				1				309		16.3
					62				54				332				37	15.2
(A)	Total -	39,	39,	73,	62,	69,	22,	137,	54,	149,	210,	1,	332,	247,	218,	309,	37	
	Average	4.9	4.9	9.1	7.8	8.6	2.8	17.1	6.8	18.6	26.3	.1	41.5	30.3	27.3	28.5	4.6	
(B)	Totals -				213				282				692				806	
	Average				53.2				70.5				173				201.5	

(A) Totals - Represents the relationship to orange direction.
(B) Totals - Represents the relationship to tree direction.

Table 15. Citrus Rust Mite Orientation (Week 2). Values for citrus rust mite orientation on both the citrus tree and on the individual fruit.

Week	Treatment	N				E				S				W				Average
		N #1	E 2	S 3	W 4	N 5	E 6	S 7	W 8	N 9	E 10	S 11	W 12	N 13	E 14	S 15	W 16	
2	10	200	100	7	100	9	0	7	22	25	16	23	7	1	2	3	0	
2	11	8	10	0	10	3	1	9	0	0	0	0	0	140	54	130	0	
2	11	1	1	1	7	2	0	0	4	5	1	0	1	0	1	2	0	
2	10	3	4	49	50	108	1	42	88	30	60	5	5	20	3	26	1	
2	10	17	21	28	87	3	3	43	11	40	65	200	30	0	1	1	0	
2	11	48	20	46	35	4	2	0	0	0	20	10	40	78	58	57	100	
2	10	200	82	200	200	0	0	0	0	1	0	0	1	0	0	1	0	
2	11	4	200	200	200	37	6	12	19	27	200	100	15	100	8	26	28	
		481				167				128				339				278.75
			438				13				362				127			235
				531				113				338				246		307
					689				144				99				129	265.25
(A)	Total -	481,	438,	531,	689,	167,	13,	113,	144,	128,	362,	338,	99,	339,	127,	246,	129	
	Average	60.1	54.8	66.4	86.1	20.9	1.6	14.1	18	16	45.3	42.3	12.4	42.4	15.9	31	16.1	
(B)	Totals -				2139				438				927				841	
	Average				535				109				232				210	

(A) Totals - Represents the relationship to orange direction.
(B) Totals - Represents the relationship to tree direction.

Table 16. Citrus Rust Mite Orientation (Week 3). Values for citrus rust mite orientation on both the citrus tree and on the individual fruit.

Week	Treatment	N				E				S				W				Average
		N #1	E 2	S 3	W 4	N 5	E 6	S 7	W 8	N 9	E 10	S 11	W 12	N 13	E 14	S 15	W 16	
3	11	30	200	5	100	6	2	24	2	1	9	19	1	0	0	9	17	
3	10	3	2	0	4	5	0	2	2	0	0	0	0	0	0	0	0	
3	11	27	200	100	100	100	200	200	33	26	13	5	10	7	5	12	11	
3	10	100	200	50	100	30	24	0	12	0	0	0	0	11	24	23	100	
3	10	10	15	2	3	50	50	100	27	200	200	150	100	0	5	0	0	
3	11	12	36	16	20	7	0	3	4	13	50	13	18	20	4	15	2	
3	10	10	15	3	7	3	1	3	3	100	50	17	100	5	2	5	2	
3	11	1	1	5	0	200	200	100	200	5	100	6	1	50	10	30	100	
		193				401				345				93				258
			673				477				422				50			406
				181				432				210				94		229
					344				283				230				232	272
(A)	Total -	193,	673,	181,	344,	401,	477,	432,	283,	345,	422,	210,	230,	93,	50,	94,	232	
	Average	24.1	84.1	22.6	43.0	50.1	59.6	54.0	35.4	43.1	52.8	26.3	28.8	11.6	6.3	11.8	29.0	
(B)	Totals -				1391				1593				1207				469	
	Average				348				398				302				117	

(A) Totals - Represents the relationship to orange direction
(B) Totals - Represents the relationship to tree direction.

Table 17. Citrus Rust Mite Orientation (Week 4). Values for citrus rust mite orientation on both the citrus tree and on the individual fruit.

Week	Treatment	N				E				S				W				Average
		N #1	E 2	S 3	W 4	N 5	E 6	S 7	W 8	N 9	E 10	S 11	W 12	N 13	E 14	S 15	W 16	
4	11	79	100	45	47	0	6	0	2	1	0	6	0	100	32	100	100	
4	10	10	0	1	2	0	1	4	1	0	0	0	3	0	0	0	0	
4	11	3	10	4	7	1	4	5	1	0	5	9	1	1	0	2	0	
4	10	30	0	45	15	0	3	5	0	100	100	200	150	100	23	100	7	
4	10	0	0	8	16	100	42	100	100	85	200	30	100	2	2	1	0	
4	11	5	100	4	31	33	2	86	100	10	10	14	6	30	33	100	1	
4	10	2	0	0	0	10	0	2	0	100	14	100	100	0	0	0	1	
4	11	12	30	10	15	44	16	24	25	7	14	62	12	14	100	100	100	
		141				188				303				247				220
			240				74				343				190			212
				117				226				421				403		292
					133				229				372				209	236
(A)	Total -	141,	240,	117,	133,	188,	74,	226,	229,	303,	343,	421,	372,	247,	190,	403,	209	
	Average	17.6	30.0	14.6	16.6	23.5	9.3	28.3	28.6	37.9	42.9	52.6	46.5	30.9	23.8	50.4	26.1	
(B)	Total				631				717				1,439				1,049	
	Average				158				179				360				262	

(A) Totals - Represents the relationship to orange direction.
(B) Totals - Represents the relationship to tree direction.

Table 18. Citrus Rust Mite Orientation (Week 5). Values for citrus rust mite orientation on both the citrus tree and on the individual fruit.

Week	Treatment	N				E				S				W				Average
		N #1	E 2	S 3	W 4	N 5	E 6	S 7	W 8	N 9	E 10	S 11	W 12	N 13	E 14	S 15	W 16	
5	11	0	0	0	0	2	0	0	0	0	0	0	0	20	20	23	0	
5	10	7	15	6	6	100	7	27	0	0	0	1	4	0	0	0	0	
5	11	32	8	35	0	15	5	8	6	40	50	23	20	19	15	11	11	
5	10	25	50	25	23	28	0	37	12	5	5	27	16	27	32	30	27	
5	10	10	18	8	0	10	12	1	3	1	2	37	1	7	0	0	1	
5	11	50	0	1	11	17	10	2	0	11	1	1	9	0	4	1	0	
5	10	7	3	19	6	11	0	16	4	8	3	7	0	13	0	2	0	
5	11	2	7	7	1	0	12	16	5	7	9	30	16	6	1	7	10	
		133				183				72				92				120
			101				46				70				72			72
				101				107				126				74		102
					47				30				66				49	48
(A) Total -		133,	101,	101,	47,	183,	46,	107,	30,	72,	70,	126,	66,	92,	72,	74,	49	
Average		16.6	12.6	12.6	5.9	22.9	5.8	13.4	3.8	9.0	8.8	15.8	8.3	11.5	9.0	9.3	6.1	
(B) Total					382				366				334				287	
Average					96				92				84				72	

(A) Totals - Represents the relationship to orange direction.
(B) Totals - Represents the relationship to tree direction.

Table 19. Citrus Rust Mite Orientation (Week 6). Values for citrus rust mite orientation on both the citrus tree and on the individual fruit.

		N				E				S				W				
Week	Treat-ment	N #1	E 2	S 3	W 4	N 5	E 6	S 7	W 8	N 9	E 10	S 11	W 12	N 13	E 14	S 15	W 16	Aver-age
6	11	1	0	0	0	0	1	0	0	0	0	10	0	0	0	2	0	
6	10	10	10	1	10	0	5	0	0	4	3	0	2	3	3	0	1	
6	11	1	3	5	0	10	10	1	6	5	18	12	9	19	12	10	30	
6	10	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	2	
6	10	50	15	12	25	0	0	0	0	2	0	2	2	7	3	4	3	
6	11	0	0	4	0	1	3	0	0	1	1	0	0	6	0	2	0	
6	11	0	2	0	0	0	0	0	0	0	0	2	0	0	2	0	0	
6	10	5	4	0	0	3	2	0	1	3	0	5	15	4	12	1	5	
		67				14				15				40				34
			34				21				22				32			27
				22				1				31				19		18
					36				7				29				41	28
(A)	Totals -	67,	34,	22,	36,	14,	21,	1,	7,	15,	22,	31,	29,	40,	32,	19,	41	
	Average	8.4	4.3	2.8	4.5	1.8	2.6	.1	.9	1.9	2.8	3.9	3.6	5.0	4.0	2.4	5.1	
(B)	Totals				159				43				97				132	
	Average				40				11				24				33	

(A) Totals - Represents the relationship to orange direction.
(B) Totals - Represents the relationship to tree direction.

by the orange direction by tree direction interaction (Odir * Tdir) suggested that no one direction on each fruit was dominant.

Tree direction was not significant indicating that no single tree direction maintained a superior mite population. On the contrary, a directional preference seemed to shift from week to week indicating a possible interaction with some abiotic factor not accounted for here. Totals (B) on Tables 14-19 display the shifting directional dominance on the tree. Totals (A) demonstrate the averaged value of mites on each direction of each orange.

To test for the relationship of the peripheral (that portion of the orange furthest from the center of the tree), internal (that portion of the fruit closest to the center of the tree), and the marginal (semi-shaded) areas, the average of the mite populations relating to these areas were compared (Table 20). The peripheral surface had 147.125 mites or 29.8% of the overall population. The internal surface comparatively was close having 144.71 mites or 29.3% of the overall population.

The marginal area, which took into account that it was the product of two sums, had an average population of 200.604 mites or 40.7% of the total population. This is an increase of at least 11% over the other two surfaces.

These data defend the earlier theories by Watson and Berger (1937) that the mite populations tend to clump about the marginal aspect of the fruit. No indication of a unilateral tree direction was discernable and it is believed to be dependent on abiotic factors.

Table 20. Citrus Rust Mite Orientation as to Microenvironmental Parameters on the Citrus Fruit. This table allows for numeric inspection of three aspects of the citrus fruit surface.

Week	Peripheral (P)	Internal (I)	Marginal (M)
1	24.75	123.50	175.000
2	240.25	232.50	306.625
3	278.00	214.75	336.125
4	211.25	209.75	269.000
5	88.50	68.75	92.500
6	40.00	19.00	24.375
Average	147.125	144.71	200.604
	29.8%*	29.3%*	40.7%*

P = That area of orange surface always facing away from the center of the tree.

I = That area of orange surface always facing towards the center of the tree.

M = The marginal area of the fruit between the P and I areas.

* = % of total population.

CHAPTER III
EFFECT OF DIFFERENT ACARICIDES ON CITRUS RUST MITES

Introduction

The purpose of this study was to evaluate rust mite control by some newly developed chemicals and to determine their effectiveness by comparison to known standards.

Materials and Methods

During the summer of 1975 and 1976, several compounds were evaluated for control of the citrus rust mite, P. oleivora, on Valencia orange trees. The compounds were mixed with water according to the manufacturer's directions, and applied to run off. A brief description of each compound is found in Table 21.

1975 field acaricide spray tests. Acaricidal tests were conducted on Valencia variety of C. sinensis at Orange Lake, Florida. The following chemicals and concentrations were evaluated:

PP199 at .005, .01, .02, and .04%

PP067 at .01, .02, .04%

Dicofol at .03% (commercial standards)

Oxamyl at .001%

A water control and an unsprayed control were incorporated into this test.

Table 21. Information on chemicals evaluated for Acaricide use in 1975-76 field tests.

Name to be Used	Chemical Name	Compound Number	Manufacturer	Rates Evaluated
Banex R	Cytex		Atlantic & Pacific Research, Inc.	1.6%
Oxamyl	Methyl N',N'-Climethyl-N-(Methylcarbomoyl)oxy - 1-thiooxamimidate	DPX-1410	DuPont	.001%
Chlorobenzylate*	Ethyl 4, 4'-dichloro-benzilate	G-23992	Cieba-Geigy	.01%
Dicofol	1,1-bis(Chlorophenyl)-2 2,2-triChloroethanol		Rohm & Haas	.03%
	N 2-Chloro-5-(trifluoro-methyl)phenyl -2, 4-nitro-6 - trifluromethyl)benzena-mine.	PP199	I.C.I. Ltd.	.005%, .01%, .02%, .04%
	(Analogue of PP199)	PP067	I.C.I. Ltd	.01%, .02% .04%

* - Accepted commercial standards.

Four blocks (rows) of trees were selected with one row spacing between blocks. Single tree treatments were assigned randomly within the blocks, with an unsprayed buffer tree between treatments. Application was with a 30 gallon, water agitated spray tank. A hand held nozzle was used to spray to run off at 200 p.s.i. Spray solutions were premixed earlier that day and stored in two gallon stainless steel canisters. The spray tank was rinsed with water between the different treatments.

Counts were made at pre-treatment (day of application), then at weekly intervals for six weeks. All treatments were sampled by locating an orange on each of the four major compass headings (N, E, S, W). Each orange was then marked with a rubber stamp at four points along the equatorial belt of the fruit corresponding to N, E, S, W quadrants. A 10X Bausch & Lomb^{R} hand lens was used to count the number of citrus rust mites per 1 cm^2 area.

1976 field acaricide spray tests. The 1976 field acaricide tests were evaluated at the same grove site, using the same block (rows) as in the 1975 test (see 1975 tests). Blocks were set up in an east-west orientation with a buffer row between blocks. Eight trees for evaluation per block were identified as having more than 60% mite infestation per ten random cm^2 observations on fruit per tree. As eight trees were identified, with at least one tree buffer within the block, treatments were randomly assigned with each tree being sprayed to run off.

The chemicals evaluated in the 1976 acaricide test are as follows:

PP199	.01, .02, .04%
Banex^{R}	1.6%

Oxamyl	.001%
Chlorobenzylate	.01%

Again a water check and unsprayed check were incorporated into the test.

A 10X Bausch & Lomb^{R} hand lens fitted with a 1/8" thick plexiglass plate enscribed with a one cm^2 grid divided into 25 units, replaced the 1 cm^2 rubber stamp and hand lens method previously used (Allen, 1976). One sample was taken from ten randomly selected fruit on each test tree. This was repeated for each treatment and replication over a five week period.

The rating of the chemicals was determined by Duncan's new multiple-range test. As the chemicals lost efficiency they were rated at least, moderate, or best control. The labels, poor or good control, were not used since no effort was made to correlate the control to economic thresholds or crop production yields.

Results and Discussion

1975 field acaricide spray tests. A Poisson distribution existed, so transformed data were generated. Table 22 displays the average transformed ($\bar{Y}$) and untransformed ($\bar{X}'$) value for each treatment. An analysis of variance (ANOVA) for significant differences between the control data and the chemicals proved statistically significant. This significance permitted further statistical evaluations using just the toxicants. By using the chemical data and excluding the controls from further ANOVA more sensitive tests were possible. The control data (both water control and unsprayed control) were tested by ANOVA for statistically significant differences (Table 23). No statistically significant difference (SSD) was found so, for the sake of this experi-

Table 22. Calculated $\overline{Y}$ and $\overline{X}'$ Values for 1975 Acaricide Tests.

Number of Observations (N)	Treatment #	$\overline{Y}$	$\overline{X}'$
384	1	.3046	.5928
384	2	.5963	-.1444
384	3	.0937	-.4912
384	4	.0625	-.4960
384	5	1.3515	1.3265
384	6	.7788	.1062
384	7	.4947	-.2552
384	8	.2369	-.4438
384	9	1.8802	3.0351
384	10	20.7890	431.6825
384	11	22.4531	503.6416

$\overline{Y}$ = Transformed mean for data

$\overline{X}'$= Untransformed mean for data

$Y = X' + .5$ $\qquad \overline{Y} = \frac{X' + .5}{N}$

$X' = Y^2 - .5$

Table 23. ANOVA for 1975 Acaricide Control Data. This was used to test for differences between the two control sets.

Source	DF	Sum Squares	F Value
Block (R)	3	170.70	6.99**
Treatment (T)	1	8.95	1.10
Week (W)	5	888.70	21.84**
BxW	15	515.91	4.23**
TxB	3	330.64	13.54**
TxW	5	50.26	1.24
TxBxW	15	663.02	5.43**

** - Highly significant (.01)

ment, all the controls were the same for the entire experiment. These control data points were averaged later and used for comparison to each chemical independently (Figures 25-33; Table 24). These graphic comparisons (Figures 25-33) demonstrate the large differences between the control and treatment data.

ANOVA on the control treatments in the 1975 test (Table 25) gave a high degree of significance (.01 level) for the following sources: blocks (b), treatments (T), weeks (W), BxW, TxB, TxW, TxBxW. Differences between blocks were expected. For this reason the blocks were incorporated in the test. The difference between the treatments simply meant that all the chemicals were not controlling the same. The SSD with the treatment by weeks interaction meant that a Duncan's new multiple range test on the chemicals had to be run on a week to week basis. The Duncan's test gave an ordered comparison between treatments and denoted the statistical significant differences (SSD), if any.

ANOVA for block, treatment, and TxT for each week demonstrated that only weeks 2, 3, and 6 were significantly different (Table 26). Week 1 gave total control by all chemicals tested. Weeks 2 and 3 proved to have SSD for the treatment. Weeks 4 and 5 should have had varying treatment control, but this was not evident. Clos analysis of the data (Figures 25-33) indicates that a natural population decline, possibly by an epizootic by Hirsutella thompsonii, Fisher, drove the populations back down, not allowing for any differences to develop. During week 6, even with this epizootic-like condition, significant differences did exist within populations of mites on the chemically treated treatments. In many cases the treated populations exceeded the control populations.

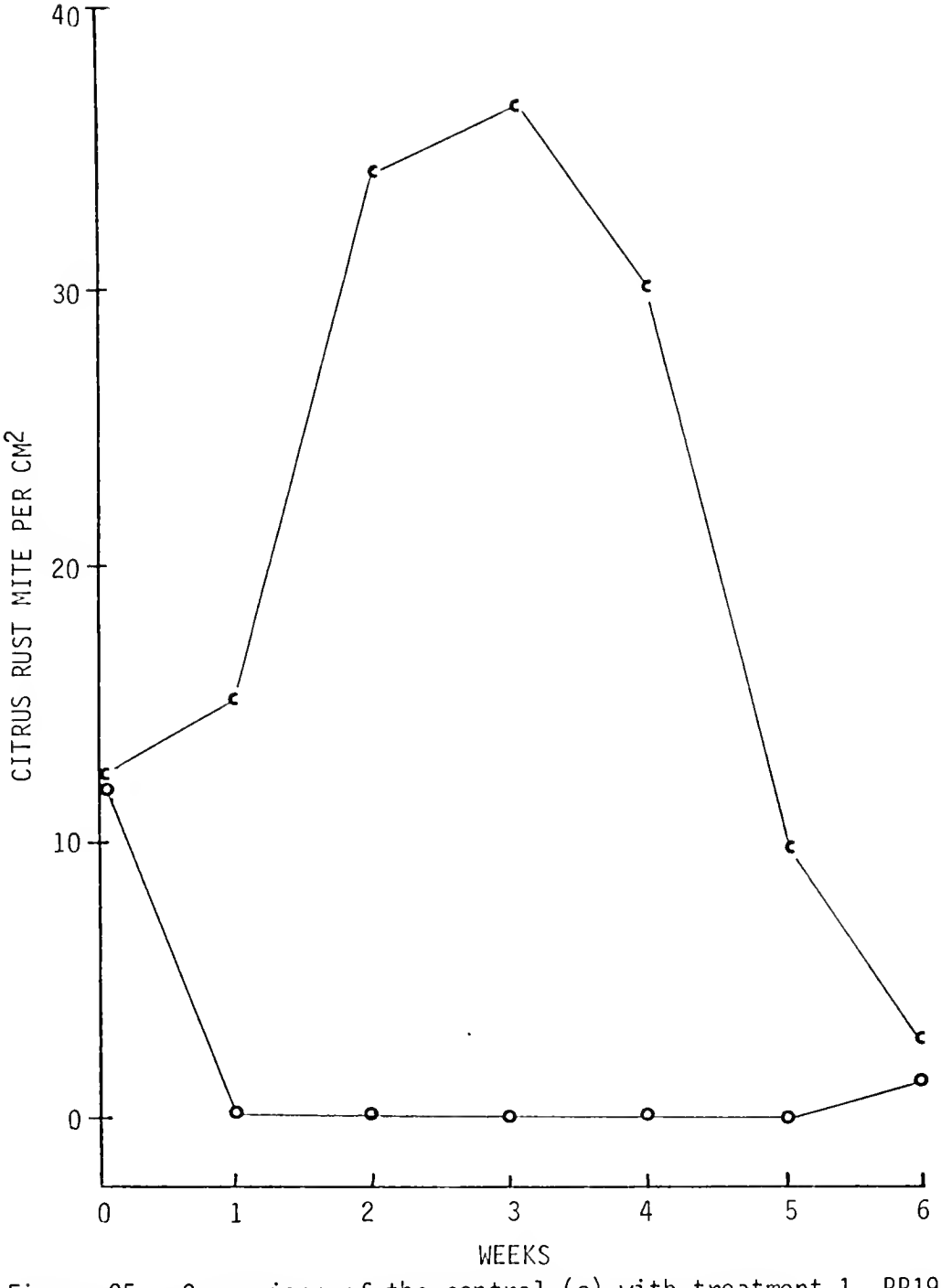


Figure 25. Comparison of the control (c) with treatment 1, PP199 at .005% in the 1975 acaricide evaluations.

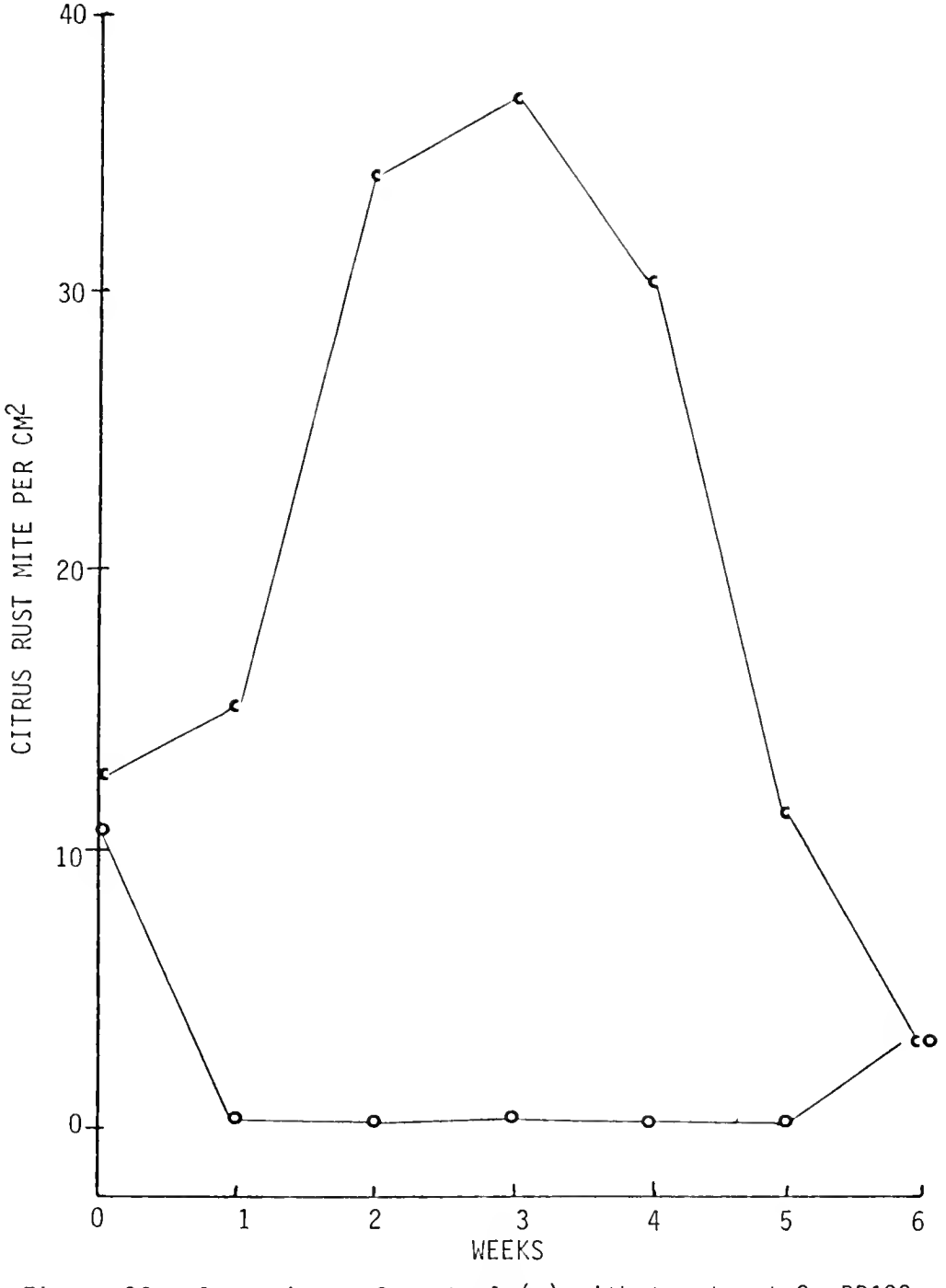


Figure 26. Comparison of control (c) with treatment 2, PP199 at .01% in the 1975 acaricide evaluations.

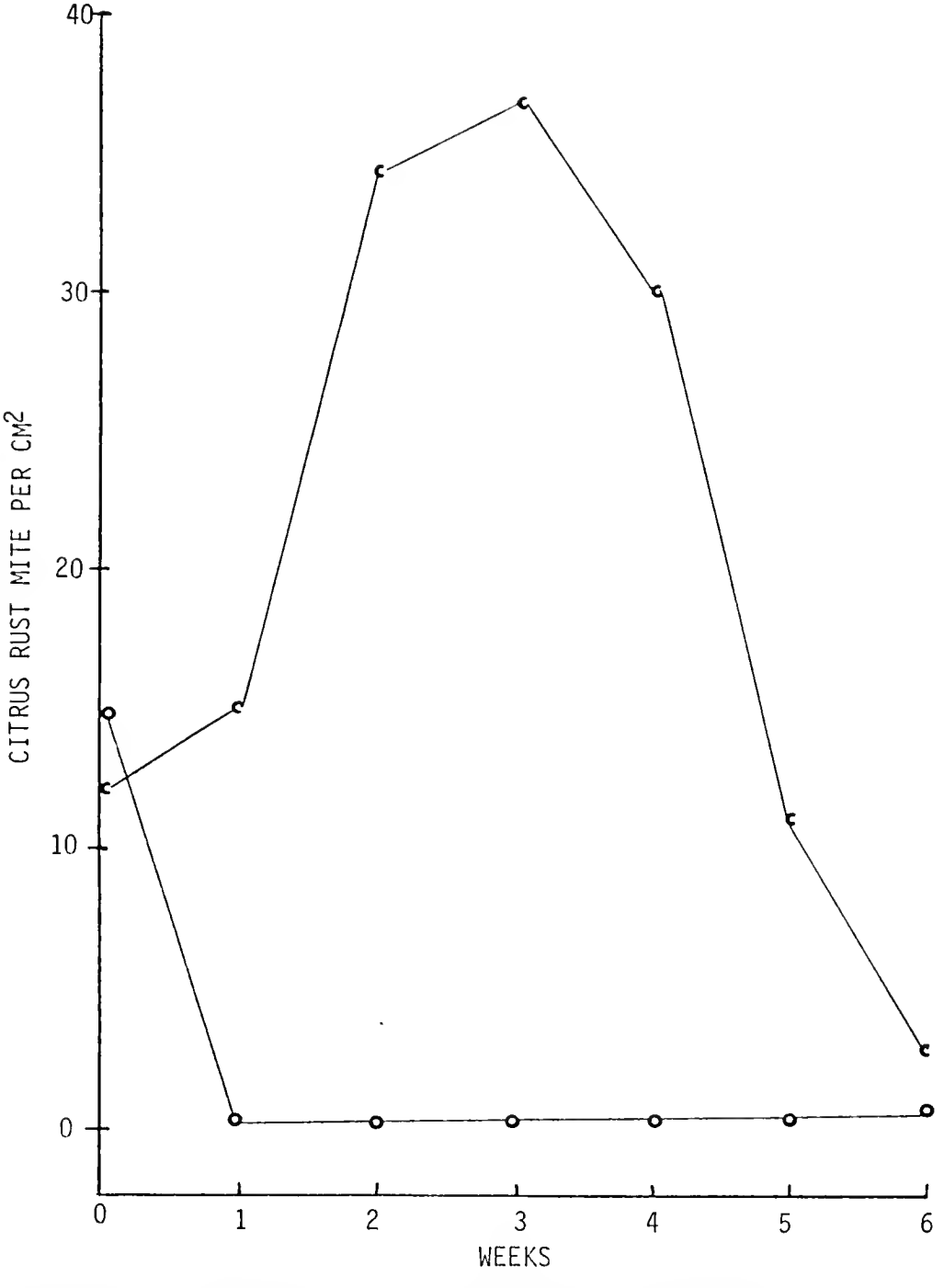


Figure 27. Comparison of control (c) with treatment 3, PP199 at .02% in the 1975 acaricide evaluations.

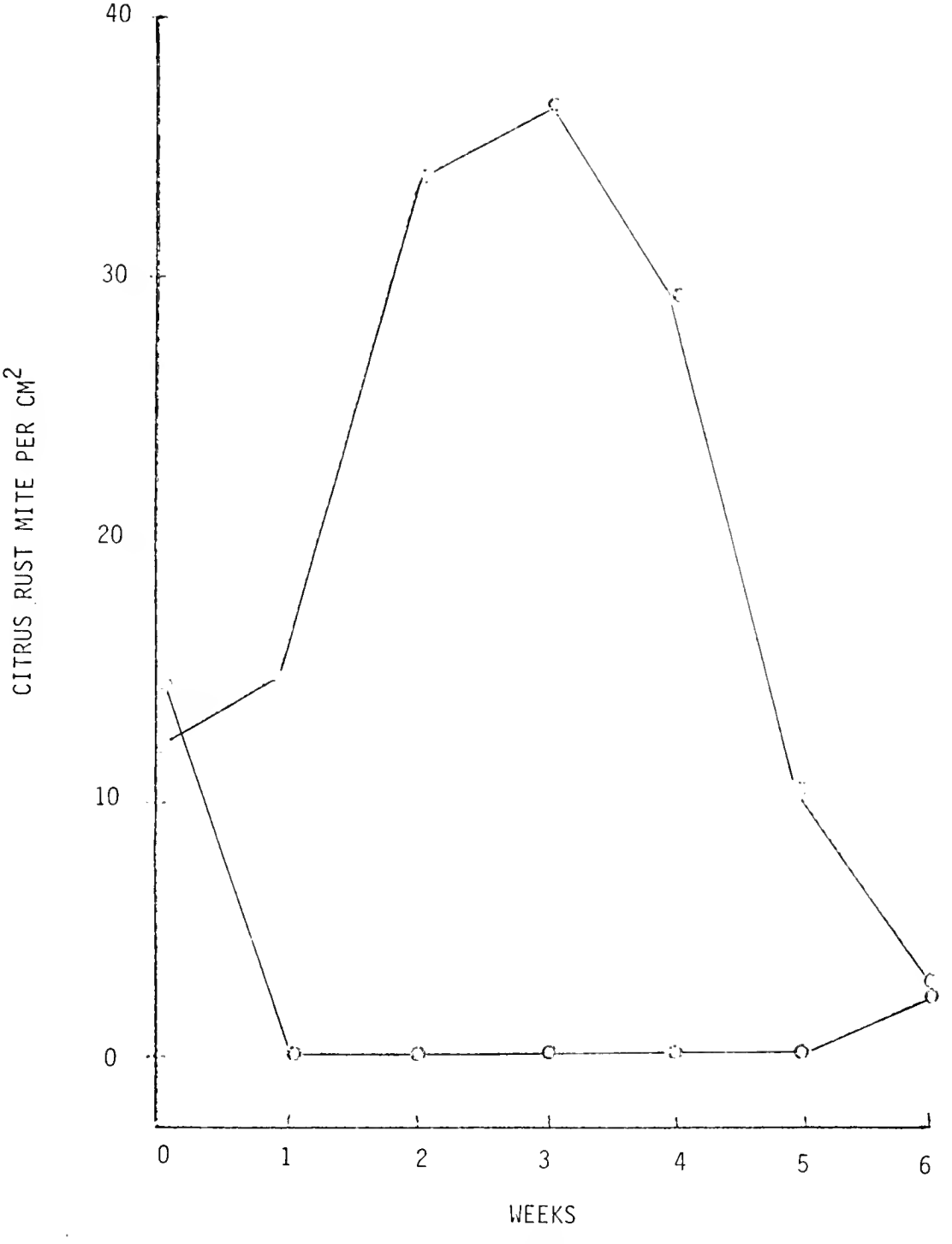


Figure 28. Comparison of control (c) with treatment 4, PP199 at .04% in the 1975 acaricide evaluations.

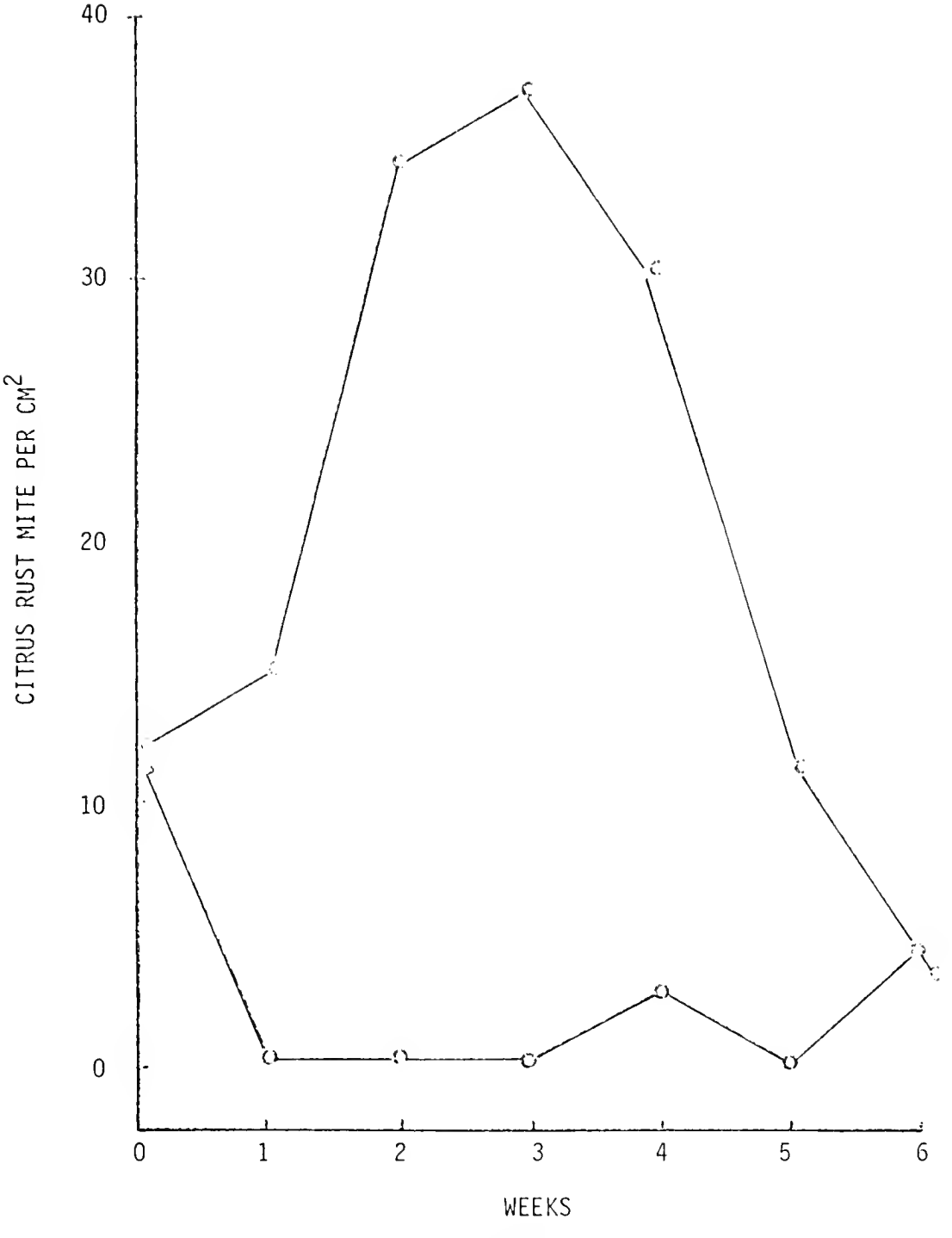


Figure 29. Comparison of control (c) with treatment 5, PP067 at .01% in the 1975 acaricide evaluations.

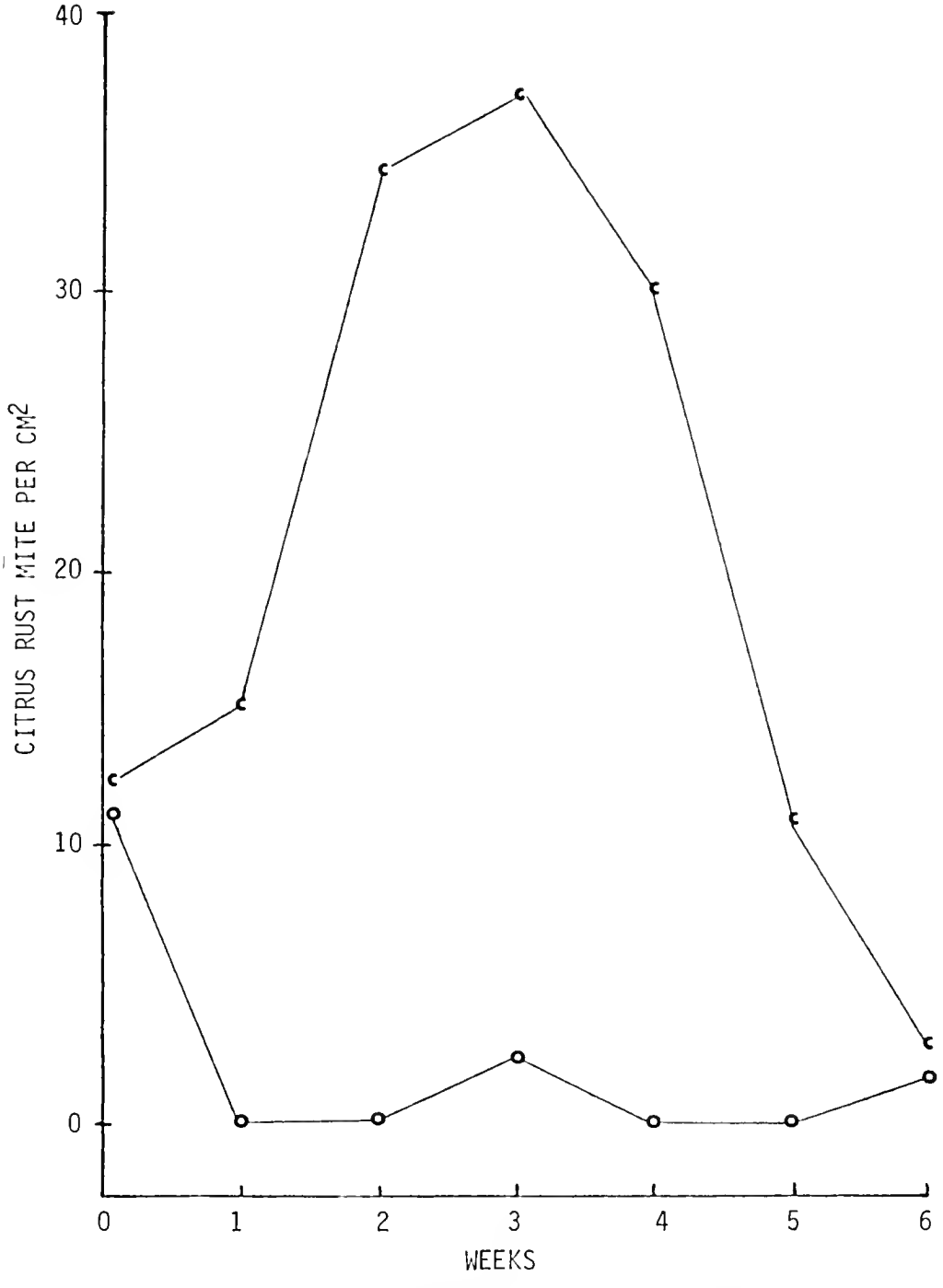


Figure 30. Comparison of control (c) with treatment 6, PP067 at .02% in the 1975 acaricide evaluations.

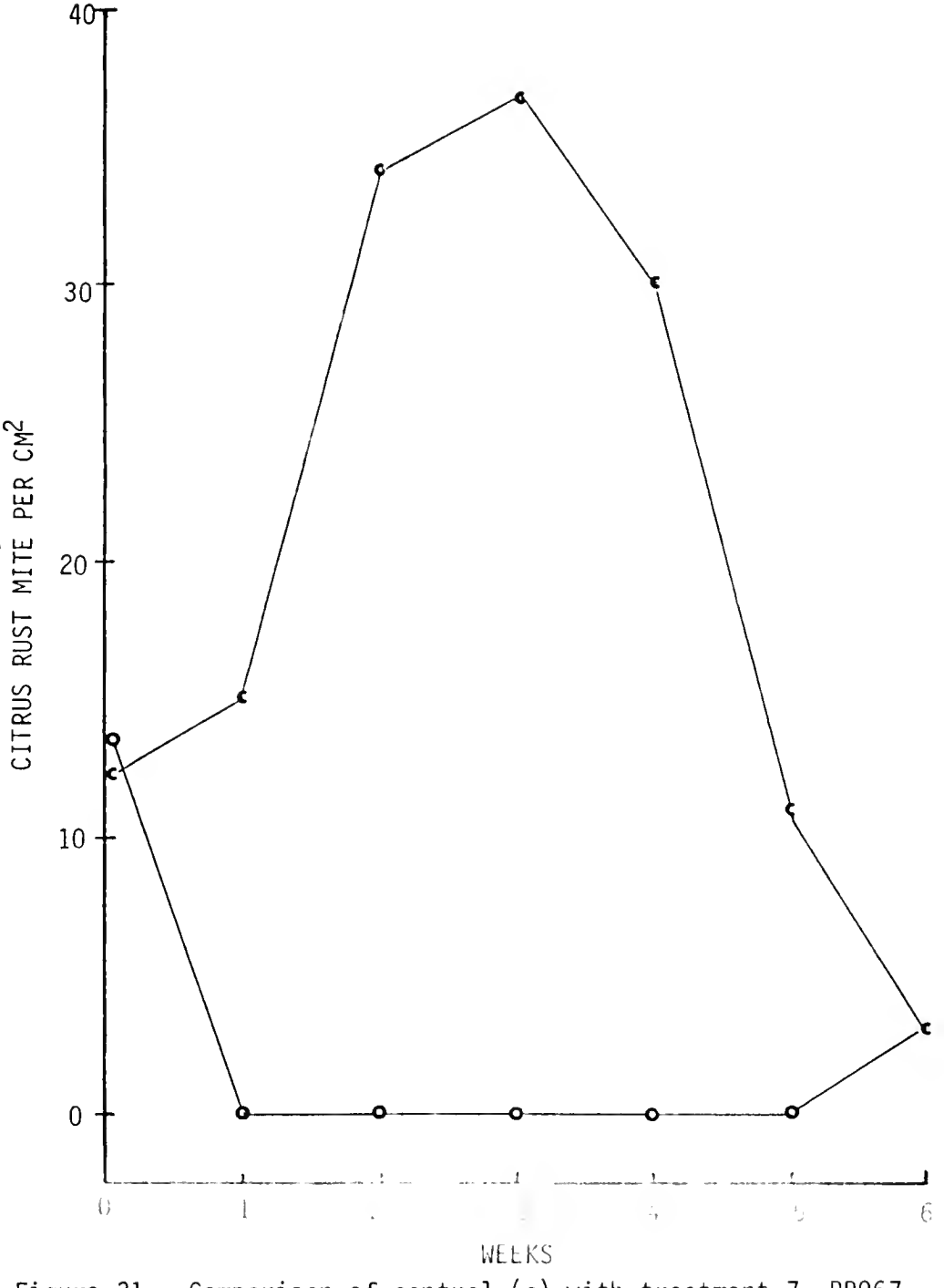


Figure 31. Comparison of control (c) with treatment 7, PP067 at .04% in the 1975 acaricide evaluations.

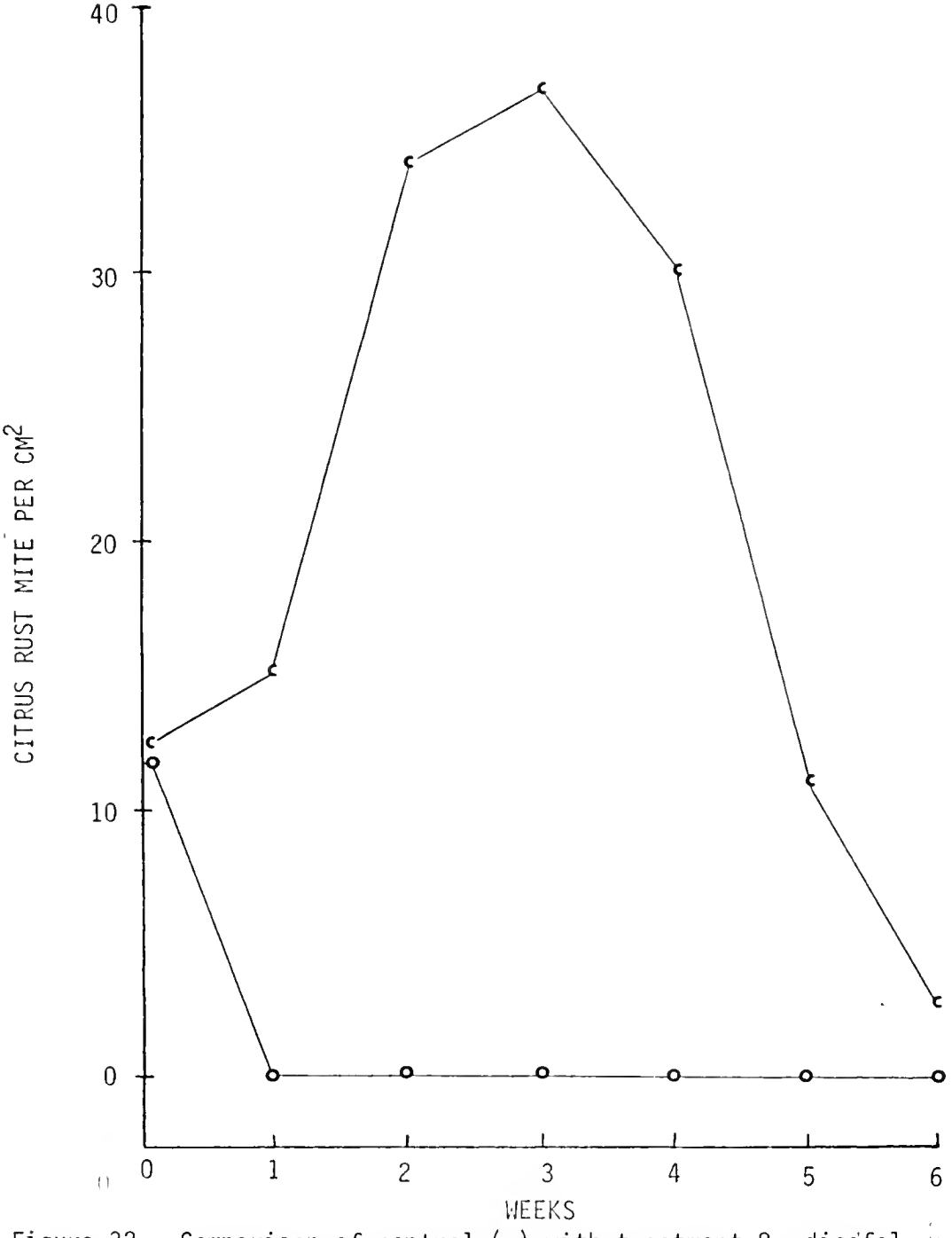


Figure 32. Comparison of control (c) with treatment 8, dicofol at .03% in the 1975 acaricide evaluations.

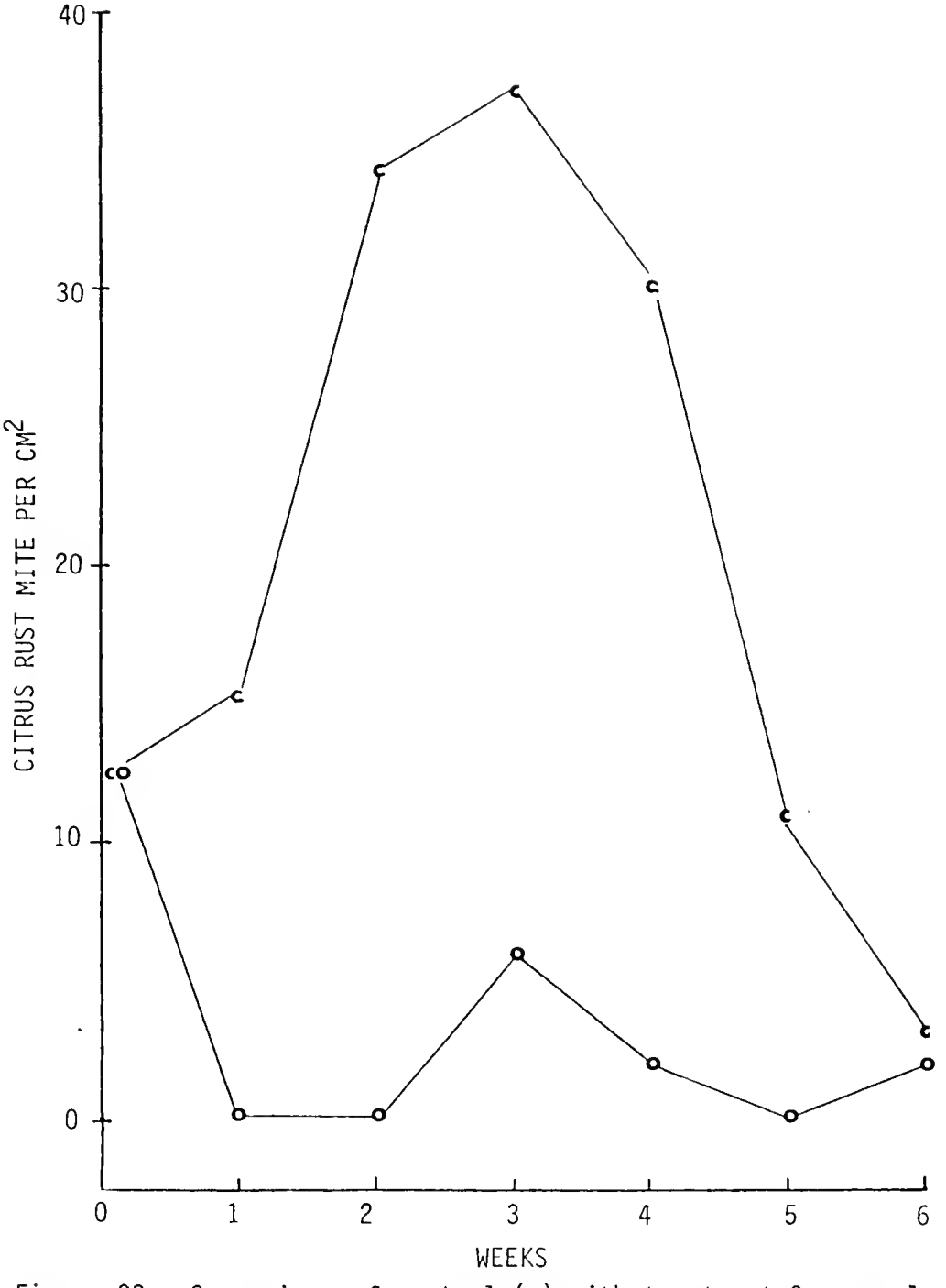


Figure 33. Comparison of control (c) with treatment 9, oxamyl at .001% in the 1975 acaricide evaluations.

Table 24. 1975 Acaricide Test. Sums and Averages for Transformed (Y) and Untransformed (X') Data.

Week	Treatment	(Y) Total Mites	(X') Total Mites	$\overline{Y}$ Average/cm^2	$\overline{X}$ Average/cm^2
0	P	802	643203.50	12.53	10050.05
1	1	16	255.50	.25	3.99
2	1	2	3.50	.03	.05
3	1	10	99.50	.16	1.55
4	1	5	24.50	.08	.38
5	1	0	-.50	.00	-.01
6	1	84	7055.50	1.31	110.24
0	P	700	489999.50	10.93	7656.24
1	2	7	48.50	.11	.75
2	2	0	-.50	.00	-.01
3	2	27	728.50	.42	11.38
4	2	2	3.50	.03	.05
5	2	0	-.50	.00	-.01
6	2	193	37248.50	3.02	582.01
0	P	925	855624.50	14.45	13369.13
1	3	3	8.50	.05	.13
2	3	1	.50	.02	.01
3	3	5	24.50	.08	.38
4	3	0	-.50	.00	-.01
5	3	0	-.50	.00	-.01
6	3	27	728.50	.42	11.38

Table 24. - Continued

Week	Treatment	(Y) Total Mites	(X') Total Mites	$\overline{Y}$ Average/cm^2	$\overline{X}$ Average/cm^2
0	P	850	722499.50	13.28	11289.05
1	4	9	80.50	.14	1.25
2	4	4	15.50	.06	.24
3	4	1	.50	.02	.01
4	4	1	.50	.02	.01
5	4	0	-.50	.00	-.01
6	4	9	80.50	.14	1.25
0	P	765	585224.50	11.95	9144.13
1	5	5	24.50	.08	.38
2	5	25	624.50	.39	9.75
3	5	22	483.50	.34	7.55
4	5	180	3239.50	2.81	50.61
5	5	0	-.50	.00	-.01
6	5	287	82368.50	4.48	1287.00
0	6	725	525624.50	11.33	8212.88
1	6	17	288.50	.27	4.50
2	6	7	48.50	.11	.75
3	6	155	24024.50	2.42	375.38
4	6	8	63.50	.13	.99
5	6	0	-.50	.00	-.01
6	6	112	12543.50	1.75	195.99

Table 24. - Continued

Week	Treatment	(Y) Total Mites	(X') Total Mites	$\overline{Y}$ Average/cm^2	$\overline{X}$ Average/cm^2
0	P	915	837224.50	14.29	13081.63
1	7	9	80.50	.14	1.25
2	7	4	15.50	.06	.24
3	7	7	48.50	.11	.75
4	7	8	63.50	.13	.99
5	7	0	-.50	.00	-.01
6	7	162	26243.50	2.53	410.
0	P	780	608399.50	12.19	9506.24
1	8	60	3599.50	.94	56.24
2	8	3	8.50	.05	.13
3	8	14	195.50	.22	3.05
4	8	1	.50	.02	.01
5	8	0	-.50	.00	-.01
6	8	13	168.50	.20	2.63
0	P	810	656099.50	12.66	10251.55
1	9	22	483.50	.34	7.55
2	9	5	24.50	.08	.38
3	9	423	178928.50	6.61	2795.70
4	9	149	22200.50	2.38	346.88
5	9	0	-.50	.00	-.01
6	9	123	15128.50	1.92	236.38

Table 24. - Continued

Week	Treatment	(Y) Total Mites	(X') Total Mites	$\overline{Y}$ Average/cm^2	$\overline{X}$ Average/cm^2
0	P	915	837224.50	14.29	13081.63
1	10	802	643203.50	12.53	10050.05
2	10	2252	507153.50	35.18	79242.24
3	10	1930	3724899.50	30.15	58201.55
4	10	2005	4020024.50	31.32	62812.80
5	10	752	565503.50	11.75	8835.99
6	10	242	58563.50	3.78	915.05
0	P	715	511224.50	11.17	7987.88
1	11	1188	1411343.50	18.56	22052.24
2	11	2091	4372280.50	33.67	68316.88
3	11	2716	7376655.50	42.43	115260.24
4	11	1821	3316040.50	28.45	51813.13
5	11	617	380688.50	9.64	5948.25
6	11	189	35720.50	2.95	558.13

Table 25. ANOVA for 1975 Acaricide Treatment Data. This was used to test for differences between treatments.

Source	DF	Sum Square	F Value
Block (B)	3	11.68	14.11**
Treatment (T)	8	43.28	19.60**
Week (W)	5	95.06	68.86**
BxW	15	15.12	3.65**
TxB	24	52.24	7.88**
TxW	40	99.63	9.02**
TxBxW	120	118.23	3.57**

** - Highly significant (.01)

Table 26. F Tests for Treatment by Week Interactions for All Six Weeks. Only the 2nd, 3rd and 6th weeks proved significantly different.

Week	Source	D. F.	Sum Squares	F Value
1	Treatment	8	2.10	.68
2	Treatment	8	.82	3.11*
3	Treatment	8	64.97	2.93*
4	Treatment	8	18.25	1.13@
5	Treatment	8	0	99999.99@
6	Treatment	8	56.75	3.76**

* - Significant (.05)

** - Significant (.01)

@ - Believed to be affected by naturally occurring epizootic.

Duncan tests were conducted on the treatments for weeks 2, 3, and 6 (Tables 27, 28, 29, respectively). Results of these tests indicated the (treatment 5) PP067 at .01% was significantly less effective than the other chemicals. At week 2 no other chemical treatment began to lose effectiveness (Figure 34; Table 27).

The Duncan test for week 3 showed oxamyl (treatment 9) as not being significantly different from (treatment 6) PP067 at .02% (Table 28). These two chemicals began losing their effectiveness during the 3rd week. PP067 at .01% ranked very close to oxamyl and PP067 at .02%, but proved statistically better than oxamyl.

During weeks 4 and 5 a naturally occurring population decline suppressed the mite population so that no differences within the treatments were statistically discernable.

For week 6 the Duncan test basically divided the chemicals into three divisions; least, moderate, and best control. Because the treatments were not analyzed as to actual damage to the fruit, it would be unrealistic to designate any of the treatments poor or good, but rather the terms least control and best control were used.

Those chemicals belonging to the least control group for week 6 were PP067 at .01%, PP199 at .01%, and PP067 at .04% level. These chemicals gave the least control of the citrus rust mites (Table 29).

The second group of chemicals in the moderate region were as follows: oxamyl at .001%, PP067 at .02%, and PP199 at .005% level. The Duncan test was not sensitive enough to place these chemicals into the other categories. Because of this ambivalence the author hestitates to recommend these for use.

The final group includes PP199 at .02%, dicofol at .03%, and

Table 27. Duncan's New Multiple-Range Test for 1975 Acaricide Treatments for Week 2.

Grouping	Mean	N^2	Treatment Number	Chemical Name/No.	Percent
A	.8424	64	5	PP067	.01%
B	.7586	64	6	PP067	.02%
B	.7450	64	9	Oxamyl	.001%
B	.7369	64	4	PP199	.04%
B	.7333	64	7	PP067	.04%
B	.7313	64	8	Dicofol	.03%
B	.7232	64	1	PP199	.005%
B	.7151	64	3	PP199	.02%
B	.7071	64	2	PP199	.01%

Table 28. Duncan's New Multiple-Range Test for 1975 Acaricide Treatments for Week 3.

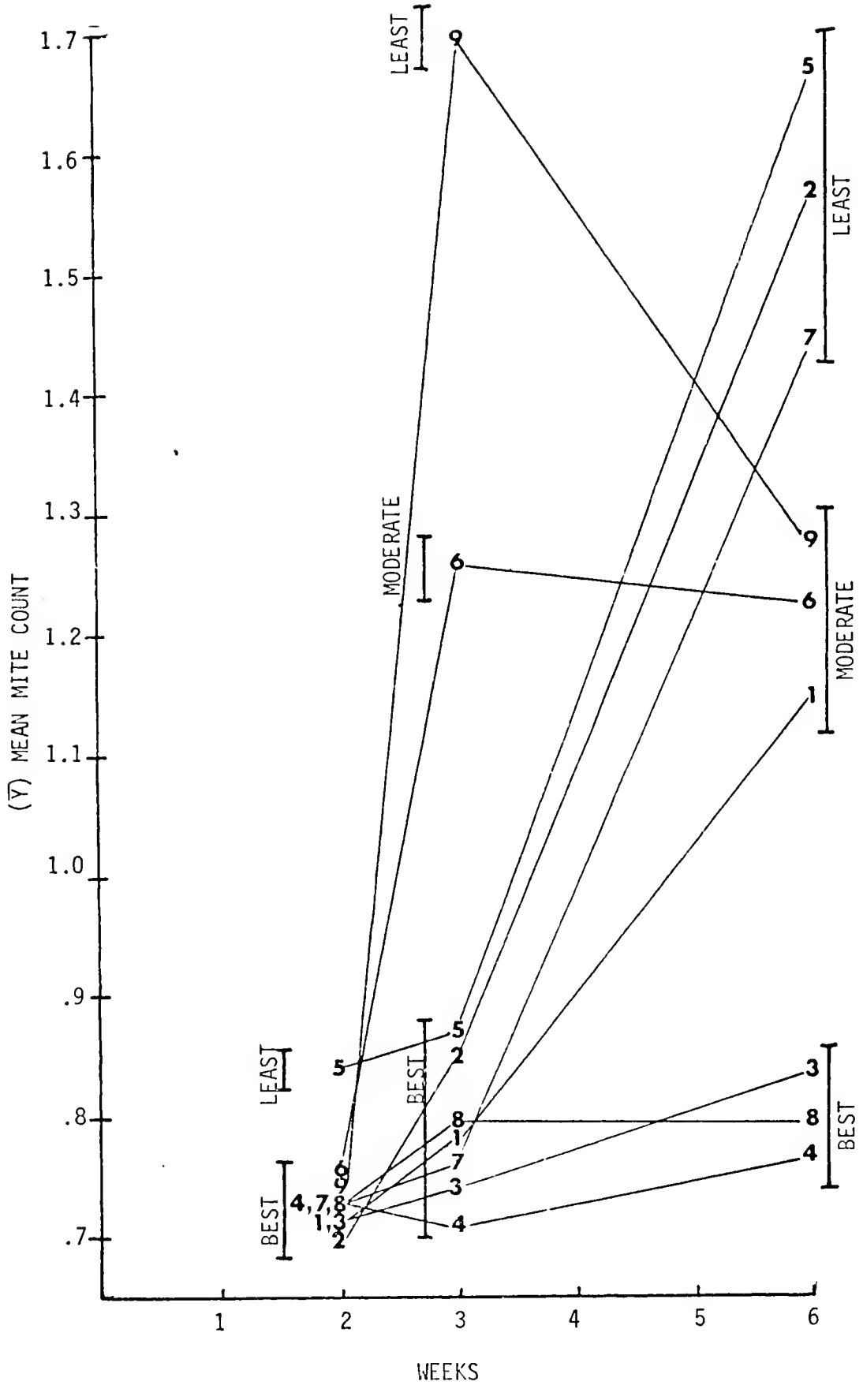
Grouping	Mean	N	Treatment Number	Chemical Name/No.	Percent
A	1.7968	64	9	Oxamyl	.001%
B A	1.2635	64	6	PP067	.02%
B	.8714	64	5	PP067	.01%
B	.8527	64	2	PP199	.01%
B	.7933	64	8	Dicofol	.03%
B	.7829	64	1	PP199	.005%
B	.7637	64	7	PP067	.04%
B	.7450	64	3	PP199	.02%
B	.7151	64	4	PP199	.04%

Table 29. Duncan's New Multiple-Range Test for 1975 Acaricide Treatments for Week 6.

Grouping	Mean	N	Treatment Number	Chemical Name/No.	Percent
A	1.6784	64	5	PP067	.01%
A	1.5608	64	2	PP199	.01%
A	1.4414	64	7	PP067	.04%
B A	1.2864	64	9	Oxamyl	.001%
B A	1.2386	64	6	PP067	.02%
B A	1.1567	64	1	PP199	.005%
B	.8596	64	3	PP199	.02%
B	.7975	64	8	Dicofol	.03%
B	.7748	64	4	PP199	.04%

Figure 34. 1975 Acaricidal tests are graphically displayed and rated as to effectiveness. Weeks 2, 3 and 6 were shown to have statistical differences for the tested treatments. Ratings of best, moderate, and least control were based on a Duncan's analysis of the treatments.

The following chemicals were evaluated:
PP199 at .005% (1), PP199 at .01% (2),
PP199 at .02% (3), PP199 at .04% (4),
PP067 at .01% (5), PP067 at .02% (6),
PP067 at .04% (7), Dicofol at .03% (8),
Oxamyl at .001% (9).


1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
(Y) MEAN MITE COUNT
1
2
3
4
5
6
WEEKS
LEAST
MODERATE
BEST
LEAST
MODERATE
BEST
LEAST
BEST

PP199 at .04% level. These chemicals proved statistically inferior to the first group of compounds, while the test was not sensitive enough to establish differences with the second.

A comparison across the weeks (Figure 34) shows I.C.I.'s product PP067 at .01% as losing its effectiveness as early as during the second week. This control continued to decline until the sixth week when it was found to be the least effective chemical tested. No significant difference could be found for any of the other chemicals during week 2. Dupont's chemical, oxamyl, was significantly less effective in control of the citrus rust mite during week 3 than any of the other chemicals. Oxamyl at the sixth week demonstrated moderate control (Figure 34). PP067 at .02% was a moderate control chemical during weeks 3 and 6. I.C.I.'s products PP199 at .01% and PP067 at .04% both maintained effective control for the first 3 weeks. At week 6, however, both chemicals had lost effectiveness and were rated least effective. PP199 at .005% lost effectiveness only after the third week and became moderately effective. Only three treatments maintained their effectiveness throughout the six week period. The chemicals were PP199 at .02%, dicofol, and PP199 at .04%. No statistical difference could be found between these chemicals for control of the citrus rust mite.

1976 field acaricidal spray test. As in the 1975 evaluations an analysis of variance, ANOVA, for significant differences between the control data and the chemicals proved statistically significant. This significance allowed for further statistical evaluations using only the toxicants which increased the sensitivity of the tests. A Poisson distribution existed so all data was transformed prior to evaluation (Table 30).

Table 30. Average $\overline{Y}$ and $\overline{X}'$ values for 1976 Acaricide trials for each treatment.

Treatment	%	Treatment Number	Number of Observations	$\overline{Y}$ Transformed Mean Mite Count	$\overline{X}'$ Untransformed Mean Mite Count
PP199	.01	1	240	2.2737	3.146
PP199	.02	2	240	1.6674	1.3628
PP199	.03	3	240	1.7034	1.4481
Oxamyl	.001	4	240	2.1431	2.6997
Chlorobenxylate	.01	5	240	3.1054	6.7881
Banex ^{R}	1.6	6	240	3.9773	12.0916
Check	--	7	240	4.8423	18.8633
Water Check	--	8	240	5.9880	30.1181

ANOVA for the untreated control (Table 31) was run to determine if there was a difference between the controls. The analysis determined a significant difference at the .05 level. The two untreated control populations were different, though there were no treatment (T) x week (W) interactions. The lack of a TxW interaction meant that, even though the controls continued to have different population levels, this level was maintained from week to week and did not vary significantly between controls. The paralleling values of the control data were averaged for comparison to the treatments. The averaged control data with each treatment are given to demonstrate the response of each chemical (Figures 35-40).

The chemical standard, chlorobenzylate, was included in the ANOVA of the treatments (Table 32). The blocks (rows) were found to be highly significant. For this reason, blocks and replicates are employed. The treatments were significantly different at the .05 level. This demonstrated the fact that there was a difference between the effects the chemicals had on the rust mite. The treatment x week interaction, TxW, failed to show any significance at the .01 or .05 levels. This demonstrated the test's inability to detect any differences between the treatments as the weeks went on. That is to say, the treatments were different but this difference did not significantly vary from week to week in the 1976 study.

The Duncan's new multiple-range test (Table 33) was tested across the weeks instead of by week (as in 1975) due to the inability to demonstrate any treatments by week interaction. Banex^{R} (Treatment 6) at 1.6% concentration, demonstrated the least control of the products

Table 31. ANOVA for control data in 1976 Acaricide evaluations. The check and water check were evaluated for variations between checks.

Source	Df	Sum Squares	F Value
Block (B)	3	448.06	6.08**
Treatment (T)	1	157.52	14.53@*
Week (W)	5	3639.23	13.61@@**
BxW	15	763.03	2.07**
TxB	3	32.53	.44
TxW	5	224.36	.84@@
TxBxW	15	802.23	2.18**

** - Highly significant (.01 level)

* - Significant

@ - Tested using M.S. for (TxB) as error term

@@ - Tested using M.S. for (TxBxW) as error term

Figure 35. Comparison of the control with treatment 1, PP199 at .01% in the 1976 acaricide evaluations.

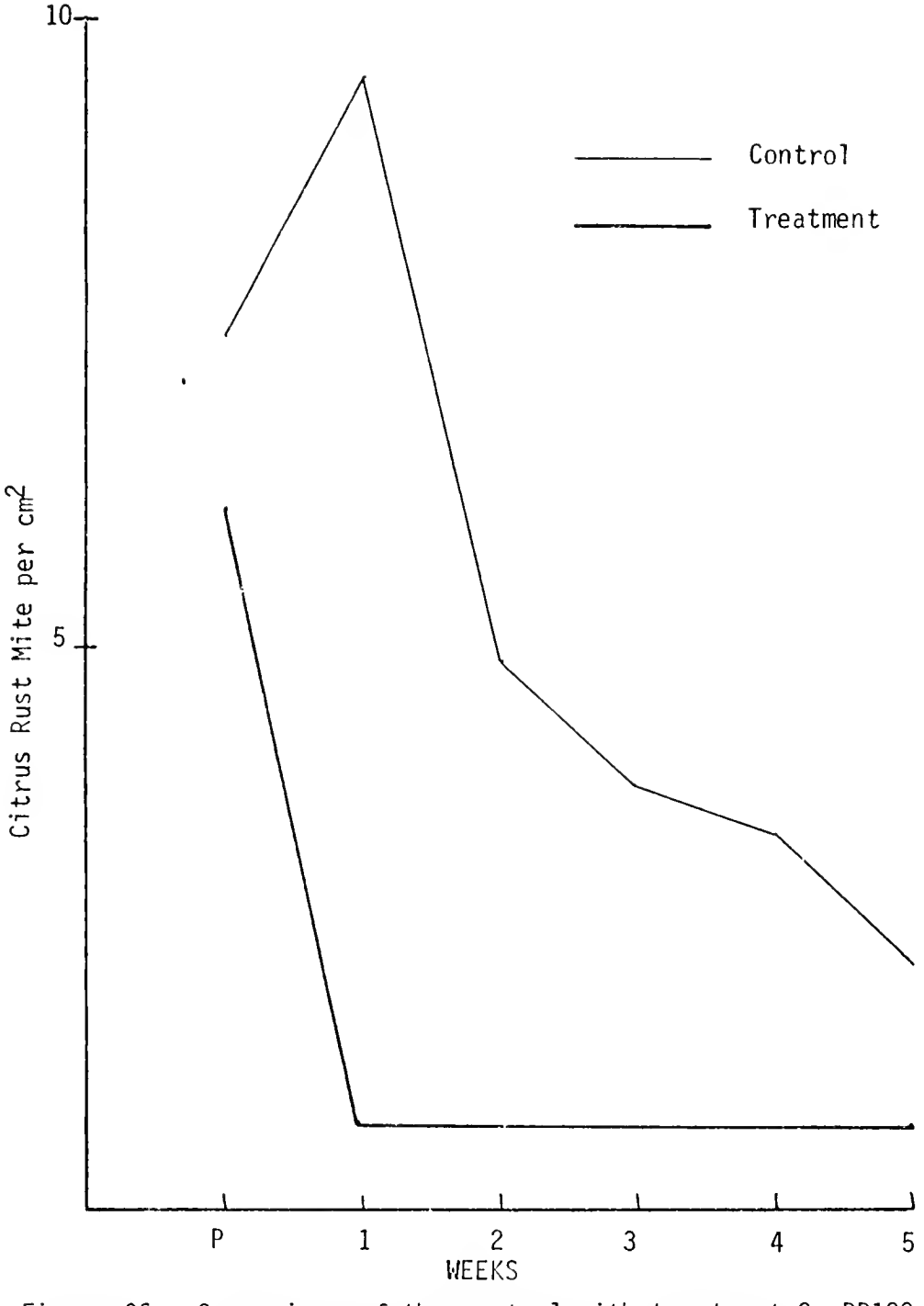


Figure 36. Comparison of the control with treatment 2, PP199 at .02% in the 1976 acaricide evaluations.

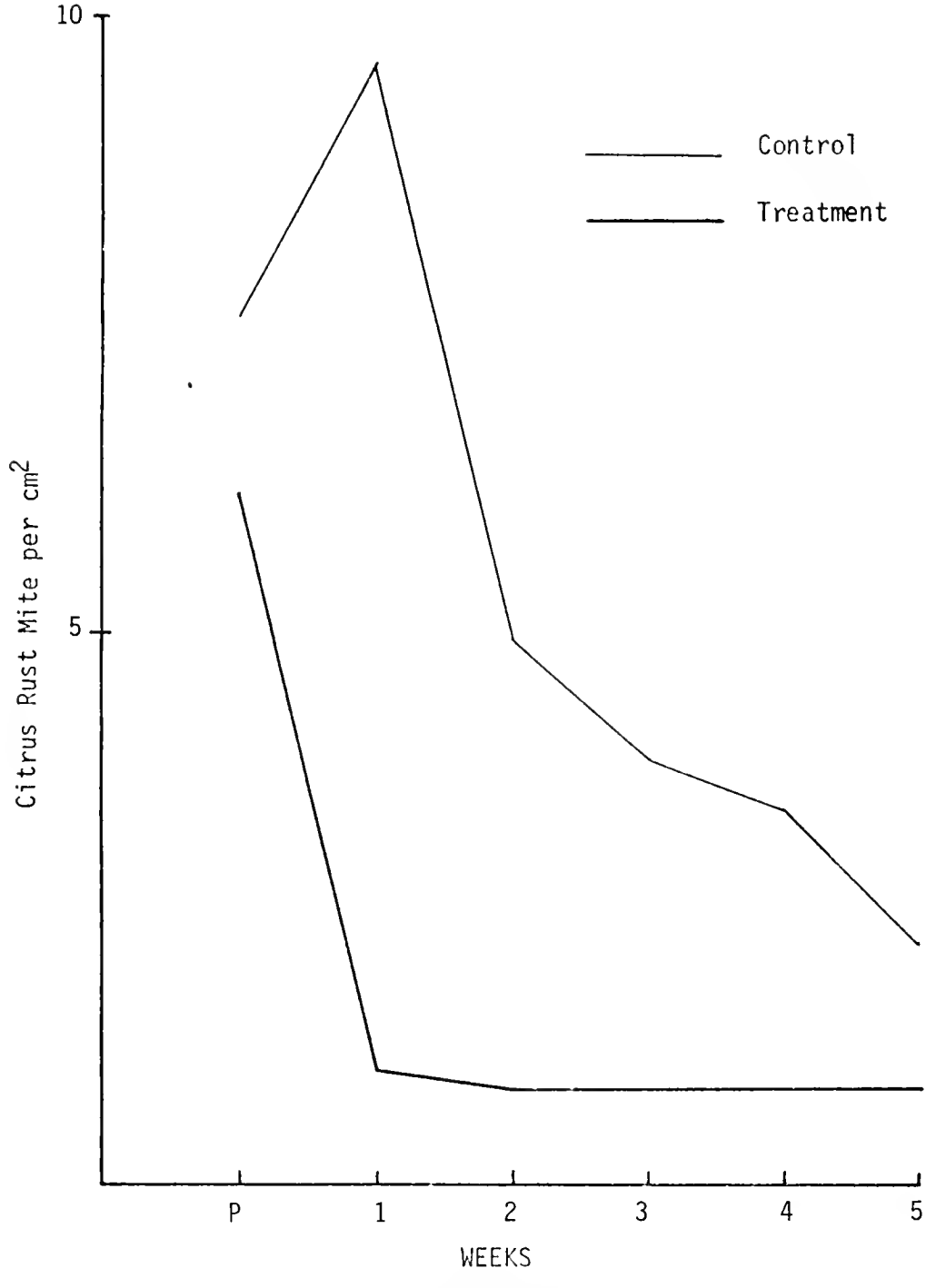


Figure 37. Comparison of the control with treatment 3, PP199 at .03% in the 1976 acaricide evaluations.

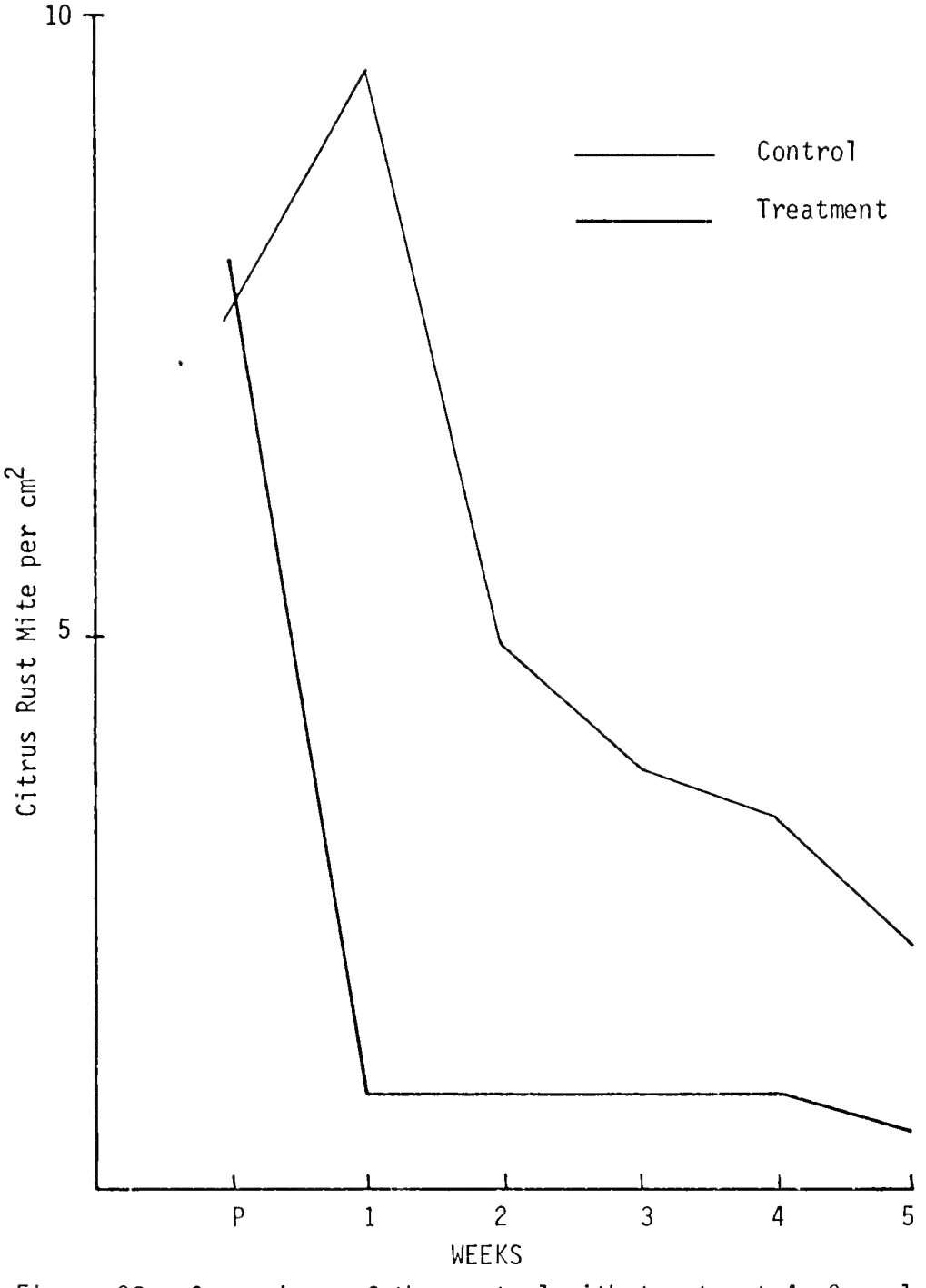


Figure 38. Comparison of the control with treatment 4, Oxamyl at .001% in the 1976 acaricide evaluations.

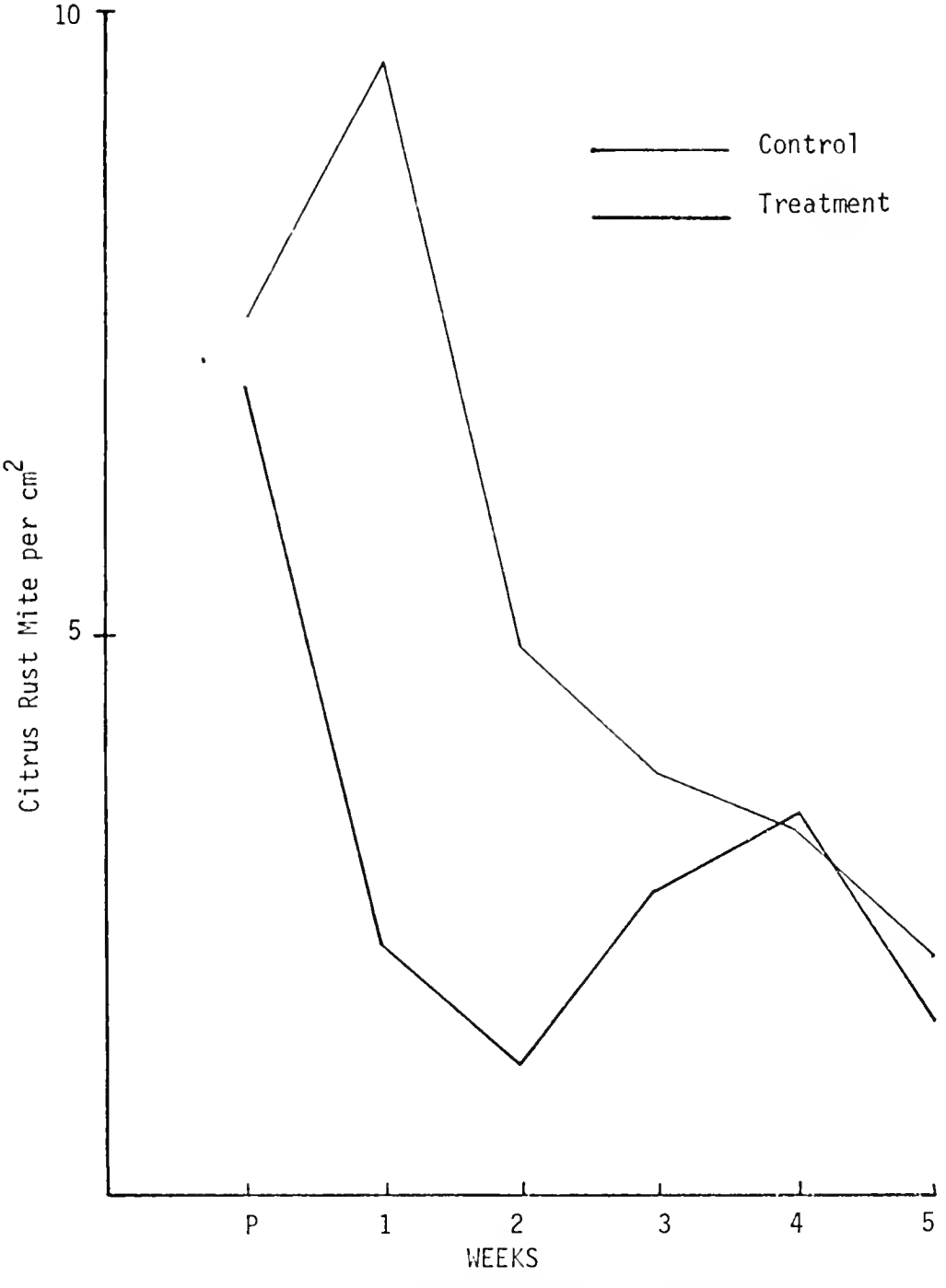


Figure 39. Comparison of the control with treatment 5, Chlorobenzylate at .01% in the 1976 acaricide evaluations.

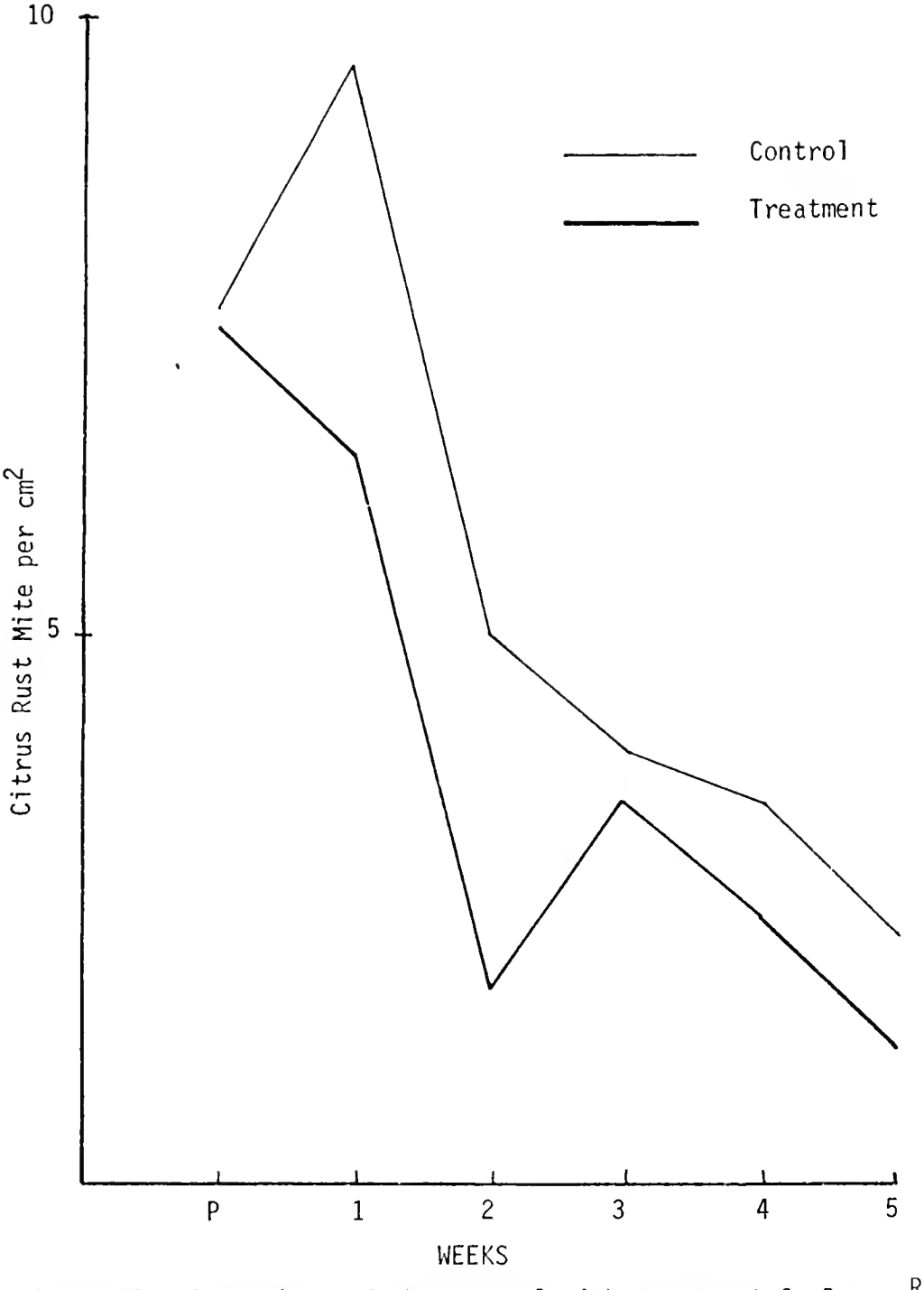


Figure 40. Comparison of the control with treatment 6, Banex R at 1.6% in the 1976 acaricide evaluations.

Table 32. ANOVA for treatment data in 1976 Acaricide evaluations. The treatments were evaluated for differences between the various compounds.

Source	Df	Sum Squares	F Value
Block (B)	3	106.45	3.65**
Treatment (T)	5	971.91	3.47@*
Week (W)	5	7693.10	46.12@@**
BxW	15	2047.38	14.04**
TxB	15	840.86	5.77**
TxW	25	1109.26	1.33@@
TxBxW	73	2435.30	3.43**

** - Highly significant

* - Significant

@ - Tested using M.S. for (TxB) as error term

@@ - Tested using M.S. for (TxBxW) as error term

Table 33. Duncan's new multiple-range test for 1976 Acaricide treatments. The greater the mean ($\bar{Y}$) value the lesser the control.

Grouping	Mean ($\bar{Y}$)	Number of Values	Treatment Number	Treatment	%
A	3.97	240	6	Banex^{R}	1.6
AB	3.11	240	5	Chlorobenzylate	.01
B	2.27	240	1	PP199	.01
B	2.14	240	4	Oxamyl	.001
B	1.70	240	3	PP199	.03
B	1.67	240	2	PP199	.02

tested (Figure 41). Although Banex^{R}, a non-toxic chemical, displayed the least control of rust mites, there was no significant difference between it and the accepted commercial standard chlorobenzylate.

Banex^{R}, also known as Choken, is characterized by A and P Research, Inc. as not acting as a toxicant but rather as an agent that possibly interferes with feeding. Regardless of how the material works, it is of extreme importance to point out that this non-toxic material gave as good control as did chlorobenzylate which is recommended for use for the citrus industry.

Chlorobenzylate at .01% was rated as moderately effective in mite control when compared to those chemicals used in the 1976 evaluations (Figure 41).

PP199 at .01%, .02%,and .03% and oxamyl at .001% were found to be statistically more effective than Banex^{R}. No difference between these chemicals and the standard was statistically discernable. These chemicals were rated as displaying best control (Figure 41).

Comparison of 1975 and 1976 Evaluations

Treatment data. The 1975 field evaluation trial period coincided exactly with the initial mite build-up and its decline (Figures 25-33). During 1976, the field evaluations were started after the initial mite population build-up had begun. It is believed that the 1976 evaula-tions began two weeks later into the population cycle than 1975 (Figures 35-40).

The 1975 epizootic-like mite decline during weeks 4 and 5 made treatment by week interactions undiscernable for this period permitting Duncan's test only on weeks 2, 3,and 6.

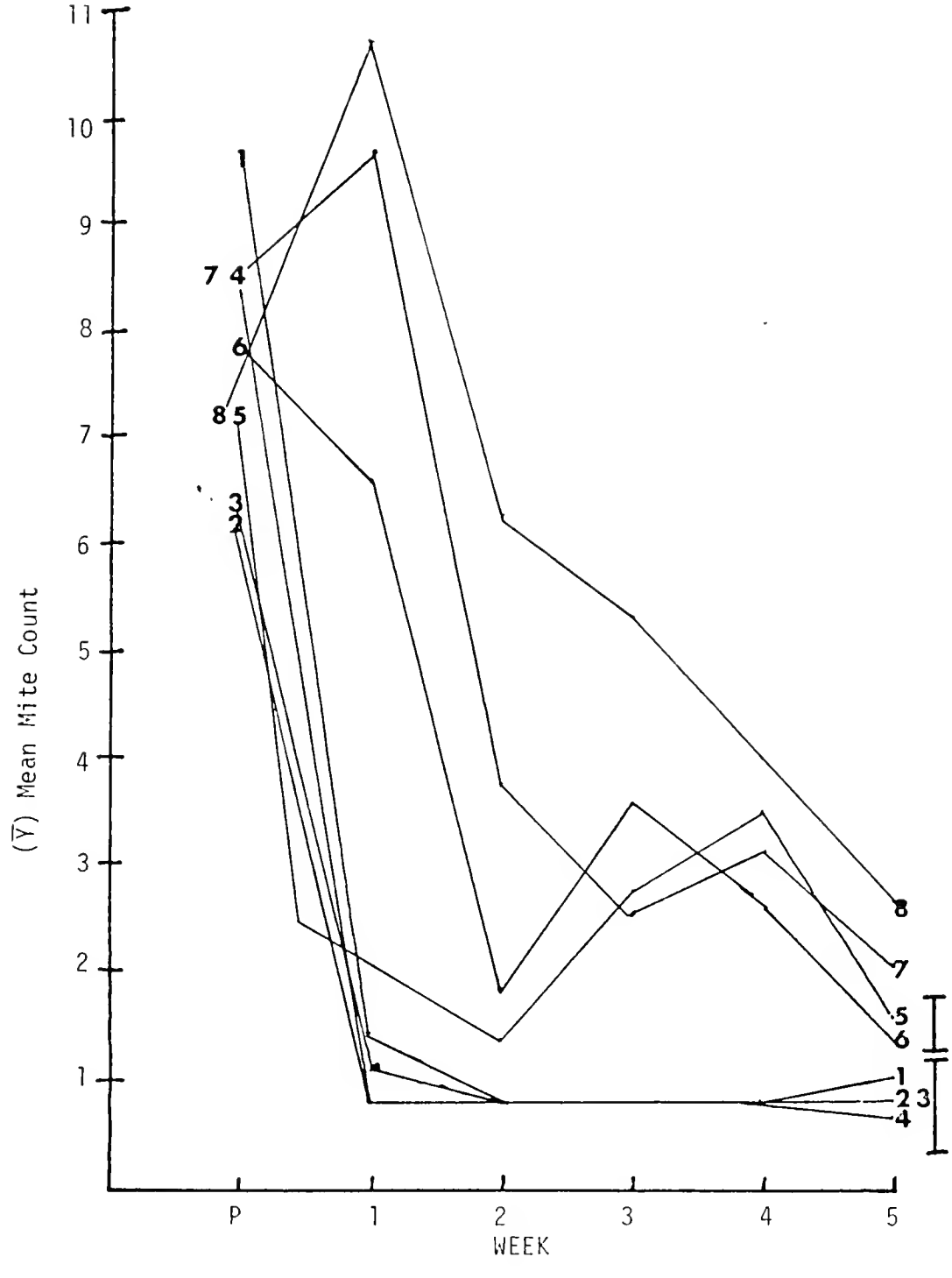


Figure 41. Treatments 1-8. The 1976 chemical evaluation for citrus rust mite control. Treatments 1-4 were evaluated as displaying best control while treatments 5 and 6 gave less control. The following chemicals were evaluated; PP199 at .01% (1), PP199 at .02% (2), PP199 at .04% (3), Oxamyl at .001% (4), Chlorobenzylate at .01% (5), Banex^{R} at 1.6% (6).

The 1976 mite population was in a declining state from week 2 through 5. This could account for the fact that no treatment by week interaction was discernable during the 1976 trials. As with the earlier tests, as the population was declining, the ANOVA could not detect a statistical TxW interaction.

For the aforementioned reasons, the Duncan's new multiple-range test for 1976 data was tested across the weeks instead of weekly as in the 1975 evaluations. This multiple week comparison gave only an overall breakdown as to the chemical's effectiveness.

Chemical standards. Different chemical standards were used for the 1975 and 1976 acaricide tests. Dicofol at .03% was used in 1975 and chlorobenzylate at .01% in 1976. These chemicals are recommended for use on citrus in the 1977 edition of the Florida citrus spray and dust schedule.

SUMMARY

The distribution of D. dormani within a grove at Orange Lake, Florida, was determined to be localized and not dispersed randomly within the grove. Several factors such as canopy density and availability of calcium and water were believed collectively responsible. A decline in the citrus tree snail population from 45 snails/snail tree in 1976 to 3 snails/snail tree in 1977 was attributed to altered cultural practices and severe environmental factors.

Snail activity is confined to periods of 100% relative humidity. Also it is noted that the rate of initiation of snail population activity follows the same rate of increase in relative humidity to 100%. The ability to predict periods of snail activity made it possible to quantify activity from hygrographic records.

The scanning electron microscopic (S.E.M.) inspection of the surface of the citrus fruit confirms that the snail does not damage the fruit surface. In addition, the S.E.M. investigations demonstrated that the citrus tree snail had the ability to suppress the citrus rust mite population. Areas of snail activity, where no feeding occurs, were characterized by a surface coating of mucilage as well as destruction of surface spermatophores.

Grazed areas were completely free of microbiota. All surface material had been removed by the feeding action of the snail.

Snail fecal content studies were conducted to support the scanning electron microscope study which indicated the ingestion of P. oleivora and its spermatophore. The fecal content consisted primarily of mycelia. Other materials such as all stages of P. oleivora and its spermatophore, and Dialeurodes citri Ashm. pupae were identified within the feces. The identification of insects, insect parts, mites, and spermatophores in the feces was the first time the snail had been demonstrated to have ingested any arthropod. The fecal pellets were encrusted and tightly bound so no reinfestation by previously ingested mites or fungi is believed possible.

The determination of the snail feeding potential on citrus rust mite and sooty mold supported the premise that suppression could be attained if the snails were in appropriate concentration. Extrapolations were made from these data demonstrating the need for at least twice the number of snails than were present in the Orange Lake, Florida, grove in 1976.

In reviewing the ability of the citrus tree snail for rust mite suppression, it is doubtful that the snail will become ever again as predominant a biological control agent as it has in the past. The author believes that sufficient data have been presented to warrant the title of biological control when referring to the snails' suppression of the citrus rust mite and certainly sooty mold. An entire cultural practice designed around the snail colony is needed to maintain a healthy, viable snail culture. Such modern techniques as row hedging, clean ground cover, removal of dead wood, and pesticide sprays are all detrimental to the snail colony. The only areas where snail-like culture is actively maintained today is at Leesburg, Florida, at

the Kramer groves. These groves are maintained in an attempt to support these colonies for future use.

Future use of the snail easily could lend itself to the dooryard citriculturist. Here the homeowner could stock a few mature citrus trees with the seeds for a snail colony and practice the necessary cultural measures to maintain the colony. The author believes that dooryard citrus is the only avenue for future snail usage. Only with the total removal of chemical acaricides would there be sufficient interest generated to reactivate use of D. dormani for commercial use.

A citrus rust mite monitoring method was developed using a rubber stamp to identify the field of view as one square centimeter. Fruit diameter, circumference, and volume were used to extrapolate citrus rust mite counts along the equatorial belt of the fruit to entire populations per citrus fruit. These extrapolations corresponded to the alcohol wash technique, indicating that a representative sample of rust mites could be found along the equatorial belt. The distribution of P. oleivora on the equatorial belt of the Valencia orange was determined to be associated with the marginal (semi-shaded) areas, supporting Watson and Berger (1937) who found russet damage corresponding to these areas.

The acaricidal tests of 1975 rated PP199 at .02%, PP199 at .04%, and dicofol at .03% as the best effective chemicals over the six week period for citrus rust mite control. Oxamyl at .001%, PP199 at .005%, and PP067 at .02% were rated as moderately effective chemicals while PP067 at .01, PP199 at .02%, and PP067 at .04% were least effective at 6 weeks post-treatment. From the results of this test,

dicofol and PP199 at .02% would be recommended if available. Dicofol, as a standard, gave excellent control. PP199 will not be used in this country for some years due to acute mammalian toxicity.

The 1976 acaricidal tests again displayed PP199 as an effective acaricide. This time the .01% concentration was statistically indistinguishable from the .02% concentration. PP199 at .01% had a higher mite population and because of lack of control in the 1975 tests would not be recommended. Oxamyl was unstable in its efficacy as a citrus rust mite suppressent. For this reason, further testing would be recommended prior to making a recommendation. In 1976 it showed control equal to or better than chlorobenzylate, a recommended standard.

Banex^{R} consisting of cytex, an extract of seaweed and polybutenes, has an acute oral LD50 34,600 mg./kg. and an acute dermal LD 50 greater than 10,250 mg./kg. of body weight. This chemical was statistically indistinguishable from chlorobenzylate, the standard. Normally it would not be economically justified to develope a chemical which is no better than the standards. Banex^{R} may be the exception to the rule. Here is an extremely safe non-petroleum extracted chemical which has the same potential as chlorobenzylate, which has a much lower LD50 value. This chemical is currently under testing. It is hoped that it will be made available commercially in the future.

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BIOGRAPHICAL SKETCH

Michael Edward Bledsoe was born on February 26, 1951, in Cincinnati, Ohio. He moved to Melbourne, Florida, in 1961. There he attended elementary and junior high and graduated from Melbourne High School in 1969. September 1969 he was awarded an athletic scholarship for basketball at Florida Institute of Technology in Melbourne, Florida. There he earned two varsity crew and four varsity basketball letters. He was captain of the basketball team his junior and senior years. He served as Chief Justice of the Student Court, was a member of Blue Key, and was listed in Who's Who Among Students in American Universities and Colleges. He was a member and officer of the Pi Kappa Alpha Fraternity. In 1973 he received his B.S. in Biological Sciences from Florida Institute of Technology. On August 11, 1973, he married Janette Arlene Barger.

He received his Master's of Science degree from the University of Florida in 1975. He served as Vice President of the Newell Entomological Society in 1976-77.

On March 15, 1977, he took the position of Product Development Specialist with Chevron Chemical Company and is currently living in Atlanta, Georgia.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. D. R. Minnick, Chairman
Associate Professor of Entomology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. C. W. McCoy
Associate Professor of Entomology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. F. A. Johnson
Assistant Professor of Entomology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. L. C. Kuitert
Professor Emeritus of Entomology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. F. W. Zettler
Professor of Plant Pathology

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1977

Jack L. Fry
Dean, College of Agriculture

Dean, Graduate School