Condition of the Phloem and the Persistence of Mycoplasmalike Organisms Associated with Apple Proliferation and Pear Decline

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ABSTRACT

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The persistence of mycoplasmalike organisms (MLO) associated with apple proliferation and pear decline depends on the condition of the host's phloem. In October, the secondary phloem of the stem of both species was largely functional, MLO could be readily found in the sieve tubes of diseased trees, and the pathogen could be transmitted easily. In November and December, the sieve tubes of both species were degenerating, but MLO were still detectable in infected trees and could still be transmitted. In January and February, however, in the majority of apple trees and in all pear trees that were investigated, only a few intact sieve tubes were present. In apple trees with a pronounced replacement phloem, there were phloem areas with a large number of functional sieve tubes adjacent to the cambial zone. Accompanying the degeneration of sieve tubes in the fall and winter there was a complete disappearance of MLO from the stem phloem of most trees and a sharp reduction in the levels present in the remainder. Relatively high numbers of MLO still were observed in diseased apple trees with replacement phloem that contained many functional sieve tubes. Stem scions from this latter group of trees gave the only positive results in grafting transmission experiments performed in February. In roots, functional sieve tubes were present in relatively high numbers in all samples taken from apple and pear trees during the winter. On the monthly average, living sieve elements were present in 40–55% of the phloem in the time between October to March. For both diseases, MLO could always be observed in the roots of diseased trees during this period and both diseases could be transmitted readily by root-grafting. It is concluded that the MLO survive mainly in the root systems of affected plants during the winter and that they spread from there to the aerial parts in the spring. Overwintering of the organisms in the stem seems to occur only in exceptional cases.

Mycoplasmalike organisms (MLO) are probably responsible for apple proliferation and pear decline (2,8,10,12). Results from grafting experiments indicate that the persistence of MLO in different parts of trees may vary with seasonal fluctuations. Apple proliferation can be transmitted readily by grafting of stem scions in summer or fall (15) or—with somewhat variable results—by budding (1,3). On the other hand, Seidl and Komarkova (23) could not transmit it by grafting dormant scions, although Schuch (19) claimed limited success for stem-grafting experiments performed in winter. Transmission by root-grafting in winter is generally successful (11,22) and for this reason, has become an established method for indexing trees.

Working with pear decline, Griggs (9) could not transmit the pathogen by grafting dormant scions during the month of March. Schneider (16,17) obtained no transmission in one experiment and limited success in another, when he attempted to transmit pear decline by grafting stem scions from dormant plants after storage in a cold room at 4C. However, he was able to transmit the pathogen when he used roots from dormant plants or shoots from plants in leaf. Seemüller et al (21) confirmed that pear decline could be transmitted by grafting roots from dormant plants.

Schaper (15) used fluorescence microscopy for direct examination of the MLO within the phloem of apple and pear trees. She found that the numbers of MLO in the stem varied considerably during the year; they increased to a maximum from summer to fall, completely disappeared during the winter in almost all cases, and declined to very low numbers in all others. They were always present in the roots of the trees in winter.

There are several ways in which this decline in the detectability and transmission of MLO in the upper part of apple and pear trees during winter could be explained. Westwood and Cameron (26) proposed that the causal agent of pear decline is so sensitive to low temperature that it cannot persist in the tops of the trees over the winter. Another possibility is that the persistence of the MLO is linked to the condition of the phloem. Since it is probable that MLO can persist only in functional sieve tubes (13), the organisms would not survive if these elements degenerated. According to Evert (5,6) a complete degeneration of sieve tubes of the phloem occurs in both apple and pear at the end of the growing season. If there is a link between phloem condition and persistence of MLO, the phloem in the roots must remain functional during winter. Such a situation has been reported by Braun and Sinclair (4), who found functional sieve tubes in roots, but not in the tops, of elm trees. No such study has yet been performed with apple and pear or with other woody plants.

The relationship between the condition of the phloem in roots and upper parts of apple and pear and the persistence of MLO in these species is reported in this paper.

MATERIALS AND METHODS

Histological studies. Two apple cultivars (Golden Delicious and Cox's Orange Pippin) and two pear cultivars (Beurre Hardy and Bartlett) were studied. The apples were grafted onto rootstocks M 4, M 11, or MM 104 and the pears onto seedlings of cultivar Kirchensaller Mostbirne. All the trees were located in the experimental orchard at Dossenheim.

Samples for microscopical studies were taken from 3- to 15-mo-old shoots, from branches or trunks several years old, and from roots (2–20 mm in diameter) between October and March over a 3-yr period. The material was fixed in 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0. For anatomical studies of the phloem, fixed samples were embedded in glycol methacrylate, and sections 1- to 2-μm thick were stained with toluidine blue. The phloem was considered functional if the sieve tubes were turgid and the adjacent companion cells contained a protoplast that was not plasmolyzed

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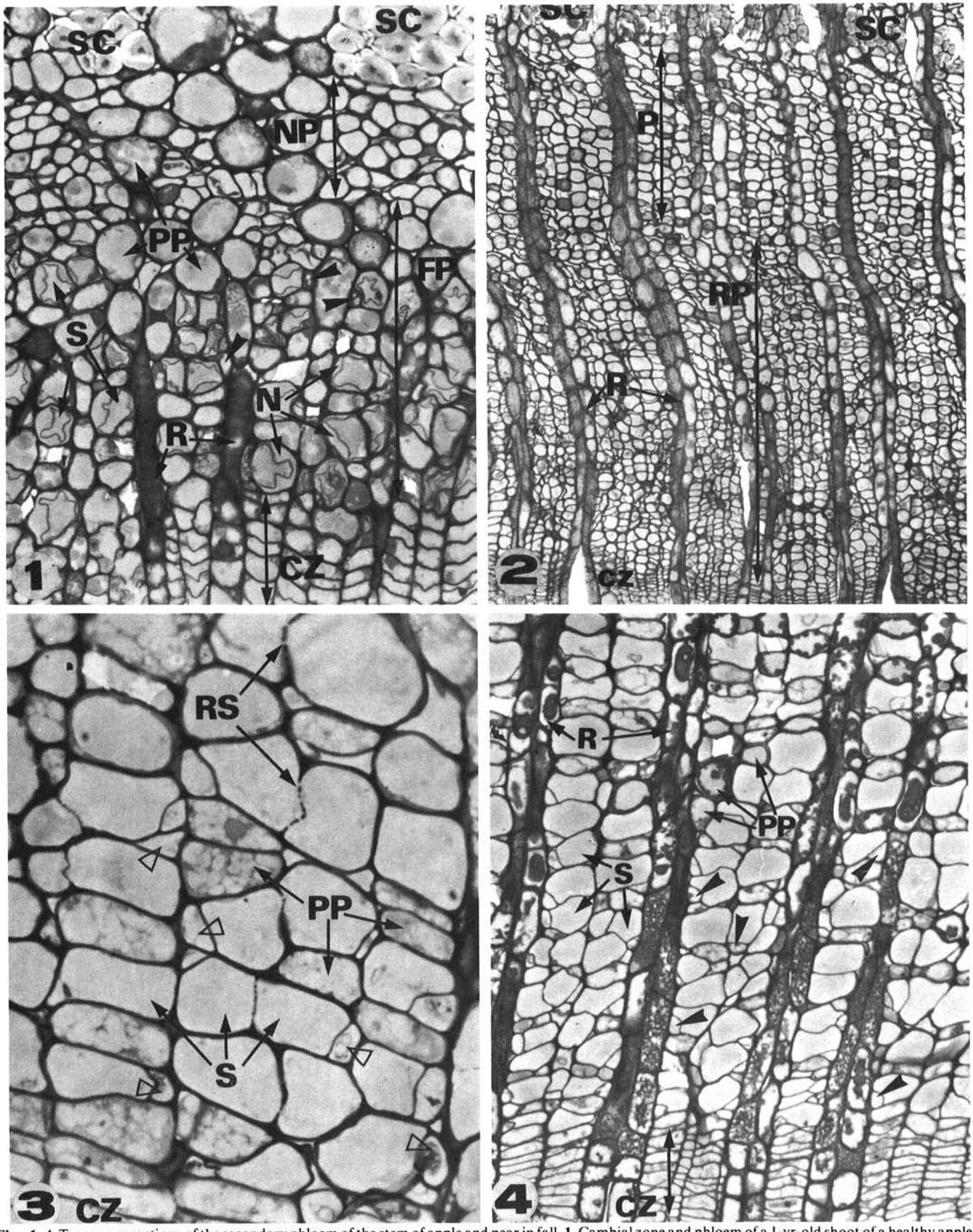


Figs. 1–4. Transverse sections of the secondary phloem of the stem of apple and pear in fall. **1,** Cambial zone and phloem of a 1-yr-old shoot of a healthy apple tree at the beginning of October. The largest part of the phloem shows functional sieve tubes (FP), most of which have nacreous walls (N). In the oldest part of the phloem the sieve tubes are collapsed (NP). ×460. **2,** Current season's phloem of the trunk of a diseased apple tree consisting of a ring of spring-formed phloem with necrotic sieve tubes (P) and a broad ring of replacement phloem (RP). Collected in November. ×120. **3,** Phloem with degenerating sieve tubes of the trunk of a healthy apple tree towards the end of November. Indications of degeneration are the plasmolysis and the large vacuoles of the companion cells (►). RS, radial sieve areas. ×740. **4,** Phloem with degenerating sieve tubes of the trunk of a diseased pear tree, collected in December. The conditions of the phloem is similar to that given in Fig. 3 for healthy apple. ×290. ⊳ = companion cell; CZ = undifferentiated cambial zone; PP = phloem parenchyma; R = ray; S = sieve tube; SC = fiber-sclereids.

or strongly vacuolated (5,7). To determine the presence of MLO in the sieve tubes, the fixed material was sectioned with a freezing microtome, stained with a DNA indicator (4'-6-diamidino-2-phenylindole [DAPI]; Serva, Heidelberg, West Germany), and examined by using fluorescence microscopy (Leitz epifluorescence illuminator) according to the methods of Ploem (20).

Transmission experiments. Transmission graftings of roots and stems from infected trees were performed in October, November, December, and February from the fall of 1978 to the spring of 1981. Trees from which the infected grafting material was taken had all shown typical symptoms and yielded positive results in the DAPI test. Material from infected apple trees was grafted onto 1- or 2-yr-old plants of rootstock M 11, and that from pear trees onto similar-aged seedlings of cultivar Kirchensaller Mostbirne. Root grafts were made as whip-and-tongue grafts, and shoot grafts as side grafts. Scions from an indicator plant were top-grafted onto the same plant. Cultivars Golden Delicious and the seedling Precocious (18) were used as indicators for apple and pear, respectively. When Golden Delicious was the pathogen source, an indicator graft was not necessary. Ten to 15 test plants were grafted with tissue from each donor tree that was investigated. Plants used as sources of indicator and check material were always kept isolated in a screened house.

After being grafted, the test plants were kept in an insectproof greenhouse for several weeks until the grafts showed signs of growth. The plants were chilled in a cold room at 4 C for 8–12 wk then returned to the greenhouse where they remained until the end of the growing season. Usually they were chilled again and observed for another season.

RESULTS

Stem phloem of apple and pear in the fall. The sieve tubes of the secondary phloem of branches and trunks of apple and pear trees degenerated during the fall, thus confirming the observations of Evert (5,7). The degeneration began in the oldest part of the tissue and proceeded towards the cambial differentiation zone. Most of the sieve tubes that had not been damaged by infection were still functional in October (Fig. 1). If, however, the trees were seriously diseased, then sieve tubes of the phloem formed in spring were necrotic and somewhat collapsed and a ring of replacement phloem was present as shown in Fig. 2 for apple.

The highest numbers of MLO in affected apple and pear trees were observed in October, when both diseases could be transmitted easily by grafting. The diseases developed in 26 of 29 apple trees and to 19 of 20 pear trees inoculated by shoot-grafting.

From November to the end of December, a more or less complete degeneration of the functional sieve tubes took place (Figs. 3 and 4). Usually it was less pronounced in the replacement phloem of diseased trees than in the spring-formed phloem. This was true for all stem parts that were investigated.

The numbers of observed MLO decreased during November and particularly in December. However, the organisms could still be detected in both functional and nonfunctional sieve tubes until the end of December; the pattern of their distribution within the sieve tubes was unchanged and typical. The MLO were usually detectable as single fluorescent particles, but where their numbers were sufficiently high, part or all of the sieve tube fluoresced (Fig. 9). The MLO fluorescence also was present in nonfunctional sieve tubes; thus, it seems either that the MLO died after the tubes had degenerated, or that they continued to fluoresce after death. The pathogens were readily transmissible and infective until the end of December.

Stem phloem of apple in winter. In January and February, 23 orchard trees (17 diseased and six healthy) were examined. In 13 of the diseased trees, and in all six healthy ones, a few small sieve tubes with cytoplasm-rich companion cells were observed at the margin of the undifferentiated cambial zone (Fig. 5). Probably not all of these young sieve tubes were fully differentiated. The remaining four diseased trees had a greater number of functional sieve tubes; these were located within several layers of the youngest part of a broad replacement phloem (Fig. 6). The average width of the phloem in these four trees was 535 μm compared with 333 μm for the other 13 diseased trees and 260 μm for the healthy ones. In the 13 diseased trees without these greater numbers of functioning sieve tubes, only one had typical replacement phloem. It would appear that the prolonged function of a larger number of sieve tubes was associated with the presence of replacement phloem.

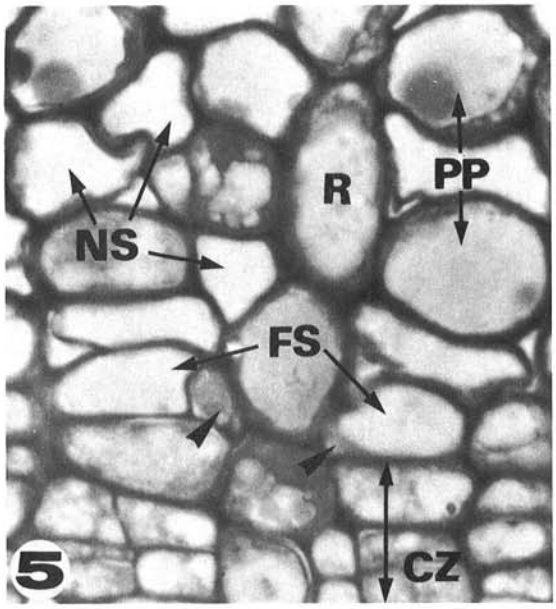
The persistence and pattern of distribution of MLO within infected trees changed during January and February. MLO viewed by fluorescence microscopy appeared to aggregate into 'stringlike' structures (Figs. 10 and 11). The 'strings' were always located along the cell walls of the sieve tubes. Of 24 trees which in the previous autumn had MLO with typical colonization pattern, 15 contained the 'stringlike' structures and three gave no reaction in February. There were further changes during March and April. At this time a typical DAPI reaction could only be found in a few exceptional cases and in many cases the 'stringlike' structures had also disappeared.

February transmission trials using stem pieces from orchard trees proved positive for one donor tree in 1979, for one of three trees in 1980, and for two of five trees in 1981. About 50, 90, and 30%, respectively, of the recipient indicator trees showed symptoms. All the orchard trees from which transmissions were obtained had replacement phloem with several layers of functional sieve tubes adjacent to the cambium and they gave a positive DAPI test. Moreover, the morphology of the MLO in the functional sieve tubes was like that observed in the fall. In contrast, trees with negative transmission results had only a few intact sieve tubes adjacent to the cambial zone. One of them had replacement phloem. All these trees contained MLO as assessed by the DAPI test—in one case typical, in the others 'stringlike'—but in both cases the fluorescing bodies were restricted to the degenerated sieve tubes.

The persistence of the MLO in the upper parts of the trees appeared to be uninfluenced by winter temperatures, which differed widely in southwestern Germany in the winters of 1978/1979 and 1979/1980. In the winter of 1978/1979 there was a 20-day period of severe frost in January, when the temperature was never above 0 C and the minimum recorded temperature was −19 C. Another 3 days of continuous frost occurred in February. The winter of 1979/1980 was much milder with only 7 days of continuous frost, all of which occurred in January. The minimum recorded temperature was −11 C.

Stem phloem of pear in winter. Seven healthy and 10 diseased trees were examined anatomically in January and February. Only one diseased tree had replacement phloem. All of the samples had functional sieve tubes, which were always few in number, small in

Figs. 5–11. Transverse sections of the secondary phloem of the stem of apple and pear in February, and patterns of sieve tube infection by mycoplasmalike organisms (MLO). **5,** Part of the cambial zone and youngest part of previous season's phloem of a diseased apple. The phloem contains two intact sieve tubes adjacent to the cambial zone. The others are degenerated. ×1,170. **6,** Youngest part of a wide ring of replacement phloem of a diseased apple tree with several functional sieve tubes. Most of them have nacreous walls (N). ×1,170. **7,** Cambial zone and youngest part of previous season's phloem of a diseased pear tree. Shows two intact sieve tubes at the margin of the cambial zone. The others are degenerated. ×1,170. **8,** Youngest part of previous season's phloem of a diseased pear tree with no functional sieve tubes. ×460. **9,** MLO fluorescence after DAPI staining in the phloem of a diseased apple shoot in October. Several sieve tubes are heavily infected and show the MLO as single particles or small aggregates (MLO), or as larger fluorescent areas (MA). The oldest sieve tube shows "stringlike" structures along the cell wall. ×790. **10,** Fluorescence patterns of a degenerating MLO population in a diseased apple shoot in February. In three sieve tubes the MLO are aggregated along the cell walls to form "stringlike" structures. ×790. **11,** "Stringlike" structures in a diseased apple shoot in February. The degeneration of MLO is more advanced than in Fig. 10, as is evidenced by the stronger aggregation and the disappearance of the "strings." ×790. ▶ = companion cell; CZ = cambial zone; FS = functional sieve tube; MS = MLO containing sieve tube; NS = nonfunctional sieve tube; NU = nucleus; PP = phloem parenchyma; R = ray; S = sieve tube; and ST = "stringlike" structures.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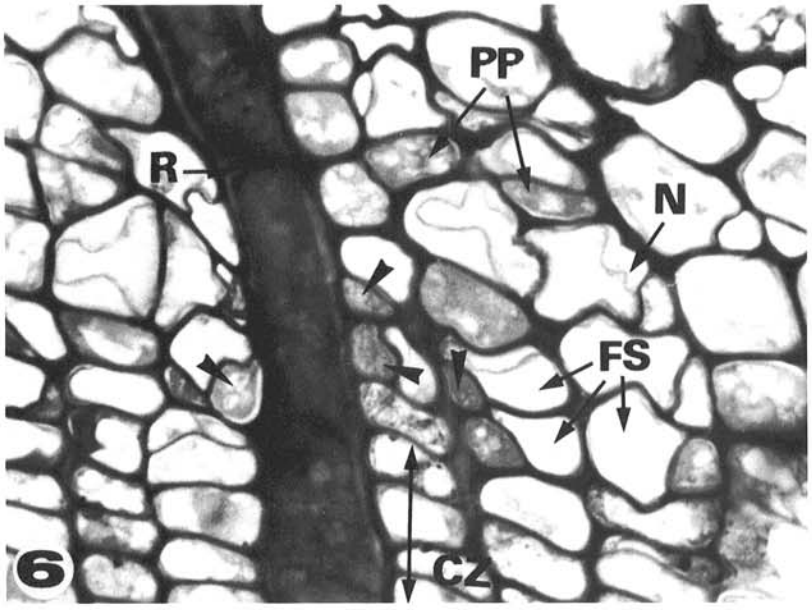
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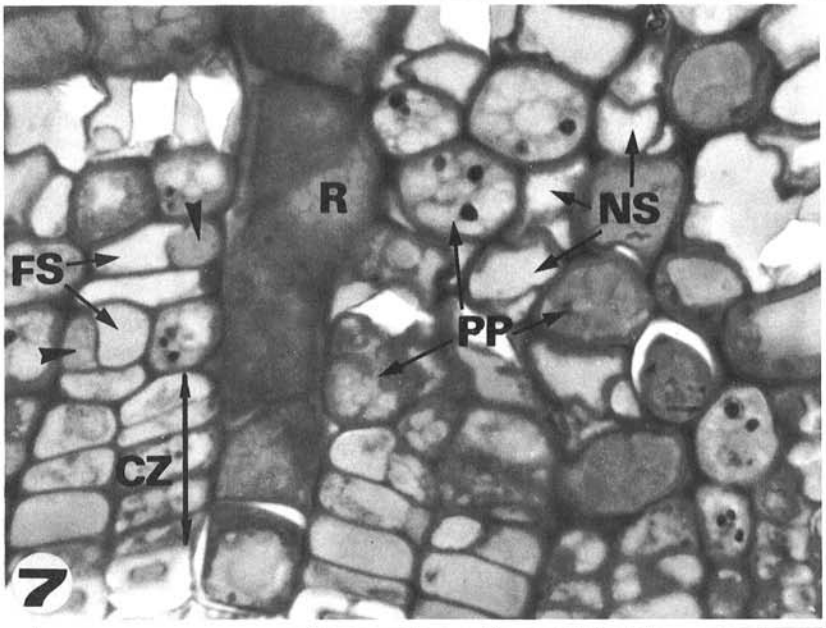
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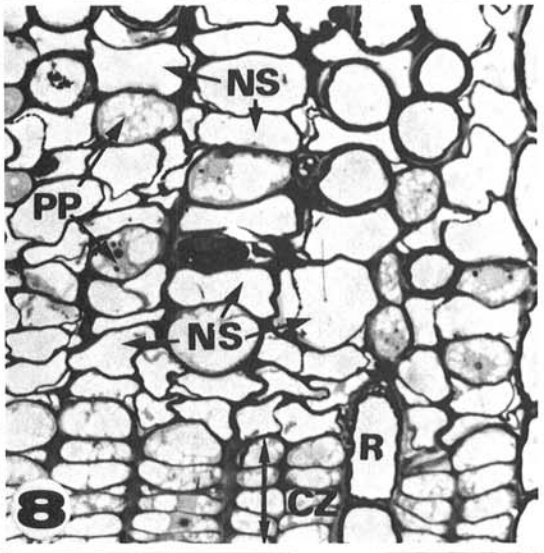
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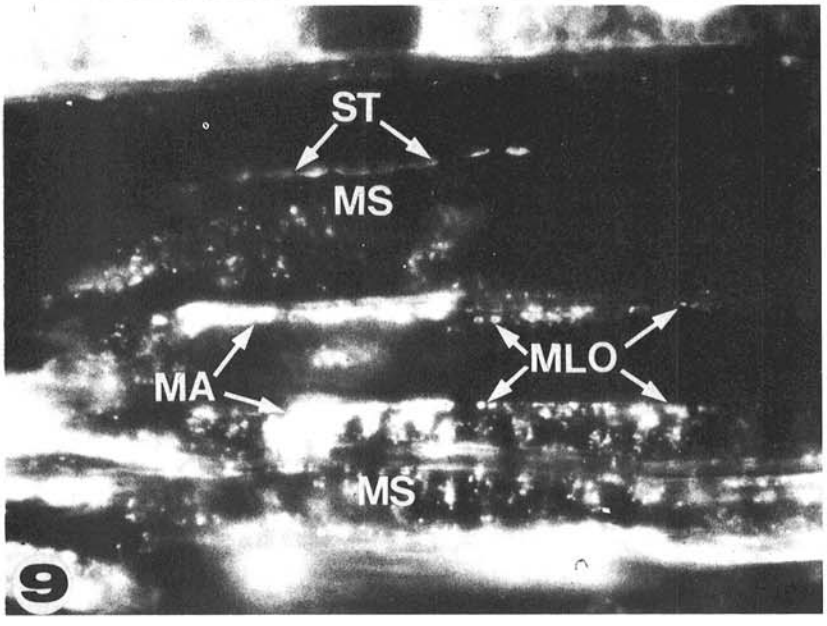
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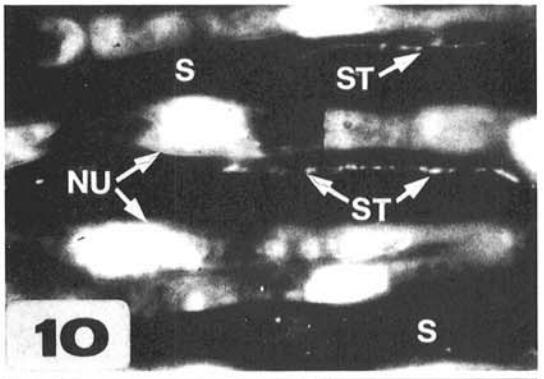
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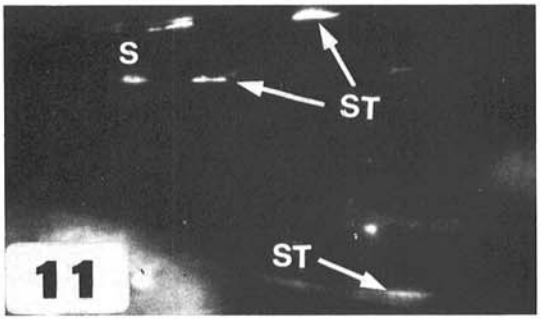
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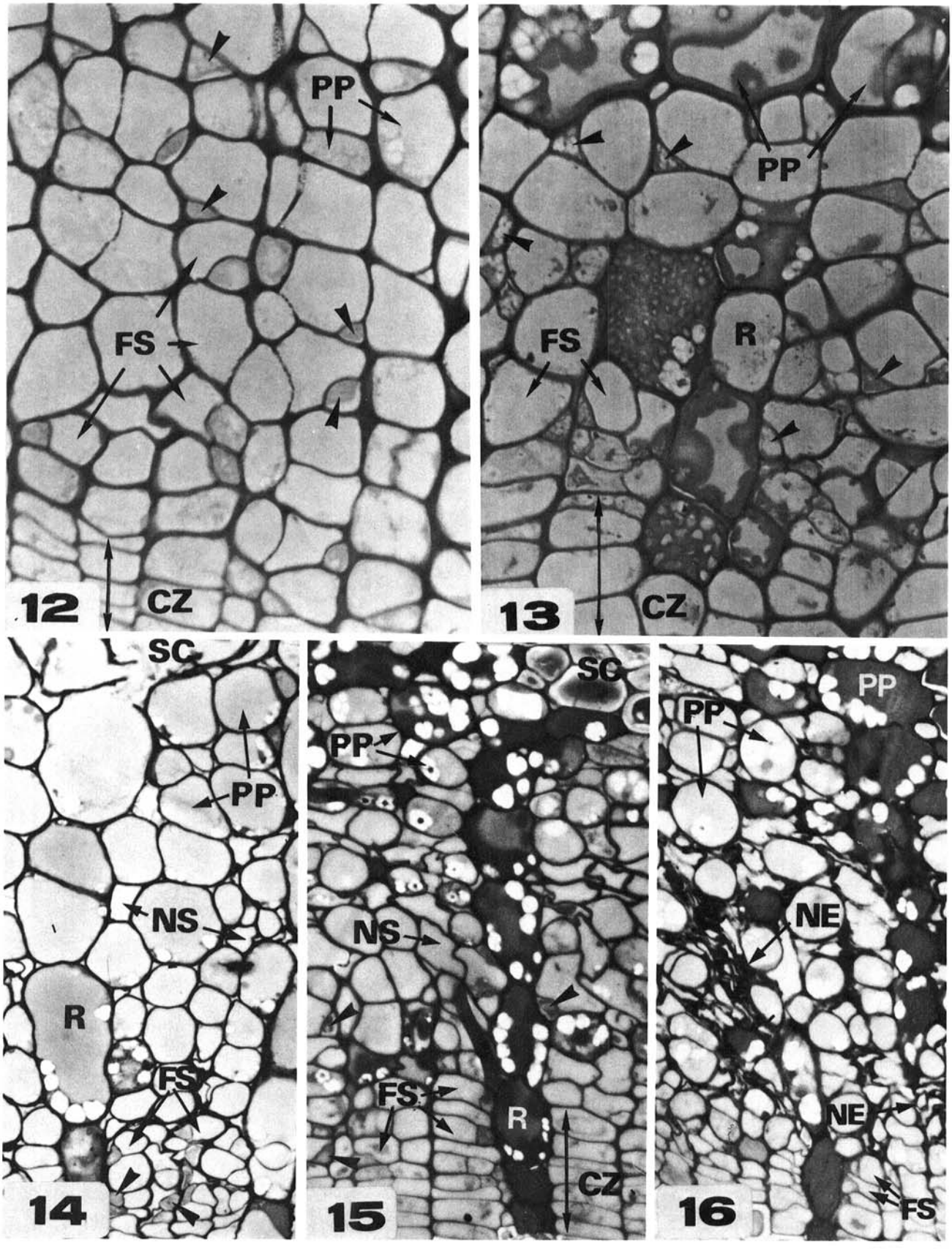

Figs. 12–16. Transverse sections of the root phloem of apple and pear trees in winter. **12,** Part of the phloem of a diseased apple tree in February with many functional sieve tubes. ×790. **13,** Part of the phloem of a diseased pear tree in January with many functional sieve tubes ×790. **14,** Phloem of a diseased apple tree in February. Functional sieve tubes are present in a few cell layers adjacent to the narrow cambial zone. In the older phloem the sieve tubes are partially collapsed due to enlargement of parenchyma cells. ×400. **15,** Cambial zone and phloem of a diseased pear tree in December. Functional sieve tubes are present in six to eight of the youngest cell layers. In the older phloem the sieve tubes are degenerated and partially collapsed. ×400. **16,** Cambial zone and phloem from a healthy pear tree in December. In this particular part of the root only a few intact sieve tubes are present. They are small and are located adjacent to the cambial zone. Note the pronounced phloem necrosis (NE) which extends from the functional part to about the middle of the phloem. ×400. ▶ = companion cell; CZ = cambial zone; FS = functional sieve tube; NS = nonfunctional sieve tube; PP = phloem parenchyma; R = ray; and SC = fiber-sclereids.

size, and located directly adjacent to the cambial zone (Fig. 7). In these respects, they were similar to those in the majority of the apple trees. There were no cases of the type occasionally found in diseased apple trees, in which larger numbers of functional sieve tubes occurred. In addition, most of the samples had areas in which all the sieve tubes had degenerated (Fig. 8). This applied to all stem parts of pear trees.

The degeneration of MLO was more pronounced in orchard pear trees than in apples. In February, 21 trees (all of which had been rated as positive by the DAPI test the previous autumn) were reexamined. In only two was the typical fluorescence reaction recorded. 'Stringlike' structures as described for apple in Figs. 10 and 11 were found in six others, and nothing was observed in the remainder. Tests in March showed that the two trees that gave a typical reaction in February were still positive, 'stringlike' structures were present in three trees, and nothing could be found in the others. Transmission experiments were performed in mid-February 1979, 1980, and 1981 on two, three, and three of the above trees, respectively. In all 3 yr one of the trees tested negative for MLO, six contained 'stringlike' structures, and one showed a typical DAPI reaction. The disease was not transmitted in either year.

Root phloem of apple and pear in winter. Root samples were taken monthly from October to March from 1978 to 1981, and a total of 16 diseased and eight healthy trees of each species was sampled. All samples had rings of phloem with functional sieve tubes adjacent to the cambium, but the width of these rings varied considerably. At one extreme only three to four cell layers with intact sieve tubes could be found, while at the other extreme over 90% of the phloem from the cambium to the fibers contained functional sieve tubes (Figs. 12–16). The monthly averages for both species ranged between 40 and 55%. No differences were detected between samples from healthy and diseased trees—the extremes and averages were similar for both. These results indicate that from some to most of the root phloem remained functional throughout the winter unlike the nearly complete degeneration of sieve tubes that occurred regularly in the stem.

No major changes were noted in the persistence of MLO in the root systems of apple and pear as winter progressed. MLO were always present in relatively high numbers. The tissue in which the typical fluorescence patterns occurred were those in which the phloem was still functional, while 'stringlike' structures were occasionally observed in degenerated sieve tubes in older parts of the phloem.

The results of transmission experiments generally corresponded with the above observations. Using roots from two diseased pear trees in 1979, from one apple and one pear in 1980, and from two apple trees and two pear trees in 1981, the disease was successfully transmitted in each case to about half of the inoculated plants.

DISCUSSION

There are previous studies that support the idea that MLO may overwinter in the roots of their hosts. Apart from the already mentioned transmission experiments with apple (11,22) and pear (17,21), studies with other woody plants indicate that such overwintering may be a general phenomenon. Thus, Stoddard (24) and Rosenberger and Jones (14) obtained good transmission of the X-disease of stone fruits in winter by grafting root parts, but transmission by shoot grafting was either unsuccessful or only successful in a small number of cases. From electron microscopy studies and observations in disease development, Tahama (25) concluded that the agent responsible for mulberry dwarf survived in the roots of the host plant during winter, and that it spread to the aerial parts in spring. These various studies are in general accord with Schaper's (15) results obtained by fluorescence microscopy. She found that although the MLOs that cause apple proliferation and pear decline were always present in the roots during winter, they either disappeared completely, or declined to very low numbers in the upper parts of the plants.

Of all who have studied the persistence of MLO in host plants, only Braun and Sinclair (4), who investigated phloem necroses of elm, related the condition of the phloem to the survival of the causal organisms. They found no functional sieve tubes in the stems of elms in winter, but did find intact sieve tubes in the roots. They concluded that the pathogen overwintered in the roots, and later reinfected the top of the plants. Results of our studies suggest that apple proliferation and pear decline are somewhat similar to elm phloem necrosis. Phloem areas with functional sieve tubes were present in the roots of healthy and diseased apple and pear trees from October to March, and MLO could always be observed by fluoresence microscopy in diseased trees. The distribution of MLO within roots corresponded well with the distribution of functional sieve tubes and their fluorescence pattern within the tubes was typical. This evidence, together with the successful transmission of the agents by root-grafting, means that the MLO must persist in the roots over the winter. Such survival in the root systems of apple and pear applies to all other times of the year, not just winter (15).

Although MLO survival in the roots of affected woody hosts appears to be a general phenomenon, no such consistent view emerges from studies done on the stem. Rosenberger and Jones (14), working with X-disease of stone fruits, found that the agent could survive the winter in shoots, but that their numbers appeared to be significantly smaller than in the summer. On the other hand, as already mentioned, there is no indication that the organisms that cause mulberry dwarf and elm phloem necrosis survived at all in the upper parts of their hosts (4,25). In the case of elm phloem necroses there was a clear relationship between the survival of the MLO and condition of the phloem.

Under different climatic conditions, Evert (5,7) found that the sieve tubes of the stem phloem of apple and pear trees degenerate completely at the end of the growing season. In all of our samples collected in February, intact sieve tubes were present whether the trees were healthy or diseased. These sieve elements could be grouped into two categories on the basis of number and location. In the first category, there were only a few young elements adjacent to the undifferentiated cambial zone. The sieve tubes were mostly relatively narrow and had cytoplasm-rich companion cells. This type of phloem was present in the majority of apple trees and in all pear trees examined. Neither disease could be transmitted in winter from trees with this phloem arrangement. This may be because the young elements were not yet infected by MLO or that the sieve tubes may not have been completely differentiated, thus were not yet a suitable environment for MLO development. In the second category, functional sieve tubes were present in greater numbers than in the first. They appeared to be fully differentiated and were located in the younger parts of wide rings of replacement phloem of diseased apple trees. Their occurrence can therefore be considered a consequence of the disease. As the replacement phloem develops after the destruction of the normal phloem, the sieve tubes in this tissue were younger than in normal phloem. This may explain: why they were functioned longer during the growing season and could possibly persist throughout the winter; why MLOs persisted in the stem of these trees; and why the disease could only be transmitted by stem-grafting in February when this type of phloem was present.

The second type of phloem arrangement and successful transmission of MLO in winter from stems was observed only in apple, but a similar situation may occur in pear. Under conditions different from that described in this paper, Schneider (17) could transmit pear decline by grafting stem scions from dormant greenhouse plants that had been held at 4 C for dormancy chilling. Similar results from comparable transmission experiments performed in February with a diseased pear were obtained by Schaper (15). The tree used in her study did not show a replacement phloem. Therefore, it seems to be possible that the degeneration of sieve tubes of trees grown in the greenhouse and kept in a cold room for dormancy chilling proceeds slower and/or is not as complete as in orchard trees during the winter. The extensive degeneration usually occurring in orchard trees might be caused by the lower temperatures in the field or by other factors. But the degeneration of MLO in the stem of orchard trees during winter is unlikely to be due to the sensitivity of the organisms to cold as suggested by Westwood and Cameron (26) for pear decline. Even after the low

temperatures in January 1979, the apple proliferation agent, which seems to be closely related to the pear decline organisms, could be transmitted by stem-grafting when the scions showed larger areas with functional sieve tubes.

LITERATURE CITED

1. Baumann, G. 1965. Die Übertragung der Virösen Triebsucht auf Apfelsämlinge im Gewächshaus. Nachrichtenbl. Dtsch. Pflanzenschutzdienstes (Braunschw.) 17:73-75.
2. Behnke, H.-D., Schaper, U., and Seemüller, E. 1980. Elektronen mikroskopischer Nachweis von mykoplasmaähnlichen Organismen bei Birnbäumen mit pear-decline-Symptomen in der Bundesrepublik Deutschland. Phytopathol. Z. 97:89-93.
3. Bovey, R. 1963. Observations and experiments on apple proliferation disease. Phytopathol. Mediterr. 2:111-114.
4. Braun, E. J., and Sinclair, W. A. 1976. Histopathology of phloem necrosis in *Ulmus americana*. Phytopathology 66:598-607.
5. Evert, R. F. 1960. Phloem structure in *Pyrus communis* L. and its seasonal changes. Univ. Calif. Publ. Bot. 32:127-194.
6. Evert, R. F. 1963. Ontogeny and structure of the secondary phloem in *Pyrus malus*. Am. J. Bot. 50:8-37.
7. Evert, R. F. 1963. The cambium and seasonal development of the phloem in *Pyrus malus*. Am. J. Bot. 50:149-159.
8. Giannotti, J., Morvan, G., and Vago, C. 1968. Micro-organismes de type mycoplasme dans les cellules libériennes de *Malus sylvestris* L. atteint de la maladie des proliférations. C. R. Acad. Sci. Paris 267:76-77.
9. Griggs, W. H. 1965. Topgrafting potted trees. Pages 10–11 in: Progress report on pear decline. University of California, Div. Agric. Sci., Berkeley.
10. Hibino, H., and Schneider, H. 1970. Mycoplasmalike bodies in sieve tubes of pear trees affected with pear decline. Phytopathology 60:499-501.
11. Kunze, L. 1972. Untersuchungen zum Nachweis der Triebsucht des Apfels im Serientest. Mitt. Biol. Bundesanst. Land- Forstwirtsch., Berlin-Dahlem 144:35-46.
12. Marwitz, R., Petzold, H., and Kunze, L. 1973. Elektronenmikroskopische Untersuchungen über das Vorkommen mykoplasmaähnlicher Organismen in triebsuchtkranken Apfelbäumen. Phytopathol. Z. 77:84-88.
13. McCoy, R. E. 1979. Mycoplasmas and yellows diseases. Pages 236–239 in: R. F. Whitcomb, and I. G. Tully, eds. The Mycoplasmas. Vol. III. Plant and Insect Mycoplasmas. Academic Press, New York. 351 pp.
14. Rosenberger, D. A., and Jones, A. L. 1977. Seasonal variation in infectivity of inoculum from X-diseased peach and choke-cherry plants. Plant Dis. Rep. 61:1022-1024.
15. Schaper, U. 1981. Untersuchungen zum Nachweis und Besiedlungsverhalten sowie zur Bekämpfung der Erreger der Triebsucht des Apfels und des Birnenverfalls. Diss. Univ. Göttingen. 147 pp.
16. Schneider, H. 1970. Graft transmission and host range of the pear decline causal agent. Phytopathology 60:204-207.
17. Schneider, H. 1971. Infectivity of stem scions versus root scions from pear decline-infected *Pyrus* sp. 'Variolosa' trees following dormancy chilling. (Abstr.) Phytopatholgy 61:1324.
18. Schneider, H. 1977. Indicator hosts for pear decline: Symptomatology, histopathology, and distribution of mycoplasmalike organisms in leaf veins. Phytopathology 67:592-601.
19. Schuch, K. 1962. Untersuchungen über die Triebsucht des Apfels. Phytopathol. Z. 43:37-47.
20. Seemüller, E. 1976. Investigations to demonstrate mycoplasmalike organisms in diseased plants by fluorescence microscopy. Acta Hortic. 67:109-112.
21. Seemüller, E., Kunze, L., and Schaper, U. 1980. Übertragung des europäischen Birnenverfalls (pear decline) auf die amerikanischen Indikatorsorten 'Magness' und 'Precocious.' Nachrichtenbl. Dtsch. Pflanzenschutzdienstes (Braunschweig) 32:33-35.
22. Seidl, V. 1965. The possibility of using root grafting method of testing for apple proliferation virus disease. Zâstita Bilja 16:323-327.
23. Seidl, V., and Komarkova, V. 1973. On some problems of apple proliferation disease. Pages 487–493 in: Proc. 7th Conf. Czechoslov. Plant Virologists, 1971, Slovak. Acad. Sci. Bratislava.
24. Stoddard, E. M. 1947. The X-disease of peach and its chemotherapy. Conn. Agric. Exp. Stn. Bull. (New Haven) 506. 19 pp.
25. Tahama, Y. 1975. Studies on the mulberry dwarf disease. XXIII. Electron microscopic examination on the mycoplasmalike organisms in the overwintered mulberry trees. Bull. Hiroshima Agric. Coll. 5:151-158.
26. Westwood, M. N., and Cameron, H. R. 1978. Environment-induced remission of pear decline symptoms. Plant Dis. Rep. 62:176-179.