Peach Rosette: the Morphology of an Associated Mycoplasmalike Organism and the Chemotherapy of the Disease

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ABSTRACT

Prunus tomentosa was the only cherry host susceptible to peach rosette. Almond (*P. dulcis*) cultivar Jordanolo was not superior to peach (*P. persica*) in symptom expression or duration of incubation period. Nevertheless, it did persist more vigorously as an indicator plant, and appeared to be more resistant to the lethal effect of rosette after dormancy. Mycoplasma-like organisms (MLO's) were found in the sieve elements of rosette-infected leaves of *Vinca rosea, P. persica,* and *P. dulcis,* but not in healthy leaves of those hosts. The dimensions of the pleomorphic organisms from electron micrographs were 80 × 1,000 nm. They were found most abundantly in *V. rosea*. The partial remission (37.5%) of rosette symptoms occurred after treatment with tetracycline hydrochloride and the lesser remission (9.1%) with chlorotetracycline hydrochloride and oxytetracycline dihydrate, support the belief that the MLO is the causal agent of peach rosette. Tetracycline hydrochloride inhibited rosette symptoms four-to-five times more effectively than the other compounds. Remission occurred when the chemicals were injected under the bark or into the wood, but not when sprayed on the foliage.

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Additional key words: phloem, host-range, tetracyclines.

Peach rosette, until now thought to be caused by a virus, has caused serious losses in peach orchards of the southeastern United States. Peach trees seldom survive more than one season after becoming infected and generally are self-eradicating. Natural spread is presumed to result from transmission by unknown vectors from infected wild plums. Wild plum stands that were once eradicated around peach-growing areas have developed and flourished since 1967, when the eradication program to control the spread of phony peach disease was suspended.

In the expectation of a higher incidence of peach rosette in the southeast, we further studied the problem. This paper reports a mycoplasma-like organism (MLO) associated with peach rosette, extends the host range, details the search for better indicators, and reports the results of chemotherapy.

MATERIALS AND METHODS.—Field-test trees of peach [*Prunus persica* (L.) Batsch 'Elberta'] in the second or third leaf were grafted with rosette-diseased buds about 1 m above ground on two of three scaffold limbs. Also, potted test trees of *Prunus* spp. in the greenhouse were grafted 15-25 cm above the soil line. Two or three inoculum buds were used as either T-buds or patch buds, depending on the condition of the bark of test trees. None of the natural sources of rosette have been free of ringspot; however, symptoms of ringspot have been mild to nonexistent. The rosette source used for inoculum in the host-range tests had shown little or no symptoms in serial passages through peach and almond. Seed of dodder, *Cuscuta campestris* Yunck, and *C. subinclusa* Dur. & Hilg. were germinated and established on Turkish tobacco (*Nicotiana tabacum* L.). Cuttings of the dodder from established sources were re-established on rosette inoculum and allowed to parasitize test plants for 3-5 months. All the test plants were indexed back by bud grafting to peach. Potted trees that had set terminal buds were stored for 800 hours at 0-5 C to break dormancy.

Rosette-infected and healthy leaves of almond [*Prunus dulcis* (Mill.) D. A. Webb], peach, and *Vinca rosea* L. were used in electron microscopy studies. Parts of midveins were excised from the leaves, submerged in glutaraldehyde solution, and cut into segments 1-2 mm thick. The tissues were further prepared for electron microscopy as described previously (6).

The chemotherapy studies were conducted with seedlings of peach cultivar Elberta and almond cultivar Nonpareil grown in the greenhouse in 20-cm diameter clay pots. The plants were in their first leaf and had a trunk caliper of 1 cm or more when inoculated. Bud inoculations were made from a common rosette source in peach. The chemotherapy trials were started after basal sprouts 10-15 cm long with symptoms typical for rosette had appeared. The rest of the test plants appeared normal. A total of 500 μg/ml tetracycline hydrochloride, chlorotetracycline hydrochloride, or oxytetracycline dihydrate was applied to each treated tree. For the spray applications, 10-ml volumes were sprayed on foliage with a fine mist sprayer: five sprays of 100 μg/ml weekly; two sprays of 250 μg/ml in a 14-day interval; and a single spray of 500 μg/ml. Injections of 10 ml of 500 μg/ml were made either under the bark or into the center of the trunk 10-15 cm above the groundline. The solutions were allowed to flow, until they drained through hypodermic needles into the tissue from an open plastic reservoir 10 cm above the injection site.

The sprayed trees were kept in the greenhouse until dormant, placed at 0-5 C for 800 hours, and returned to the greenhouse for observation. Injected trees were planted in the field 30 days after treatment.

RESULTS.—*Host range studies.*—Peach seedlings of cultivars Elberta, Lovell, Rutgers Red, and Nemaguard expressed typical rosette symptoms within 4-11 months after inoculation (Table 1). The symptoms were expressed first as sprouts with shortened internodes and small densely-rosetted leaves that developed below the site of inoculation. In the field, the first indication of infection in inoculated peach trees was the prolific development of basal sprouts with rosetted leaves below the inoculation site. Field trees did not develop rosette above the

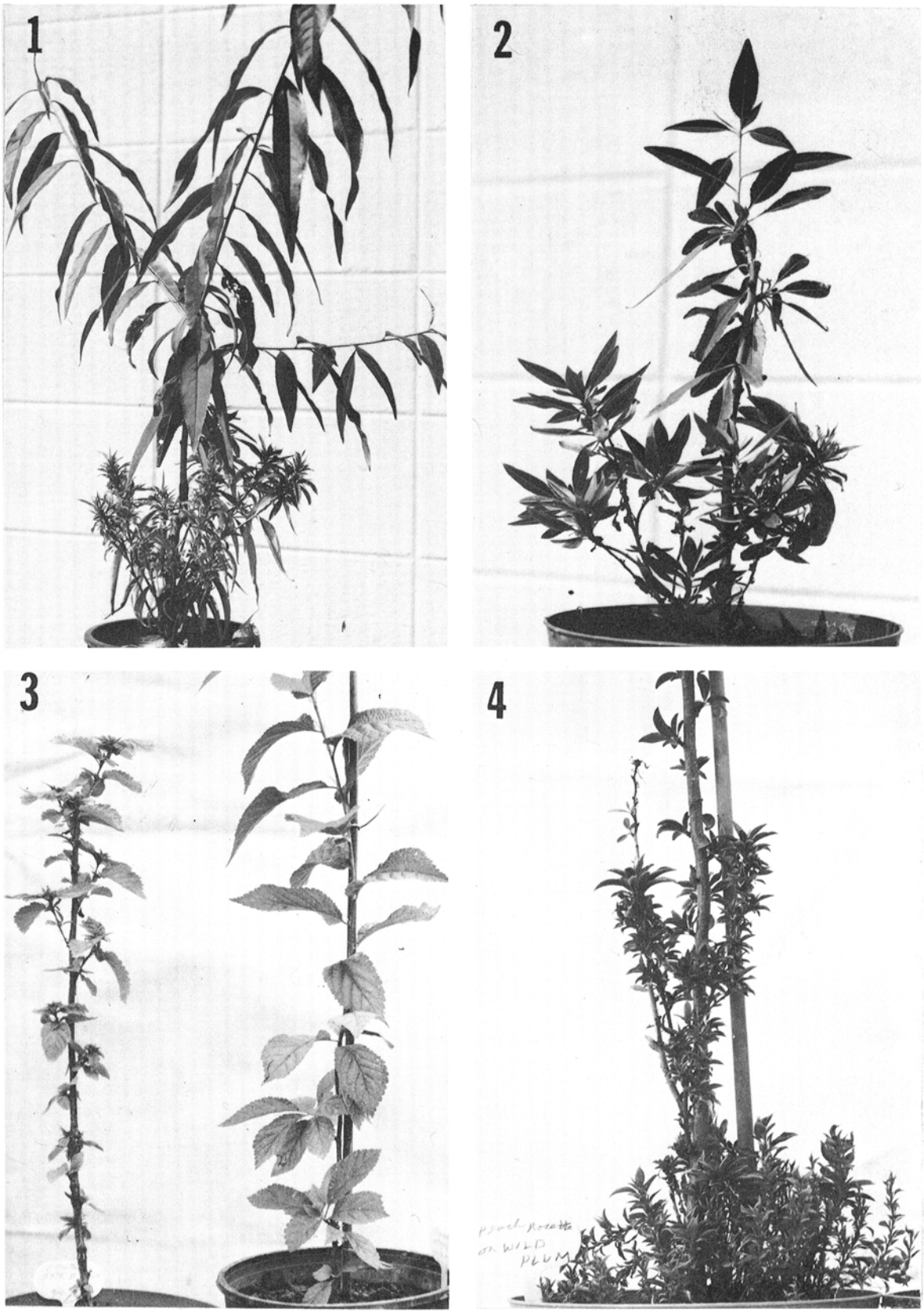


Fig. 1-4. Seedlings of various *Prunus* spp. expressing peach rosette symptoms after inoculation with diseased buds: **1)** Elberta peach; **2)** Jordanolo almond; **3)** Manchu cherry with a healthy control; and **4)** wild plum.

TABLE 1. Transmission of peach rosette by budding to various species of greenhouse-grown *Prunus*

Test plants	Inoculated (no.)	Transmitted (no.)	Observed time of transmission (months)	Index to peach
Peach seedlings				
Elberta	37	27	4	+
Rutgers Red	2	2	4	+
Lovell	10	5	7	+
Nemaguard	4	3	11	+
Plum				
Shiro	2	2	5	+
P. angustifolia Marsh.	3	1	10	+
Almond				
Jordanolo	13	7	5	+
Jordanolo	48	...	7	+
Jordanolo	6	3	8	+
Cherry				
Mahaleb seedling	10	0	15-17	[a]
Manchu cherry seedling	4	3	9	+
Montmorency	6	0	12-17	[a]
P. serotina Ehrh seedling	10	0	12	[a]
Kwanzan	10	0	12	−
Shirofugen	10	0[b]	−	−

[a] + = rosette transmission to index host; indexed 30 May 1973 and 9 July 1973, and to be indexed again.
[b] Inoculum sources of rosette that have been tested have also contained ringspot, which killed the trees early.

inoculation site until the year after inoculation. Trees of hybrid plum (cultivar Shiro) and seedlings of native plum (*P. angustifolia* Marsh.) and Jordanolo almond also developed the rosetted basal sprouts (Fig. 1, 2, 4). In the greenhouse, these hosts lost the leaves above the inoculation site, except for a continuous tuft of relatively normal leaves at the shoot terminals. Symptoms were well-developed within 4-8 months in most peach cultivars, in Shiro plum, and in Jordanolo almond, but were slower to develop in Manchu cherry, wild plum, and Nemaguard peach seedlings (Table 1).

'Manchu' cherry was the only near relative of the cherry that developed rosette symptoms or that indicated infection when indexed back to peach (Fig. 3).

None of the indicator plants proved superior to peach in expression of symptoms or duration of incubation period. However, Jordanolo almond was adopted as a host for the stock culture of the peach rosette agent for our study. This cultivar is more tolerant to infection and is fairly compatible when grafted to other species of *Prunus*. Also, it is more resistant than peach to death from rosette after dormancy (Table 2).

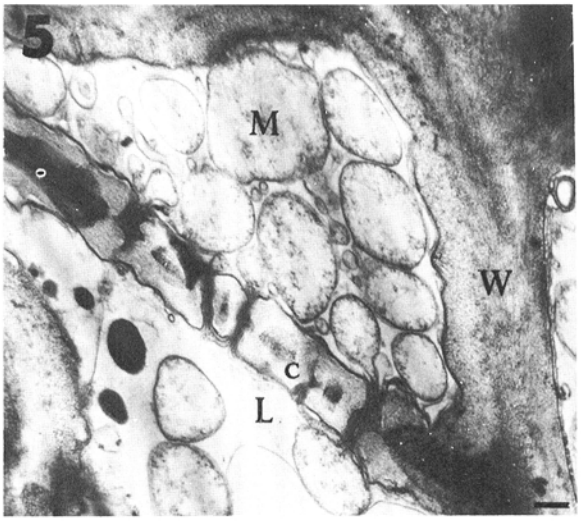
After 5 months, the infectious agent of peach rosette was transmitted from peach to three of four plants of *Vinca rosea* through *Cuscuta campestris,* but not through *C. subinclusa.*

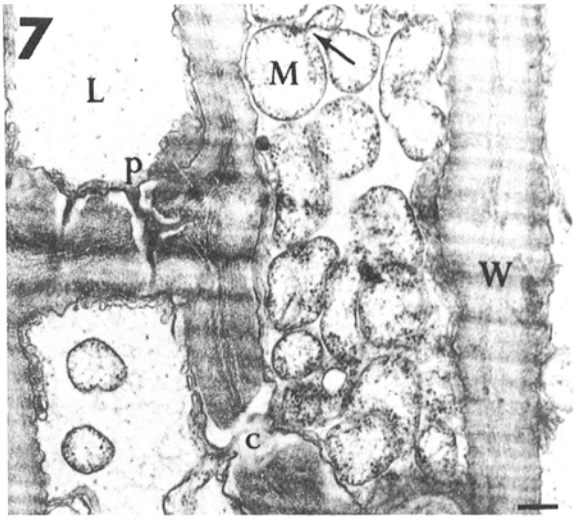
Electron microscopy.—MLO's were found only in the sieve elements of phloem of leaves of the diseased *V. rosea,* peach, and almond, but not in the healthy leaves. We can only presume the causal relationship of MLO's to the disease; definitive proof must await the completion of Koch's postulates.

The pleomorphic organisms which were found had the typical characteristics described for mycoplasmas: lack of rigid cell wall, presence of a unit membrane, peripheral distribution of ribosome-like particles, and a DNA-like network in the central region (4, 8, 11). We observed morphological variants which corresponded to previous reports of elementary bodies, and also filamentous and ovoid forms (12) that measured 80 × 1,000 nm in diameter or length (Fig. 5, 7).

The greatest number of MLO's was observed in *V. rosea,* followed by peach and almond. Sieve elements were often seen filled with them.

Fig. 5-9. Electron micrographs showing pleomorphic mycoplasmalike organisms (MLO) in the sieve elements of mid-vein of leaves affected with peach rosette disease. Scale bars = 0.2 μm. All views are in cross-sections: **5)** *Vinca rosea.* Host cell filled with three types of MLO: elementary bodies, filamentous forms, and ovoid forms. ×20,064. **6)** *Vinca rosea.* Part of a lateral sieve area in high magnification: a constricted organism appears to be passing through a pore. The electron-lucent body near the pore (arrow) might be a part of the filamentous organism entering from the opposite side. ×64,748. **7)** *Prunus persica.* Host cell filled with mycoplasma-like organisms (MLO's). One MLO (arrow) appeared to be budding. ×25,536. **8)** *Prunus persica.* Part of a lateral sieve area with MLO passing through the pores. Electron-dense parts of MLO's congregate on one side of the sieve area and electron-lucent parts on the opposite; an ultrathin filament (arrow) connects through the pore. ×38,400. **9)** *Prunus persica.* Simultaneous passage of a pair of MLO's through pores of a lateral sieve area. Note ultrathin filament in each pore. ×47,424. Legend: c = callose; L = lumen of sieve element; M = MLO; p = pore; Pp = P-protein; W = cell wall. Scale bar = 0.2 μm.


5
M
W
c
L


7
L
M
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W
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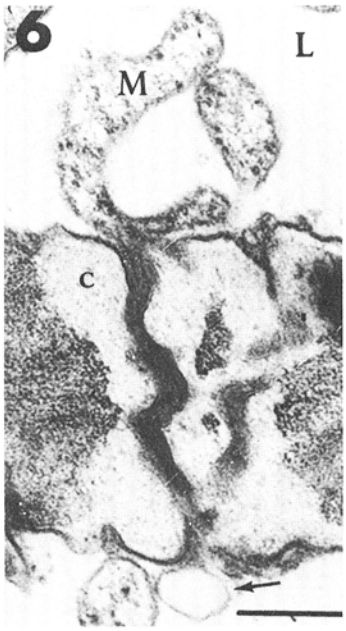
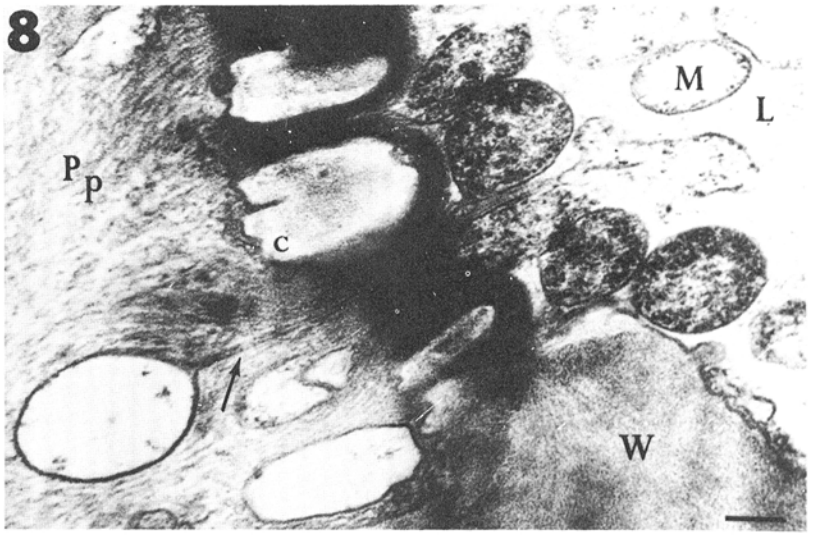

6
L
M
c


8
M
L
P
p
c
W


9
L
M
P
W

TABLE 2. Response of rosette-affected peach and almond seedlings to tetracycline-HCl (T), chlorotetracycline HCl (C), and oxytetracycline dihydrate (O) applied by foliar spray and trunk injection

Chemical	10 ml Applications (no. × μg/ml)	Test plant	Test plants (no.) Total	Dead[a]	Survivors with rosette	Remission fraction[b]	Remission (%)	Remission for each chemical[c] (avg %)
Foliar spray (greenhouse)								
T	5 × 100	Peach	5	3	2	0/2	0	
	2 × 250	Peach	5	3	2	0/2	0	
	1 × 500	Peach	5	4	1	0/1	0	
	1 × 500	Almond	2	0	2	0/2	0	T = 0(0/7)
C	5 × 100	Peach	5	5	0	...	...	
	2 × 250	Peach	5	4	1	0/1	0	
	1 × 500	Peach	5	4	1	0/1	0	
	1 × 500	Almond	2	0	2	0/2	0	C = 0(0/4)
O	5 × 100	Peach	5	2	3	0/3	0	
	2 × 250	Peach	5	4	1	0/1	0	
	1 × 500	Peach	5	3	2	0/2	0	
	1 × 500	Almond	2	1	1	0/1	0	O = 0(0/7)
Control	(no treatment)	Peach	5	3	2	0/2	0	
Trunk injection into bark (b) or wood (w) (field)								
T	1 × 500	Peach (b)	5	1	1	3/4	75	
		Peach (w)	5	1	3	1/4	25	
		Almond (b)	5	2	3	0/3	0	
		Almond (w)	5	0	3	2/5	40	T = 37.5(6/16)
C	1 × 500	Peach (b)	5	1	4	0/4	0	
		Peach (w)	5	2	3	0/3	0	
		Almond (b)	5	2	2	1/3	33	
		Almond (w)	5	4	1	0/1	0	C = 9.1(1/11)
O	1 × 500	Peach (b)	5	1	3	1/4	25	
		Peach (w)	5	1	4	0/4	0	
		Almond (b)	5	4	1	0/1	0	
		Almond (w)	5	3	2	0/2	0	O = 9.1(1/11)
Control	(no treatment)	Peach	5	5	0	...	...	
		Almond	6	4	2	0/2	0	

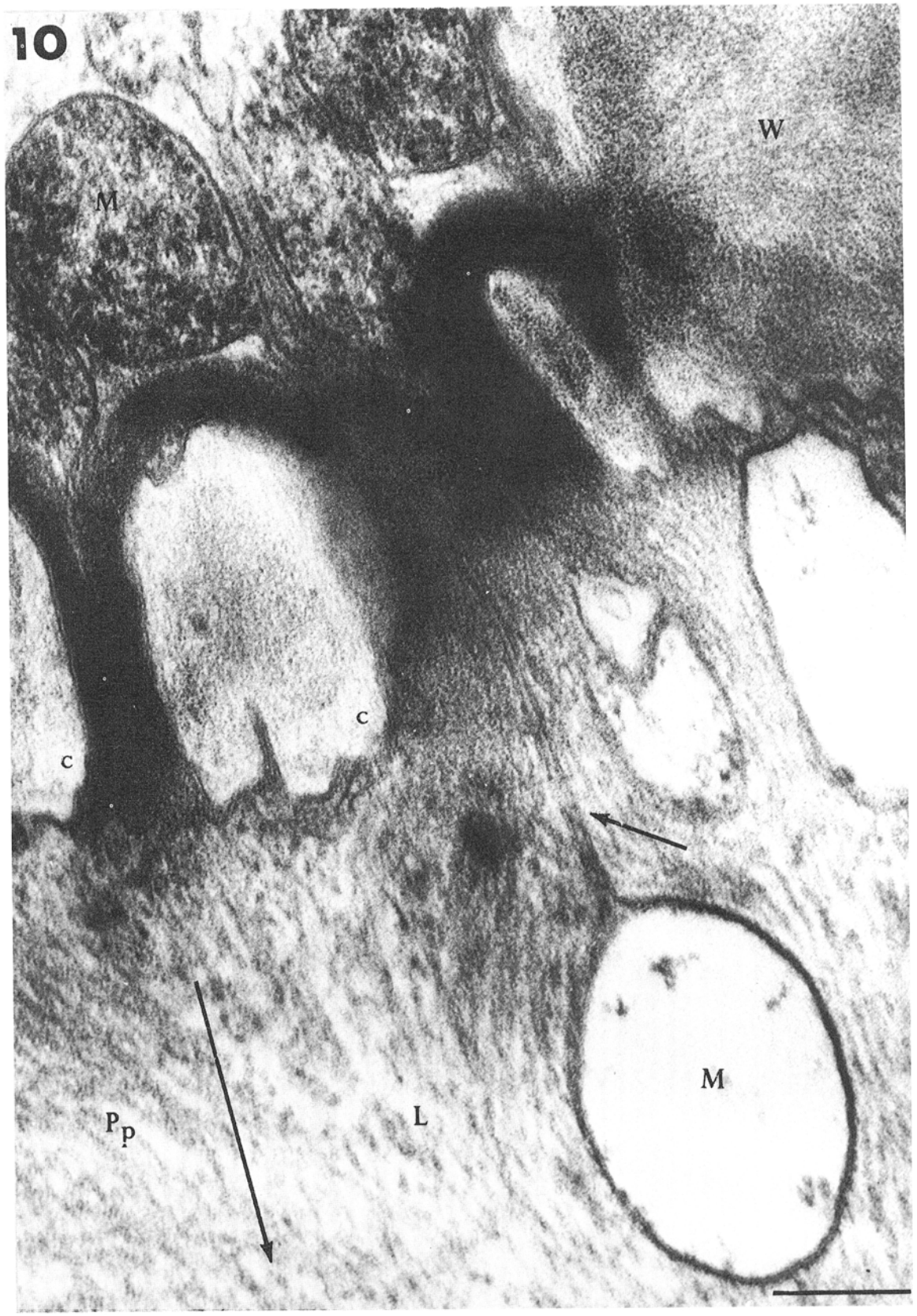
[a]Test plants dead after dormancy: almond, 43%; peach, 55%.
[b]Number of plants with remission/total surviving plants.
[c]Overall average of T, C, and O: bark injection, 22.2%; wood, 10.9%; peach, 21%; almond, 12%.

Chemotherapy.—Trees sprayed with tetracycline hydrochloride (T), chlorotetracycline hydrochloride (C), and oxytetracycline dihydrate (O) were all living and showing rosette symptoms when put into cold storage to break dormancy. The field-planted trees injected with the chemicals were also living but showed some leaf scorch, both in the terminals of the trees and in the areas of the injections. Phytotoxicity was observed most often with T and C and least with O.

There was no remission of symptoms from spray applications with T, C, or O (Table 2). However, basal trunk injections of these antibiotics caused some remission in both peach (21%) and almond (12%). Four of eight peach and two of eight almond trees treated with T, or 37.5% showed no rosette symptoms after dormancy. In contrast, all untreated controls either were dead or showed systemic symptoms. Treatments C and O induced only 9.1% remission of symptoms.

DISCUSSION.—The development of basal rosetted sprouts as the first symptom after bud inoculation was noted in our studies. KenKnight (9) reported the same result when he removed bark rings at various time intervals after inoculation to show movement of the causal agent in the phloem. The combination of the electron microscopic evidence of MLO's in the phloem and the partial remission of symptoms due to antibiotics, support the presumption that the MLO were responsible for the rosette symptoms.

Fig. 10. *Prunus persica.* Highly magnified view of Fig. 8. A number of MLO's appear to enter one pore, one unit membrane is triple-layered (upper left), and a stretched ultrathin filament appears to come through the pore (arrow). The long arrow indicates the direction in which the MLO might have moved. ×96,110.


10
W
M
C
C
M
L
Pp

We noted in electron microscopy studies, as have others (7), that when an MLO appeared to be passing through a sieve pore, one end of the MLO had more electron-dense ribosome-like particles than the opposite end in the adjoining cell (Fig. 6, 8, 9). We suggest that the direction of movement of the body with its cellular contents is from the electron-dense end to the electron-lucent end for two reasons: (i) The elongated ultra-thin filament of the unit membrane suggests stretching with the flow pattern in the sieve elements (Fig. 8, 10); (ii) fluid molecules in the MLO migrated through the partly plugged sieve pore first, leaving behind particulate matter too large to pass readily. The configurations of the MLO's at the sieve tube area suggest a force exerting pressure in one direction in the phloem at that moment. The arrangement of the P-protein mass also suggests movement in the same direction. Moreover, the unit membrane of the MLO is clearly extremely pliable since it can constrict to about 27 nm in diameter, or three times the thickness of its unit membrane. This ability would enable MLO's to pass through small pores, but if masses of MLO's accumulate at the pores, conceivably they could close or partly close the sieve areas (Fig. 8 or 10).

The tiny electron-lucent body situated immediately at the pore (Fig. 6, lower center) is thought to be a part of the filamentous form entering the pore from the adjoining lumen. In Fig. 8 and 10, some jamming is evident at the pores, due to simultaneous entry of at least three MLO's and P-proteins (3) at the one pore. The extreme constriction of the unit membrane and the force of the assimilate stream could account for the stretching of the fine filament seen in Fig. 8 and 10.

The aster yellows agent was passed through a millipore filter with 0.45-μm diameter pores (1); Staphylococcal L-forms passed through millipore filters with 0.05-μm pores (10). The width of the stretched filament of the organism shown in sieve pores in Fig. 8 and 10 was about three times the thickness of the unit membrane, or 27 nm. The average pore diameter in sieve plates was 1.5-2.5 μm, and that of the lateral sieve areas was about 0.5 μm in dicotyledons (5). Callose deposition around the pores, presence of slime or filamentous reticulum (2, 3) in the lumina of the sieve elements, and the number of MLO's (Fig. 8 or 10) could influence the functional size of the pores and thus affect the ease of movement of MLO's and of assimilate streams in the phloem. Such plugging in the phloem could be responsible for some of the symptoms described for "yellows" disorder.

LITERATURE CITED

1. COHEN, R., R. PURCELL, and R. L. STEERE. 1969. Ultrafiltration of the aster yellows agent. Phytopathology 59:1555 (Abstr.).
2. CRAFT, A. S., and C. E. CRISP. 1971. Phloem transport in plants. W. H. Freeman, San Francisco. 48 p.
3. CRONSHAW, J., and K. ESAU. 1967. Tubular and fibrillar components of mature and differentiating sieve elements. J. Cell Biol. 34:801-816.
4. DAVIS, R. E., and R. F. WHITCOMB. 1971. Mycoplasma, rickettsiae and chlamydiae: possible relation to yellows diseases and other disorders of plants and insects. Annu. Rev. Phytopathol. 9:119-154.
5. ESAU, K., and V. I. CHEADLE. 1959. Size of pores and their contents in sieve elements of dicotyledon. Proc. Nat. Acad. Sci., U.S.A. 45:156-162.
6. GOHEEN, A. C., G. NYLAND, and S. K. LOWE. 1973. Association of a rickettsialike organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63:341-345.
7. HIRUKI, C., and P. SHUKLA. 1973. Mycoplasmalike bodies associated with witches'-broom of bleeding heart. Phytopathology 63:88-92.
8. HULL, R. 1971. Mycoplasma-like organisms in plants. Rev. Plant Pathol. 50:121-130.
9. KENKNIGHT, G. 1957. Translocation of peach rosette virus. Phytopathology 47:20 (Abstr.).
10. MOLANDER, C. W., H. J. WEINBERGER, and B. M. KAGAN. 1965. Growth of staphylococcal L-forms on and through membrane discs of various pore sizes. J. Bact. 89:907.
11. RAZIN, S. 1969. Structure and function in mycoplasma. Annu. Rev. Microbiol. 23:317-356.
12. SINHA, R. C., and Y. C. PALIWAL. 1969. Association, development and growth cycle of mycoplasma-like organisms in plants affected with clover phyllody. Virology 39:759-767.